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FACULTY OF FOOD TECHNOLOGY OSIJEK, CROATIA**

**ICC – INTERNATIONAL ASSOCIATION FOR
CEREAL SCIENCE AND TECHNOLOGY**

**Proceedings of the
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FLOUR – BREAD '13**

**9th CROATIAN CONGRESS
OF CEREAL TECHNOLOGISTS**

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FLOUR – BREAD '13
9th CROATIAN CONGRESS
OF CEREAL TECHNOLOGISTS

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PREFACE

The 7th International Congress and the 9th Croatian Congress of Cereal Technologists "Flour-Bread '13" was held from 16-18 October 2013 in Opatija, Croatia. The "Flour-Bread" Congress has been organized traditionally every second year since 1997 and become one of the most significant European scientific meetings in the field of cereal science and technology. The research results presented at "Flour-Bread" Congresses provides faster acceptance of the latest scientific and professional achievements in cereal production and processing, development of new products and quality enhancement.

The "Flour-Bread '13" Congress topics were the following: breeding and quality of cereal grains, grain storage and milling technology, analytical and rheological methods, baking technology, improvers and additives, starch and modified starch, extrusion and pasta production, biscuit and pastry products, nutritional quality of cereals, cereal food safety and cereal based functional foods.

Despite the economic recession worldwide, over 200 experts from 25 countries took part in the Congress (Austria, Bosnia and Herzegovina, Brazil, Czech Republic, France, Iraq, Iran, Italy, Jordan, China, Latvia, Lithuania, Hungary, Malaysia, Mexico, Netherlands, Germany, Poland, Saudi Arabia, Slovakia, Slovenia, Serbia, Spain, Turkey, the United Kingdom and of course Croatia). There were 43 oral presentations and 68 posters at the Congress. Eleven national and international companies presented their production programmes, process and laboratory equipment and cereal industry products. Many new scientific achievements were introduced to the Congress participants and they also had opportunity to exchange their personal experiences with the experts from various institutions and industry.

Editors

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SCIENTIFIC PAPERS

POSSIBILITIES OF PROCESSING LEFTOVER BREAD FROM AN ECONOMIC AND TECHNICAL POINT OF VIEW

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ABSTRACT

Bread returns and leftovers as well as leftover doughs in bakeries are valuable raw materials for the production of food and feed products. The in-line use of bread and dough leftovers in bakeries, e. g. processing it to obtain sourdoughs or syrups, is common practice. Bread returns and leftovers are also dried and milled to produce feed meals. All of these are also used for energy production. They serve as a carbon source for the production of ethanol or methane and can also be burned to produce heat. In their enzymically digested form, they can replace molasses as a substrate in the production of baker's yeast. In addition to their reuse, a substantial proportion of leftover bread is disposed of as waste.

In Germany, the primary use of bread as food accounts for 85% of the total production of 4.7 million t/a. The remaining 15% are not consumed and represent a monetary value of 1.7 billion EUR/a. Half of the uneaten bread finds a secondary use, mainly by making it available as feed products. The proceeds of this secondary use are less than the price of a comparable amount of fodder cereal. The greatest gain can be made when dried bread returns and/or leftovers are directly burned.

Besides the in-line reuse of bread returns and leftovers, all other methods result in sharp reductions in the proceeds originally gained by processing flour to make bread. Therefore, bread returns place a constant burden on the economical production of bread. This burden can only be marginally reduced by the current prevailing conditions for bread production and marketing.

In addition, bread that is sold but not eaten is a substantial source of food waste. The losses in income caused by its disposal amount on average to about 10 EUR/consumer/a in Germany. These losses are an unwanted tribute to our modern life.

Keywords: bread, dough, returns, leftovers, processing, economic evaluation

INTRODUCTION

The issue of food waste has been the subject of public interest in many countries for ethical, social, environmental and economic reasons. A study entitled “Determining how much food is thrown away and proposals for reducing the amount of food that is thrown away in Germany” has been conducted by the Institute for Sanitary Engineering, Water Quality and Solid Waste Management at the University of Stuttgart on behalf of the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) in order to demonstrate the magnitude of the problem [1]. The amounts of food waste were determined over the value-added chain for food – from food production via the food trade to the consumption of food by consumers or bulk users (restaurants and canteens) as end users – but without taking agriculture into account.

To enable food waste to be quantified, a definition of the term was first established. According to the definition, food waste is classified as “unavoidable”, “partly avoidable” or “avoidable” waste. Thus waste occurring during the preparation of food is unavoidable, leftovers are partly avoidable and unsaleable food is avoidable waste (Figure 1).

Food waste	Kind
Unavoidable	Waste during preparation of food
Partly avoidable	leftovers
Avoidable	Un-consumed food; saleable but unsold food

¹⁾ Source: ISWA-Studie, Stuttgart, Februar 2012; Kranert, M. et al.:
Ermittlung der weggeworfenen Lebensmittelmengen und Vorschläge zur
Verminderung der Wegwerfrate bei Lebensmitteln in Deutschland.

Figure 1. Food waste by definition ¹⁾

The study showed that a total of 11 million tonnes of food are wasted in Germany every year (Table 1). Households account for 6.7 million t/a, for example, of which 2.5 million t/a are unavoidable waste produced during the preparation of food. This reduces the amount of needless food waste for which consumers are responsible to 53 kg food per head per year. The value of the food waste is around 234 EUR per head per year. Another study [2] has put the value of all the food wasted by consumers at 310 EUR per

head per year. The nominal market value of food waste in Germany is thus around 25 billion EUR per year.

Table 1. Food waste along the value-added chain from processing and selling to consuming food ¹⁾

Chain-link	Amount of food waste (10⁶ t/a in Germany)
Processing / trading	2.4
Consuming	
- household	6.7 (2.5) ²⁾
- large-scale consumer	1.9
Total	11.0

¹⁾ Source: ISWA-Studie, Stuttgart, Februar 2012; Kranert, M. et al.: Ermittlung der weggeworfenen Lebensmittelmengen und Vorschläge zur Verminderung der Wegwerfrate bei Lebensmitteln in Deutschland.
²⁾ Including unavoidable waste during the preparation of food.

Compared with the food wasted by consumers (households and bulk users), the amount of food wasted during production and in the food trade is considerably lower, at 2.4 million tonnes per year. For example, only 1.1% of all food purchased by food retailers, with a market value of 1.2 billion EUR per year, is wasted [3]. This amounts to 310,000 t/a, including 70,000 t/a bread, most of which ends up in organic waste bins. Apart from the baked goods given to charities, the waste bread occurring in the food retail trade has a nominal turnover value of 330 million EUR per year. These figures form the basis for studying the problem of food waste in the baked goods sector and ways of reducing the quantity of food waste, taking economic and technical considerations into account in the context of the overall issue outlined above.

Food waste in the baked goods sector

According to information published by the Association of German Industrial Bakeries (Verband Deutscher Großbäckereien e.V.), the turnover for bread, rolls, other baked goods and products from baked goods in the bakery sector in Germany totals 17 billion EUR per year (Table 2) [4]. The overall production of baked goods amounts to 85 kg per head per year. This includes 57 kg bread and rolls per year that are actually eaten and 10 kg per head per year that are not eaten, partly because they are thrown away or are unsaleable. According to these figures, 85% of the bread and rolls produced are consumed. As bread and rolls are fresh products that do not keep the consumption rate

of 85% calculated from the figures can be regarded as high. If the current technology for producing, distributing and selling baked goods is considered, the scope for any further increase in the consumption rate of such baked goods can be regarded as limited. Thus the issue that essentially needs to be addressed is whether there are economically and ecologically appropriate ways of reducing the quantities of waste baked goods rather than throwing them away.

Table 2. Basic data for food waste in the baked goods sector

Turnover of the bakery sector (EUR/a)	17 billion
Production of bakery products (kg/head/a)	85
Consumption of baked goods (kg/head/a)	
– Bread and rolls = termed as bread	57
– Other baked items	15
– Products of baked goods	3
Uneaten bread (kg/head/a)	10

Source: Verband Deutscher Großbäckereien e.V., Press conference of Sept. 10, 2010, Düsseldorf.

To this end, a distinction first has to be made between the different types of waste in the baked goods sector. From the point of view of the production process, waste baked goods are the leftover doughs and baked goods occurring in factories as well as spoilt remnants of doughs and baked goods. From the distribution point of view, waste baked goods comprise the waste produced by consumers and the bread returns in shops and supermarkets. This paper deals specifically with waste bread and rolls which are referred to below as “bread” in accordance with the German nomenclature [5].

A particular feature of the German baked goods sector is that bread delivered to shops and supermarkets but not sold within the period of time that is customary in the trade - and which is thus unsaleable - is sent back to the manufacturers unless otherwise agreed. The quantity of unsold bread returned to manufacturers is estimated by the author to be equivalent to the quantity of bread that is sold but not eaten (Table 3). According to this estimate, the quantities of bread returns and uneaten bread each amount to 0.34 million tonnes per year, each with an actual or fictitious annual turnover value of 0.85 billion EUR.

Table 3. Economic evaluation of the problem of food waste in the baked goods sector

Produced bread ¹⁾	Amount (10⁶ t/a)	Monetary value (10⁹ EUR/a)
Sold bread ²⁾	5.5	13.40 ⁴⁾
Unsold bread ³⁾	0.34	0.85 ⁵⁾
Sold, but uneaten bread ³⁾	0.34	0.85 ⁶⁾

¹⁾ Calculated from the information released by the Verband Deutscher Großbäckereien e.V., press conference of Sept. 10, 2010, Düsseldorf, and the size of the population in Germany of around 82m inhabitants.
²⁾ Without taking into account the cash value of the bread returns of around 50m EUR/a.
³⁾ Divided and allocated according to a rough estimate.
⁴⁾ Actual turnover.
⁵⁾ Fictitious turnover.
⁶⁾ Included in the actual turnover.

At this point it can be seen that unsold bread and its turnover value must be taken into consideration in the overall turnover. Under conditions that can be otherwise assumed to be constant, e.g. a fixed market share and a constant consumption of bread, profits can only be maximised by reducing the percentage of bread returns or by finding a profitable secondary use for them. Bread manufacturers have above all a considerable economic interest in a reduction in the quantities of bread returns as the resulting opportunity to minimise production costs enables them to strengthen their market position.

Ultimately, it is the behaviour of consumers who not only always expect baked goods to be fresh but also expect a wide variety of baked goods to be available which has given rise to the problem of the need to achieve a balance between the amount of bread produced and the amount of saleable bread. In spite of efforts on the part of manufacturers, including the use of methods of producing fresh products close to the time of consumption, the quantity of unsaleable bread has remained almost constant for a long period of time.

On the face of it, the aim should be to achieve a considerable reduction in the large quantity of unsaleable bread and bread wasted by consumers for the reasons stated at the beginning of this paper. As already mentioned, bread manufacturers have a great economic interest in a reduction in the amount of unsaleable bread. They are less interested in the consumption rate rising as a result of changes in consumers' behaviour. By contrast, suppliers to bakeries have neither an interest in reducing the quantities of bread returns nor in increasing the rate of consumption. This is due to the fact that there are different ways of assessing bread leftovers depending on whether bread production is seen from the point of view of business management or the national economy.

From an economic point of view, it must be considered that a reduction in the use of raw materials as a result of a decrease in the quantity of bread returns and an increase in the rate of consumption would have a negative impact on the contribution of the suppliers, i.e. of the millers and machinery producers, to the value added. This would have a detrimental effect on the employment systems both within and outside the baked goods sector. These consequences are linked to the gross value added which results from processing the wheat to make flour, to baking bread from that flour and distributing and selling the bread.

It can be seen from the simplified illustration of the gross value added from wheat to bread in Figure 2 that the gross value added from wheat to flour is only 10% while it is 900% from wheat via flour to bread [6,4]. The sharp increase in the gross value added from flour to bread is a result of the costs incurred when processing flour to make bread. These costs include the cost of the other raw materials, the use of premises, machinery, transport, energy and work. In this case, the gross value added refers solely to the saleable bread, i.e. the bread that is sold and accounts for the turnover.

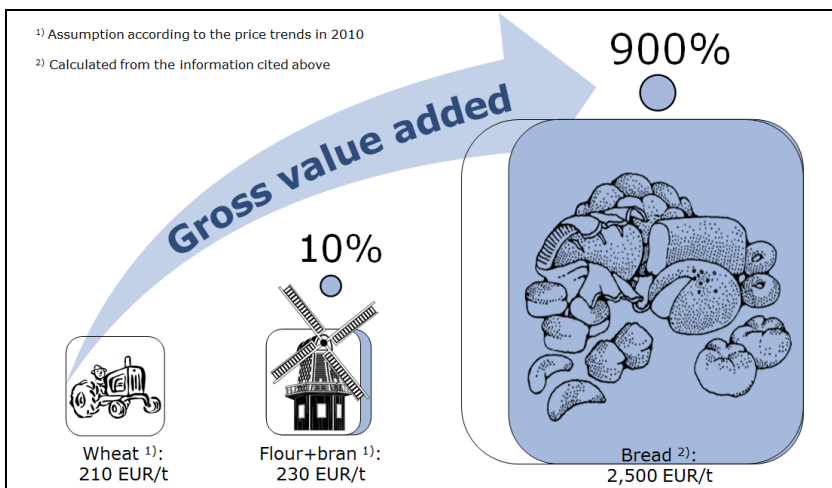


Figure 2. Simplified illustration of the gross value added from grain to bread

The considerable value added between saleable flour, which is not yet in a form that is ready to eat, and saleable bread, which is ready to eat, means that bread returns, and their nominal turnover value, have a detrimental effect on the turnover. The effect of the turnover value of the bread returns on the operating results is only more or less neutral because all manufacturers are faced with virtually the same problem. For manufacturers, bread returns are thus a cost factor which has to be included in the production costs. These costs have a detrimental effect on the selling or retail price of bread and are borne

by the consumer. As bread returns – i.e. unsaleable bread – no longer have the gross value added of saleable bread they are waste from the manufacturer's point of view. Such waste can be reused, either in a way that is self-financing or in a way that yields a return, or has to be disposed of, which involves costs, if the manufacturer does not satisfy the legal requirements for reusing bread returns.

From an economic point of view, the gross value added between bread and bread returns according to this account is around fifteen times greater than the value of the flour in the bread. It is therefore evident that bread production must be considered as of far greater importance to the national economy than the production of the raw material. With regard to the economic and social aspects of the importance of food waste, the debate on leftover bread must be relativised with relation to overall economic activity, especially regarding ethical and ecological aspects, as a large proportion of leftover bread has a secondary use.

Use of leftover bread

Leftover bread should always be used in accordance with its potential for generating added value, as is the case for other types of food waste. The highest level of the potential for generating added value from food waste, especially non-saleable food, is to reuse it by processing it to make other foods. Below this is the level at which food waste is either used directly as feedstuff or to produce feed products. The third level is the use of food waste as fuel or as a raw material for the production of gaseous or liquid fuels (biogas, ethanol), which also creates by-products. This is followed by the level at which the waste is disposed of. The waste no longer has any potential for generating added value as the cost of its disposal is greater than its calorific value.

The flow diagram in Figure 3 illustrates the paths of bread and bread leftovers during production and distribution. The diagram summarizes the figures for the quantities of bread and leftover bread referred to above. There may, of course, be considerable differences between the mass flows in individual factories, depending on the ranges of products and the methods used for their production and distribution. All companies, however different their capacities, ranges of products and the technical equipment required for distribution may be, endeavour of course to minimise the amount of leftover bread, both internally and externally. In this context, a great deal of progress has been made with regard to the sustainability of production, particularly over the past two decades [7,8]. First and foremost, this includes the success in limiting the amount of internal waste.

Technical developments have also contributed to this success. Two of those developments will be dealt with in greater detail below as, firstly, they have come about against the background of general advances in technology in the baked goods sector and, secondly, because they have become the starting point for further developments, in particular with regard to the use of bread returns. Although both developments strictly speaking only concern the use of bread remnants, they may also be applied to a certain

extent to leftover dough, depending on the very different requirements of individual companies.

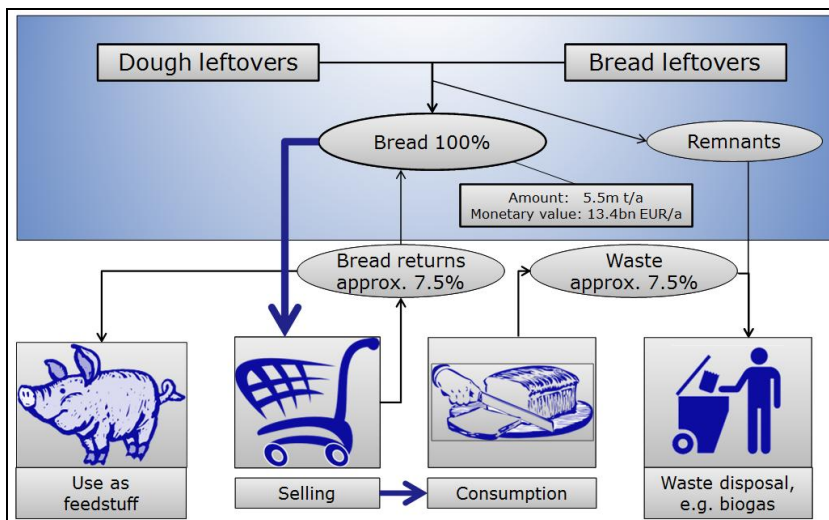


Figure 3. Flow diagram for the use of dough and bread leftovers and of bread returns

The use of leftover dough will not be dealt with in any greater detail in this paper as it does not have the same significance as leftover bread for reprocessing owing to its place at the very beginning of the value added chain. As far as possible, leftover dough is reworked. The remainder can be used as feedstuff or disposed of.

Bread sourdough

One of the processes is the Isernhäger bread sourdough fermentation in which a special type of sourdough is produced. The development of the Isernhäger bread sourdough is based on the invention of the Isernhäger sourdough process [9] and the well-known use of bread remnants in dough production. Using bread remnants in dough is a time-honoured method of increasing the moistness of the crumb, while at the same time enhancing the flavour of the bread. The use of bread in doughs is limited in Germany to 6% in the case of brown and white bread and 20% in the case of wholemeal bread [10]. Moreover, it must not be possible to notice the added bread remnants in the crumb.

The improvement in the qualitative properties of bread achieved by adding bread remnants, including enabling the bread to stay fresh longer, can be regarded as an extra benefit which could otherwise only be obtained by adding swelling and/or flavouring

agents. Hence the bread remnants acquire a value which is greater than that of the flour they replace in the bread.

Apart from the ecologically valuable advantage of using bread remnants at the level of the highest potential for generating added value, the additional benefit created by using bread remnants has the advantage that it can generally be realised at a lower cost than if comparable products, if available, were to be used. Of course, the cost of processing the bread remnants must be considered when the additional benefit is evaluated economically. In such calculations, the capital outlay for the requisite apparatus and machinery is frequently set against the realisation of the in-line processing of bread remnants at the exploitation level of food.

As sourdough is always required for the production of rye and rye-based bread, the additional capital outlay required for the specially equipped fermenters needed for the Isernhäger sourdough process is low (Figure 4) [11]. However, the far greater advantage of using the Isernhäger sourdough process rather than simply adding bread remnants to the dough is that the bread remnants in the sourdough are altered both qualitatively and quantitatively to a far greater extent owing to the long fermentation period and the acidic reaction environment [12]. The resulting homogeneous material can be used to bake well-leavened, aromatically flavoured bread which stays fresh longer than bread baked with conventional sourdough.

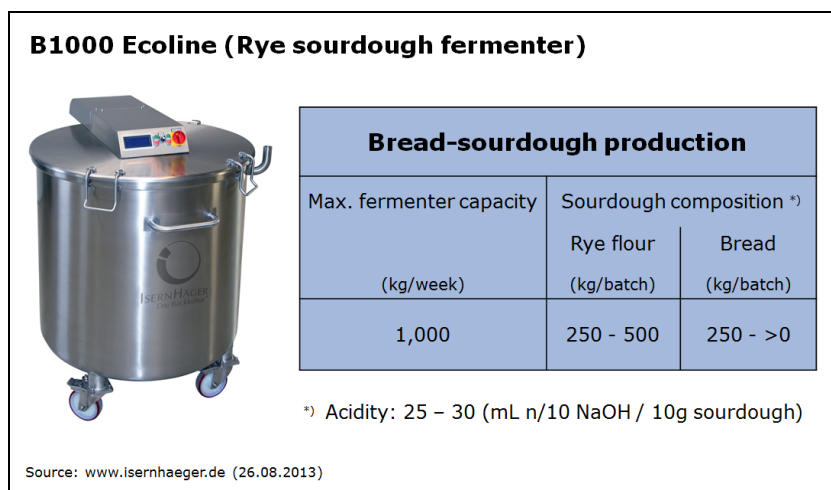


Figure 4. Rye sourdough fermentation with bread as a recipe component

The other advantages of Isernhäger sourdough, such as storage and the hygienic use of the bread remnants, are also significant. Considering the possible capacity of the fermenters, the method is particularly suitable for small bakeries whose range of

products mainly comprises baked goods that are usually made with sourdough made from rye milling products.

Bread syrups

The other process is the production of syrups from wholemeal bread remnants. The products, which are known as bread syrups, can be used above all as ingredients in wholemeal bread in order to lend it particular sensory properties. These include the flavour first and foremost, but also the firmness and colour of the crumb.

Bread syrups are made by enzymatic fermentation of coarsely crumbled bread remnants which have been mashed in water. Essentially, the starch in the bread is partially degraded by amylolytic hydrolysis with α -amylase and amyloglucosidase to form glucose and glucose polymers [13]. The protein in the bread can also be partially hydrolysed with proteases [14]. The enzymatic hydrolysis is interrupted by inactivating the enzymes by boiling the syrup. The resulting bread syrup is yellowish-brown and has a sweet taste and a creamy consistency. The syrup can be pressure-cooked to produce brown components, after which it is dark brown in colour.

The syrups can be used to produce wholemeal bread with a wide range of quality characteristics by varying the colour, flavour and consistency of the crumb. This provides great scope for developing and modifying products to suit the sensory preferences of consumers.



Figure 5. Yeastless sponge doughs and bread syrups for the production of wholemeal bread varieties

A relatively high capital outlay is required to purchase the thick matter fermenters equipped with an agitator which are required to produce the syrups (Figure 5) [15]. As the industrial production of wholemeal products is considered to be the principal application, this technology is suitable for processing bread remnants in the relevant industrial bakeries.

Closed loop processing of bread returns

Both examples show that the gradual advances in the microbiological fermentation of doughs and the enzymatic saccharification of the crumb have resulted in significant technical applications in the baked goods industry. These advances eventually led to my working group developing a concept for an environmentally friendly use of leftover bread in industrial bakeries which makes use of a closed loop method of processing bread returns (Figure 6) [16,17].

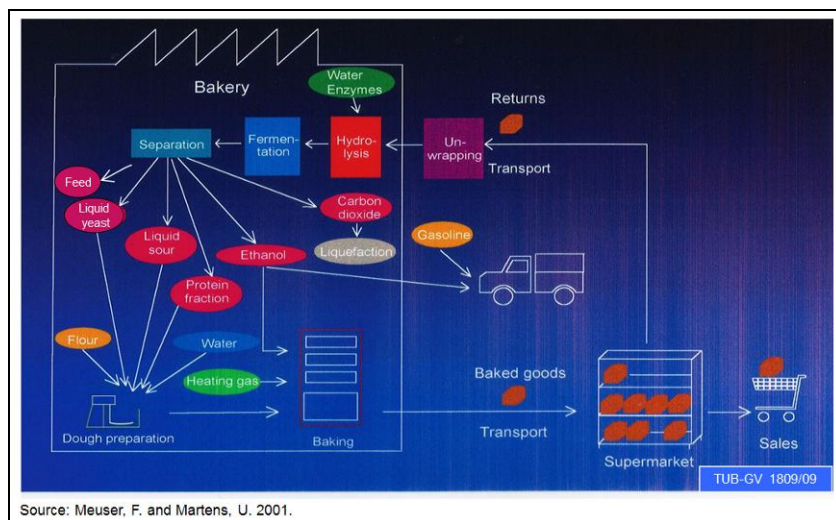


Figure 6. Concept for an environmentally-friendly use of bread returns in industrial bakeries

The idea was to transform the dry matter of the returns into components which could either be used as recipe components or production resources. The specific aim was to put the cycle of organic materials and the pattern of their energetic use in part on the level of the energy intake by human food consumption. Prolonging the utilisation of materials was also considered in order to exploit existing recovery potentials, e.g. the waste heat of baking ovens. All these objectives aimed at achieving a sustainable industrial production

of bread by careful and environmentally friendly use of the resources in raw materials and energy.

We ended up with a rather complicated process by which the bread returns are treated enzymatically to produce a syrup which serves as a substrate for fermentations. The end products of the fermentations are liquid baker's yeast, liquid sour, ethanol, carbon dioxide and an insoluble fraction rich in protein and dietary fibre. All of these products can be used for bread production, with the ethanol serving as heating fuel and the liquefied carbon dioxide being used to cool the bread. We successfully carried out on a pilot scale the main process steps required to obtain the relevant products for the environmentally friendly processing of bread returns and use of said products. However, the economic viability of this process has still to be demonstrated in practice.

It would only have been necessary to process a limited amount of the daily bread returns to obtain this range of products in the large industrial bakery of the project partner, the former Müller Brot GmbH, Neufahrn, as much more yeast could have been produced from the total amount of bread returns than would have been needed for bread production. Therefore, the daily consumption of yeast in the factory under investigation served as a calculation quantity for the dimensioning of the process and its product streams.

It is very important to note that the liquid yeast, liquid sour and protein fraction that were obtained could be used for the production of the bread varieties at the bread factory. There was no significant difference between the quality properties of the bread varieties which were baked according to the standard recipes and the recipes which included the new products.

Considering these positive results, the question arises as to why this technology has not yet become state of the art in large industrial bakeries. There is one main reason which is standing in the way.

It is essentially the fact that it would require large-scale investment and skilled personnel for its realisation and it would operate without offering the opportunity to significantly improve revenues.

Compared with this obstacle, environmental friendliness alone is not very high on the agenda in plans for the economic survival of bakeries confronted with ever-increasing competition. Owing to this less favourable situation, we proposed a step-by-step realisation of the whole process, starting with the production of liquid baker's yeast in an industrial bakery of an appropriate size [18, 17].

Baker's yeast

In the meantime, the production of baker's yeast from saccharified waste bread in combination with molasses as substrates has been realised (Figure 7) [19]. The technology was developed by Dr. Fricko, Fricko Design GmbH, Vienna, Austria, for the Special Yeast AB in Malmö, Sweden. Since 2006, the largest industrial bakery in Sweden

has been supplied with an amount of liquid yeast (yeast cream) which is equivalent to 3,000 t fresh yeast – equivalents/a (H30).



Figure 7. Production of baker's yeast by applying saccharified waste bread in combination with molasses as substrates

The special feature of the yeast production is that the fermentation substrate consists of a mixture of 80% hydrolysed bread (membrane-filtered, with 23% d.m. glucose) and 20% molasses. The molasses mainly add minerals and vitamins to the substrate. The yield of yeast from the bread and molasses is around 72% and 28% respectively. 15 tonnes of yeast cream, equivalent to 10 tonnes of fresh yeast, are produced per batch.

The advantage of producing yeast from bread remnants is that processing takes place at the same exploitation level as feedstuffs and the waste water pollution is much lower than when producing yeast from molasses. In addition to its cost-saving environmental soundness, the economic viability of the method is determined above all by the capital outlay, the operating costs and the potential revenue from the use of the bread remnants as feedstuff. The basis for comparing the cost of the substrate is the price of molasses at the relevant time. The process appears to be economically viable as the plant has already been in operation for many years. From this it can be concluded that the entrepreneurial courage needed to invest in new processing technology can be worthwhile [20].

Feedstuffs

It can be seen from the examples of the use of leftover bread in bakeries described above that a concept by which all bread remnants can be used to obtain products for in-line use

has hitherto only been developed and tested on a pilot-plant scale for large industrial bakeries. However, this concept has not yet been put into practice. Apart from the important reason referred to above, this is first and foremost due to the great advances in methods of processing leftover bread to make feedstuffs and the logistics involved [21]. Moreover, the amendment to the EU regulation on the marketing of feedstuffs in 2012 [22] has played a major part in the trend towards bread producers no longer marketing waste bread as feedstuffs themselves but leaving the marketing to the feedstuff producers instead.

According to the EU regulation, bread producers must declare the waste bread as “waste to be disposed of”, a “feedstuff” or a “product for the production of feedstuff” before selling it [21]. It is not possible to alter the declaration at a later date so that bread manufacturers who declare the leftover bread as “feedstuff” bear the full legal responsibility, including the responsibility for animal health, when placing it on the market themselves. However, if the leftover bread is declared as a “product for the production of feedstuff”, the responsibility for complying with legal requirements will be borne by the feedstuff manufacturer instead.

If leftover bread is processed by feedstuff producers to obtain feed, there is the additional significant advantage for bakeries that the market for feed, owing to its size, is easily able to absorb the leftover bread in its entirety. The leftover bread can therefore be used at the second highest exploitation level. This has at least an ecological and thus environmental advantage that can be considered greater than the advantage that would be achieved at the third level.

The disadvantage of using leftover bread to obtain a “product for the production of feedstuff” – if this is done completely separately from baking operations – is the high cost of processing it, in the simplest case by drying the bread to make feed meal. As a result, the revenue from selling the leftover bread has virtually no economic significance for the gross value added during the production of bread. After the cost of drying has been deducted, the market value of the leftover bread for a bakery falls to a value far below that of the flour or cereal. By contrast, the feedstuff producer can obtain the cereal price as feedstuff made from leftover bread virtually retains the nutritional value of the cereal.

Evaluation of the possibilities for exploitation

The first important finding with regard to evaluating the possibilities of using leftover bread is that the energy contained in flour, or the nutritional energy from the original product, the cereal, is transferred to the baked bread. The nutritional energy is only lost virtually without generating any value added when bread is disposed of as waste. By contrast, it is maintained in all other forms of exploitation with a separate potential for generating added value. This is shown by the figures in Table 4 which give a rough idea of the potential revenue that can be earned by the various methods of using leftover

dough and bread at the various exploitation levels, although the figures are only intended as a guide.

Table 4. Evaluation of the exploitation of dough and bread leftovers and bread returns

Level of exploitation ¹⁾	Exploitation as / for	Comparison		Potential revenue (EUR/t; 85% d.m.)
		Product	Value (EUR/t) ²⁾	
1	Food	Glucose syrup	380	≥ 300
2	Feedstuff	Feed grain	200	≤ 180
	Production of feedstuff	Feed grain	200	> 60 - < 120
	Production of baker's yeast	Molasses	150	≥ 150
3	Production of bioethanol	Wheat	200	≥ 180
	Production of biogas	Maize silage	90 ³⁾	≈ 90
	Fuel	Wood pellets	180	> 60 - ≤ 160
4	Waste	Biowaste	—	< 0

¹⁾ 1 = highest, 4 = lowest level of exploitation from an environmental point of view.
²⁾ Based on market price trends in 2011/12 including VAT.
³⁾ Calculated for 85 % d.m.; original silage: 33 % d.m.; price: approx. 30 EUR/t.

The potential revenues are contrasted with the cost of comparable products for each actual or possible type of exploitation. In each case, the approximate cost of processing the leftover bread to obtain the various types of products has been taken into consideration. This can be relatively high in certain cases, for example the cost of logistics and drying the leftover bread. With regard to maximising the potential revenue, the user of the products may also be an important factor. This applies in particular to the energetic use of the products.

The figures clearly show that the greatest value added can be achieved if the waste bread is processed to obtain products that are valuable for baking bread. The calculated contribution of the waste bread to the value added cannot be less than the price of the flour, but may exceed it considerably. For all other types of use at the subsequent exploitation levels, it is even considerably lower than the price of cereal which is used as a comparison while it is approximately the same for the other comparable products. This is of particular interest when comparing the use of waste bread with the use of wood pellets.

With regard to the energy costs for baking, using waste bread directly to heat the bakery ovens is cheaper than using wood pellets. The energy costs are definitely much lower than the current cost of heating oil or gas (Table 5). It is a curious fact that this would also be true if a bakery bought feed pellets produced from bread returns and used them to heat its ovens. However, if that bakery had its own unsold bread processed to obtain

feed pellets it would have to bear the cost of drying and logistics. From this it follows that burning such pellets to obtain heat would even exceed the cost of heating oil.

Table 5. Use of leftover bread as fuel in bakeries

Fuel	Calorific value (MJ/kg)	Comparison (Cents/MJ) ¹⁾
Bread ²⁾	17	0.98 ³⁾
Wood pellets	18	1.26
Fuel oil	43	2.33

¹⁾ Based on current market price trends; feed wheat: 167 EUR/t, wood pellets: 227 EUR/t, fuel oil 1000 EUR /t as per August, 2013.
²⁾ Taking into account the heat needed for drying bread to 95% d.m.
³⁾ Assuming direct burning.

If ethical considerations are added to these economic considerations regarding the use of leftover bread it can be seen that, on the bread production side, market constraints alone mean that every effort is being made to minimise the loss of nutritional energy and to make up for the loss of added value, mainly by bread returns, by integrating it into the calculation of the gross value earned. Thus bread production satisfies all the ethical, social, economic and ecological criteria referred to at the beginning of this paper when business management and national economy are considered. The same does not apply to the waste bread produced by consumers as it is thrown away. The resulting costs are higher than the potential yield from burning rubbish or other uses of this type of waste which is difficult to collect separately.

Thus, while the waste bread produced by consumers is ethically and ecologically undesirable, this drawback is less important economically than avoidable losses caused by waste in the primary production of food, for example. This can be seen in the prorated amount of value added tax paid by the consumer for bread that he or she does eat. Calculated on the basis of the figures presented at the beginning of this paper (cf. Table 2), the amount concerned is higher than the cost of the flour used to produce the bread that is thrown away, at around 60 million EUR. As this part of the value added tax revenues results solely from the nominal value added in the unconsumed bread, the state therefore has no interest in increasing the rate of consumption as this would reduce the amount of bread produced.

This economic evaluation of the leftover bread that is actually disposed of as waste leads to the conclusion that bread that is thrown away can be considered as an unwanted tribute to our

“brave new world”.

REFERENCES

1. Kranert, M. (Projektleiter) 2012. Ermittlung der weggeworfenen Lebensmittelmengen und Vorschläge zur Verminderung der Wegwerfrate bei Lebensmitteln in Deutschland. Institut für Siedlungswasserbau, Wassergüte- und Abfallwirtschaft, Universität Stuttgart. Studie erstellt im Auftrag des BMELV.
2. Rosenbauer, J. (Studienleiter) 2011. Save Food Studie. Das Wegwerfen von Lebensmitteln – Einstellungen und Verhaltensmuster. Quantitative Studie in deutschen Privathaushalten. Durchgeführt von The Consumer View GmbH für Cofresco Frischhalteprodukte Europa.
3. Horst, F. 2013. EHI – Studie analysiert Nahrungsmittelverluste im Lebensmitteleinzelhandel. Paper presented at the “42. Wissenschaftliche Informationstagung“ der Berlin-Brandenburgischen Gesellschaft für Getreideforschung e.V., 17.01.2013, Berlin, (www.getreideforschung.de).
4. Verband Deutscher Großbäckereien e.V. 2010. Press conference of September 10, 2010, Düsseldorf.
5. Leitsätze für Brot und Kleingebäck vom 19.10.1993 (Beilage zum BAnz. Nr. 58 vom 24.3.1994, GMBL. Nr. 10 S. 346 vom 24.3.1994), zuletzt geändert am 19.9.2005 (BAnz Nr. 184 vom 28.09.2005, GMBL. Nr. 55 S. 1125).
6. Getreide, Raps – Landwirtschaftskammer Schleswig Holstein. 2012. www.lksh.de (10.12.2012).
7. Scheich, A. 2013. Inner- und außerbetriebliche Maßnahmen zur Verminderung des Anfalls von Restbrot und Restteig aus der Sicht einer Filial-Bäckerei. Paper presented at the “42. Wissenschaftliche Informationstagung“ der Berlin-Brandenburgischen Gesellschaft für Getreideforschung e.V., 17.01.2013, Berlin, (www.getreideforschung.de).
8. Weyland, B. 2013. Inner- und außerbetriebliche Maßnahmen zur Verminderung des Anfalls von Restbrot und Restteig aus der Sicht einer Großbäckerei. Paper presented at the “42. Wissenschaftliche Informationstagung“ der Berlin-Brandenburgischen Gesellschaft für Getreideforschung e.V., 17.01.2013, Berlin, (www.getreideforschung.de).
9. Menge, W. Verfahren zur Herstellung eines natürlichen flüssigen Sauerteiges für die Bereitung von Brot und Backwaren. Deutsches Patent 2611972.
10. N.N. 2004. Rückbrot. Page 249 in: IREKS ABC der Bäckerei, 6. Auflage. IREKS GmbH (ed.).
11. B1000 Ecoline (Rye sourdough fermenter). www.isernhaeger.de (26.08.2013)
12. Meuser, F., Suckow, P. and Fischer, G. 1987. Einfluss der Fermentation auf Zusätze von Brot zu Isernhäger Sauerteigen. Getreide, Mehl und Brot 41 : 112-116.
13. Lieken – Batscheider Mühlen- und Backbetriebe GmbH. Verfahren zum Herstellen eines Brotteiges unter Verwendung eines Brühstückanteils. Deutsches Patent 3402778.

14. B. Wendeln jr. GmbH. Verfahren zum enzymatischen Abbau von Restbrot und Verwendung des erhaltenen Abbauproduktes. Europäische Patentschrift 0229979.
15. Schäfer, R. and Meuser, F. 1991. Developments in the production of high-quality German wholemeal breads. 1991 Paper presented at the AACC, Annual Meeting, Seattle.
16. Meuser, F. and Martens, U. 2001. Aufarbeitung von Rückbrot unter umweltrelevanten und wirtschaftlichen Gesichtspunkten. Forschungsvorhaben AZ 07377, Deutsche Bundesstiftung Umwelt, Osnabrück; Final report: Technische Universität Berlin, Berlin, Germany.
17. Meuser, F. and Martens, U. 2010. Use of leftover bread for the production of baker's yeast in industrial bakeries. Pages 17-29 in: Proceedings, 5th International Congress Flour – Bread '09, Opatija, Croatia, October 21-23, 2009. ISBN 978-953-7005-21-4, Osijek.
18. Meuser, F., Martens, U. and Roick, Th. 2004. Aufbereitung von C-Quellen für die Hefeherstellung auf der Basis stärkehaltiger Produkte. Pages 63-74 in: Vortragstexte der VH-Hefetagung in Potsdam, 26. u. 27. April 2004. Versuchsanstalt der Hefeindustrie e.V. (ed.)
19. Fricko, P. 2013. Restbrotverwertung zur Backhefeherstellung in einer Großbäckerei. Paper presented at the "42. Wissenschaftliche Informationstagung" der Berlin-Brandenburgischen Gesellschaft für Getreidewissenschaft e.V., 17.01.2013, Berlin, (www.getreidewissenschaft.de).
20. N.N. "42. Wissenschaftliche Informationstagung" 2013. Zusammenfassung der Vorträge. *Cereal technology* 67: 34-47.
21. Böttinger, R. Technik und Logistik der Verarbeitung der Retouren von Brot und Backwaren zu Futtermitteln. Paper presented at the "42. Wissenschaftliche Informationstagung" der Berlin-Brandenburgischen Gesellschaft für Getreidewissenschaft e.V., 17.01.2013, Berlin, (www.getreidewissenschaft.de).
22. Commission Regulation (EU) No 225/2012 of 15 March 2012 amending Annex II to Regulation (EC) No 1831/2003 of the European Parliament and of the Council. *Official Journal L* 077, 16.03.2012, Pages 001-005.

TECHNOLOGICAL SOLUTIONS PREPARATION AND MILLING WHEAT IN LJUBAČE MILL TUZLA

UDC 664.71 (497.6)

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ABSTRACT

Preparation of grain for milling during storage is one of the important processes in the production of flour. Storage conditions are extremely important for the process of grain milling. The process of milling in Ljubače Mill and its technological solution have been valid and from the solutions brought forth of many modern plants for grain milling. In this paper will be possible to demonstrate a new improved technological solution of part of line production of flour from storage to vibrating sieves.

Keywords: preparation, storage, grain, milling

INTRODUCTION

Ljubače Mill was founded in 1949th when it was built and put into operation in an industrial mill for wheat processing capacity of 60 t/24 h. Technology for processing, constantly perfected, and capacity increased by so that in 1980 reached a size of 120 t/24 h. Newly built mill was put into operation in 1986 year with modern technology that allows the production of a wide range of high-quality wheat.

The old mill is well adapted and customized for the processing of corn with the primary objective of producing quality brewers semolina, and with a newly purchased equipment 1998th also has the latest line to produce all kinds of animal food. Simultaneously with the construction of a new mill, preparations were done and performed work on the construction of new large storage spaces - silos for reception, cleaning and storage of wheat. The mill has suddenly stopped working 2010th.

Today, there is already a great experience in technologies of wheat milling, and provide an exceptional contribution to the improvement of bakery industry product quality.

Equipment used in the milling process is constantly being refined, new equipment is put into the product lines, so that the modern mill is almost completely automated system, based on the input and output characteristics of the of raw materials and products.

The main characteristic of the internal transport is the fact that it increases the price of the finished product, and its value in use remains the same. For this reason, are

necessary research aimed at finding optimal solutions of internal transportation, important for technical and economic planning.

This is important in order of shortening the length of the internal transport, and reducing energy consumption.

MATERIALS AND METHODS

Storage of wheat in practice depends greatly upon moisture at the entrance. Theoretically allowed moisture is to 15%, an internal standard is to 14%, due to the deviation of the results of Moisture. This is extremely useful measure because of wheat warming. Hectoliter mass must be over 76, meets from 76-80, over 80 is good, over 84 ideal. Hectoliter mass is correlated with the moisture content of wheat. Permitted content impurity is to 4%, higher content of impurity entails lower wheat price. Silo storage are equipped with temperature sensors at multiple locations in silos. If the temperature exceeds 30 degrees, especially if it is constantly increasing lead to a warming of wheat mass (self-heating), ignition (moist wheat - humidity over 15%) as well as the microbiological activity. Very important is a ventilation because of removal of a certain amount of moisture, cooling wheat and insertion of oxygen.

The basic parameters of the silo are:

Capacity silo cca: 4.000.000 kg.

Cells number: 20 pieces.

Cell height: 17.50 m.

Cell diameter: 4 x 4 m.

Cell height without hopper: 15.50 m.

Hopper height: 2.00 m.

Calculation of weight:

$$V = [(a^2 \cdot h) \cdot (HL \cdot 10)] + [(a^2 \cdot h / 2) \cdot (HL \cdot 10)] - [(a^2 \cdot h) \cdot (HL \cdot 10)]$$

cells in the rectangular without hopper	hopper in pyramid form	reduction of the cell gap
-----------------------------------------------	---------------------------	------------------------------

where is:

a^2 = cell width,

h = height,

HL = mass kg · 10,

V = the volume.

To ensure these conditions in the storage area with silos of this type, it is extremely important internal transport. In modern mills, where hygienic limits permit, pneumatic transport, with high energy consumption, as well as mechanical and gravitational transport, both are used in internal transport. Mechanical transport can be achieved by using many types of transporters which are selected according to different criteria, but in almost every situation can use two structural types, with different expenditures of energy.

Pneumatic transport, according to the principle of operation, can be:

- flying,
- suppressing,
- transport material in a fluidized state

(transport fluidized material in pneumatic channels - troughs).



Figure 1. Total internal transport in Ljubače Mill Tuzla

Table 1. The content and conditions in the silo N^o. III

N ^o .	Date offloading	Quantity			Quality			Offloading	
		loaded weight kg	offloaded weight kg	difference kg	moisture %	impurities %	HL weight kg.	silos	cell
1	13.10.09.	25.220	25.480	260	13.20	3.00	77.65	3	6
2		25.060	25.120	60	13.40	3.00	77.65	"	"
3		25.140	25.140	0	13.80	3.50	77.05	"	"
4		26.220	26.340	120	12.20	3.50	78.90	"	"
5		25.840	25.720	-120	13.80	3.50	79.10	"	"
6		25.040	25.000	-40	14.00	4.00	78.50	"	"
		152.520	152.800	280					

The parameters of the internal transport in Ljubače Mill:

- the total energy requirements in Ljubače Mill - 1036.61 KW,
- internal transport energy requirements – cca. 300 KW,
- total length of internal transport - cca. 1000 – 1500 m.

In the use are the following types of transporters: conveyers, elevators, pneumatic. Transport of the wheat carried out horizontal using transporters and vertical transportation using elevator.

Vertical transport of flour is pneumatic and horizontal using transporters.

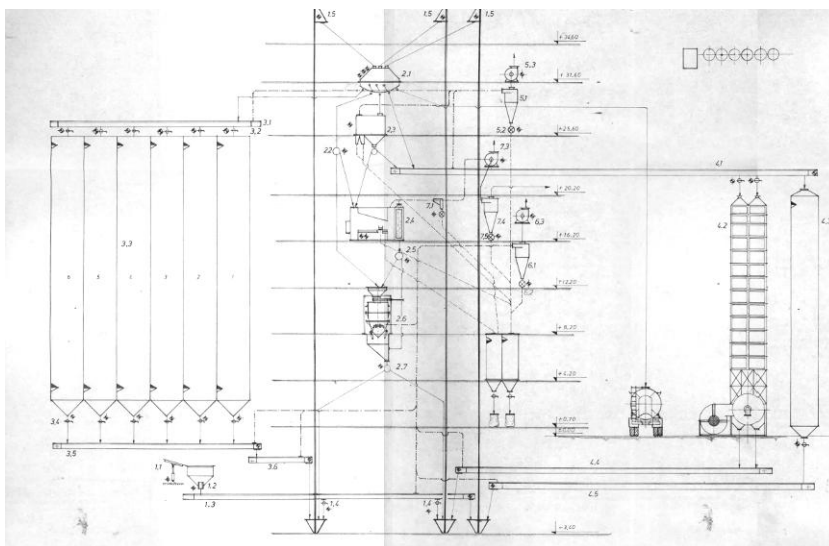


Figure 2. Internal transport in storage area in Ljubače Mill Tuzla

On the line of the internal transport of production of flour from storage to vibrating sieves exist 11 horizontal conveyors, 5 vertical elevators and two lines of pneumatic transport.

- total length of conveyors - 61.5 m,
- total length of elevators - 140 m,
- total length of pneumatic transport - cca. 100 m.

All equipment is, according to the purpose, of the same type, as late makes it easier the estimation of investment and exploitation costs. Example and review of internal transport by storage and preparation of wheat is given to the previous scheme, which represents only a part of the plant. It is evident that only in this part exist a large number of horizontal transporters at various plant cotes, with inappropriate positions, and a small number of splitters, and with little use of gravitational movement.

Improvements and cost savings are possible on the way to reposition the internal transport equipment, with calculation of new transport units.

The calculation is based on the capacity of 150 t/24 h.

The calculations are performed as follows:

- conveyors - numerical method and comparison,
- elevators - numerical method and comparison,
- pneumatic transport - numerical method.

RESULTS AND DISCUSSION

By reducing the number of units of equipment, including aggregates which drive them, as well as reducing the overall length of transport, it is possible to reduce investment costs for 10-12%.

As noted, because the equipment of the same type, exploitation costs for this part of the plant to reduce to 8-10%.

Additionally, it is necessary repositioning of new equipment in relation to the current situation.

CONCLUSIONS

Reconstruction of the Ljubače Mill is an ideal opportunity for the redesign of the internal transport. The result will be a reduction in investment costs during repairs, as well as the later reduction of exploitation costs (energy consumption, technical maintenance of equipment etc.).

It will also result in easier management of process equipment by staff.

REFERENCES

1. Šef, F., Olujić, Ž. 1988. Projektiranje procesnih postrojenja. Savez kemičara i tehnologa Hrvatske, Kemija u industriji, Zagreb, Croatia.
2. Beer, E. 1985. Priručnik za dimenzioniranje uređaja kemijske procesne industrije. Savez kemičara i tehnologa Hrvatske, Kemija u industriji, Zagreb, Croatia.
3. Martinov, M. 2009. Mlinske mašine i uređaji. Mlinpek Zavod Doo, Novi Sad, Serbia.
4. Meissner, W. 2012. Vademekum: deo 3 - Tehnike transporta. Mlinpek Zavod Doo, Novi Sad, Serbia.
5. Strakosch, G., Caporale, R. 2010. The Vertical Transportation Handbook, Fourth Edition, John Wiley & Sons, Inc., Hoboken, USA.
6. Mills, D. 2004. Pneumatic Conveying Design Guide, Second Edition. Elsevier, Oxford.

MICROSTRUCTURE ANALYSIS TOOLS TO SUPPORT BREAD RESEARCH

UDC 664.64.016.3

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ABSTRACT

Analytical tools have been the support of great advances in the area of cereals and cereal based products, and microstructure studies have been conclusive for confirming and supporting research hypothesis. An overview about the microstructure analysis tools applied at bread level to follow crumb and crust features and the effect of recipes and processing on those will be presented. Cereal proteins and starch involvement in breadmaking has been followed by light microscopy and scanning electron microscopy (SEM) revealing protein network formation besides gelatinization and gelification. Particular emphasis will be made on enzymes acting on proteins and their role building a protein network when needed, and bakery additives like hydrocolloids, along with the correlation between microstructure with biochemistry and rheology. Recently, microtomography has been also applied to assess changes in crust and crumb of bread. In addition, image digital analysis of bread crumb allowed quantitatively discriminating recipe or process changes. Overall, an overview about the microstructure analysis tools applied to bread quality evaluation will be presented.

Keywords: bread; microstructure; SEM; digital analysis; light microscopy; quality

INTRODUCTION

Cereals and cereal processing have always encouraged numerous researches due to their enormous impact on human nutrition worldwide. Knowledge about the changes associated to cereals processing or even directly related to cereals have been gained through the development of analytical tools that provide information about inner composition, processing behavior, quality of final products and so on. Chemical and rheological studies are the most often used for studying either biopolymer changes during cereal processing or the impact of formulations (additives and technological aids) on the final product quality (instrumental or sensorial). Nevertheless, microstructure has received less attention, likely due to the highly-cost devices that are necessary for undertaking those studies. Despite that, among the different analytical tools available for giving information about cereals and their derivatives, the study of the microstructure has been very useful for obtaining a clear picture of the role of the main cereals

biopolymers (starch and proteins) and their possible interaction between them or with other polymers.

Different approaches to the microstructure of bread dough have been carried out using light microscopy [1], transmission electron microscopy (TEM), and scanning electron microscopy (SEM) [2]. Less work has been done on the microstructure of bread, although lately increasing number of studies are using the microstructure analysis to correlate with instrumental quality parameters [3-4]. SEM provides an appropriate means for characterizing the physical properties and textural attributes of food ingredients in a formulated product. The advantages that make the scanning electron microscope an extremely useful investigative tool for examination of the flour–dough transition include its very large depth of focus and the possibility of obtaining three-dimensional images of sample surfaces with minimal preparation [1]. The use of other techniques as X-ray microtomography has been used to characterize cellular morphologies [5]. The technique, which is non-invasive, requires no sample preparation and allows studying in three-dimensional (3D) the inner microstructure of food materials.

This report will give an overview about the application of the microstructure studies on both cereal based food processing and a quality assessment.

Microstructure of cereals based products. Recipes effect on bread microstructure

Cereal and cereal-based foods are a significant segment of the food industry. Independently of the cereal microstructure, many of the processes that transform the cereal into food products involve the development and change of its microstructure in the food, due to the mechanical and thermal constraints that occur during processing. Microstructure of cereal-based foods, like bread, biscuits and snacks are strongly dependent on the size and distribution of the bubbles within them. Hence, the nature, as well as interaction of components and the manufacturing process, will affect the microstructure of final product.

To illustrate the utility of microstructure studies when setting up cereal based formulations, the effect of some technological or processing aids is described. Improvers or additives, such as hydrocolloids, surfactants, stabilizers, emulsifiers, oxidants, gums and supplementary enzymes (e.g. amylases, proteases, glucose oxidase, lipases or transglutaminase), are very often added in bread formulations or recipes with the purpose to obtain better quality bread and provide consumers with products that meets their needs. The use of enzymes is very common in breadmaking [6], and they could be added in the mill (adapted flour) or in bakeries (during the mixing step). Apart from the starch hydrolyzing enzymes, the structuring action enzymes have been very useful for giving strength to the protein structures. Transglutaminase (TG) has been used as cross-linking enzymes for joining endogenous flour proteins or even to create linkages between flour proteins and exogenous proteins [7]. Rheological and biochemical studies have confirmed that this enzyme promoted changes in the proteins pattern and dough properties, nevertheless only detailed microstructure analysis can reveal the real picture

of the enzyme action on the dough. SEM micrographs confirmed that more compact and homogeneous protein network is obtained in the presence of transglutaminase, and it appeared that linkages could be created within proteins of different sources [7].

Gluten free products are cereal based products where the role of microstructure is even more crucial in the quality of baked goods. The absence of the protein viscoelastic biopolymer (gluten) forces to build an internal "artificial" structure for holding the carbon dioxide released during fermentation, and also for food appearance, and crumb structure. Some sort of gums, emulsifier and proteins, together with other cereal sources, have been reported for reaching that purpose. Enzymes have been proposed as an alternative for creating internal linkages, and in fact the rheological studies revealed that viscoelastic properties changed and even the instrumental quality parameters of the gluten free bread [8-9]. Specifically, Gujral and Rosell [8] used TG with rice flour to generate a protein network through the formation of covalent cross-links between the polypeptide chains of the rice flour that was able to retain the dough structure during proofing and baking. Later on, Marco and Rosell [9] confirmed that TG was even able to act between soybean and rice proteins, by the formation of new intermolecular covalent bonds catalyzed by TG, leading to better quality gluten free bread. SEM micrographs confirmed that a protein network resembling the gluten structure was created with very tiny filaments joining starch granules evenly distributed and proteins. Furthermore, other structuring agent like (HPMC) was a very well supplement for improving that network. A systematic study about the effect of each ingredient or additive showed that the compact rice dough structure (Fig. 1a) could be expanded by HPMC addition, obtaining a more continuous matrix structure with a bigger size of cell gas (Fig. 1b).

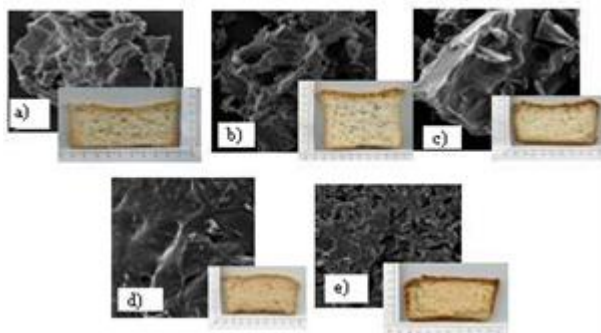


Figure 1. Scanning electron micrographs and images of the crumb of different rice based bread. (a): rice flour; (b): rice flour + HPMC; (c): rice + TG; (d): rice flour + soybean protein isolate (SPI); (e): rice flour + SPI+ HPMC + TG

Concerning to TG treatment, smaller gas cells probably to crosslinking protein were obtained leading to a uniform crumb structure (Fig. 1c), whereas in the presence of

soybean protein, the structure was disaggregated and the gas cells were irregular (Fig. 1d). The combination of HPMC, soybean protein and TG led to rice bread with better dough structure and more open and aerated crumb structure similar to the wheat bread structure (Fig. 1e).

Usually, the production of gluten free bread requires very complex formulations, containing the necessary combination of ingredients and additives for giving a network matrix. Therefore, microstructure in those products plays a fundamental role at dough and bread level. Garzón [10] screened in rice based gluten-free bread formulation the role of corn starch, vegetal protein, animal protein and different gums (xanthan and guar gum, UltraCel™ and HPMC). Using different recipes, breads presented modifications in their crumb structure and appearance. For example, the breads with vegetal protein showed larger cells and less uniform crumbs (Fig.2 a,b,c). While breads with animal protein displayed best crumb with more homogenous and smaller cells closely related to the wheat bread structure (Fig.2. d,e,f).

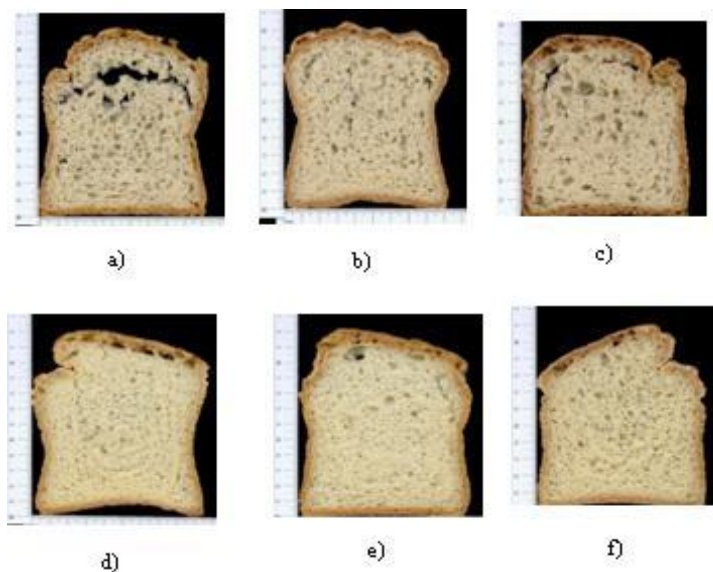


Figure 2. Bread slices obtained from optimized gluten-free bread formulation. (a): rice flour + corn starch + vegetal protein + HPMC; (b): rice flour + corn starch + vegetal protein + xanthan and guar gum; (c): rice flour + corn starch + vegetal protein + UltraCel™; (d): rice flour + corn starch + animal protein + HPMC; (e): rice flour + corn starch + animal protein + xanthan and guar gum; (f): rice flour + corn starch + animal protein + UltraCel™

According to the above, the microstructure of dough and bread result is completely dependent on the recipes and the interaction among ingredients, additives and processing aids.

Process effect of bread microstructure

Fresh bread typically presents an appealing brown crust, a pleasant aroma, a soft and elastic crumb texture and a crispy crust. Nevertheless, those characteristics are greatly dependent on the breadmaking process. For instance the crispy characteristics of the crust are highly dependent on the baking process. In addition, it has a relatively short shelf life since during their storage, a number of physical and chemical changes occur. This problem, coupled with the complex processes involved in conventional breadmaking and increasing consumer's demands, have led to search for efficient methods to produce bakery products without undesirable changes and extending their shelf life.

During the baking occur physical and chemical changes that lead to a change from a closed cell foam structure to an open cell sponge structure. However, the crumb and crust structure is affected by different factors such rate and amount of heat application or amount steaming, which resulting in different types of bread. Altamirano-Fortoul et al. [5] investigated the effect of the amount of steaming (100, 200 and 40 ml) during baking on bread crust feature. Results indicated that amount of steam affected significantly the colour, permeability, mechanical properties and crust microstructure. With respect to microstructure, X-Ray microtomography gave a detailed picture of the crust and the sub-zone located under the crust, displaying different cell structures depending on the amount of steam applied. Breads baked with 100 ml of steam showed more homogeneous and spherical crust cellular structure. Contrarily, breads baked with 200 ml of steam presented more heterogeneous cellular structure and cell size distribution. However, when much steam was applied during baking (samples with 400 ml), bubbles were more elongated, with a distorted appearance; it seems that their resistance to expansion was exceeded, and protein-starch matrix network was disturbed and broken in several places. Also, higher porosity was observed for 100 ml steamed samples than in the case of 200 and 400 ml. Therefore, the extent of steaming amount could be followed by the impact of the crust, which basically resulted from the extension of starch gelatinization and the internal water pressure within the cells.

A food preservation method like high hydrostatic pressure (HHP) has been used for different vegetables and also animal based foods. HHP treatment is a non-thermal process that reduces the microflora and do not affect the organoleptic and nutritional quality of the product. This treatment has been also proposed for obtaining innovative sensorial and functional properties. Bárcenas et al. [11] investigated the effect of different levels (50, 100, 150, 200 or 250 MPa) of HHP on microbiological, physical and structural characteristics of dough, as well as their potential for creating wheat breads with novel characteristics. With respect to the structural characteristics, they found that microstructural organization in wheat dough was affected when subjected to pressure

levels higher than 50 MPa; untreated wheat dough presented a continuous structure with the intact starch granules embedded in the protein matrix structure (Fig. 3a). Nevertheless, dough treated at pressure of 50 and 150 MPa showed well defined starch granules with diverse size, and the surrounding structures were progressively reduced (Fig. 3 b-c). The lowest level (50 MPa) treated dough originated crumbs with an uneven distribution of the gas cells, and increased size of the alveoli (Fig. 3b) compared to the control (Fig. 3a). While that dough treated with at 250 MPa drastic changes were observed, the protein was greatly affected and starch granules disappeared as individual structures adopting a discontinuous (Fig. 3 d) film like organization similar to what happen after swelling and gelatinization, this likely due to protein unfolding.

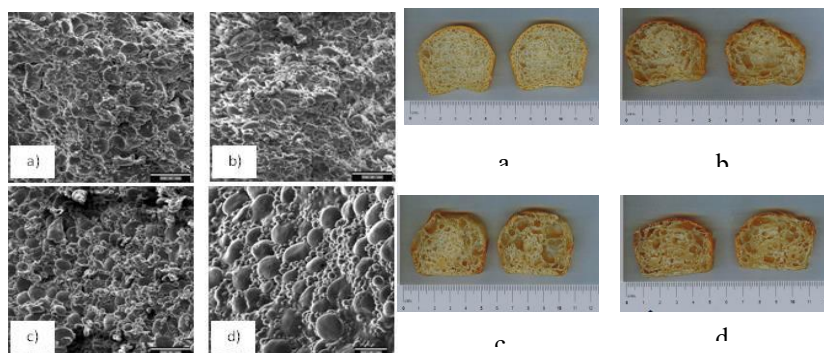


Figure 3. Scanning electron micrographs (SEM) of wheat dough (500×) and the resulting breads, exposed to different levels of high pressure processing (a): 0MPa; (b): 50 MPa; (c): 150 MPa; (d): 250 MPa for 4 min

Bread crust microstructure

Crust attention has been generally conducted to its crystalline or rubbery state, because it is responsible of the crispness. The importance of the crust is understandably because crispness is lost within a few hours after baking, and causes the rejection of the product. However, lately, bread surface have been proposed as the way to produce probiotic baked products. In fact, different types of probiotic coatings have been applied onto the surface of partially baked breads before full baking stage [12]. Overall results show that *L. acidophilus* included in microcapsules can survive after baking, also changes in physico-chemical and sensory properties as well as microstructure, due to probiotics coatings, were presented in breads. SEM micrographs revealed differences between control crust (without probiotic coating) and the crust with probiotic coating (Fig. 4). Control crust showed a continuous veil-like film that revealed a dominant presence of the partially gelatinized starch granules (Fig. 4a). While, crust with starchy coating containing microcapsules, showed a continuous background of gelatinized starch covering the microcapsules (Fig. 4b). With a double starchy layer less microcapsules

became evident (Fig. 4c). Finally, a double coating where microcapsules were sandwiched presented a high concentration of microcapsules over surface due to the treatment (Fig. 4d).

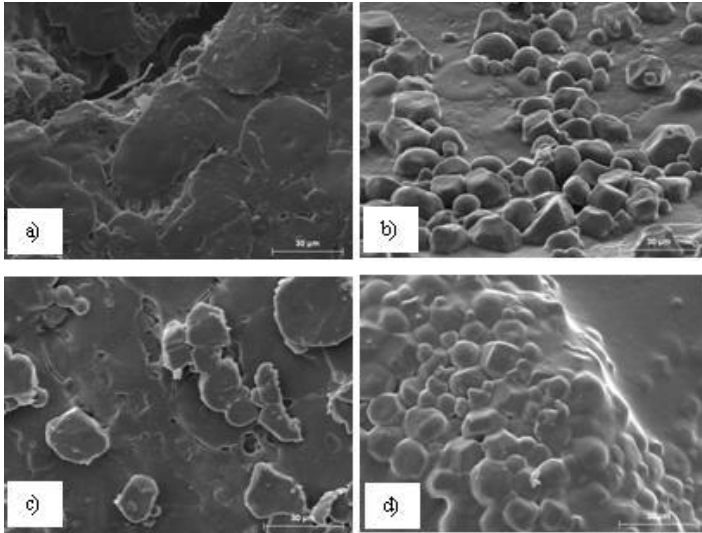


Figure 4. Scanning electron micrographs of bread crust surface (900x). Images correspond to the following probiotic coatings treatments: (a): Crust without probiotic coating; (b): crust with microcapsules suspended in a starchy coating; (c): crust with double starchy coating containing microcapsules; (d): crust containing a double coating with microcapsules sandwiched

CONCLUSIONS

Food microstructure reveals the organization of constituents and their interaction. Most solid foods, including bakery products are micro-structured. Many properties of dough (rheological behavior, textural) and bread (transport properties, textural and sensory properties) are related to their microstructure. During the breadmaking process different changes in the microstructure occur; existing structures are destroyed and new ones are created. Considering that fact, it could be stated that microscopy techniques are necessary to understand the relationship of microstructure, physical properties and bread quality. Therefore, with the purpose to support the analytical methods and to provide complete and meaningful information in bread research, microstructure analysis tools are used. Recent advances in microscopy analysis like X-Ray micro-tomography allow inspecting the microstructure nondestructively and in 3D. This tool has been used to assess the bread crust structure and obtain quantitative information about the cellular matrix.

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REFERENCES

- Rosell, C.M., Altamirano-Fortoul, R., Don, C., and Dubat, A. 2013. Thermo-mechanically induced protein aggregation and starch structural changes in wheat flour dough. *Cereal Chem.* 90:89–100.
- Rojas, J.A., Rosell, C.M., Benedito, C., Pérez-Munuera, I., and Lluch, M.A. 2000. The baking process of wheat rolls followed by cryo scanning electron microscopy. *Eur. Food Res. Technol.* 212: 57-63.
- Altamirano-Fortoul, R., and Rosell, C.M. 2011. Physico-chemical changes in breads from bake off technologies during storage. *LWT- Food Sci. Technol.* 44: 631-636.
- Matos, M.E., and Rosell, C.M. 2012. Relationship between instrumental parameters and sensory characteristics in gluten-free breads. *Eur Food Res Technol.* 235: 107-117.
- Altamirano-Fortoul, R., Le Bail, A., Chevallier, S., and Rosell C.M. 2012. Effect of the amount of steam during baking on bread crust features and water diffusion. *J. Food Eng.* 108 (1): 128–134.
- Rosell C.M., and Collar C. 2008. Effect of various enzymes on dough rheology and bread quality. In: *Recent Research Developments in Food Biotechnology. Enzymes as Additives or Processing Aids*, R. Porta, P. Di Pierro and L. Mariniello (Eds). Research Signpost, Kerala, India.
- Bonet, A., Blaszcak, W., and Rosell, C.M. 2006. Formation of homopolymers and heteropolymers between wheat flour and several protein sources by transglutaminase catalyzed crosslink. *Cereal Chem.* 83 (6): 655-662.
- Gujral, H.S. and Rosell, C.M. 2004. Functionality of rice flour with a microbial transglutaminase. *J. Cereal Sci.* 39: 225–230.
- Marco, C., and Rosell, C.M. 2008. Breadmaking performance of protein enriched, gluten-free breads. *Eur. Food Res. Technol.* 227(4): 1205-1213.
- Garzón R. 2012. Engineering Final Dissertation Diseño y Desarrollo de Panes de Arroz Nutricionalmente Enriquecido. Estudio Reológico de Masas y Calidad de productos. Polytechnical University of Valencia (UPV). Spain.
- Bárceñas, M.E., Altamirano- Fortoul, R., and Rosell C.M. 2010. Effect of high pressure processing on wheat dough and bread characteristics. *LWT. Food Sci. Technol.* 43 (1): 12-19.
- Altamirano-Fortoul, R., Terrazas, R., Quezada-Gallo, A., and Rosell, CM. 2012. Viability some probiotic coatings in bread and its effect on the crust mechanical properties. *Food Hydrocoll.* 29 (1): 166–174.

PRODUCTION SPONGE CAKE FROM HARD WHEAT FLOUR

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ABSTRACT

The availability of soft wheat flour is an important factor in cakes production. The aim of this study is to identify a specification for production cake flour from hard wheat. The result of analysis for protein %, ash %, wet gluten %, dry gluten %, gluten index, falling no, acidity %, damaged starch, sedimentation values and particles size are 8.7, 0.53, 24.9, 8.5, 99, 205, 0.15, 7.7, 74 and 9.9 respectively. The development time, stability, elasticity, softening, water absorption, resistance, extensibility and R: F values for dough are 1.5, 2.5, 100, 120, 56, 98.6, 450, 170 and 2.64 respectively. The average of cakes volume is 672 c.c. and overall acceptability is 7.3 of 9 hedonic scales. Adding of 300 ppm L-cysteine to cake batter improve the quality parameters positively ($P<0.005$) of both of cakes volume is 672 c.c. and overall acceptability. Cake volume and overall acceptability of sensory evaluation test correlate positively ($P<0.005$) with protein and gluten content, sedimentation rate and extensograph parameters. Hard wheat cake flour could be substituted for soft wheat flour as well as the cake producers will advise to add 300 ppm L-cysteine to cake batter improves the quality.

Keywords: cake, flour, baking, quality, rheology, L-cysteine

INTRODUCTION

Sponge cake is produced from flour, sugar, lipids, eggs, and baking powder which have a firm, yet well aerated structure [1-3]. Cake flour is characterized, low ash and low protein content produced for best cake and fine bakery products by milling of soft wheat grains that highly refined flour which is taken from the centre of the kernel of wheat [1-3]. The common specifications for typical for cake flour are recommend; moisture 13.50%, protein 8.00% and ash content of 0.35%. The highest quality cakes are prepared from low-protein flour (7-9%) with small particle size and little starch damage which will mix easily to give a smooth cake batter and tender cakes. Hard wheat yields sturdy flour with higher protein content, is generally used in the production of yeast leavened bakery products such as bread, whereas the soft flour of weak protein is found appropriate for the production of cookies, cakes, biscuits and crackers [1-3]. Weak flour proteins don't form constant gluten network when flour is mixed with water due to smaller quantity and basic quality characteristics of gluten proteins in weak flour [1-3]. Gluten provides the structure in baked goods which affects by the quality and quantity

of gluten. Higher amounts of gluten proteins are not desirable for cake production because large amounts of gluten proteins prevent spread of dough and hampers molding of dough to specific dimension and shape [4-6]. The larger amounts of gluten proteins make the dough stronger and elastic that contracts/recoils after sheet formation [4-6]. Starch gelatinization during baking plays an important role in producing internal texture of cakes. Starch also gives to the crust colour formation [5-7]. Damaged starch is one that has been physically damaged during the wheat milling process which differs between flours milled from different wheat varieties [5-7]. A high quality cake should have high volume with a fine uniform moist crumb. Starch gelatinization, protein denaturation together with carbon dioxide formation gives cake its porous, fine and soft microstructure [5-6]. The degree of expansion is dependent on the viscosity of the batter. If the batter is thick, it would be difficult for the air bubbles to escape, which would result in a high volume [4-7].

It is possible to modify rheological properties of wheat flour via cysteine during processing, in particular shear viscosity and viscoelastic performance of wheat flour dough through protein-protein reaction mechanisms and other interactions in extruded cereal-based snack foods [9]. The addition of 200 or 300 ppm L-cysteine to cake batter made with unchlorinated, heat-treated flour also produced cakes with volumes and crumb grain scores equal to those of cakes made with the chlorinated control flour [10]. L-cysteine as reducing agent can be used as a dough softener in baking. L-cysteine reduces the disulfide bonds to two -SH bonds to weaken the gluten network and to relax the dough to a more workable dough [9,10]. The softening of the dough can easily be shown in an extensogram. Dough softeners are mainly added to increase the extensibility of the wheat gluten, which, for example, is very important for automated production lines, as it has a positive effect on machinability. Cake flour needs a combination of strength, extensibility and tolerance that depends mainly on application, flour quality, water absorption and mixing conditions. Cake flour is generally treated with different methods such as heating, chlorine or ozone treatments, addition of xanthan gum, L-cysteine, hydrogen peroxide and peroxidase to enhance its baking potential and produce cakes with volumes that were slightly greater than those of cakes made with the chlorinated control flour [8-12]. The purpose of this study is to categorize a specific standard specification for production and using cake flour produced from hard wheat by adding L-cysteine which gives a high quality of cake products.

MATERIALS AND METHODS

Cake flour production and Selection of the extraction rate

Cake flour produced from milling of hard wheat is determined by on skills and/or practical experience of miller as well as extraction rate, particle size of flour and other laboratory tests. Three samples of hard wheat grains were milled by 50% extraction rate.

Flour analysis

Moisture of flour was determined by AACC 44-15A method. Ash determined by AACC 08-3 method. Protein of flour was determined by AACC 46-13 Micro-Kjeldahl method. Starch determined by spectrophotometer using perchloric acid / anthrone reagent at 625 nm. Total sugar determined of by volumetric method (the Lane–Eynon official method. Lipids Wet, dry and Gluten Index determined by Glutomatic Method (ICC 137) (ICC, 1998). Starch damage determined by AACC Method 76-31 (ICC Method No. 164). Sedimentation values were determined by using (AACC method 56-60.01). Titratable acidity determined by AACC Method 02-31.01. Determination of the particle size of milling products by using sieve analysis (ICC Method No. 207) [13, 14].

Measurements of water absorption %, peak time, dough stability and mixing tolerance index MTI were determined by using a Farinograph (Brabender OHG, Duisburg, Germany) according to AACC 54-21 method, constant flour weight procedure. Dough extensibility, maximum resistance to an extension, Area of Extensogram and the Ratio were determined using an Extensograph (Brabender OHG, Duisburg, Germany) according to AACC 54-10 method [13, 14].

Cake making

The ingredients used were flour (300 g), egg (300 g), sugar (250 g), milk powder (30 g), shortening (200 ml), emulsifier (20 g), 300 ppm L-cysteine and water (60 ml). Creaming mixing procedure was used. All ingredients except for the flour and milk were mixed for 5 minutes using a mixer, after addition of the milk and flour, the mixing process was continued for 3 min at speed 8. Individual 150 mm diameter, 50 mm height, metallic, butter coated pans were filled with 150 g of cake batter and baked in an electric oven at 250 °C for 20 min. Six cakes of each batter were baked. Three were chosen for volume analysis and three for sensory evaluation measurement. After baking, the cakes were removed from the pans, left for 1 h at room temperature to cool and then packed hermetically in plastic bags to prevent drying. Analysis of cakes was performed after 24 h storage at room temperature.

Cake volume

Cake volumes were measured by using the rape seed displacement in the AACC method [13].

Sensory evaluation

Cakes were evaluated by 60 untrained panelists for overall acceptability using a hedonic rating test was also performed to assess the degree of acceptability of cakes. The taste panelists were asked to rate the sample for color, flavor, texture and overall acceptability on a 1-9 point scale where 1=dislike extremely; 2=dislike very much; 3 dislike

moderately; 4=dislike slightly; 5=neither like nor dislike; 6=like slightly; 7=like moderately; 8=like very much and 9=like extremely [15].

Statistical Analyses

Values in the text and tables are expressed as Means \pm SD. Experiments were performed in three replicates and the data analyses were performed using SAS software (SAS Institute, 2004). LSD tests for significant differences at $P \leq 0.05$ among the wheat varieties [16].

RESULTS AND DISCUSSION

Table 1 shows the flour components at 50% rate of extraction of flour hard wheat. The percentages of protein, starch, ash and sugars are suitable for cake making and specification standard of cake flour.

Table 1. Hard wheat flour components at 50% Extraction rate

Component	%
Moisture	13.6 \pm 0.3
Proteins	7.8 \pm 0.4
Starch	73 \pm 2
Ash	0.41 \pm 0.06
Sugars	1.2 \pm 0.2

*Average of three samples \pm SD

Table 2 shows average results of protein %, ash %, wet gluten %, dry gluten %, gluten index %, falling no, acidity %, damaged starch %, sedimentation values and particles size of cake flour of the studied samples of hard wheat with and without adding of 300 ppm L-cysteine to the flour before the tests. The values of the studied samples display no significant differences at $P \leq 0.05$ between moisture, proteins, ash gluten index, acidity, particles size and damaged starch for the studied samples of hard wheat with and without adding of 300 ppm L-cysteine. While wet gluten, dry gluten, sedimentation and falling number values were significantly different at $P \leq 0.05$ for both studied samples of hard wheat flour with and without adding of 300 ppm L-cysteine. Compared with what is known about soft wheat flour properties, the results obtained in table 2 approaches to specification parameters for cake flour by milling soft wheat adding of 300 ppm L-cysteine as reducing agent seemingly effect on the values of wet and dry gluten, gluten index and sedimentation values. This is may be due to the changes in interactions

between proteins polypeptides and the likelihood of increased loss of proteins during the process of washing with water by during the test.

Table 2. Some properties of cake flour of hard wheat*

	Hard flour without L-cysteine	Hard flour with L-cysteine
Moisture (%)	14±0.8 a**	14.1±0.5 a
Protein (%)	8.7±0.3 a	8.8±0.2 a
Ash (%)	0.53±0.07a	0.51±0.03a
Wet gluten (%)	24.9±0.7 a	21.3±1.4 b
Dry gluten (%)	8.5±0.4 a	7.5±0.4 a
Gluten Index (%)	99±0.2 a	99.3±0.3 a
Falling number (s)	205±5 a	395±5 b
Acidity (%)	0.15±0.03 a	0.17±0.15 a
Damaged starch (%)	7.7±1.2 a	7.2±1.1 a
Sedimentation values (ml)	74±3 a	62±4 b
Particle size (µm)	9.9±0.5 a	9.5±0.2 a

*Average of three samples ± SD

**Means within rows with different letters significantly different according to LSD at $P \leq 0.05$.

Table 3 shows the average results of the development time, stability, softening, water absorption, resistance, extensibility and R:F for dough rheological properties of cake flour of the studied samples of hard wheat with and without adding of 300 ppm L-cysteine to the flour before the tests. The values of the studied samples show no significant differences at $P \leq 0.05$ between development time and water absorption whereas stability, softening, resistance, extensibility and R:F values of rheological properties of cake flour samples of hard wheat with and without adding of 300 ppm L-cysteine to the flour before the tests show significant differences at $P \leq 0.05$. Compared with the soft wheat flour properties, the results obtained by farinograph and extensograph without adding of 300 ppm L-cysteine were higher than to the specification parameters for cake flour by milling soft wheat. These rheological properties results in the table 3 results may explain the difficulties of using of hard wheat cake flour in cake making which cake producers is facing. So that they should apply different ways to solve this problem such as apply special treatments such as using of L-cysteine by the miller or producers of cake to improve the rheological properties cake flour and the final cake product manufactured from hard wheats

Table 3. Some rheological properties of hard wheat cake flour*

		Hard flour without L-cysteine	Hard flour with L-cysteine
Farinograph	Development Time(min)	1.5±0.1 a**	1.4±0.3 a
	Stability (min)	2.5±0.2 a	1.8±0.4 b
	Softening (BU)	120±10 a	90±10 b
	Water absorption (%)	56±2 a	58.5±2 a
Extensograph	Resistance (BU)	450±10a	240±10 b
	Extensibility (mm)	170±3 a	140±5 b
	R:E	2.6±0.2 a	1.7±0.1 b

*Average of three samples ± SD

**Means within rows with different letters significantly different according to LSD at $P \leq 0.05$.

The results of cake volumes presented in table 4. The average of hard flour cake without the adding of L-cysteine measurements is 672 cm³ which is significantly differences or affected to volume of cakes samples that average 753 cm³ which produced by the adding of 300 ppm L-cysteine at $P \leq 0.05$. These values of the cake volume of the samples studied were due to differences in the properties of the gluten, which was particularly evident in the previously mentioned tests such as sedimentation and rheological tests obtained by farinograph and extensograph. These results are consistent, logical and emphasizes the role of reducing agents and effect on relaxing of gluten network which led to an increase in the volume of the resulting cake is also emphasizes the importance of adding such agents to improve the properties of the resulting when using cake flour hard wheat.

Table 4. The volume and overall acceptability of cake

	Hard flour without L-cysteine	Hard flour with L-cysteine
Volume (cm ³)*	672 ± 5 a***	753 ± 3 b
Average of overall acceptability**	7.3 a	8.3 b

*Average of three samples ±SD

**Average of 60 panelists of a 1-9 point scale using a hedonic rating test

***Means within rows with different letters significantly different according to LSD at $P \leq 0.05$.

Table 4 also shows the sensory measures using a hedonic rating to evaluate the overall acceptability of cake flour of the studied samples of hard wheat with and without adding of 300 ppm L-cysteine. The average of overall acceptability for the tested samples produced without adding of 300 ppm L-cysteine were 7.3 of 9. While The average of overall acceptability for the tested samples produced with adding of 300 ppm L-cysteine were 8.3 of 9 which were particularly evident in the previously mentioned tests and values of the cake volume which affected significantly at ($P < 0.05$) by the adding of L-cysteine, due the effect of reducing agents on relaxing of gluten network and the cake volume. The result suggested that cake flour of hard wheat could be substituted for soft wheat flour in cake production. But for getting more high values of the overall acceptability bakeries may apply to add L-cysteine a special treatment or adding suitable additives or improvers.

Table 5. The correlation between the cake volume and overall acceptability with some properties of cake flour of hard wheat

	Cake volume hard flour without L-cysteine	Overall acceptability hard flour without L-cysteine	Cake volume hard flour with L-cysteine	Overall acceptability hard flour with L-cysteine
Protein	0.84587 ^{S*} 0.0123	0.75473 ^S 0.0735	0.85967 ^S 0.0124	0.84763 ^S 0.0145
Moisture	0.01497 ^{NS} 0.9735	0.02653 ^{NS} 0.7288	0.03257 ^{NS} 0.9335	0.03525 ^{NS} 0.9508
Ash	0.46651 ^{NS} 0.1647	0.66893 ^S 0.0741	0.49651 ^{NS} 0.1464	0.64783 ^S 0.0781
Wetgluten	0.75672 ^S 0.0176	0.73467 ^S 0.0312	0.79382 ^S 0.0187	0.77788 ^S 0.0812
Dry gluten	0.85341 ^S 0.0045	0.69552 ^S 0.0430	0.89851 ^S 0.0018	0.79472 ^S 0.0680
Gluten index	0.45750 ^{NS} 0.1553	0.33436 ^{NS} 0.3650	0.64760 ^{NS} 0.1653	0.65036 ^{NS} 0.3616
Falling No	-0.73434 ^S 0.0176	-0.84705 ^S 0.0014	-0.56534 ^S 0.0676	-0.77005 ^S 0.4314
Acidity	0.47432 ^{NS} 0.6585	0.23849 ^{NS} 0.0623	0.34134 ^{NS} 0.3483	0.32859 ^{NS} 0.5673
Damagedstarch	0.54662 ^S 0.3679	0.45442 ^{NS} 0.2404	0.63452 ^S 0.1449	0.58042 ^{NS} 0.8146
Particle size	-0.05624 ^{NS} 0.6068	-0.36566 ^{NS} 0.4583	-0.04554 ^{NS} 0.5078	-0.35576 ^{NS} 0.5553

* ^{NS}Not Significant; ^S Significant at P -value < 0.005

Table 5 displays the correlation between the cake volume and overall acceptability with some properties of hard wheat flour with and without adding of 300 ppm L-cysteine. The results show both of cake volume and overall acceptability of sensory evaluation test correlate positively at ($P<0.005$) or influenced by protein and gluten content in flour with and without adding of 300 ppm L-cysteine. As expected, in hard wheat, there is a strong relationship between protein and gluten content and volume of backed products which effect quality of cake products with and without adding of 300 ppm L-cysteine. However, only ash showed a significant correlation at ($P<0.005$) with overall acceptability of sensory evaluation test correlate positively with and without adding of 300 ppm L-cysteine. To the contrary, both of falling number and particle size were negatively correlated ($P<0.005$) with the overall acceptability and cake volume in both tested samples with and without adding of 300 ppm L-cysteine. Results also showed that there is a moderate positively correlation ($P<0.005$) between the damaged starches and cake volume while it has not significant correlation with overall acceptability of sensory evaluation test. Although, the results found all of moisture, gluten index, acidity and particle size of flour have not significant correlation ($P<0.005$) with cake volume and overall acceptability of cake flour of hard wheat with and without adding of 300 ppm L-cysteine. Therefore, the values in the table 5 of the correlation between hard wheat cake flour and cake quality can increase positively at ($P<0.005$) with adding of 300 ppm L-cysteine. The correlation between the cake volume and overall acceptability with sedimentation rate and some rheological properties of hard wheat flour with and without adding of 300 ppm L-cysteine are shown in Table 6. The results show both of cake volume and overall acceptability of sensory evaluation test correlate positively ($P<0.005$) with sedimentation rate. Although, it was found both of cake volume and overall acceptability correlate positively ($P<0.005$) with softening of dough which determined with farinograph with and without adding of 300 ppm L-cysteine. In addition to the resistance, extensibility and R:E values of dough that measured via extensograph parameters correlate positively ($P<0.005$) with cake volume and overall acceptability of sensory evaluation with and without adding of 300 ppm L-cysteine. To the contrary, farinograph dough stability parameter values were negatively correlated ($P<0.005$) with both of the overall panelists acceptability and cake volume of hard wheat cake flour with and without adding of 300 ppm L-cysteine. The results show all of water absorption of flour, development time and elasticity of dough which determined with farinograph have not significant correlation ($P<0.005$) with both of cake volume and overall acceptability of cake flour of hard wheat with and without adding of 300 ppm L-cysteine. However, it is seem the correlation values at ($P<0.005$) for sedimentation rate and both of farinograph and extensograph parameters without adding of 300 ppm L-cysteine were not high. This give an indication about cake flour of hard wheat could be substituted for soft wheat flour in cake production. Therefore, the values in the table 6 of the correlation between hard wheat cake flour and cake quality can increase positively at ($P<0.005$) with adding of 300 ppm L-cysteine. So that cake producers should apply a special treatment or adding suitable additives or improvers to improve the quality of cake such adding 300 ppm L-cysteine.

Table 6. The correlation between the cake volume and overall acceptability with sedimentation rate and some rheological properties of hard wheat cake flour

	Cake volume hard flour without L-cysteine	Overall acceptability hard flour without L-cysteine	Cake volume hard flour with L-cysteine	Overall acceptability hard flour with L-cysteine
Sedimentation rate	0.64536 ^S 0.0245	0.74627 ^S 0.0089	0.78553 ^S 0.0141	0.84623 ^S 0.0066
Development time	-0.24867 ^{NS} 0.3562	-0.25786 ^{NS} 0.4342	-0.24586 ^{NS} 0.3987	-0.26758 ^{NS} 0.4568
Stability	-0.4967 ^S 0.0697	-0.5679 ^S 0.1213	0.6456 ^S 0.0745	0.6578 ^S 0.1453
Softening	0.64582 ^S 0.0543	0.86781 ^S 0.0657	0.92232 ^S 0.0460	0.85650 ^S 0.1324
Water absorption	-0.67453 ^{NS} 0.3678	-0.37894 ^{NS} 0.4544	-0.36784 ^{NS} 0.3652	-0.37667 ^{NS} 0.3569
Resistance	0.87345 ^S 0.0285	0.84231 ^S 0.0033	0.92985 ^S 0.0186	0.86131 ^S 0.0096
Extensibility	0.54595 ^S 0.0714	0.63771 ^S 0.0987	0.56584 ^S 0.0514	0.62751 ^S 0.0688
R:E	0.73516 ^S 0.0240	0.81572 ^S 0.0240	0.76476 ^S 0.0830	0.85872 ^S 0.0140

* ^{NS}Not Significant; ^S Significant at *P*-value < 0.005

CONCLUSIONS

The study resolute the miller can produce flour with nearly percentage of soft wheat flour but the rheological properties may be good for cake making or productions .but for produce high quality cake the study recommend to apply a way to improve or modify the strength of gluten by adding food additives permitted for improving flour characteristics such as enzymes, reducing agents such as adding 300 ppm L-cysteine or heat treatments to alter the chemical bonds that affect intensity and strength of gluten, therefore affect the final size of the typical cake request and give internal and external structure typical cake request.

REFERENCES

1. Cauvain, S.P. and Cyster, J.A. 1996. *Sponge Cake Technology*. 1st ed. CCFRA Rev. 2, CCFRA, Chipping Campden.
2. Cauvain, S.P. and Young, L.S. 2006. *Baked Products: Science, Technology and Practice*. 1st ed. Wiley-Blackwell, Oxford.
3. Al-Dmoor, H. 2013. Cake flour: Functionality and quality. *Europe. Scient. J.* 9:168-180.
4. Sahi, S.S. and Alava, J.M. 2003. Functionality of emulsifiers in sponge cake production. *J. Sci. Food Agric.* 83:1419-1429.
5. Sakiyan, O., Sumnu, G., Sahin, S. and Bayram, G. 2004. Influence of fat content and emulsifier type on the rheological properties of cake batter. *Eur. Food Res. Technol.* 219:635-638.
6. Sumnu, G. and Sahin, S. 2008. *Food Engineering Aspects of Baking Sweet Goods*. 1st ed., Taylor and Francis, Boca Raton.
7. Kim, C.S. and Walker, C.E. 1992. Interactions between starches, sugars and emulsifiers in high ratio cake model systems. *Cereal Chem.* 69:206-212.
8. Lambert, I. A. and Kokini, J. L. 2001. Effect of L-cysteine on the rheological properties of wheat flour. *Cereal Chem.* 78:226-230.
9. Thomasson, C.A., Miller, R.A. and Hosene, R.C. 1995. Replacement of chlorine treatment for cake flour. *Cereal Chem.* 72:616-620.
10. Donelson, J.R., Gaines, C.S. and Finney, P.L. 2000. Baking formula innovation to eliminate chlorine treatment of cake flour. *Cereal Chem.* 77:53-57.
11. Dubois, M., Coste, A.G., Despres, T., Efstathiou C. Nio, E. Dumont, and Parent-Massin, D. 2006. Safety of Oxygreen, an ozone treatment on wheat grains. Part 2. Is there a substantial equivalence between Oxygreen-treated wheat grains and untreated wheat grains? *Food Additives Contaminants.* 23:1-15.
12. Finnie, S.M., Bettge, A.D. and Morris, C.F. 2006. Influence of flour chlorination and ingredient formulation on the quality attributes of pancakes. *Cereal Chem.* 83:684-691.
13. *Approved Methods of the American Association of Cereal Chemists AACC*. 2000. 11th ed. St. Paul, MN.
14. Young, L.S. 2009. *The ICC Handbook of Cereals, Flour, Dough and Products: Methods, Tests and Applications Assurance and Safety of Crops & Foods*.
15. Lawless, H. and Hildegarde, H. 1999. *Sensory Evaluation of food: Principles and Practices*. 1st ed., Springer. New York.
16. *Statistical Analysis System*. 2008. *SAS User's Guide*. SAS Institute Inc., Cary, NC. USA.

SALT INTAKE THROUGH BAKERY PRODUCTS IN SLAVONIA REGION

UDC 664.66 : 664.644.1(497.54)

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ABSTRACT

The reduction of salt intake is a public health issue in many countries because it has been identified as the main cause of several diseases such as hypertension, heart and kidney diseases, stomach cancer, osteoporosis, stroke and obesity. The WHO has set a worldwide target of a maximum salt intake of 5 g/day for adults as a recommendation to reduce daily salt intake. Of all foodstuffs, bread has been identified as the single highest contributor to the total daily salt intake. Therefore, the aim of this paper was to estimate salt intake through bakery products in Slavonia Region and their dependency on different parameters. The quantity of bakery product intake derived from national food consumption survey. The results showed that consumers in Slavonia eat about 148 g/day of various bakery products, and consequently intake 2.43 g/day of salt, which is half of the recommended daily intake. Research has shown that salt intake by bakery products are almost the same in villages and towns. Related to gender, men's intake is higher than women. The intake is highest at low body mass index, and is higher in younger than in elderly population.

Keywords: salt intake, bakery products consumption, national food consumption survey

INTRODUCTION

One third of all global deaths can be attributed to cardiovascular disease (CVD) including heart attack, stroke and related diseases [1]. Elevated blood pressure is a major, modifiable, causal factor of CVD, and salt (sodium chloride) is the primary cause of raised blood pressure. The main source of salt in the diet is processed foods (about 70-75% of the total intake), with cereal and cereal products contributing 30% of overall intake [2].

The vast majority of people have no idea about the salt contents in the food they eat. Therefore, they do not know how much salt they ingest. As a result of this, high sodium intake occurs without the consumer's knowledge and they cannot control their salt

intake. Therefore people in their households need to reduce the salt they add to food, but most importantly, the food industry needs to lower the salt contents [3].

In UK in 1996, a number of experts set up an action group, the Consensus Action on Salt and Health (CASH) with the aim to negotiate with food manufacturers and suppliers a universal and gradual reduction of the salt content of processed foods as well as to increase community awareness about excess salt intake. Following the example of CASH, a World Action Group on Salt and Health (WASH) was established in 2005, with participation of over 300 international experts. WASH aims at reduction of salt in the diet worldwide by exerting pressure on multi-national food companies to reduce the salt content of their products [4].

In 2006, the First Croatian Congress on Hypertension announced Declaration of salt reducing programme in Croatia, and in 2007 at the 6th Croatian Congress on Atherosclerosis Croatian Action on Salt and Health (CRASH) the national programme for reducing salt intake was launched [5].

The World Health Organization (WHO) has set a worldwide target of a maximum salt intake of 5 g/day for adults as a recommendation to reduce daily salt intake [6].

The aim of this study was to estimate salt intake through bakery products in Slavonia Region and their dependency on different parameters (place of living, gender, BMI and age).

MATERIALS AND METHODS

Croatian Food Agency conducted National Food Consumption Survey in Croatia. The survey was carried out in accordance with EFSA's guidance [7]. It was conducted among the adult population (18-64 years) in two parts (1st part – autumn 2011, 2nd part – summer 2012) on representative sample of 1000 respondents in each part. A 24-hour recall method was used and survey was conducted in two non-consecutive working days (with at least two weeks interval between them) and one day of weekend. Data were collected by face-to-face interview at participants home. Representative sample covered different socio-demographic parameters (regional coverage, the ratio of rural-urban environment, education, monthly income, employment status, family status, level of physical activity, body weight and height). Also, the questionnaire on the frequency of consumption, with classification from several times a day to once a year, was used to determine chronic exposure. The manual from Senta et al [8] was used to specify the amount of consumed food. The household measurement and then weighing of particular food were used for food that is not included in the manual [8]. All participants were asked additionally questions to describe food and drink they consumed (place of consumption, where food was bought, type of preparation, brand, origin of food...) in order to help in identifying specificity of food and drinks in classification.

This research comprises only data from 1st part of the survey and does not include consumption of filled bakery products.

The salt content in bakery products was calculated on the basis of two data sources: data obtained in questionnaire regarding bread and rolls consumption and salt content from paper Ugarčić-Hardi et al [9].

RESULTS AND DISCUSSION

From 175 participants in Slavonia Region, 9 participants are excluded because of missing data. Only consumers of bakery products are included in this paper, and there are 158 consumers in this Region. They consumed approximately 148 grams of bakery products per day.

If we look at the data of Croatian Bureau of Statistic for 2011 we can see that the consumption of bread and other bakery products was 73.9 kg per year which is approximately 202 g/day per household member [10]. This difference in consumption may be the result of various research methodologies, and because of the fact that we didn't include filled bakery products in our research.

Data from EFSA Comprehensive European Food Consumption Database [11] shows that in each country more than 95% of participants consume bread and rolls, except in Finland where the percentage of consumers is 35%. According to the Database [11] consumption in countries such as Belgium, Germany and Spain, which have used the same method as in our study, consumption was 129.4 g/day; 138.1 g/day and 90.2 g/day, respectively.

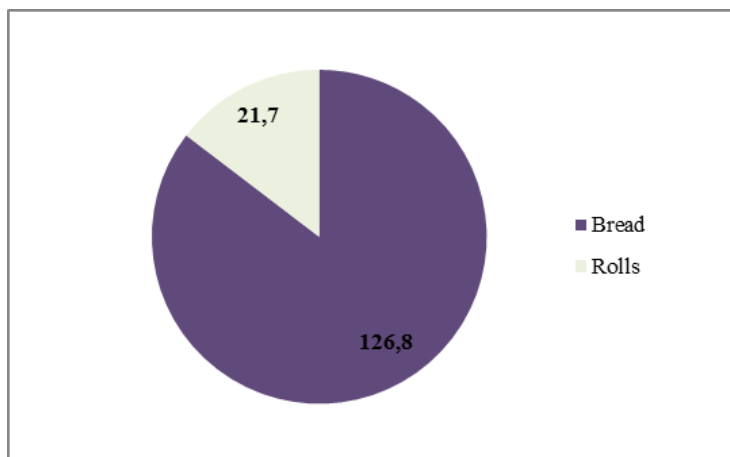


Figure 1. Consumption of bakery products (g/day)

From total daily bakery products intake (148 g/day) bread represent 85.4% (126.8 g/day) and the rest are rolls in quantity of 21.7 g/day (Figure 1).

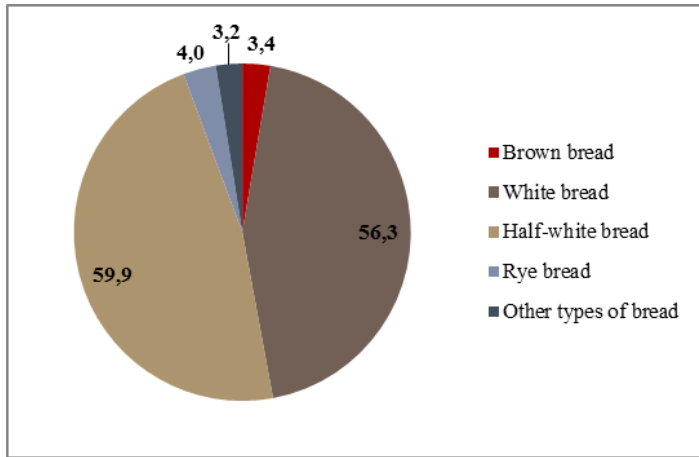


Figure 2. Consumption of bread (g/day)

When we take into account different types of bread, there are two the most common types: half-white bread (47.3%) and white bread (44.4%). It is interesting that brown bread is present in only 2.7%.

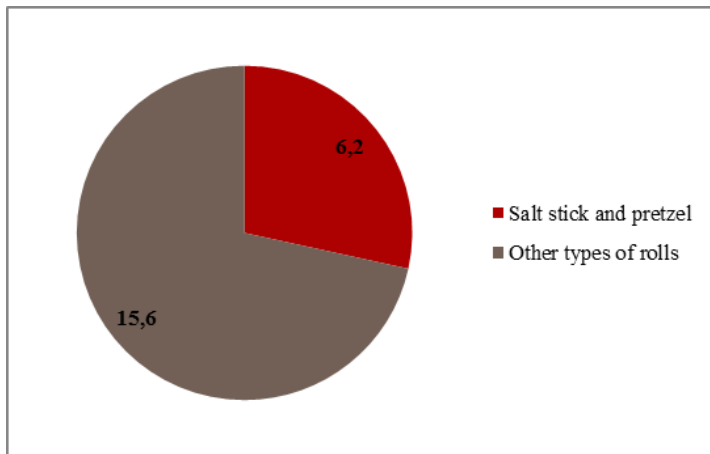


Figure 3. Consumption of rolls (g/day)

Figure 3 shows the consumption of rolls and rolls strewed with salt in g/days, where consumption of salt sticks and pretzel, which have additional salt on the surface, is 1/3 of all consumed rolls.

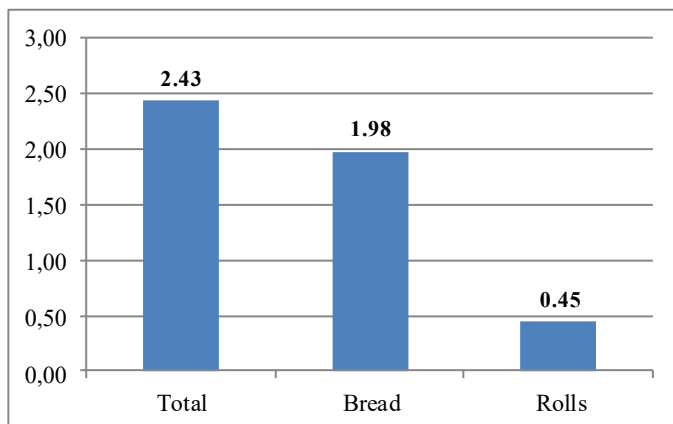


Figure 4. Salt intakes (g/day) from bakery products

Figure 4 shows that salt intake through bread is 1.98 g/day, what is almost 4.5 times more than through rolls, where intake is 0.45 g/day. This is expected when we take into account quantity of consumption of these products.

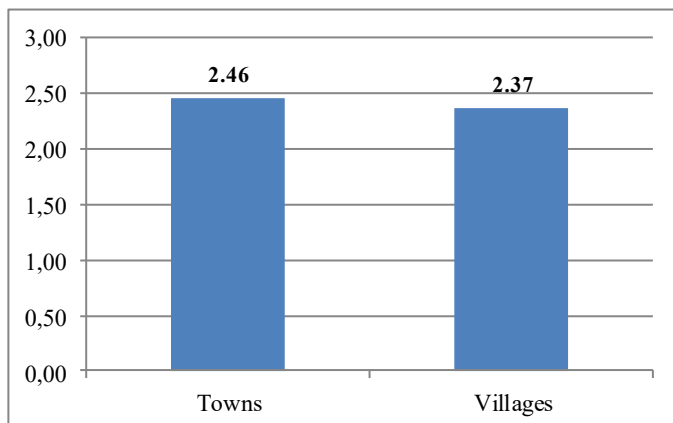


Figure 5. Salt intakes (g/day) in towns and villages

From a total of 158 consumers, 102 (64.6%) consumers live in towns and 56 (35.4%) in villages. Average salt intakes are 2.46 g/day in towns and 2.37 g/day in villages that shows that salt intake is almost the same.

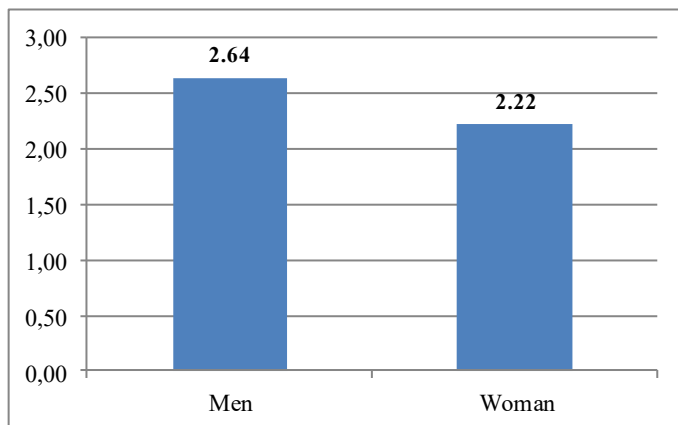


Figure 6. Salt intakes (g/day) according the gender

77 men and 81 women consume bakery products. Their average salt intakes are 2.64 g/day and 2.22 g/day, respectively. Results show that salt intake is higher in male population. But when we consider higher average body weight for men (84 kg) in relation to women (69 kg), we can see that for both gender the average salt intake is 0.03 g/kg of body weight, which means that this difference is not significant.

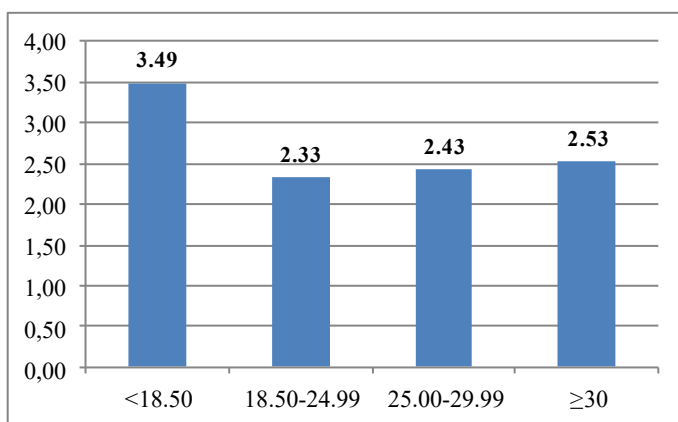


Figure 7. Salt intake (g/day) according BMI

BMI scale is divided in four categories. First category is underweight (<18.50), second is normal weight (18.50-24.99), third is overweight (25-29.99) and fourth is obesity (≥ 30). The results show that salt intake is highest at low BMI. It could mean that they eat more bakery products than other BMI categories or that they eat more products stewed with salt. Nevertheless, this result should be taken with a reserve, because the number of respondents in this category is very low. Additionally, these should be analyzed more detailed to see which products they consumed, which age classes are at lowest BMI and their monthly income, in order to explain this result.

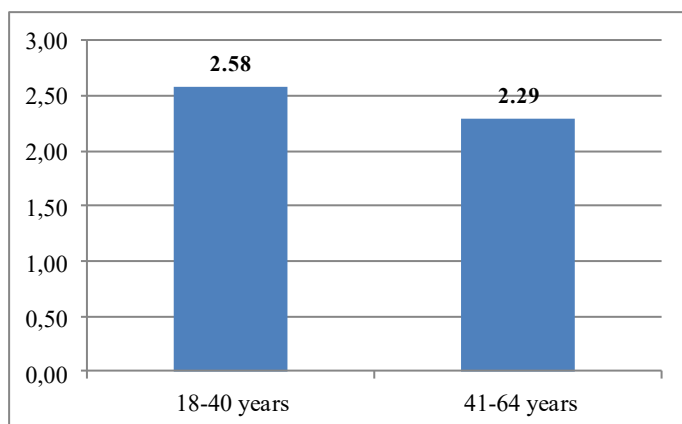


Figure 8 Salt intakes (g/day) according the age

Regarding age there are 2 categories. The first category represents 18-40 years old consumers and the second 41-64 years old. There are 75 consumers in 1st category and 83 consumers in 2nd category. Salt intake is higher at younger population. This also, partly, can be explained by quantity of food that younger people can eat in relation to older people, who eat less quantity of food per day.

CONCLUSION

Our results show that salt intake through bakery products in the Slavonia Region is 2.43 g/day. That can indicate that the total salt intake significantly exceeds the limit of 5 g of salt/day set by the WHO.

Salt intake through bakery products represent half of the recommended amount and various studies show that the amount of salt in bakery products can be reduced without affecting the quality and organoleptic characteristics of the final product. Therefore is

necessary to encourage manufacturers, or it should be legally regulated, to reduce the amount of salt in bakery products as much as possible.

In meantime, it is necessary to raise awareness of this problem through campaign for consumers' education and to influence industry in order to reduce quantity of salt in their products and so become recognized as producers that take care about consumers' health.

REFERENCES

1. WHO/FAO. 2003. Diet, nutrition and the prevention of chronic diseases. In WHO Technical Report Series 916. Geneva, Switzerland. http://whqlibdoc.who.int/trs/who_trs_916.pdf [15.09.2013.]
2. Lynch E.J., Dal Bello F., Sheehan E.M., Cashman K.D., Arendt E.K. 2009. Fundamental studies on the reduction of salt on dough and bread characteristics. *Food Res. Int.* 42:885-891.
3. Plácido A., Kupers R., Paíga P., Magalhães J., Nouws H. P. A., Delerue-Matos C., and Oliveira M. B. P. P. 2012. Salt content in bread and dough from north Portugal: Method development and comparison. *J. Food Compos. Anal.* 27:14-20.
4. Strazzullo P., Cairella G., Campanozzi A., Carcea M., Galeone D., Galletti F., Giampaoli S., Iacoviello L., and Scalfi L. 2012. Population based strategy for dietary salt intake reduction: Italian initiatives in the European framework. *Nutr. Metab. Cardiovasc. Dis.* 22:161-166.
5. Jelaković B., Kaić-Rak A., Milčić D., Premuzić V., Skupnjak B., and Reiner Z. 2009. Less salt – more health. Croatian action on salt and health (CRASH). *Liječ Vjesn*, 131(3-4):87-92.
6. World Health Organization (WHO). Guideline: Sodium intake for adults and children. Geneva. 2012.
7. EFSA (2009). General principles for the collection of national food consumption data in the view of pan-European dietary survey. *EFSA Journal.* 7(12):1435.
8. Senta A., Pucarín-Cvetković J., and Doko Jelinić J. 2004. Kvantitativni modeli namirnica i obroka. *Medicinska naklada*, Zagreb.
9. Ugarčić-Hardi Ž., Dumančić G., Pitlik N., Koceva Komlenić D., Jukić M., Kuleš A., and Sabo M. 2010. The salt content in bakery products in Osječko-Baranjska County. Page 551 in: *Proceedings of 5th International Congress Flour-Bread 2009.*, Opatija, Croatia.
10. Državni zavod za statistiku: Statistički ljetopis Republike Hrvatske 2012. Zagreb, 2012.
11. EFSA (2011). The EFSA Comprehensive European Food Consumption Database. <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm> [17.09.2013.]

ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA AND THEIR APPLICATION ON WHEAT SEEDS DECONTAMINATION

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ABSTRACT

This paper presents the results of the in vitro experiments aimed to evaluate the antimicrobial activity of lactic acid bacteria (LAB) against seed-borne pathogens in spring and winter wheat. The evaluation of antimicrobial activities of *Lactobacillus sakei*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* strains producing organic acids and bacteriocins like inhibitory substances against *Fusarium culmorum*, *Fusarium poae* and *Penicillium* spp. microorganisms widely common on wheat grains, were performed using an overlay assay method. *Fusarium* spp. was effectively inhibited by *P. pentosaceus* KTU05-10, whereas *P. acidilactici* KTU05-7 effectively suppress the growth of *F. culmorum*, *F. poae* and *Penicillium* spp. Wheat seeds pre-treatment with *L. sakei* KTU05-6, *P. acidilactici* KTU05-7 and *P. pentosaceus* KTU05-10 decreased the damage of wheat seedlings respectively, 33.6, 23.6 and 15.6%, whereas *L. sakei* KTU05-6, *P. acidilactici* KTU05-7, *P. pentosaceus* KTU05-8 and KTU05-10 decreased rot of wheat roots respectively, 6.0, 11.0, 7.9 and 11.5%. The significantly higher antifungal activity was recorded after LAB application at 20 and 30 ml per 100 g seeds compared with 10 ml per 100 g seed dose. According to the obtained results 20 ml of LAB suspension dose may be used for successful *Fusarium* contaminants removal from wheat grains surface.

Keywords: Lactic acid bacteria, *Fusarium*, wheat seeds, decontamination

INTRODUCTION

Microscopic fungi and their produced metabolites such as mycotoxins are often found as contaminants in agricultural products. Mycotoxins are secondary metabolites produced by a wide variety of filamentous fungi, including species from the genera *Aspergillus*, *Fusarium* and *Penicillium*. They cause nutritional losses and represent a significant

hazard to the food and feed chain [1]. Environmental factors, especially temperature and humidity, play a very important role in growth of fungal and mycotoxin production in cereals especially on the *Fusarium* species occurrence and disease severity [2]. The same factors affect the content and composition of mycotoxins in cereal grain [3,4]. The *Fusarium* spp. predominantly found in association with seedling blight, in small-grain cereals all over Europe. The diseases are commonly caused by *Fusarium culmorum*, *F. graminearum*, *F. avenaceum* and [5]; however *F. poae*, *F. tricinctum*, *F. sporotrichioides*, *F. equiseti* and *F. langsethiae* are also very common [6, 7]. The *Fusarium* grain infection decreases the germination and quality of kernels in connection with mycotoxic contamination. The most economically important mycotoxins occurring in food and feed are aflatoxins, ochratoxin A, patulin, and fusariotoxins that cause serious human illness are deoxynivalenol (DON), diascetoxyscirpenol (DAS), T-2 toxin, HT-2 toxin, nivalenol, zearalenone (ZEN), fumonisins [5, 8-10]. Principally, there are three possibilities to avoid harmful effect of contamination of food and feed caused by mycotoxins: (1) prevention of contamination, (2) decontamination of mycotoxin-containing food and feed, and (3) inhibition of absorption of mycotoxin content of consumed food into the digestive tract [11]. Seed infection can be controlled in different ways: chemical control and alternative methods as natural preservatives. Recently, natural agents produced using microorganisms with antimicrobial properties have received considerable interest in the international practice. One of the major compounds that are currently used to produce safe products are bacteriocins, released by lactic acid bacteria (LAB). LAB are generally recognized as safe (GRAS) and play an important role in food and feed fermentation and preservation either as the natural microflora or as starter cultures added under controlled conditions [12]. The antimicrobial effect of LAB is mainly related to the production of organic acids, however some strains are able to synthesize antimicrobial substances – bacteriocins [13]. LAB during growth releases molecules that potentially inhibit growth of fungi [14, 15] and therefore lead to a lower accumulation of their mycotoxins [16]. Gourama, 1991 [17], using a dialysis assay, demonstrated the occurrence of a metabolite that inhibits aflatoxin accumulation in *Lactobacillus* cell-free extracts. Coallier-Ascah and Idziak, 1985 [18] reported a significant reduction of aflatoxin biosynthesis by *Lactobacillus*-cell free supernatants and suggested that this inhibition was related to a heat stable, low-molecular-weight inhibitory compound.

The creation of bioproduct produced by LAB is necessary to increase the resistant of the plant against developing phytopathogens such as fungi producing secondary metabolites (e.g. mycotoxins) during the growth of the plant as well as with that to increase the efficiency of organic farming. The aim of these studies was to evaluate antifungal activity of LAB and the ability to use LAB for the detoxification of wheat seeds in an organic system.

MATERIALS AND METHODS

LAB preparation for antifungal activity testing

Lactobacillus sakei KTU05-6, *Pediococcus pentosaceus* KTU05-8, KTU05-9 and KTU05-10, *Pediococcus acidilactici* KTU05-07, able to produce BLIS designated as sakacin 05-6, pediocin 05-8, 05-9, 05-10 and pediocin 05-7 respectively, were originally isolated from Lithuanian sourdoughs [19]. All LAB were grown in de Man Rogosa Sharpe (MRS) medium (Oxoid, Milan, Italy) at 25 °C (KTU05-8 and KTU05-9), 30 °C (KTU05-6) or 35 °C. 2% of LAB cells were inoculated into a fresh medium and propagated for 18 h. Fresh LAB suspension including supernatants and LAB cells cultivated in MRS media was used for grain (wheat and rye) detoxication.

Antimicrobial activity determination using overlay assay method

LAB antimicrobial activity against *F. culmorum*, *F. poae* and *Penicillium* spp. was evaluated using overlay assay method. 1 µl of overnight LAB suspension was inoculated on 20 mm length line on MRS agar media surface on Petri dishes and incubated 3 days at optimal LAB temperatures. 15 ml of potato dextrose agar media with inoculated 10⁴ spores/ml of *F. culmorum* and *F. poae* and *Penicillium* spp. were overlaid on surface. The plates were inoculated 11 days at 25 °C. Antifungal activity evaluated if clear zone was found around line of LAB. Length and width of the clear zone was measured.

The seed treatment with LAB

The research involved laboratory tests on the seeds of spring wheat cvs. 'Granary' naturally infected by *Fusarium* pathogens and winter wheat *Triticum aestivum* L. naturally contaminated with *Fusarium* spp, *Mucor* spp. *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* spp. Seed treatment was done by hand in glass bottles and seeds were accurately shaken for 10 min. LAB suspension doses: 10, 20 and 30 ml was applied. The seeds were plating on Petri dishes with previously prepared fresh potato dextrose agar (PDA) media. The petri dishes with seeds were incubated in thermostat at 25 °C from 4 to 11 days when overlay assay method was used. Temperature effect on LAB efficiency was evaluated when the fungi was cultivated 7 days at 15, 20 and 25 °C. The inhibition of fungi was expressed (%) in comparison with control sample that was not pre-treated with LAB.

RESULTS AND DISCUSSION

Antimicrobial activity of LAB against Fusarium and Penicillium spp.

Antimicrobial activity of LAB against *F. culmorum*, *F. poae* and *Penicillium* spp. was evaluated using overlay assay method and presented in Table 1. The highest antifungal activity against *F. culmorum* show *P. pentosaceus* KTU05-9 (23.0/38.4 mm) after four days

growth. *F. poae* and *F. culmorum* was effectively inhibited by *P. pentosaceus* KTU05-9 (16.0/26.2 and 23.0/38.4 mm, respectively), *P. acidilactici* KTU05-7 (16.2/28.2 and 12.4/24.2 mm, respectively) and *L. sakei* KTU05/6 (9.6/22.8 and 8.0/22.4 mm, respectively) after four days incubation. *Penicillium spp.* growth was suppressed by *P. acidilactici* KTU05-7 and *L. sakei* KTU05-6. The highest antifungal activity spectrum show *P. acidilactici* KTU05-7 that inhibit the growth of fungi of all tested indicator microorganism after 11 days of cultivation. Antifungal activity of *P. pentosaceus* KTU05-9 against *F. culmorum* after 4, 7 and 11 days incubation is shown in Fig.1.

Table 1. Antimicrobial activity of LAB against fungi

LAB	Inhibition zone (width/ length), mm								
	<i>Fusarium culmorum</i>			<i>Fusarium poae</i>			<i>Penicillium spp.</i>		
	Fungi cultivation time, days								
	4	7	11	4	7	11	4	7	11
Pp KTU05-9	23.0/	13.4/	4.0/	16.0/	14.2/	10.4/	-	-	-
	38.4	21.2	20.6	26.2	24.8	20.8			
Pa KTU05-7	12.4/	9.2/	7.2/	16.2/	15.0/	12.2/	18.0/	16.6/	14.4/
	24.2	21.4	20.6	28.2	22.6	21.0	24.6	23.2	22.0
Ls KTU05-6	8.0/	5.0/	-	9.6/	2.4/	-	13.8/26.6	12.0/	9.2/
	22.4	20.8		22.8	20.2			24.2	23.8

Lactobacillus sakei (Ls), *Pediococcus pentosaceus* (Pp) and *Pediococcus acidilactici* (Pa)



Figure 1. *P. pentosaceus* KTU05-9 antifungal activity against *F. culmorum* after 4 (a), 7 (b) and 11 (c) days of incubation

Wheat seeds decontamination using bacteriocins producing LAB

Additionally, antifungal activity of LAB was evaluated against various fungi present on wheat seeds. Decontamination effect of LAB was evaluated against *A. flavus*, *Fusarium* spp., *Penicillium* spp., *A. niger* and *Mucor* spp. fungi that was found on naturally contaminated wheat seeds (Fig. 2). The highest inhibition of *Fusarium* spp., *Mucor* spp. and *A. niger* was observed using LAB for wheat grains decontamination. The highest antifungal activity show *P. acidilactici* against *Fusarium* spp. and *A. niger* (respectively, 57 and 83% reduction of fungal contamination). Moreover *P. acidilactici* inhibit the growth of *A. flavus* and *Mucor* spp. by 21 and 43%, respectively. The lowest inhibition of *P. acidilactici* was observed against *Penicillium* spp. (2%). The highest antifungal activity of *P. pentosaceus* KTU05-9 was observed against *Fusarium* spp. (48%). *Mucor* spp., *A. flavus*, *A. niger* and *Penicillium* spp. growth on wheat seeds were decreased by *P. pentosaceus* KTU05-9 up to 48, 21, 35 and 23%, respectively. *L. sakei* KTU05-6 decreased *A. niger*, *Fusarium* spp. and *Mucor* spp. growth by 83, 49 and 42%, respectively. Low antimicrobial activity of *L. sakei* was observed against *A. flavus* and *Penicillium* spp. Studies show that bacteriocins producing LAB with antifungal activities are suitable to use for fungi such as *Fusarium* spp., *Mucor* spp. and *A. niger* inhibition and cereals decontamination, moreover LAB combinations could be used to achieve higher decreasing of pathogens and increase control of microbiological contamination.

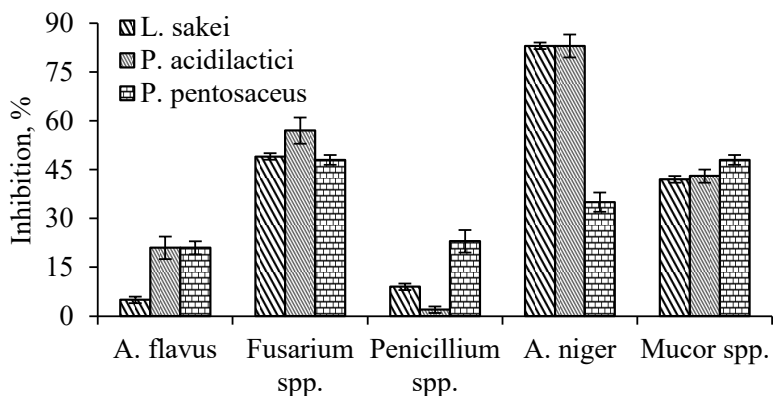


Figure 2. LAB antifungal activity (%)

LAB influence on *Fusarium* infection and wheat seeds decontamination

LAB used for strongly infected wheat seeds by *Fusarium* fungi decontamination at 15, 20 and 25 °C temperatures gave different antimicrobial activity against *Fusarium* spp. (Fig.3). *L. sakei* and *P. pentosaceus* KTU05-10 were most effective at 25 °C, while

P. acidilactici showed the best efficacy at 15 °C. *P. pentosaceus* KTU05-8 gave similar efficacy at both temperatures – 15 and 20 °C. *P. pentosaceus* KTU05-9 reduced *Fusarium* infection at all temperatures by 12-16%. *P. pentosaceus* KTU05-10 show the best antimicrobial activity against *Fusarium* fungi and it significantly reduced *Fusarium* infection level independent of cultivation temperature. Dalie et al., 2010 [13] reported that LAB have the highest antimicrobial effect when cultivated at 25 °C. However, antifungal activity of LAB may be affected not only by temperature, but also by used LAB species, time of incubation, growth medium, pH, and nutritional factors [20].

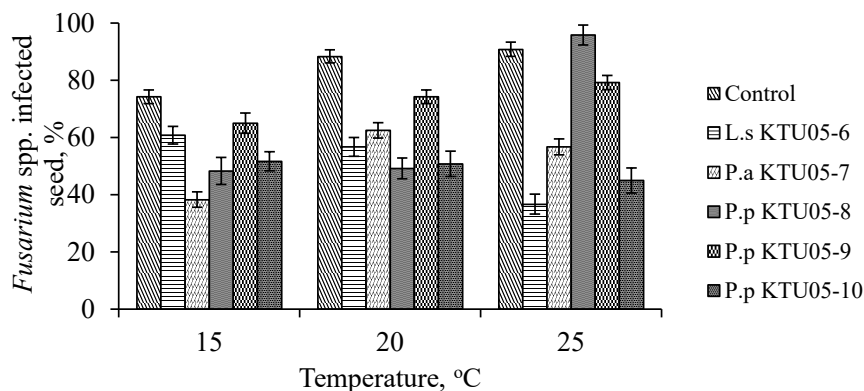


Figure 3. Temperature influence on antifungal activity of LAB for *Fusarium* spp. infection decreasing on wheat seeds at different

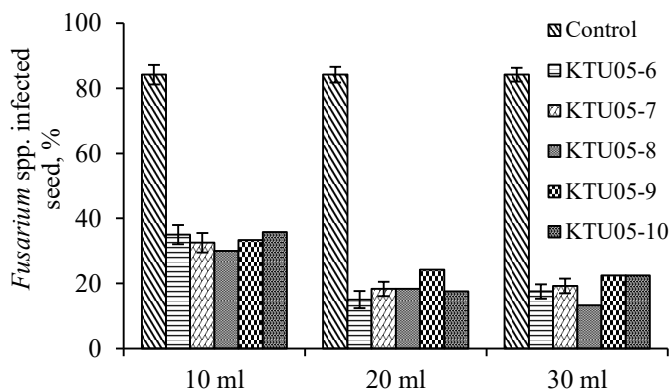


Figure 4. LAB dose influence on *Fusarium* spp. infection decreasing on wheat seeds

Dose of product is an important factor as well, therefore different concentrations of LAB suspension (10, 20 and 30 ml) was applied for cereals decontamination.

LAB applied at different doses (10, 20 and 30 ml) after one week incubation at 20 °C significantly reduced *Fusarium* infection on spring wheat seeds (Fig. 4). The significantly higher antifungal activity was recorded after LAB application at 20 and 30 ml per 100 g seeds compared with 10 ml per 100 g seed dose. According to the results 20 ml dose may be used for cereals decontamination.

Was found significantly positive influence of LAB treatment on the lower part of wheat seedlings and roots (Table 2). Wheat seeds pre-treatment with *L. sakei* KTU05-6, *P. acidilactici* KTU05-7 and *P. pentosaceus* KTU05-10 significantly decreased the damage of wheat seedlings respectively, 33.6, 23.6 and 15.6%, whereas *L. sakei* KTU05-6, *P. acidilactici* KTU05-7, *P. pentosaceus* KTU05-8 and KTU05-10 significantly decreased rot of wheat roots respectively, 6.0, 11.0, 7.9 and 11.5%.

Table 2. LAB influence on wheat seeds seedlings and roots diseases

Applied LAB	Seedlings diseases, %	Roots diseases, %
Control	54.6	24.7
<i>L. sakei</i> KTU05-6	36.2	16.5
<i>P. acidilactici</i> KTU05-7	41.7	17.5
<i>P. pentosaceus</i> KTU05-8	54.1	26.8
<i>P. pentosaceus</i> KTU05-9	52.3	21.4
<i>P. pentosaceus</i> KTU05-10	46.1	22.2

Bonestroo et al., 1993 [21] reported that fungi are sensitive to fermentation products, lactic and acetic acids. Propionic acid reduces fungal growth especially at lower pH [22] and inhibit amino acid uptake [23]. Schnürer & Magnusson, 2005 [24] research show that number of antifungal metabolites, e.g. peptides, phenyllactic acid, proteinaceous compounds and 3-hydroxylated fatty acids have also been isolated from lactic acid bacteria.

CONCLUSIONS

Tested LAB *L. sakei* KTU05-6, *P. acidilactici* KTU05-07, *P. pentosaceus* KTU05-8, KTU05-9 and KTU05-10, show antimicrobial activity against toxins producing fungi, especially *Fusarium* species. These LAB could be applied as alternative for the bioproduct creation to increase the resistant of seeds and plants against pathogens such as *Fusarium* fungi able produce mycotoxins, in organic farming.

ACKNOWLEDGMENTS

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REFERENCES

1. Juodeikiene, G., Bartkiene, E., Viskelis, P., Urbonaviciene, D., Eidukonyte, D. and Bobinas, C. 2012. Fermentation processes using lactic acid bacteria producing bacteriocins for preservation and improving functional properties of food products. Page 63-100 in: *Advances in Applied Biotechnology*, M. Petre (Ed.). InTech, Rijeka, Croatia.
2. Parry, D.W., Jenkinson, P. and Mc Leod, L. 1995. Fusarium ear blight (scab) in small grain cereals – a review. *Plant Pathol.* 44:681-686.
3. Perkowski, J., Basiński, T., Wiwart, M., Kostecki, M., Buško, M. and Matysiak, A. 2008. The effect of environmental conditions on ergosterol and trichothecene content of naturally contaminated oat grain. *Ann. Agric. Environ. Med.* 15:271-276.
4. Ramirez, M.L., Chulze, S. and Magan, N. 2006. Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. *Int. J. Food Microbiol.* 106:291-296.
5. Bakutis, B., Baliukoniene, V. and Lugauskas, A. 2006. Factors predetermining the abundance of fungi and mycotoxins in grain from organic and conventional farms. *Ekologija.* 3:122-127.
6. Bottalico, A. and Perrone, G. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *Eur. J. Plant Pathol.* 108:611-624.
7. Parry, D.W., Jenkinson, P. and Mc Leod, L. 1995. Fusarium ear blight (scab) in small grain cereals – a review. *Plant Pathol.* 44:681-686.
8. Brennan, J.M., Fagan, B., Maanen, A., Cooke, B.M. and Doohan, F.M. 2003. Studies on in vitro growth and pathogenicity of European *Fusarium* fungi. *Eur. J. Plant. Pathol.* 109:577-587.
9. Samson, R.A., Hoekstra, E.S. and van Oorschot, C.A.N. 1988. *Introduction to food-borne fungi*. Centraalbureau voor Schimmelcultures, Institute of the Royal Netherlands, Academy of Arts and Sciences, Baarn, The Netherlands.
10. Chassy, B.M. 2010. Food safety risk and consumer health. *New Biotechnology.* 5:534-544.
11. Halász, A., Lásztity, R., Abonyi, T., Bata, A. 2009. Decontamination of Mycotoxin-Containing Food and Feed by Biodegradation. *Food Rev. Int.* 25:284-298.
12. Diop, M.B., Dibois-Dauphin R., Tine, E., Jacqueline, A.N., Thonart, P. 2007. Bacteriocin producers from traditional food products. *Biotechnol. Agron. Soc. Environ.* 11:275-281.
13. Dalié, D.K.D., Deschamps, A.M., Richard-Forget, F. 2010. Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food control.* 21:370-380.
14. Cizeikiene, D., Juodeikiene, G., Paškevičius, A. and Bartkienė, E. 2013. Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. *Food Control.* 31:539-545.

15. Digaitiene, A., Hansen, A., Juodeikiene, G., Eidukonyte, D. and Josephsen, J. 2012. Lactic acid bacteria isolated from rye sourdoughs produce bacteriocin-like inhibitory substances active against *Bacillus subtilis* and fungi. *J. Appl. Microbiol.* 112:732–742.
16. Gourama, H., Bullerman, L.B. 1995. Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacillus* species. *J. Food Prot.* 58:1249-1256.
17. Gourama, H. 1991. Growth and aflatoxin production of *Aspergillus flavus* in the presence *Lactobacillus* species. Ph.D. thesis, University of Nebraska-Lincoln.
18. Coallier-Ascah, J., Idziak E., 1985. Interaction between *Streptococcus lactis* and *Aspergillus flavus* on production of aflatoxin. *Appl. Environ. Microbiol.* 49:163-167.
19. Digaitiene, A., Hansen, A., Juodeikiene, G., & Josephsen, J. 2005. Microbial population in Lithuanian spontaneous rye sourdoughs. *Ekologia i Technika.* 13:193-198.
20. Batish, V.K., Roy, U., Lal, R. and Grover, S. 1997. Antifungal attributes of lactic acid bacteria – A review. *Crit. Rev. Biotechnol.* 17:209-225.
21. Bonestroo, M.H., Dewit, J.C., Kusters, B.J.M. and Rombouts, F.M. 1993. Inhibition of the growth of yeasts in fermented salads. *Int. J. Food Microbiol.* 17:311-320.
22. Woolford, M.K. 1984. The antimicrobial spectra of some salts of organic acids and glutaraldehyde in respect to their potential as silage additives. *Grass Forage Sci.* 9:53-57.
23. Eklund, T. 1989. Organic acids and esters. Page 161-200 in: *Mechanisms of action of food preservation procedures*, G.W. Gould (Ed.), Elsevier Applied Sciences, New York, USA.
24. Schnürer, J. and Magnusson J. 2005. Antifungal lactic acid bacteria as biopreservatives. *Trends Food Sci. Tech.* 16:70-78.

COMPETITIVITY OF CROATIAN MILLING INDUSTRIES; DOMESTIC RESOURCE COST APPROACH

UDC 664.7 (497.5)

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ABSTRACT

The aim of this study was to evaluate, is it existing competitiveness of the milling industry in Croatia, ready for the market environment in the European Union. The research methodology is based on the application of a group of indicators trade openness and discovered competitive advantages, as well the overall analysis of the structure of the milling industry in Croatia. Analysis of competitiveness of Croatian milling industries using the domestic resource cost (DRC) method, taking into account opportunity costs of not only input factors, but also the foreign exchange rate and the price of final product in the EU market, which refers to the ratio between opportunity costs of domestic production and value added. The conducted research has shown some specifics of the perception of the competitiveness of Croatian milling industries.

Keywords: competitiveness, domestic resource cost, milling industries, Croatia

INTRODUCTION

This paper examines the competitiveness of Croatian companies milling industry. Competitiveness as a success factor is present in every company, regardless of whether the company at the national or international market. DRC method was applied to the sample companies separate sector for the period since 2011 to 2012th year. Competitiveness of enterprises is modelled as a function of their activities, the characteristics and features of their environment. The research results are consistent with theoretical predictions about the behaviour of not competing companies. In building their international competitiveness of the Croatian mill sector relies mainly on reducing costs, improving productivity, reducing the number of employees and expanding supply of finished products. Mill sector in Croatia is characterised by severe business conditions with pronounced challenges ruthless market that additional burden supernumerary mills, and grey economy.

GENERAL INFORMATION ABOUT CROATIAN MILLING INDUSTRY

The milling industry is actively 167 mills - users of registry stamps for the purposes of Republic Croatia, 11 producers of mixtures and concentrate, 47 importers of flour, 35

importers of mixtures and concentrates, and 7 legal entities engaged in the pre-packaging of flour. Annual production is 450-550000 tones of flour, the total milling wheat is 75% (bread flour), meal participates in the structure of milling wheat with 20% and bran with 4-5%.

Table 1. Production of flour in Croatia by type

Type of flour	The amount (tonnes)	The structure of production (%)
T-400	15.000	3
T-550	250.000	53
T-850	75.000	16
T-700	1.500	0,3
T-1100	5.000	0,3
T-1600	500	0,1
Integrated	1.500	0,3
Bran	25.000	5
Meal	100.000	21

Source: Croatian Chamber of Economics, Zagreb, Annual report 2013

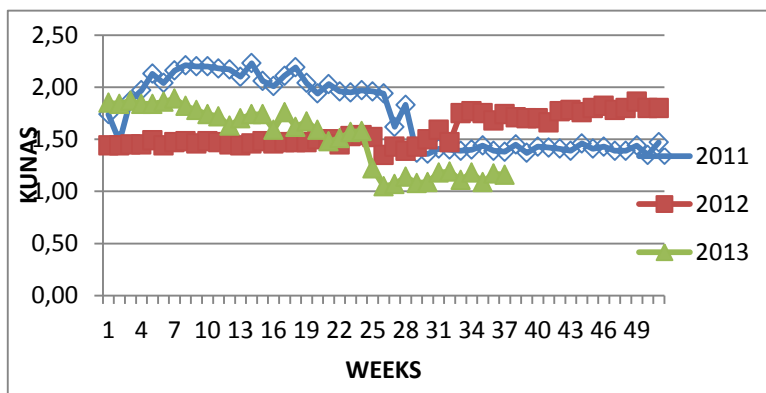


Figure 1. Movement of wheat prices in 2011, 2012 and 2013. year, Croatia (Ministry of agriculture 2013)

According to the records of the Ministry's 10 largest millers produce 70% of domestic flour (from approximately 400,000 tones), and the 5 biggest importer imports 73% of imported flour (approximate 2,200 tones). By analysing the level of the average production per production unit-mill for the Republic of Croatia is 2,423 tones, or utilisation of production capacity is 40%.

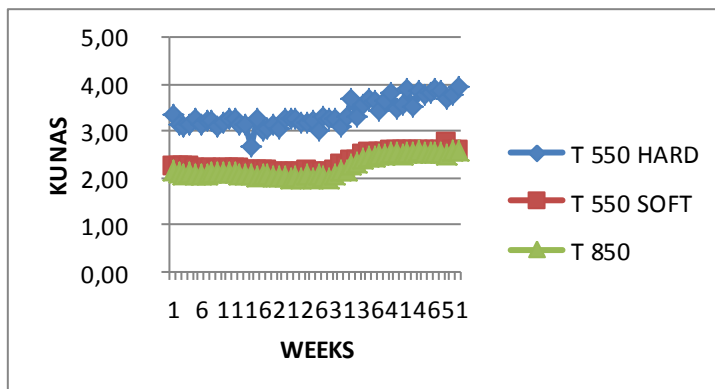


Figure 2. Movement of prices for 3. type of flour in 2012. year, Croatia (Ministry of agriculture 2013)

RESEARCH

For the purposes of this study were interviewed subjects in milling production in Republic Croatia, research was carried out in 2013 years, in order to collect data on the current economic and social situation in isolated producers. Of the total number of active mills 167, listed demanding questionnaire completed by the 18 subjects, with a focus on biggest producers from eastern Croatia (Slavonia and Baranja).

And in order to collect the necessary parameters for calculating the costs of the application of domestic resources (DRC). Collected data of the impact of politics, technology, economics, social impact, information on subcontractors, storage capacities and competition for the mill sector in Croatia. Based on data collected from the field, and the available data on the total production of the mill sector in Croatia, made the corresponding calculation of this coefficient competitiveness (DRC).

Domestic Resource Cost ratio (DRC)

The DRC method is presented as a ratio of domestic costs to the real value added. Though the DRC method is one of the best methods for measuring comparative

advantages, it contains some particular credible requirements, such as data actuality and technological exactness and uniformity in defining production conditions of comparative technologies.

DRC method is mostly used in practise to assess the general agricultural competitiveness, which is based on an analysis of DRC indicators. DRC compares the opportunity costs of domestic production with the resulting added value. This study applied this ratio in order to obtain a clearer picture of the situation in the analysed sector.

The *Domestic Resource Cost* ratio is usually presented in the form

$$DRC_i = \frac{\sum_{j=k+1}^n a_{ij} P_j^D}{P_i^B - \sum_{j=1}^k a_{ij} P_j^B}$$

with

- a_{ij} quantity of the j -th traded (if $j \leq k$) or non-traded (if $j > k$) input ($j = 1, 2, \dots, n$) used to produce one unit of output i ;
- P_j^D social price of non-traded input j ,
- P_i^B border price of output i ,
- P_j^B border price of traded input j .

The formula shown in our example in the numerator is put sum of labour costs, capital and maintenance costs (inputs that are not subject to international trade), set in the relationship of accounting and domestic prices, and the denominator is the added value of production, less the costs of market inputs per production unit, expressed in border prices. DRC can be incorporated into policy analysis matrix (PAM-Policy Analysis Matrix), and consistent accounting framework that incorporates ambiguous competitiveness factors.

When the resulting value DRC greater than 1, the domestic mill sector is competitive and effective speaking at an international level, (the opportunity cost of domestic resources spent is less than the potential value of international exchange earned by exports or the resulting value savings result of import substitution of domestic production).

When the value of the DRC is less than one, then the result is considered an indicator of long-term competitiveness. Where the DRC more than one valid counter value for all the criteria. When the resulting value DRC is the 1, the domestic mill sector is neutral, neither the competitive, and neither the not competitive.

Research results analysis

The study was conducted to investigate the potential competitiveness of isolated milling sector in Croatia, in order to obtain results that pointed to possible problems or advantages of separate sectors, considering that Croatia is a full member of the European Union from 01 July 2013. year.

Table 2. Total income and expense dissenting milling sector, for the 2011th and 2012th years

Variables/year	2011.	2012.
Sales revenues	1.435.447.000	1.500.775.000
Other income	62.952.000	46.790.000
Value of production/domestic prices	1.498.399.000	1.547.565.000
Cost of raw materials	551.916.000	530.101.000
Value of work	143.139.000	144.714.000
Other expenses	761.382.000	839.168.000
The total cost of marketable inputs	1.456.437.000	1.513.983.000

Source: Authors using data from available documents issued by the Croatian bureau of statistics, Ministry of agriculture and Croatian Chamber of Economics

The analysis of domestic cost of resources is based on Competitiveness study, within is completed the Policy Analysis Matrix (PAM), for each of two years in milling sector production in Croatia.

Table 3. Total revenues, expenses and profits of milling sector in Croatia for the 2011th and 2012th years

Variables/year	2011.	2012.
Total revenue	1.498.399.000	1.547.565.000
Expenses	1.456.437.000	1.513.983.000
Profit	41.962.000	33.582.000

The policy analysis matrix is a product of two accounting identities, one defining profitability as the difference between revenues and costs and the other measuring the effects of divergences (distorting policies and market failures) as the difference between observed parameters and parameters that would exist if the divergences were removed.

By filling in the elements of the PAM, an analyst can measure both the extent of transfers occasioned by the set of policies acting on the system and the inherent economic efficiency of the system, according to prices and different types of expenses.

Table 4. PAM-Policy Analysis Matrix for milling sector in Croatia, for the 2011th and 2012th years

Year	Variables/ Expenses	Incomes	Inputs	Resources	Profit
2011.	Actual prices	1.498.399.000	761.382.000	695.055.000	41.962.000
	Economic prices	1.155.755.000	600.586.000	552.579.000	2.590.000
	Effect of variation efficiency policy	342.644.000	160.796.000	142.476.000	39.372.000
2012.	Actual prices	1.547.565.000	839.168.000	674.815.000	33.582.000
	Economic prices	1.290.201.000	760.520.000	527.071.000	2.610.000
	Effect of variation efficiency policy	257.364.000	78.648.000	147.744.000	30.972.000

Table 5. Coefficients according to PAM, milling sector in Croatia, 2011th and 2012th years

Coefficient / year	2011.	2012.
Private cost ratio (PCR)	0,09	0,95
Nominal protection coefficient on tradable inputs (NPCI)	1,27	1,1
Nominal protection coefficient on tradable outputs (NPCO)	1,3	1,2
Effective protection coefficient (EPC)	1,32	1,34
Profitability coefficient (PC)	16,2	12,9
Subsidy ratio to producers (SRP)	0,034	0,024
Domestic resource cost ratio (DRC)	0,99	1

The DRC ratios for the 2011th year is 0.99, and for year 2012th is value 1, this results said that under prevailing conditions are not profitable and neither the profitable, for the Croatian milling sector. Both ratios are the value one, that indicate how milling sector was found to be the neutral for both calculating years.

CONCLUSION

In this paper we have tried to analyse the competitiveness of Croatian milling sector. For this reason, the methodology used is based on the calculation of the Domestic Resources Costs (DRC) index. This DRC is a measure, in terms of real resources, of the opportunity cost of producing or saving products to foreign exchange. It provides a comparison between the domestic costs to produce a given good with its value added at international price. Due to result achieved for year 2011 and 2012 Croatian milling sector was neutral. I would require mayor improvements in management, technology, and wheat and flour prices for this to be achieved. Our results indicate that the inefficient allocation of productive factors induced by the interventionist economic policy resulted in a significant loss for the milling production in Croatia. For achieved a competitive production in milling sector Croatia must decrease production cost and increase production in next few year, (utilisation of production capacity is 40%), also need to increase the quality of the final product to be lower import balance of mill products.

REFERENCES

1. Croatian Bureau of Statistics 2011. 2012. Yearbook.
2. Croatian Chamber of Economics. 2013. Annual reports.
3. Čačić J., Tratnik M., Čačić D. and Kovačević D. 2007. Influence of different technologies on the competitiveness of strawberry production in Croatia. *Agric. conspec. sci. (ACS)* 71:181-186.
4. Dhebibi B. and Frija A. 2009. Impact of domestic resource costs on the competitiveness of Tunisian fresh fruit and vegetable products exports. In: International Conference "Energy, Climate Change and Sustainable Development, Hammamet, Tunisia.
5. Franić R. and Kumrić O. 2006. Sugar sector in Croatia, competitive or not?, presentation on EAAE, Chania, Crete, Greece.
6. Franić R. 2009. Agricultural policy, competitiveness and farm income, Faculty of Economics in Zagreb, Zagreb.
7. Kapaj A., Kapaj I., Deci E., Tabaku I. and Guri F. 2010. Investigation of production opportunities and resource use efficiency in agricultural production of Albania. International Food and Agribusiness Management Association.
8. Ministry of agriculture. 2013. Annual reports.
9. Ministry of agriculture. 2013. Draft Law on Amendments to the Law on special conditions for placing on the market of flour with the final bill, Zagreb.

10. Monke E., and S.R. Pearson. 1989. The policy analysis matrix for agricultural development. Ithaca, N.Y., U.S.A.: Cornell University Press.
11. Pearson S. 1991. Introduction to the Policy Analysis Matrix, University of Stanford, Stanford, USA.
12. Ruiz E. M. 2003. Autarkic Policy and Efficiency in the Spanish Industrial Sector. An Estimate of Domestic Resource Cost in 1958, Department of Economic History, London School of Economics, United Kingdom.
13. Zmaić K., Lončarić, R. and Sudarić, T. 2007. Economic efficiency of cereal crops production by application of PAM, Cereal Research Communications, Proceedings of the VI. Alps-Adria Scientific Workshop Obervellach, Austria.
14. Yercan M. and Isikli E. 2009. Domestic resource cost approach for international competitiveness of Turkish horticultural products. Afr. J. Agric. Res. 4: 864-869.

WHEAT COMPOSITE FLOUR WITH HEMP, TEFF AND CHIA

UDC 664.64.016 : 532.135

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ABSTRACT

Non-traditional ingredients (chia, hemp, teff) in baking technology enhance the rheological properties of dough, increase of bread quality and nutritional value. The aim of the presented research was to evaluate the features of wheat flour composites with hemp (*Cannabis sativa*) (5-20%), teff (*Eragrostis tef*) (10-30%) and chia (*Salvia hispanica*) (2.5–5.0%) grinded seeds and rheological behaviour of dough. The investigation of wheat flour and dough composite characteristics has been conducted using of farinograph, amylograph and mixolab. Relation of mixolab features to others rheological characteristics are described by correlation analysis. Changing of rheological features of composite flours depends on the type of fortification. Level of addition corresponds with increasing of water absorption and dough time development. Characteristics of starch described as amylograph features are connected more with added components than their amount. Influence of tested recipe according to mixolab was reflected mainly in starch retrogradation. In case of hemp addition, extent of C5-C4 values is equal to 0.24 N-m between M and K1-20 samples.

Keywords: composites wheat-hemp, teff, chia

INTRODUCTION

Application of non-traditional components in cereal technology can extend possibilities for production of alternative cereal based goods. Hemp, teff and chia milling products can serve for wheat flour fortification. Above mentioned innovative components are known for a specific chemical composition with higher nutrition value compared to wheat. In spite of enhance the rheological properties of dough, increase of bread quality and nutritional value.

Hemp (*Cannabis sativa*) is planted as two subspecies, namely ssp. *culta* a ssp. *indica*. The latter is called hash hemp and belongs to forbidden raw material with respect to intoxicating substances production. Hemp flour composition depends on variety and planting locality, also differs according to defatting. Protein, fat and starch rates are known to be 30-33%, 7-13%, approx. 40%, respectively. Seed contains a significant level of beta-carotene and vitamins B1 and E. Considering mineral component aspect a benefit could be found in higher portion of iron and zinc. Approx. two-thirds of hemp proteins

is composed by edestin, belonging to low molecular weight globulins [1]. Content of 10–15% insoluble fiber [2] may be also reason for wheat flour fortification.

Teff (*Eragrostis tef*) is classified into cereal group of the *Poaceae* family grown largely in Ethiopia where production exceeds that of most other cereals. Flat bread injera (ingera) dominates among other culinary treatments, and it is manufactured from fermented dough with a portion of wheat. Because of its tiny seeds, whole meal flour is characterised by high rate of coating layers, resulting into higher content of insoluble polysaccharides. Proteins have non-gluten nature and owing to prevailing portion of prolamins belong to easily digestible ones. However low content of lysin was reported [3]. From a nutritional benefit viewpoint, high minerals content (mainly iron, calcium, phosphorus and copper) and B1 vitamin is cited [4].

Chia (*Salvia hispanica* L.) is an annual herb of the *Labiatae* family, producing seeds which have formed the basic food from the Aztec civilization. Now, to main producers belong Argentina, Columbia and Peru. The composition includes minerals, about 20% protein, 30-32% fat, 30-40% polysaccharides (insoluble fibre as the important portion) and 2-3% fructo-oligosaccharides. The fatty acid of the oil was found to consist mostly of C16:0, C18:0, C18:2 and C18:3 [5]. Both forms (black and white) exhibit a high antioxidant activity due to the presence of phenolic compounds and tocopherols [6]. Chia seeds have been approved by the EFSA and in the EU may be used as novel food ingredients in bakery products at 5%. This year maximum can increase to 10% (according to 2013/50/EU).

A goal of the presented work was an investigation of farinograph and amylograph versus mixolab features comparability, based on quality determination of wheat flour composites with hemp, teff or chia flour. For this purpose, principal component analysis was used, and the method should also distinguish the influences of alternative flour and used addition level.

MATERIALS AND METHODS

Wheat flour used as composites base was of commercial origin, obtained from industrial mill Delta Prague, and is characterised as bright type (ash content 0.59%) with protein content 12.9%. Hemp flour sample K1 was gained as conventional planting regime commodity (E. Citterbartová's company, CZ). Wheat flour replacement was selected on levels of 5%, 10%, 15% and 20%. Tested teff flour (R1) is obtained by laboratory milling of the dark seeds (Tobia GB). Wheat flour replacements were 10, 20 and 30%. Whole meal chia flour (CH1) obtained by milling of the black seeds (Mexico) is added of 2.5 and 5%. Mixed composites were signed by combination of alternative flour type and its addition level, e.g. K1-15 means ratio of 85%/15% (w/w) of wheat/hemp or R1-10 ratio of 90%/10% (w/w) of wheat/teff, respectively.

Using factor 5.7, protein content (PRO) was determined according to the Kjeldahl's method (ČSN ISO 1871). For protein quality is used Zeleny test (ČSN ISO 5529).

The farinograph and the amylograph test were performed following ČSN ISO 5530-1 and ICC 126/1, respectively. Observed features were dough development time and dough stability in min (DDE and DST, respectively) together with mixing tolerance index (MTI, farinograph unit) in the former and amylograph viscosity maximum (AMY, amylograph unit) in the latter case. On the Mixolab, predefined protocol “Chopin+” was used with the test settings corresponding to user’s manual and published earlier. Course of wheat and composite dough behaviour was described by five basic torque points (C1–C5, N·m) and four pair differences (C1–C2, C3–C2, C3–C4 and C5–C4, abbreviated as e.g. C12). Relation of mixolab features to basic characteristics is described by means of statistical methods.

RESULT AND DISCUSSION

Analytical features of composite flours

Changing of analytical features of composite flours is demonstrated on Fig. 1. Increase of protein contents corresponds with lowering bakery quality (tested as Zeleny sedimentation).

Addition of teff changed significantly protein quality without effect on analysed amount. Opposite effect is found with chia meal fortification but is necessary to take in account very small level.

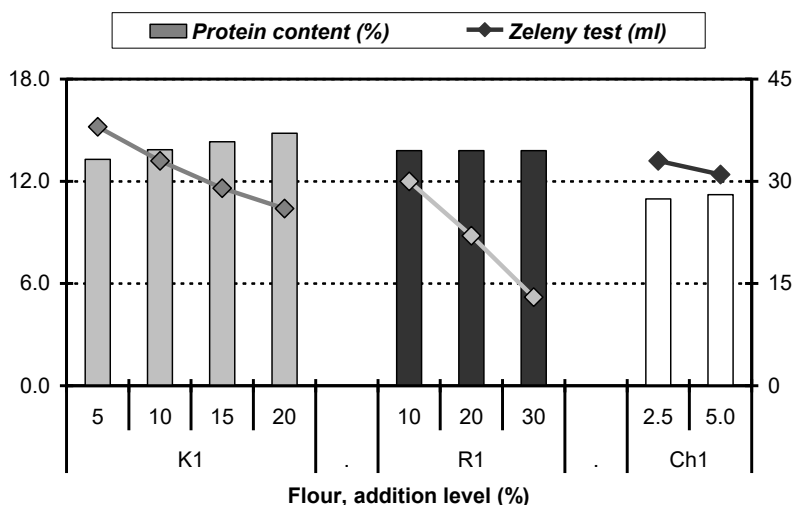


Figure 1. Protein content and Zeleny sedimentation value of composites

Rheological behaviour of composite flours

Farinograph test of K1 composites shown changes in dough rheological behaviour in correspondence with substitution rate (Table 1). Only the WAB demonstrated a practical independency on hemp flour ratio, oscillating around 62.9% of added water. Compared to standard M, time-expressing parameters (DDE, DST) were prolonged in different extent, twice in the former and almost about 50% in both latter cases. Finally, dough softening of four K1-composites could be consider as comparable to standard – recorded MTI were 40 FU for sample M, and from 20 to 50 FU for fortified samples. Effect of teff on R1 composites is observed in similar tendency. Changes of fortification level significantly lower DDE and DST parameters but dough softening increased namely in case of composite R1-30 in compared with wheat dough. Chia flour addition prolonged time of dough development and stability more than twice (Table 1).

Table 1. Farinograph and amylograph test results of wheat flour composites

<i>A. Effect of 5 and 20% hemp</i>				
Sample	Farinograph			Amylograph
	DDE (min)	DST (min)	MTI (FU)	AMA (AU)
K1-05	6.0 ^a	8.5 ^a	30 ^a	490 ^b
K1-20	5.5	11.5	40	365
<i>B. Effect of 10 and 30% teff</i>				
R1-10	9.5 ^a	11.0 ^a	60 ^b	400 ^b
R1-30	5.0	6.5	120	620
<i>C. Effect of 2.5 and 5.0% chia</i>				
CH1-2.5	7.5 ^a	19.0 ^b	20 ^a	260 ^a
CH1-5.0	9.0	19.0	20	260

DDE – dough development, DST – dough stability, MTI – mixing tolerance index;
FU – farinograph unit.

AMY – amylograph viscosity maximum; AU – amylograph unit.

a-b: column means related to alternative crop signed by same letter are not significantly different ($P < 0.05$).

Amylograph pasting properties of flour suspensions with K1, R1 and CH1 were determined in agreement with Falling Number, verifying relation between reference and screening method of amylase activity and starch stage estimation. The higher K1 hemp flour content, the lower composites viscosity (AMA) was measured; observed maximal decrease of 125 AU. Opposite changes caused the teff addition to wheat flour and recorded AMA were 325 AU for sample M and ranges of 400 to 620 AU for composites with R1. Tested level of chia seeds have not significant influence on pasting properties of composites CH1-2.5 and CH1-5.

Table 2. Mixolab test results of wheat flour composites

Sample	Mixolab torque point (N·m)				
	C1	C2	C3	C4	C5
<i>A. Effect of 5 and 20% of hemp</i>					
K1-05	0.98 ^a	0.50 ^b	2.10 ^b	1.59 ^b	2.42 ^b
K1-20	0.95	0.49	1.91	1.25	1.86
<i>B. Effect of 10 and 30% of teff</i>					
R1-10	1.20 ^a	0.37 ^a	1.79 ^a	1.26 ^a	1.71 ^a
R1-30	0.99	0.26	1.79	1.32	1.88
<i>C. Effect of 2.5 and 5.0% of chia</i>					
CH1-2.5	1.21 ^b	0.58 ^c	2.50 ^b	1.75 ^c	2.55 ^c
CH1-5.0	1.19	0.53	2.00	1.69	2.57

C1 – C5: Mixolab torque data

a-c: column means related to alternative crop signed by same letter are not significantly different ($P < 0.05$).

Mixolab curves of standard M and selected flour composites are depicted in Table 2. Owing to measurement at constant water absorption, there is a noticeable descent in C1 values – it signalizes qualitative changes in flour protein composition as the fortification degree increased. Within K1 composites, the torque has increased to the second maxima (similarly to the farinograph test). At curves mixing part, a partial closeness of samples K1-5 and K1-10 vs. K1-15 and K1-20 was observed (Fig. 2). After 1000 s of the test,

protein denaturation occurred (C2 points around 0.50 N·m), i.e. effect of different recipe composition was suppressed. This phenomenon is explained as protein unfolding, coupled with that torque decrease [7]. Starch gelatinization viscosity was the highest for the M sample (C3=1.59 N·m), and very soft insignificant decrease of the C3 values was recorded in a sequence of K1-composites (maximal difference 0.15 N·m only, Fig. 2). On the other hand, starch gel stability (C4) was dependent on dough formula. For the composite K1-20 was recorded similar value (1.42 N·m). Opposite to that, gel enzymatic hydrolysis as C34 reached approx. twice degree in the same samples comparison (0.37 and 0.66 N·m). Influence of recipe composition was reflected mainly in starch retrogradation because of extent C54 values equal to 0.24 N·m between standard and K1-20 sample flour and wheat-hemp composites starch demonstrated diversity of similar extent as starch gel stability. Curves were more differentiated together, but distance between standard and most enriched sample attained approx. 25% (C5 values 2.54 and 1.86 N·m, respectively). In partial agreement with the described trends, importance of C parameter pair differences was verified. C12 is related to proteins quality, and a drop from 0.60 to 0.48 N·m between M and K1-5 sample was provable.

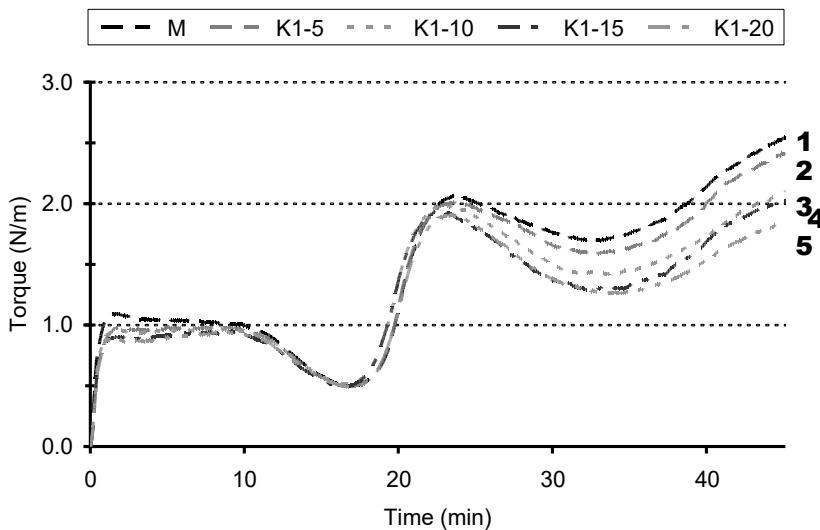


Figure 2. Mixolab curves of wheat flour M and K1-composites.
 Samples order: 1 – M (standard), 2 – K1-5, 3 – K1-10, 4 – K1-15, 5 – K1-20

Starch gelatinization (C32) was most intensive for pure wheat dough (1.56 N·m), and for composite K1-20 recorded similar value (1.42 N·m). Opposite to that, gel enzymatic

hydrolysis as C34 reached approx. twice degree in the same samples comparison (0.37 and 0.66 N·m). Influence of recipe composition was reflected mainly in starch retrogradation because of extent C54 values equal to 0.24 N·m between standard and K1-20 sample.

Effect of teff additions on mixolab profile was statistically different from hemp or chia ones (Table 2). In contrast to farinograph test results, gained mixolab data revealed very soft impact on recorded kneading profile, perhaps to tested amount. Nevertheless, viscous part of mixolab curve corresponded with amylograph prove course – higher portions of teff flour increased C4 and C5 values. C12 parameter showed higher weakening of protein up to about 0.73 N·m, the strongest one within the tested composite flour set. Also retrogradation of wheat-teff dough progressed to a different rate compared to wheat-hemp dough cases – according to smaller C54 values, faster staling of such bread could be presumed.

Chia fortification led to most different mixolab rheological behaviour of composite dough. In all five basic torque points, determined values were provably the highest, i.e. wheat-chia dough has higher tolerance to overmixing (C2 approx. 0.55 N·m, C12 0.64 N·m) and starch pasting properties were close to optimum (e.g. starch gel stability ca 1.7 N·m, C54 over 0.8 N·m) in correspondence to farinograph and amylograph test results. That positive effect should be considered in relation to the lowest fortification level within the composite flours set.

Statistical analyses

According to statistic evaluation (Table 3), parameter C1 negatively correlated with protein content compared to Zeleny test positive relation with torque points C2–C5. A negative sign for relation of Falling Number to C1 is based on physical nature of the test – indirect estimation of amylase activity via suspension viscosity evaluation.

Table 3. Correlation analysis between composite analytical and mixolab parameters

	C1	C2	C3	C4	C5
Protein content	-0.81**	<i>ns</i>	<i>ns</i>	-0.59*	<i>ns</i>
Zeleny test	<i>ns</i>	0.63*	0.71**	0.71**	0.66**
Falling Number	0.58*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

C1 – C5 – mixolab torque data.

*, **, *** – relationships significant at P = 95%, 99% and 99.9%, respectively;

ns – non-significant.

Links of farinograph and amylograph features were also affected by lower count of tested items. A quite high robustness was found for MTI feature – 7 of 9 calculated relationships were significant. Correspondence of the farinograph and mixolab tests was confirmed by positive correlation between MTI and C12. Interesting finding is connection of DST and MTI with anti-stalling effect represented by C54.

Table 4. Correlation analysis between the mixolab, farinograph and amylograph test parameters

	C1	C2	C3	C4	C5	C12	C32	C34	C54
DDE	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
DST	0.73***	0.68***	0.63**	0.63**	0.67***	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.65**
MTI	<i>ns</i>	-0.96***	-0.93***	-0.76***	-0.79***	0.77***	-0.57**	<i>ns</i>	-0.76***
AMY	-0.46*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

C1 – C5, C12 (C1-C2), C32 (C3-C2), C34 (C3-C4), C5-C4 (C54) – mixolab torque data.

DDE – dough development, DST – dough stability, MTI – mixing tolerance index.

AMY – amylograph viscosity maximum.

*, **, *** – relationships significant at P = 95%, 99% and 99.9%, respectively;

ns – non-significant.

CONCLUSION

Three different types of composites were prepared in terms of partial substitution of wheat flour by hemp, teff or chia flour. Blend analytical properties was evaluated as protein content and quality (Zeleny test) and amylase activity estimation (Falling Number). Technological quality was described by three rheological proves – farinograph, amylograph and mixolab ones. Gained data were statistically explored by correlation analysis.

Chemical composition of prepared blends was dependent on type of added alternative flour. Only the hemp one significantly increased protein content, but its technological quality was diminished by all three plant materials. Rheological behavior was affected, too, in extent corresponding to enhancement rate. By the farinograph test, twice or even triple prolongation of dough development time or stability during kneading was determined for wheat-hemp and wheat-chia samples. Further, a partial dilution of gluten structures has occurred (mainly for teff flour), resulting into dough softening. Reversal effect on pasting properties had hemp and teff flour –gradual diminishing and increase, respectively, were measured.

Based on mixolab test results, a statistically different baking quality was evaluated for teff and chia composites. The latter had better tolerance to overmixing as well as longer starch gel stability together with slower retrogradation.

Findings of correlation analysis were affected by lower number of tested samples. The Zeleny value feature and farinogram MTI parameter had the highest count of significant relationships to evaluated mixolab curve descriptors.

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REFERENCES

1. Callaway J. C. 2004. Hempseed as a nutritional resource: An overview. *Euphytica*. 140:65–72.
2. Dimic E., Romanic R., and Vujasinovic V. 2009. Essential fatty acids, nutritive value and oxidative stability of cold pressed hempseed (*Cannabis sativa* L.) oil from different varieties. *Acta Aliment.* 38(2):229–236.
3. Adebowale A. R. A., Emmambux M. N., Beukes M., and Taylor J. R. N. 2011. Fractionation and characterization of teff proteins. *J. Cereal Sci.* 54:380–386.
4. Hager A.-S., Wolter A., Jacob F., Zaninni E., and Arendt E. K. 2012. Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. *J. Cereal Sci.* 56:239–247.
5. Ciftci O. N., Przybylski R., and Rudzinska M. 2012. Lipid components of flax, perilla, and chia seeds. *Eur. J. Lipid Sci. Technol.* 114:794–800.
6. Reyes-Caudillo E., Tecante A., and Valdivia-Lopez M. A. 2008. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem.* 107:656–663.
7. Banu I., Stoenescu G., Ionescu V., and Aprodu I. 2011. Estimation of the Baking Quality of Wheat Flours Based on Rheological Parameters of the Mixolab Curve. *Czech. J. Food Sci.* 29(1):35–44.

THE STUDY OF BREAD QUALITY ENRICHED WITH PINEAPPLE (*Ananas comosus*) AGRO WASTE POWDER AS A NOVEL FUNCTIONAL INGREDIENT

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ABSTRACT

A study was focused on producing powder from pineapple waste using conventional method. Pineapple powders which contain Bromelin, was added into the bread as functional ingredients in three variables; bread 1:1.5% powder, bread 2:3.0% powder and bread 3:6.0% powder. The physicochemical analysis and sensory evaluation tests were done in this study. The result for proximate composition indicated that, moisture content showed significant difference in all the variations. The control has the highest moisture content which was 39.81%. In contrast, the result for ash, protein, fat and fiber did not differ significantly $p \geq 0.05$. For physical analysis, the mean of weight and height measurement showed significant difference since the p -value is ≤ 0.05 . The ANOVA show the hardness and crust color did not differ significantly. From qualitative descriptive analysis (QDA) test, all means for each attributes were not significantly different except for hardness, flavor and elasticity. The Hedonic Test showed that, bread 1 was more preferable than the other samples. It can be concluded that agro waste from pineapple is applicable as a Novel Functional Ingredient for Bread.

Keywords: Pineapple agricultural waste, bread quality, functional ingredient

INTRODUCTION

Malaysia, once ranked as one of the top 3 pineapple producers in the world in the 60's and early 70's, has only a relatively modest industry today. Pineapple belongs to the Bromeliaceae family and grows on the ground. Pineapple is a widely grown fruit in tropical and subtropical regions around the globe, and is a rich source of stem and fruit proteolytic bromelain. Pineapple (*Ananas comosus*) decanter / agro waste is a by-product of the pineapple processing industry and consists basically of the residual pulp, peels, and skin.

There are two types of pineapple bromelain. Stem bromelain is a cysteine proteinase within bromelain preparations derived from pineapple stem, while fruit bromelain is a glucoprotein proteinase present in pineapple juice, and enzymatically leaves with a broad substrate specificity of the internal polypeptide bonds of proteins [1].

Fruit bromelain has been used in various industrial and medical applications including brewing, meat tenderization, functional protein predigestion, as well as in the prevention of diarrhea, digestive aids, antithrombotic, edema treatment and osteoarthritis and promotes the absorption of antibiotic drugs. It also regulates and activates various immune cells and their cytokine production [2,3]. Moreover, bromelain has been used as a folk medicine, a wound healer, an anti-inflammatory, and an anti-diarrhea and digestive aid [4,5]. Because of this very wide range of applications, commercial bromelain is very expensive costing up to 2400 USD/kg.

Pineapple waste products are still under utilized while the demand for high fiber products was also increasing. Most of the researches utilized the pineapple waste by using chemical composition. This study focused on the waste of pineapple using the simple conventional procedure and can be used directly into the food product that is formulated using the waste of the pineapple parts and other ingredients.

MATERIALS AND METHODS

Fresh pineapple (*Ananas comosus*), Josephine type, was purchased from Pasar Borong, Kuala Lumpur, Malaysia. The fruit was sliced and blended using commercial blender for further process for pineapple waste to powder. Pineapple decanter wastes were further dried at 40° overnight using food dehydrator. Dried decanter wastes were grinded and sieved to a powder. The Bread prepared using various formulations. The pineapple agro waste powder was added into three variables: Bread A was 1.5% of pineapple waste powder, Bread B 3.0% of the powder and Bread C was ad 6.0% of pineapple waste powder. The pineapple waste powder was added together with other ingredients during mixing stage (Figure 1).

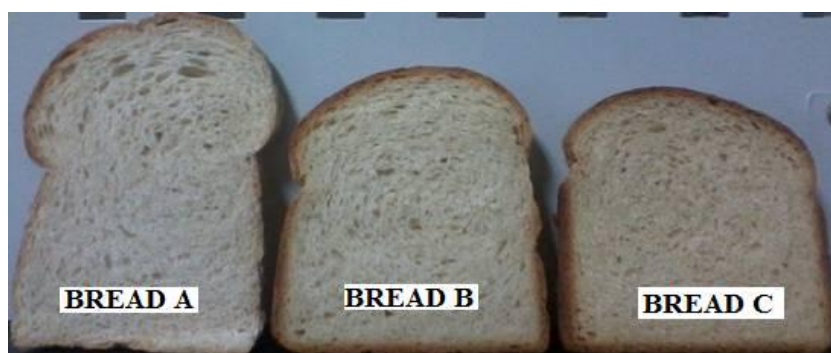


Figure 1. Bread samples

The moisture content was determined using Moisture Analyzer A&D MF-50. Ash was measured by dry combustion. Protein content (N% \times 5.7) was determined by Kjeldahl method. Crude fiber was determined according to the procedure AOAC, 2000 [6] (Method 32-07). Free fat were measured by petroleum ether extraction, followed by evaporation to constant weight [AACC, 2000 [7] (Method 32-07)]. Finally, the carbohydrate was calculated as 100%- (% moisture + % ash + % protein + % fiber + % fat). Proximate analysis of moisture, ash, protein, crude fiber and crude fat contents of pineapple waste powder were determined according to AOAC International (2000) [6] method.

The physical characteristics of bread analyzed were height, weight and texture analysis using Texture Analyzer (Shimadzu, Japan). The height to weight ratio was calculated. The L* (Lightness), a* (Redness), and b* (Yellowness) values of the crust and the crumb were measured using HunterLab LabScan XE Spectrophotometer. Sensory analysis were determined using 10 point Quantitative Descriptive Analysis method with a scale 0=lowest intensity and 10=the highest intensity and 7 point Hedonic scale rating with 1=dislike extremely and 7=like extremely were used in evaluating the sensory attributes of the bread sample. The attributes were evaluated in terms of color (crumb and crust), hardness (crust and crumb), aroma, flavor, porosity (texture of cell), and elasticity.

RESULT AND DISCUSSION

The results, as presented in Table 1, showed the proximate values of pineapple waste powder obtained in this work (Powder A) compared to previous works; Powder B represented by Mat and Abdullah [8] and Powder C by Bardiya et al. [9].

Table 1. Proximate analysis of pineapple waste powder

Proximate composition (%)	Powder A	Powder B	Powder C
Moisture	11.19 \pm 0.09	87.50	92.20
Ash	2.67 \pm 0.49	4.05	10.60
Protein	4.17 \pm 1.13	5.18	NA
Fat	1.158 \pm 0.877	NA	NA
Crude Fibre	70.301 \pm 0.884	10.57	NA
Total Carbohydrate	10.511	NA	35.00

Values are mean \pm Standard deviation

Powder A (Pineapple waste powder A): This work, used dehydrator

Powder B (Pineapple waste powder B): Mat and Abdullah, [8]

Powder C (Pineapple waste powder C): Bardiya et al., [9]

Moisture content of Powder C shows the highest percentage of moisture (92.20%) compared to Powder A (11.90%) and Powder B (87.50%). The ash content of Powder C (10.60%) is much higher than the other two. The 5.18% of protein of Powder B is considered higher compared to Powder A (4.17%) and Powder C. For crude fat, the previous works do not present any value for fat content, in this work the amount of fat content is only 1.158%. The crude fiber content (70.301%) in this work is also higher than the previous work. Powder A (10.511%) was reported to have the lowest value of carbohydrate composition while the highest value of carbohydrate composition obtained by Powder C (35.0%). The major differences found in the proximate composition of these three samples were mainly contributed by several factors such as differences of drying method uses, or differences in varieties of geographical factors [10].

As shown in table 2, control has the highest moisture content (39.807%) compared to others bread samples. Bread A shows the lowest percentage of moisture content (29.010%). Since the p-value is less than $\alpha=0.05$, we have sufficient evidence to conclude that there is significant difference between the mean value of moisture content among all variations. For the ash content, it shows that bread B has the highest amount of ash content (2.235%) compare to others bread samples. Bread B shows the lowest ash content (1.1420%). Since the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean value of ash content among all variations. From the result it showed that control has the highest protein content (15.240%) and also highest fat content (2.270%) compare to others bread samples. Since, the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean value of protein and fat content among all the variations. Based on table 2, it shows that bread A has the highest fiber content (35.370%) compare to others bread samples. Since, the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean value of fiber content among all variations

Table 2: Proximate analysis of bread enriched with pineapple waste powder

Bread	Moisture	Ash	Protein	Fat	Fibre
Bread A	29.010±0.0265a	1.42±0.212a	14.2±2.000a	1.048±0.592a	35.37±1.266a
Bread B	35.637±0.0404a	2.235±0.106a	14.905±0.021a	0.893±1.166a	26.795±0.209a
Bread C	35.047±0.0379ab	1.75±0.339a	14.675±0.021a	0.934±1.067a	25.937±0.170a
Control	39.807 ± 0.0513a	2.08±0.495a	15.24±0.057a	2.27±1.440a	25.929±0.894a

Values are mean ± standard deviation.

Means sharing the same letters in column are not significantly different from each other (Tukey's HSC test, $p > 0.05$)

From Table 3, bread A show the highest value of weight 791.67 g compared to other breads; Bread B (763.33 g), Bread C (763.33 g) and control (760.67 g). ANOVA gives the p-value 0.046 and F-value 4.21. Since, the p-value is less than $\alpha=0.05$, we have sufficient evidence to conclude that there is significant difference between the mean value of weight among all variations. While for the height, Bread A shows the highest value of height 12.87 cm among all the Bread samples. Bread B which are 12.2% less than Bread A, Bread C which are 28.3% less than Bread A and control which are 4.7% less than BreadA. Since, the p-value is less than $\alpha=0.05$, we have sufficient evidence to conclude that there is significant difference between the mean value of height among all variations.

Table 3. The value of weight, height and the ratio of weight and height of bread

Sample	Weight (g)	Height (cm)	Weight/ Height
Bread A	791.67 ± 16.07a	12.87 ± 0.06a	61.47
Bread B	763.33 ± 5.78a	11.30 ± 0.20b	67.52
Bread C	763.33 ± 5.28a	9.23 ± 0.15c	82.70
Control	760.00 ± 10.00a	12.27 ± 0.59a	61.93

Values are mean ± standard deviation

Means sharing the same letters in column are not significantly different from each other (Tukey's HSC test, $p>0.05$)

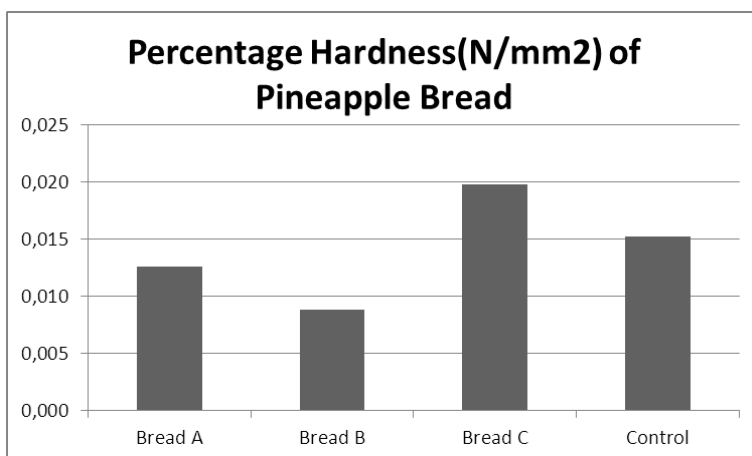


Figure 2. Percentage of hardness for bread enriched with pineapple waste powder

Based on Figure 2, it shows that bread C has the highest value of hardness (0.01979 N/mm²) compare to others bread samples; bread A (0.01260 N/mm²), bread B (0.00884 N/mm²) and control (0.01524 N/mm²). ANOVA gives the p-value 0.247 and F-value 1.68. Since, the p-value is more than $\alpha = 0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean value of hardness value among all.

Referring to table 4, bread A has the highest 'L' value (55.74) among the others bread samples. ANOVA gives the p-value 0.060 and F-value 5.89. Since, the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean of 'L' value among all. The column of 'a' value showed that control has the highest 'a' value (11.65) compared to others bread samples. ANOVA gives the p-value 0.278 and F-value 1.86. Since, the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean 'a' value among all. For the 'b' value it shows that control has the highest of 'b' value (26.84) compare to others bread samples. ANOVA gives the p-value 0.607 and F-value 0.68. Since, the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean of b value among all.

Table 4. L, a, b value for crust (control and bread enriched with pineapple waste powder)

Sample	L value	a value	b value
Bread A	55.74±3.81a	7.89±1.83a	21.88±4.39a
Bread B	49.14±3.56a	10.43±1.76a	23.66±4.94a
Bread C	41.12±4.72a	11.39±1.08a	22.91±1.27a
Control	51.46±1.34a	11.65±2.24a	26.84±2.88a

Values are mean ± standard deviation.

Means sharing the same letters in column are not significantly different from each other (Tukey's HSC test, $p>0.05$)

The crust is associated to the brown surface of bread while crumb is the inner white spongy structure beneath the crust. The crust, which is formed through Maillard reaction and caramelisation during baking, has several important functions on bread properties. The thickness and characteristics of the crust to a large extent define the product and give its name [11, 12]. In general, bread crust is referred as a marketing tool that attracts customers through its appearance, aroma, and flavour [13]. The crust formation affects the amount of moisture evaporating from wet dough during the baking process as a thicker crust is produced with a higher moisture loss in bread [11]).

This is because crust formation develops simultaneously as moisture evaporates during the baking process. Moisture loss during baking translates to weight loss of bread and this is less favourable for breads sold by its weight.

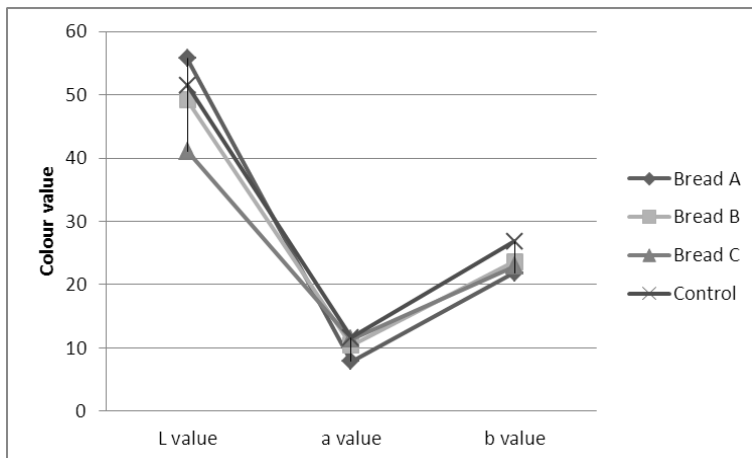


Figure 3. L, a, b value for crust bread enriched with pineapple waste powder

Table 5 showed all the data for the sensory attributes evaluated. The crust colour of bread shows that bread A has the highest intensity of crust colour. Cauvain [14] states that the crust colour in bread is principally formed by Maillard-type reactions involving reducing sugars and amino compounds (free amino acids and terminal amino-groups in soluble protein). For colour formation, it needs both factors to be present in appropriate amounts. The p-value is more than $\alpha > 0.05$; we have sufficient evidence to conclude that there is no significant difference between the mean values of crust colour attribute among all variations. For the crumb colour attribute, bread A and bread B showed the same score, 6.09 which is the highest score compared to bread 3 and bread 4. Since, the p-value is more than $\alpha > 0.05$; we have sufficient evidence to conclude that there is no significant difference between the mean values of crumb colour among all variations.

The crust hardness attribute shows the highest score is given by the bread B (7.00) and the lowest score comes from the control (5.27). Since, the p-value is more than $\alpha = 0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean value of crust hardness among all variations. For crumb hardness scores, bread B has the highest score (7.18) and the lowest score for this attribute is control (4.27). Since, the p-value is less than $\alpha = 0.05$, we have sufficient evidence to conclude that there is significant difference between the mean value of crumb hardness among all variations. Gallagher [15] reported that the absence of gluten will increase the movement of the

water from bread crumb to crust, thereby resulting in a firmer crumb and a softer crust. The control shows the lowest intensity of bread crumb and crust, it might be due to the lack of gluten present.

Table 5. Attributes score for control bread and bread enriched with pineapple waste

Attributes/ Variation	Crust's Colour	Crumb's Colour	Crust's Hardness	Crumb's Hardness	Aroma	Flavor
Bread A	6.27±2.50a	6.09±1.51a	6.09±0.94a	5.82±1.47a	6.09±2.43a	5.46±1.29a
Bread B	6.82±1.60a	6.09±1.45a	7.00±1.27a	7.18±1.40a	6.46±1.81a	4.91±2.17a
Bread C	6.09± 1.87a	6.00±2.45a	5.91±1.64a	6.73±2.20a	6.09±1.97a	7.00±2.19a
Control	6.00± 1.95a	6.00±3.19a	5.27±2.57a	4.27±2.33ab	5.36±2.46a	4.91±1.97a

Values are mean ± standard deviation.

Means sharing the same letters in column are not significantly different from each other (Tukey's HSC test, $p>0.05$)

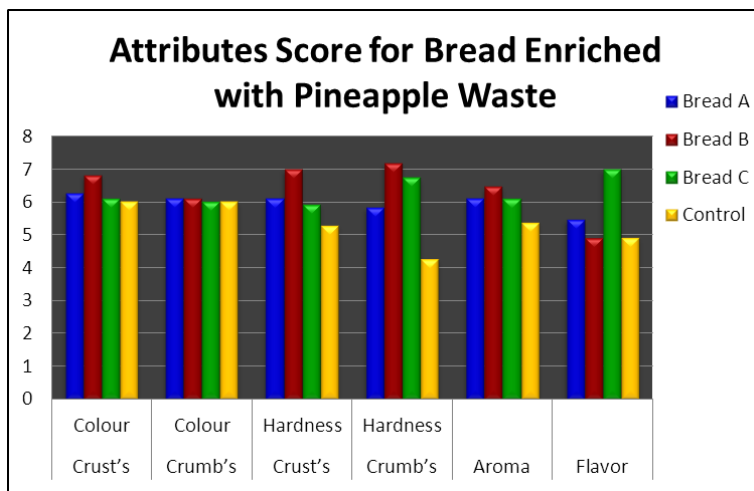


Figure 4. Attributes score for control bread and bread enriched with pineapple waste powder

For the next attributes, the aroma of bread B shows the highest intensity (6.46) while the bread control shows the lowest intensity of aroma (5.36). Since, the p-value is more than $\alpha=0.05$, we have sufficient evidence to conclude that there is no significant difference between the mean value of aroma attribute among all variations. The bread added with pineapple waste powder gives different aroma (pineapple-like aroma) compared to the control (without any added of pineapple waste). For the flavor aspect of the analysis, bread C gives the highest score 7.00 compared with other bread. Since, the p-value is less than $\alpha=0.05$, we have sufficient evidence to conclude that there is significant difference between the mean value of flavor attribute among all variations. From the results it can be concluded that when the pineapple waste powder increase, the intensity of flavor increase.

Based on the figure 5, it shows that Bread A has the highest preference, 39%. The lowest preference showed by bread C, 16%. We can say that the panelist liking preference is more toward the bread A. From the ANOVA, the p-value given is 0.000. Since, the p-value is less than $\alpha=0.05$, we have sufficient evidence to conclude that there is significant difference between the mean value of hedonic scale mean among all variations.

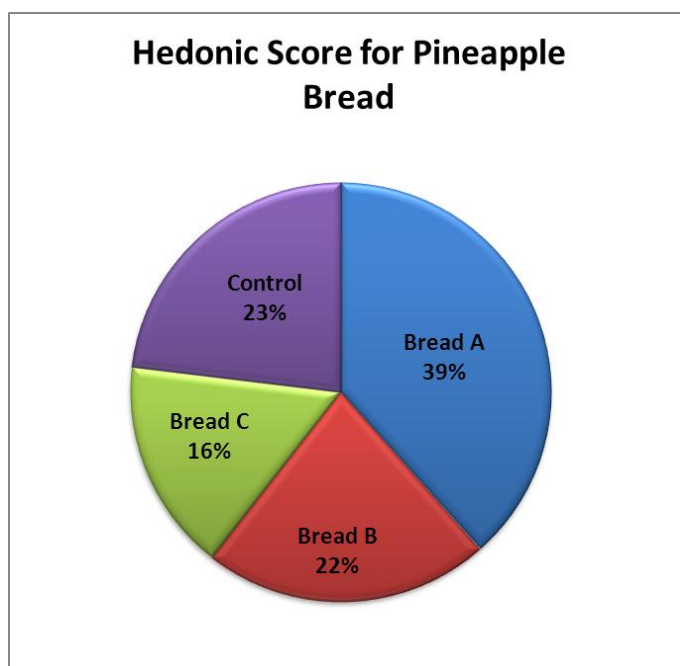


Figure 5. Hedonic score for control bread and bread enriched with pineapple waste powder

CONCLUSION

This study was focused on making bread enriched with pineapple decanter agro waste powder as a novel functional food. In this study bread A is more preferable and was chosen to be the most favourable formulation among the three variables. As a conclusion, the pineapple decanter agro waste powder can be processed by conventional method and can be utilized in bread making. This study also achieves to prove that pineapple decanter waste is applicable to be used as a functional ingredient in breads besides giving an extra added value for bread.

REFERENCES

1. Rowan, A. D., and Buttle, D. J. 1994. Pineapple cysteine endopeptidases. *Methods Enzymol.* 244:555–568.
2. Brien, S., Lewith, G., Walker, A., Hicks, S. M., and Middleton, D. 2004. Bromelain as a treatment for osteoarthritis: A review of clinical studies. *Evidence-based. Complementary and Alternative Medicine.* 1:251–257.
3. Maurer, H. R. 2001. Bromelain: Biochemistry pharmacology and medical use. *Cell. Mol. Life Sci.* 58:1234–1245.
4. Bitange, N. T., Zhang, W., Shi, Y. X., and Wenbin, Z. 2008. Therapeutic application of pineapple protease (bromelain). *Pakistan Journal Nutrition.* 7:513–520.
5. Koh, J., Kang, S. M., Kim, S. J., Cha, M. K., and Kwon, Y. J. 2006. Effect of pineapple protease on the characteristics of protein fibers. *Fiber Polymer.* 7:180–185.
6. AOAC. 2000. Official method of analysis of the AOAC. 16th Edition. Washington DC, USA.
7. AACC. 1999. Approved Method of Analysis. 11th Edition. St. Paul, MN, U.S.A.
8. Mat, H., and Abdullah, M. B. 1998. Production of Organic Acid from Local Raw Materials. Project Report. Universiti Teknologi Malaysia. (Unpublished).
9. Bardiya, N., Somayaji, D. and Khanna, N. 1996. Biomethanation of Banana Peel. *Bioresour. Technol.* 99:4346–4348.
10. Emega, T. H., Robert, C., Ronkart, S. N, Wathelet, B. and Paquot, M. 2008. Dietary Fibre Component and Pectin Chemicals Features of Peels during Ripening in Bananan and Plantain Varieties. *Bioresour. Technol.* 99:4346–4345.
11. Wiggins, C. 1999. Proving, baking, cooling, Cauvain, S.P. and Young, L.S. (Ed). *Technology of Breadmaking.* Maryland: Aspen Publishers.
12. Cauvain, S. P and Young, L. S. 2000. *Baking Problems Solved.* North America: Woodhead Publishing Limited.
13. Purlis, E. and Salvadori, V. O. 2007. Bread browning kinetics during baking. *J. Food Eng.* 80:1107–1115.

14. Cauvain, S. P. and Young, L. S. 2006. Baked products: science, technology and practice. United Kingdom: John Wiley & Sons.
15. Gallagher, E., Gormley, T. R. and Arendt, E. K. 2003. Crust and crumb characteristics of gluten free breads. *J. Food Eng.* 56:153-161.

EFFECT OF BREAD TYPE CHOICE ON NUTRITION QUALITY IN ELDERLY

UDC 613.2-053.9 : 664.66

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ABSTRACT

Physiologic and functional changes during aging result in changes in nutrient needs. Older adults have specialized requirements because of aging effects on absorption, utilization, and excretion. They need less energy but have higher needs of some micronutrients like folic acid, vitamin B₆ and B₁₂. The aim of this study was to evaluate how small choices can influence dietary intake in elderly nursing home resident with limited selection of foods.

Monthly menus in which only choice that residents have is white or brown bread were analysed and compared regarding energy and nutrient content. Obtained values were compared to recommendations for the elderly based upon the presumption that the whole portion was eaten. Gender and age of residents were also taken into consideration during these comparisons.

Brown or white bread choice did not have impact on energy, proteins or fats, but small change in carbohydrate intake was noticed. Brown bread choice positively influenced intake of dietary fibres, which is especially important in male population where white bread choice results in fibres intake below the recommendations. Among minerals brown bread choice the most positively influenced iron intake, while in the group of vitamins increase was noticed for thiamine, riboflavin, niacin, vitamin B₆ and folate.

In conclusion the simple choice of brown instead of white bread results in improved intake of dietary fibres and B group of vitamins, as well as the intake of certain minerals.

Keywords: energy value, nutrient content, food, nursing home, elderly

INTRODUCTION

Individuals aged 65 and over are generally considered to be elderly population. Their rate in the overall human population is rapidly growing in almost all parts of the world, and with the increase of elderly people number, the specific nutrition-related problems of the elderly years take on greater significance [1].

Aging brings about a progressive decline in the functioning of all organs and systems [1, 2]. Physiologic and functional changes during aging result in changes in nutrient needs [3]. Older adults have specialized requirements because of aging effects on

absorption, utilization, and excretion. Energy needs decline but requirements for many nutrients increase [4]. In elderly, adequate protein intake is essential for the prevention of muscle mass losses, fibres intake for decreasing constipation [5]. Therefore elderly should select nutrient-dense foods to meet their nutritional needs [1, 2]. Additionally, co morbidity, whether acute or chronic, presents challenges in planning the diet of elderly persons.

Malnutrition is correlated with institutionalization, hospitalization and rehabilitative care, especially in elderly [5, 6]. At the same time, due to the modern lifestyle, more and more elderly individuals relies on the nursing homes where, unlike in family, many residents depend exclusively on the food provided by the institution. Only few choices are available, and even those are limited due to the habits which are not so easy to change in this stage of life. Therefore the aim of this study was to evaluate, in nursing home residents, how small choices like bread type choice can influence total dietary intake of macro and micronutrients with the focus on those most often being deficient in the elderly population.

SUBJECTS AND METHODS

Participants of this study were indirectly residents of the nursing home. At the moment of the study nursing home had 161 residents, 65 of which were males and 96 females. Both genders were represented by individuals of age groups 50-70 (20 males and 28 females) and over 70 (45 males and 68 females) years of age.

Complete monthly menus offered to nursing home residents were assessed in order to get dietary intake of macro and micronutrients. Detailed information about the menu and food preparation processes was gathered from the kitchen staff.

In order to convert the menus to nutrients, the food composition database [7] and specialised NutriPro software were used.

Only dietary choice which residents had in this institution was to choose between white or brown bread and therefore all nutritional calculations were done with both choices to compare influence of this choice on energy and nutrient intake.

Obtained values were compared to recommendations for the elderly [4] based upon the presumption that the whole portion was eaten. Both, gender and age of residents were taken into consideration during these comparisons.

RESULTS AND DISCUSSION

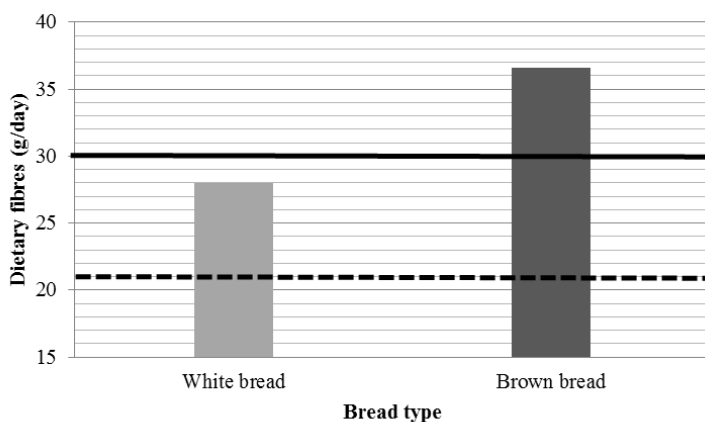
Wheat bread has to contain more than 90% of wheat flour. If it is produced of white wheat flour with the ash content up to 0.60% it is called white bread, and if it is produced of wheat flour with the ash content higher than 1.05% it is called brown bread [8]. Considering the differences in white and brown bread nutrient composition, and their quite high daily consumption, it was assumed that brown bread choice could have

an important positive effect on the overall nutrient intake in elderly, and especially on the intake of micronutrients.

Results of the study shown that brown or white bread choice did not have impact on total energy intake, proteins or fats but small change in carbohydrate intake was noticed (Table 1).

Table 1. Total daily energy intake and contribution of macronutrients through menu with white and brown bread

	Menu with white bread	Menu with brown bread
Total energy intake (kJ)	10738	10558
Total energy intake (kcal)	2568	2524
Energy intake from proteins (kcal)	421	421
Energy intake from fats (kcal)	942	942
Energy intake from carbohydrates (kcal)	1227	1184
% of total energy intake from proteins	16.2	16.5
% of total energy intake from fats	36.4	37.0
% of total energy intake from carbohydrates	47.4	46.5



Adequate intake [4] in adults of 51 years of age or older is presented for males with solid black line and for females with a dash line.

Figure 1. Average daily intake of dietary fibres through the offered menus with white or brown bread

Constipation is a common problem among the elderly population, with prevalence between 25% and 35% among the free-living individuals, and even 50% to 65% among nursing home residents. Gastrointestinal transit times are lengthened due to lack of physical activity, poor hydration and the most importantly a lack of dietary fibres [1]. Brown bread choice positively influenced intake of dietary fibres, which is especially important in male population where white bread choice results in fibres intake below the recommendations (Figure 1).

In the process of considering data obtained for minerals and vitamins intake it was stressed that recommended intakes for individuals vary in dependence on gender and age [4]. Therefore all obtained values were compared with the specific groups for which they differ.

Among minerals bread choice influenced potassium, calcium, phosphorus, iron and selenium intake (Table 2). In these entire cases dietary intake was elevated as a result of brown bread choice.

Although the primary aim of this study was to evaluate the effect of the bread type choice on the overall nutrition quality, it should be stressed that the study revealed enormously high sodium intake.

Table 2. Compliance of average daily intakes of selected minerals through the menus combined with white or brown bread to the recommended daily intakes (% DRI) [4]

Element	Bread type	All	Males	Females	51-70	>70
Na	white				1205.5	1306.0
	brown				1205.5	1306.0
K	white	69.4				
	brown	77.1				
Ca	white	73.5				
	brown	76.5				
P	white	231.9				
	brown	270.5				
Mg	white		50.5	66.3		
	brown		50.5	66.3		
Fe	white	213.1				
	brown	235.6				
Zn	white		29.8	40.9		
	brown		29.8	40.9		
Se	white	312.5				
	brown	391.1				

In the group of vitamins change was evident for thiamine, riboflavin, niacin, vitamin B₆ and folate (Table 3).

Table 3. Compliance of average daily intakes of selected vitamins through the menus combined with white or brown bread to the recommended daily intakes (% DRI) [4]

Vitamin	Bread type	All	Males	Females	51-70	>70
C	white		48.9	58.7		
	brown		48.9	58.7		
Thiamin	white		108.8	118.7		
	brown		129.8	141.6		
Riboflavin	white		130.7	154.5		
	brown		141.8	167.6		
Niacin	white		75.3	86.1		
	brown		100.0	114.4		
B ₆	white		59.9	67.9		
	brown		87.4	99.1		
B ₉	white	44.9				
	brown	53.0				
A	white		52.8	67.9		
	brown		52.8	67.9		
D	white				17.5	11.7
	brown				17.5	11.7
E	white	11.1				
	brown	8.26				
K	white		107.8	143.7		
	brown		107.8	143.7		

Although results show adequate intake through the meals served in a nursing home it should not be forgotten that due to the various reasons elderly often leave foods on the plate. Calvo in his study used three day weighted record of served meals and dietary intake and found that food intake was significantly lower than that served [9]. Villarroel et al. [6] in their study conducted in four Spanish nursing homes found that average percentage of consumption is 81.98% but with significant differences between institutions as well as between meals.

To avoid deficiencies, choices and changes in the menu should be introduced with caution. With lower energy requirements and in some cases increased requirements for other nutrients optimal diet for elderly needs to be nutrient dense [1].

CONCLUSIONS

In conclusion the simple choice of black instead of white bread results in improved intake of dietary fibres and B group of vitamins, as well as the intake of certain minerals. Therefore, small but significant choices like this one should be encouraged in elderly to achieve higher nutrient density without raising the energy intake.

REFERENCES

1. Langley-Evans, S. 2009. Nutrition: A Lifespan Approach, Wiley- Blackwell, Oxford.
2. Mitchell, M. K. 2002. Nutrition across the life span, Second Edition, Saunders An imprint of Elsevier. USA.
3. American Dietetic Association (ADA). 2000. Position of the American Dietetic Association: Nutrition, aging, and the continuum of care. J. Am. Diet. Assoc. 10(5):580-595.
4. Food and Nutrition Board, Institute of Medicine, National Academies. 2004. Dietary Reference Intakes. National Academy of Sciences, Washington DC.
5. Sharlin, J., and Edelstein, S. 2011. Essentials of life cycle nutrition. Jones and Bartlett Publishers, Sudbury, Massachusetts.
6. Villarroel, R. M., Sangrá R. A., Massaguer L. P., and Codina A. F. 2012. Assessment of food consumption, energy and protein intake in meals offered in four Spanish nursing homes. Nutr Hosp. 27(3):914-921.
7. Kaić-Rak, A., and Antonić Degač, K. 1990. Tablice o sastavu namirnica i pića. Zavod za zaštitu zdravlja SR Hrvatske, Zagreb.
8. Ministry of Agriculture, Forestry and Water Management. 2005. Regulation on the essential requirements for quality of cereals, mill and bakery products, pasta, dough and dough products. Off Gazette. 78/05.
9. Dudet Calvo, M. E. 2013. Nutritional assessment of meals served and dietary intake in a nursing home. Revista Espanola de Nutricion Comunitaria. 19(1):20-28.

DURUM WHEAT AND KAMUT® BREAD CHARACTERISTICS: INFLUENCE OF CHEMICAL ACIDIFICATION

UDC 664.641.12

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ABSTRACT

Sourdough fermentation is one of the oldest biotechnological process used in food production [1]. The application of sourdough in bread making production could improve the quality and the flavor of wheat bread; has a positive nutritional implications by increasing mineral availability and by lowering the glycemic response of baked goods [1] and improve the shelf-life of the products. However, the use of sourdough implies some disadvantages like the increasing of labor input and an higher production uncertainty [2]. Because of these disadvantages the purpose of this research was to study the modifications induced by a chemical acidification realized with lactic acid used to simulate sourdough fermentation of bread obtained with durum wheat and Kamut® flour.

Significant differences were found between the control bread and the chemical acidified one. Moreover it can be assumed that acidification induces a different modification on durum wheat and Kamut bread in a different way.

Keywords: durum wheat flour; Kamut® flour; chemical acidification

INTRODUCTION

Among the cereal flours, wheat is unique in its ability to form dough when mixed with water and to retain the gas produced during fermentation which results in a leavened product [3].

Durum wheat flour has been used in the formulation of several types of bread in the Mediterranean area, and this type of flour is often associated to sourdough fermentation to obtain products largely appreciated by the consumers for their organoleptic characteristics [4].

Nowadays, interest is growing on other cereals grains that constitute a highly nutritional ingredients for healthy food production and special dietary uses. Several are considered ancient crops and/or minor cereals (e.g. Kamut®, barley, spelt, rye, einkorn, millet, oat,

sorghum) and are often underutilised or only consumed locally; others are pseudocereals (e.g. quinoa, amaranth, buckwheat) [5].

Recently, among minor cereals, interest on Kamut® is increasing. According to Khlestkina, Röder, Grausgruber & Börner [6], the Kamut® wheat grouped together in a cluster containing three accessions of *Triticum polonicum* and three of *T. durum*. Therefore these authors suggested that Kamut® derived from a natural hybrid between *T. durum* and *T. polonicum*, which occurred in the Fertile Crescent.

The traditional process of sourdough bread production has enjoyed renewed success in recent years, due to increasing consumer demands for more natural, tasty and healthy foods [1]. The addition of sourdough during production of wheat bread can have a considerable effect on dough characteristics. Many of the effects of sourdough have been attributed to a drop in pH value caused by the production of organic acid, in particular lactic acid, the main metabolic product of the LAB (lactic acid bacteria). Furthermore, the sourdough microflora may also contribute to changes in the dough structure depending on their characteristics properties such as specific enzymatic activities or the production of substances such as exopolysaccharides [1].

The aim of acidification is to increase the shelf life and to improve the aroma and the water binding relation.

The purpose of the present work was to study the modifications induced by a chemical acidification mimicking sourdough fermentation of two different bread obtained with durum wheat and Kamut® flour.

MATERIALS AND METHODS

Bread preparation

The flours used to realize the bread samples were commercial durum wheat bread-making flour and Kamut® flour, supplied by Mulino Sima (FE-Italy). The physico-chemical characteristics of the flours are reported in Table 1.

The ingredients used to prepare the different dough formulations were: flour, deionised water, salt and compressed yeast. All the ingredients were mixed using a mixer mod. Major mixer (Kenwood Chef Major KM 005, Treviso, Italy) at the first speed for 5min. The chemically acidified dough contained, in addition, lactic acid (Fluka) at a rate of 0.55% (fwb) to yield dough with a pH value comparable with a biologically acidified dough. In order to develop the final product, after 5 h of fermentation the dough was sheeted to about 1cm thickness and then cut using a stainless steel rectangular mould (15 cmx6 cm). Afterward dough was allowed to ferment in a proofer for 1h at 30 °C and 70% relative humidity. Fermented dough was baked in a convection oven (FC61, ANGELO PO e Grandi Cucine S.p.A, Carpi, Italy) at 210 °C for 23 min and for 20 min the durum wheat and the Kamut® bread respectively. After baking, bread samples were

cooled down at room temperature (22±1 °C) before performing the instrumental analyses. Baking process was performed in triplicate.

Table 1. Physico-chemical characteristics of Kamut® and durum wheat flour

Characteristics	Kamut® Flour	Durum Wheat Flour
Moisture Content (%)	12.7	13.8
Dry Gluten (%)	13.5	8.54
<i>Alveograph parameters</i>		
P	120	167
G	15.4	11.1
P/L	2.5	6.6
<i>Farinograph Parameters</i>		
Water absorption (%)	73.1	63.3
Water absorption (14% moisture basis) (%)	70.5	62.8
Development time (min)	2	1.4
Stability (min)	4.6	2.5
Softening 10' (BU)	70	66
Softening 12' (BU)	101	74

Bread analyses

The bread volume was evaluated by using the rapeseed displacement method [7].

After measuring the volume, the loaf weight (m_{bread}) was recorded.

Specific volume (V_s) of the bread was calculated as:

$$V_{\text{specific bread}} \left[\frac{\text{ml}}{\text{g}} \right] = \frac{V_{\text{bread}}}{m_{\text{bread}}}$$

The bread mechanical characteristics were evaluated with a Texture Analyser mod. TA.HDi 500 (Stable Micro System, Godalming, Surrey, UK) equipped with a 50 mm diameter aluminium cylinder probe and a 50 kg load cell. Texture profile analysis (TPA) in a double compression cycle was performed with 40% penetration depth, 3.0 mm/s test speed and a 5 s gap between compressions [8]. Before performing the analysis, for each

sample two slices were cut from the central portion of three different bread loaves and one square test piece (20 mmx20 mm) was taken from the crumb of each slice and six pieces were obtained and analysed. The textural parameters considered were hardness (g) and chewiness (g) [9].

In order to perform the image analysis the final products were cut in regular slices of 20 mm thickness. The inner portion of the slices was considered for crumb grain features measurements. The images were captured using an image acquisition system similar to that developed by Mendoza and Aguilera [10], with slight modifications. Images from the slices were taken on the matte black background and saved in JPG format. Canon remote Capture Software (version 2.7.0) was used for acquiring the images directly in the computer. A portion of each image was considered and digital images were processed by an advanced Image Analysis software (Image Pro-Plus version 6.2 Media Cybernetics, USA). Pore size distribution analysis was performed by counting the percentages of pores that fall into six predefined area classes (cm²): 0.150<CLASS 1<0.50; 0.50 CLASS 2<5; 5<CLASS 3<10; 10<CLASS 4<50; 50<CLASS 5<150; 150<CLASS 6<200.

The Gas-chromatography mass spectrometry/solid-phase microextraction (GC-MS/SPME) analysis was performed in order to evaluate the volatile compounds. After preconditioning according to the manufacturer's instructions, the carboxen-polydimethylsiloxane coated fiber (85 µm) and the manual SPME holder (Supelco Inc., Bellefonte, PA, USA) were used. Before head space sampling, the fiber was exposed to GC inlet for 5 min for thermal desorption at 250 °C. Three grams of sample were placed into 10 ml glass vials and added of 10 µl of 4-methyl-2-pentanol (final concentration of 4 mg/l), as the internal standard. Samples were then equilibrated for 10 min at 45 °C. SPME fiber was exposed to each sample for 40 min and subsequently desorbed in GC. Both phases of equilibration and absorption were carried out under stirring condition. Identification of molecules was carried out based on comparison of their retention times with those of pure compounds (Sigma-Aldrich, Milan, Italy). Identification was confirmed by searching mass spectra in the available databases (NIST version 2005 and Wiley Vers. 1996) and literature.

Significant differences (at P-level≤0.05) between means of the breads parameters regarding the influence of the different flour used (durum wheat and Kamut®) and of the chemical acidification were explored by using analysis of variance (ANOVA) and Fisher's least significant difference (LSD); the Mann-Whitney test was used if significant differences emerged between variance means at the Levene test. The statistical analyses of data were performed using Windows software "StatSoft" (Tulsa, OK, USA), version 7.0.

RESULTS AND DISCUSSION

Loaf volume

Regarding the control breads, sample realized with Kamut® flour showed an higher specific volume value (2.00 ml/g) compared to the durum wheat flour bread (1.43 ml/g).

The gluten proteins are responsible not only for the cohesive and viscoelastic property of wheat flour dough but also for the protein-starch interaction that is related to the dough's ability to retain gas during fermentation and partly for the setting of the dough during baking [11]. Kamut® flour seems to improve the volume of the bread by allowing the entrapment of air bubbles in dough and providing stability to the dough mixture during baking, this is probably due to the higher level of gluten present in Kamut® flour (13.5%) compared to the gluten level in durum wheat flour (8.54%). The acidification had a significant influence on bread realized with Kamut® flour, in fact the acidified sample had a lower specific volume (1.58 ml/g) compared to the control one. Instead the acidification seems not to influence the specific volume of durum wheat bread in a significant way.

Texture profile analysis

Samples realized with durum wheat flour control (1578.56 g) and acidified (1520.81 g) showed an hardness values higher than Kamut® breads control (619.13 g) and acidified (906.34 g). The consumer usually assumes that a less firm, tenderer bread is most desirable. Crumb firmness arises from the integrity of the gluten network, the degree of crosslinking, the amount of gas incorporated, and from other structural components of the bread [12]. The higher hardness value is associated to the lower specific volume values of durum wheat bread samples compared to the Kamut® ones.

The increase in hardness in Kamut® bread samples following the acidification was mainly associated to the significant reduction of loaf volume as previously reported.

A similar trend was observed for the chewiness parameter. Bread chewiness was 1226.20 g and 1135.72 in control and acidified durum wheat samples respectively and 689.82 g and 470.61 g in control and acidified Kamut® samples respectively.

Image analysis

The gas cells having the smallest dimension (CLASS 1 and CLASS 2) occupied 93% of the holed area of durum wheat bread, control and acidified, and 83% and 87% of Kamut® breads, control and acidified respectively. It is evident that in all samples the highest area was occupied by the class of smallest dimension thus the bread showed a crumb fine and uniform.

The control bread sample realized with Kamut® flour showed an higher percentage of gas cell belongs to class 3 and 4 (16%) compared to durum wheat bread (7%). The acidification cause a positive decrease of these gas cells and the decrease is more evident in Kamut® bread sample. Infact the acidified Kamut® bread showed 13% of gas cells, while durum wheat bread 6%. It is known that holes of relatively small size are required in bakery products, while large voids or irregular crumb distribution are undesirable. Usually bread realized with sourdough had a greater number of small cells [1].

Volatile compounds

Regarding the volatile compounds, in control breads a greater amount of volatile molecules (aldehydes/ketone, acids, hydrocarbons, furans/ethers, esters and aromatic compounds) was observed in Kamut® bread with the exception of alcohols and phenolic compounds attaining their highest level in durum wheat bread.

The acidification decreased all the predominant volatiles retained in Kamut® breads. On the other hand in durum bread the acidification increased almost all the volatile compounds. It can be assumed that acidification induces a modification of the volatile compound interactions with dough matrix of durum wheat and Kamut® flour in a different way. Therefore wheat flour seemed more suitable to support a sourdough fermentation due to its ability to retain some volatile metabolites.

CONCLUSIONS

The control bread produced with Kamut® flour showed better structural (higher specific volume value and lower hardness) and aromatic characteristics than durum wheat bread.

The chemical acidification on Kamut® bread improve the crumb grain characteristics, but make the specific volume, the textural and aromatic characteristics worse. Instead on durum wheat bread the chemical acidification seems not to have any negative influence. For these reasons a multigrain blends could be an useful and efficient strategy to obtain a modern and innovative bread meeting structural and aromatic standards.

The effect of application of sourdough fermentation process on dough properties is more than simple acid production therefore further researches are necessary in order to evaluate the influence of sourdough on dough and bread characteristics.

REFERENCES

1. Clarke, C. I., Schober, T. J., and Arendt E. K. 2002. Effect of Single Strain and Traditional Mixed Strain Starter Cultures on Rheological Properties of Wheat Dough and on Bread Quality. *Cereal Chem.* 79:640-647.
2. Koceva Komlenić, D., Ugarčić-Hardi, Ž, Jukić, M., Planić, M., Bucić-Kojić, A., and Strelec, I. 2010. Wheat dough rheology and bread quality effected by *Lactobacillus brevis* preferment, dry sourdough and lactic acid addition. *Internationa J. Food Sci. Tech.* 45:1417-1425.
3. Gellyncka, X., Kühnea, B., Van Bockstaeleb, F., Van de Walleb, D., and Dewettinckb, K. 2009. Consumer perception of bread quality. *Appetite.* 53:16-23.

4. Chiavaro, E., Vittadini, E., Musci, M., Bianchi, F., and Curti, E. 2008. Shelf-life stability of artisanally and industrially produced durum wheat sourdough bread ("Altamura bread"). *LWT- Food Sci. Tech.* 41:58-70.
5. Angioloni, A. and Collar C. 2011. Nutritional and functional added value of oat, Kamut®, spelt, rye and buckwheat versus common wheat in breadmaking. *J. Sci. Food Agr.* 91:1283-1292.
6. Khlestkina, E.K., Röder, M.S., Grausgruber, H., Börner, A. 2006. A DNA fingerprinting-based taxonomic allocation of Kamut® wheat. *Plant Genetic Resources.* 4:172-180.
7. Approved methods of the AACC. 1988. Method 74-09. St. Paul, MN: American Association of Cereal Chemists.
8. Gàrbaro, A., Varela, P., Giménez, A., Aldrovandi, A., Fiszman, S.M., and Hough, G. 2002. Textural quality of white pan bread by sensory and instrumental measurements. *J. Texture Stud.* 33:401-413.
9. Balestra, F., Cocci, E., Pinnavaia, G.G., and Romani, S. 2011. Evaluation of antioxidant, rheological and sensorial properties of wheat flour dough and bread containing ginger powder. *LWT- Food Sci. Tech.* 44:700-705.
10. Mendoza, F., and Aguilera, J.M. 2004. Application of image analysis for classification of ripening bananas. *J. Food. Sci.* 69:471-477.
11. Demirkesen, I., Mert, B., Summu, G., and Sahin, S. 2010. Rheological properties of gluten-free bread formulations. *J. Food Eng.* 96:295-303.
12. Yi, J., and Kerr, W.L. 2009. Combined effects of freezing rate, storage temperature and time on bread dough and baking properties. *LWT- Food Sci. Tech.* 42:1474-1483.

PHYSICO-CHEMICAL PROPERTIES AND ACCEPTABILITY STUDY OF HIGH ENERGY CEREAL BAR MADE FROM MALAYSIAN PUFF GLUTINOUS RICE WITH HALAL INGREDIENTS

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ABSTRACT

Cereal bar is sweet, nutritious food due to rich content of protein, lipids, and carbohydrates. The aim of this study is to develop some formulation of cereal bar using puff glutinous rice with different binding agent; which is glucose syrup, brown sugar and honey. The dried ingredient were added with the puff glutinous rice as well as binding agent with different ratio. The cereal bar was evaluated for texture analysis, proximate value, water activity and sensory evaluation. From the result, there is no significance difference on the proximate value and water activity. Moreover, it is found that the CB 1 formulation (ratio syrup glucose to honey 1:2) has the best result in texture analysis due to its hardness and adhesiveness. However, during the sensory evaluation, the cereal bar formulate with honey to brown sugar 3:3 had the highest value on consumer acceptance.

Keywords: high energy cereal bar, puff glutinous rice, physicochemical properties

INTRODUCTION

Cereal bars are snacks foods of good sensory characteristics due to their contributing rich content of protein, lipids and carbohydrates. The most popular varieties of cereal bars among consumer are nutritious, energy, nutraceutical and diet bars (Palazzolo, 2003). Nowadays is the new trend for consumption of energy, innovative and convenience food, which has occurred recently and the market for cereal-bars are due to the consumer's awareness of health benefits of grains [2]. Previous study Esteller *et al.*, (2004) [3] cereal bars are made from processed cereal grains that can be incorporated with different ingredients, such as whole cereals, dehydrated or crystallized fruits, chestnuts, nuts, almonds, sugar, candies, and chocolates. These ingredients must be suitably combined to assure a mutual complementation or supplementation as far as flavor, texture and physical characteristics are concerned, particularly the point of balance of water activity and the physicochemical properties.

Glutinous rice is a form of short-grain rice that has higher starch content than its medium. This sticky white rice is a staple food in many Asian countries was removed

the husk, bran and germ of the rice grain by manufacturers to produce it. Moreover, the glutinous rice is relatively healthy because having low fat content but it does not offer the nutritive value of brown or wild rice [4]. Besides, the glutinous rice is a major type of cultivated rice with long-standing cultural importance in Asia. Glutinous rice exemplifies the capacity for plants to evolve phenotypic modifications in response to local cultural preferences [5].

The glutinous white rice contains 169 calories comes from 153 kcal carbohydrates, 2.8 kcal fat and 13.4 kcal protein, and they have no cholesterol [6]. The fact, glutinous white rice has low fat content but high calories content as well as supplies considerably less fibre than other types of rice and not contains gluten [4]. In addition, glutinous rice lacks the starch amylase which approximately up to 30% of the total starch in non-glutinous rice endosperm [7]. In contrast to glutinous rice, non-glutinous rice common rice show wide variation in amylose content, this quantitative variation is affected by multiple loci [8].

The greatest difficulties in obtaining a good cereal bar is a combination of several ingredients with specific functionality such as vitamins, minerals, proteins, fibres, sweeteners, binding agents, flavorings and produce a desire product with good texture as well as appearance to achieve the goals high energy cereal bars [9]. Therefore, the objectives of this study were to formulate the cereal bar as high energy bar made from puff glutinous rice and some other halal ingredients. This research attempts to determine the most suitable binding agent between brown sugar, honey and syrup glucose in the making of cereal bar. The nutritional content analysis and sensory evaluation on cereal bar will be conducted.

MATERIALS AND METHODS

The raw materials were purchased from the outlet at Kajang, Selangor, Malaysia.

Puff glutinous rice preparation

The glutinous rice flakes were soaked in the water for 1 to 2 hours, sun dried and then the glutinous rice was fried to get puffed rice.

Preparation of energy cereal bar

The dried ingredients and the binding agents were mixed together until the homogenize mixture is produced. The mixture with different type of binding agents (syrup glucose, brown sugar and honey) was coded as in Table 1. Then the mixture was baked in the oven at 180 °C for 10 minutes. The cereal bar was left to rest and to cool prior conducting the analysis of physicochemical properties and acceptability study.

Table 1. Code for different formulation of cereal bar

Code for formulation	Ratio of binding agent (syrup glucose: honey: brown sugar)
CB1	1:2:0
CB2	0:3:0
CB3	1:0:2
CB4	0:0:3
CB5	0:2:1
CB6	0:3:3

Analysis of physicochemical properties

The texture analysis, water activity and proximate analysis were done to evaluate the physicochemical properties of cereal bar. For texture analysis, the cereal bar with different formulation was run through texture analyzer by using hardness and cohesiveness test, coupled with the Software Texture Expert with probe TA-45. The water activity was analyzed by using the Aqualab Decagon 3TE [10]. Proximate analysis was done to determine the macronutrient content in the cereal bar. The mineral, moisture, fat, protein, fibre and carbohydrate content was determined (AOAC, 2000) [12].

Acceptability study

The hedonic scale was structured in nine points and 50 untrained panelists were asked to evaluate the sensorial attributes, which are appearance, color, flavor, taste, texture and overall impression (Stone & Sidel, 1993). Then, the six formulation of cereal bar were compared each other to evaluate the consumer acceptance and expectations [14].

Statistical Analysis

Physicochemical and acceptability data were analyzed using the Minitab software. Significance mean values were separated using the Tukey test. The Analysis of Variance (ANOVA) was evaluated to determine the significance difference ($p < 0.05$) among the formulation.

RESULTS AND DISCUSSION

Analysis of physicochemical properties

The texture properties of various formulation of cereal bar were presented in Table 2. The hardness of the product is the maximum test force (N) loaded on food products using a plunger. The hardness value ranged between 14.50 N to 22.68 N and the values did not statistically different among themselves. Thus, all the binding agents used in these formulations give similarity on the hardness of the product.

Table 2. Texture analysis of cereal bar

Formulation	Hardness (N)	Adhesiveness (N.mm)	Water Activity (%)
CB 1	19.79	47.0925	0.5285
CB 2	22.09	57.8446	0.5025
CB 3	22.68	9.4280	0.4770
CB 4	22.21	20.8070	0.4945
CB 5	14.57	40.0305	0.4845
CB 6	22.43	83.2500	0.5680

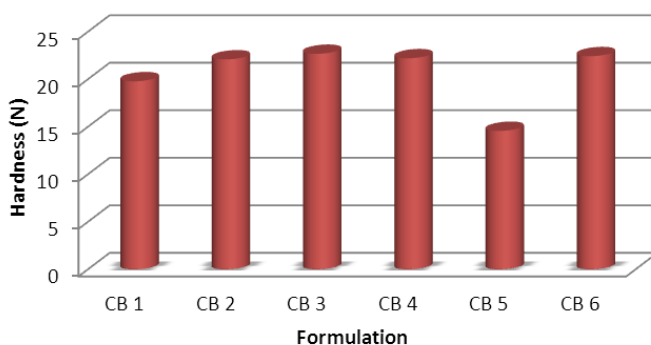


Figure 1. Hardness of cereal bar

Refer to Figure 1, the highest value of hardness was CB 3 22.68 N and the lowest was CB 5; 14.57 N. The binding agent of CB 3 was formulated with ratio of syrup glucose to brown sugar (1:2) whereas CB 5 with the ratio honey to brown sugar (2:1). The study made by Olivia (2011) [15] brown sugar is moist and sticky than white sugar. In addition, previous studied by Hull (2010) [16] stated that syrup glucose is viscous and sticky. Therefore, the combinations of brown sugar and syrup glucose give the major contribution to the hardness. These types of binding agent are ideal for use as a binder for cereal bars because the syrup holds the various ingredients together to form a solid bar. Some authors have reported hardness of cereal bars in their research. Torres *et al.*, (2009) [9] obtained an average hardness of 11.15 N and 17.60 N for cereal bars made of Jackfruit seed and Jenipapo. Paiva (2008) obtained an average hardness of 16.39 N for cereal bars based on the rice and vegetable residue. The values obtained from previous research were close in this work except the cereal bars CB 5 formulated.

The general term for adhesiveness is stickiness and this study had evaluated the force required to overcome the stickiness on the surfaces of a cereal bars. In the adhesiveness test, there were significant differences and the change was in the range 9.428 N.mm to 83.25 N.mm. Referring to the Figure 2, the highest value of adhesiveness is CB 6, 83.2 N.mm and the lowest was CB 3, 9.428 N.mm.

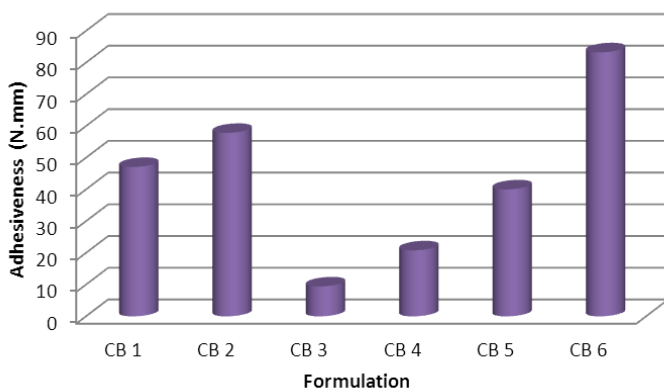


Figure 2. Adhesiveness of cereal bar

The water activity, A_w test was conducted to determine the effects of water on the texture of the products. The value obtained in Figure 4 shows the percentage of the A_w in the cereal bars that affect the texture of the product. Overall formulations were statistically different among themselves. The value of A_w was in the range 0.477% to 0.568%. Formulation CB6 has highest value of A_w which is 0.568% whereas formulation CB 3 is the lowest 0.477%. From the previous study by [17] the percentage of water

activity in cereal bars preferably about 0.45% to 0.57%. The final product must not be so dry as to give a perceptible mouth feel. Higher water activity can result in the agglomerates and hence the bars becoming too soft and less chewy and crispiness in texture and mouth feel.

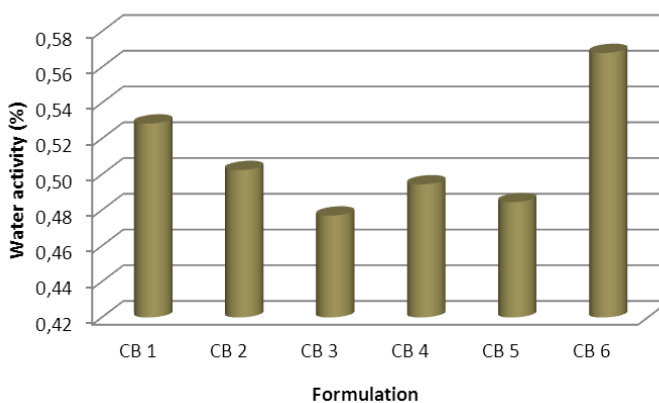


Figure 4. The water activity of cereal bar

Based on Figure 5, the values of ashes, moisture and fibre content of the six formulations found in this work were no statistically different among themselves. The range value of ashes content is 11.42% to 12.71%; moisture content 9.91% to 12.74%; whereas fibre content is 1.28% to 3.24%.

The protein content of cereal bars presented was very low in an average 1.97% to 3.32%. There were not significantly different among the six formulations. The CB 1 formulation has highest value of protein content and CB 3 was the lowest. Previous studied by Torres *et al.*, (2004) [9] shows that the protein content of cereal bar by using exotic fruit also has low protein content approximately 0.05%. Thus, the incorporation of binding agent such as brown sugar, syrup glucose and honey with dried ingredients halal foods in this project produce low protein content but higher in comparing with the previous studied [9]. The amount of fat was not significantly differing with each other. The major contribution on the fat content in cereal bar was come from butter.

Comparing the total carbohydrate content among the formulations, there were significantly differences. The cereal bar CB 6 has the highest value of carbohydrate content since the formulation ratio of honey to brown sugar is 3:3. As stated by White and Doner (1980) [18], honey is primarily a high-energy carbohydrate food as well as brown sugar also has higher total carbohydrate [6]. The honey and brown sugar contribute to high carbohydrate content in cereal bars.

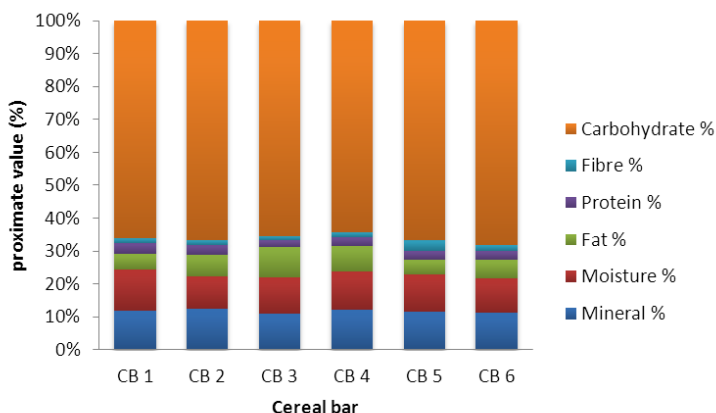


Figure 5. Proximate value of cereal bar

The total energy value was illustrated as in Figure 6. It was verified that the total energy value among the formulations did significantly differ. The range energy values in 100g cereal bar were approximately 384.33 kcal to 515.43 kcal. Cereal bars CB 1 has the highest energy value 515.43 kcal due to the protein and the carbohydrate content contributed. Moreover, previous study by White and Doner (1980) proved that the honey has 64 calories for a tablespoon. The amount of calories in honey is higher than other sugar and prefer as healthier choice because of its vitamins and minerals. Besides, the syrup glucose also has higher calories content which is provides 81 kcal for a tablespoon [19].

The energy content in CB 5 has the lowest value, 384.33 kcal due to the low calorie content of brown sugar. According to [20] brown sugar has low calorie content which is 52 kcal for 1 tablespoon, it less contributes on energy value in CB 5. Some author reports their research in physicochemical values of cereal bars. Cereal Bar formulated with jackfruit has energy values 277.02 kcal and Jenipapo with energy values 278.99 kcal. It showed the energy value of cereal bars in these study were higher than previous study by Torres *et al.*, (2004) [9] based on exotic fruit formulation.

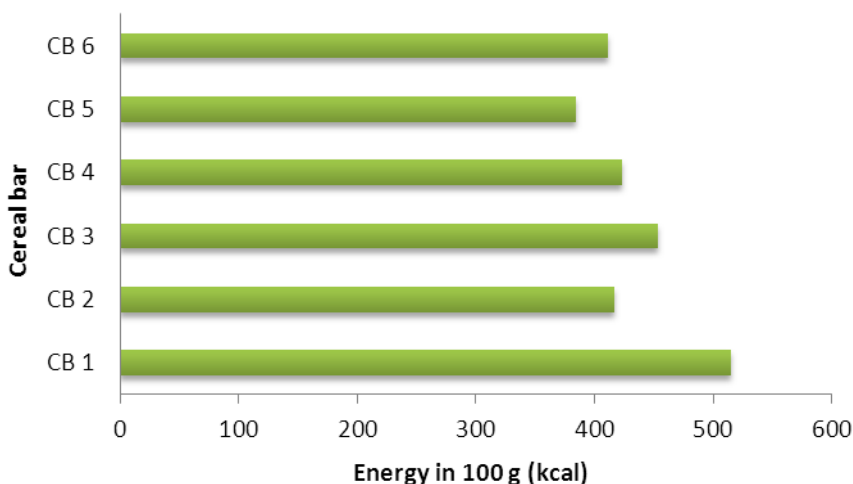


Figure 6. Energy content in cereal bar

Acceptability study

Statistical analysis showed the overall impression of cereal bar formulation with different acceptability. The attribute appearance shows significant difference between CB 1, CB 2 and CB 3 when comparing with CB 4, CB 5 and CB 6. CB 3 formulation obtained the lowest score which has 4.38. This result shows that the ratio of syrup glucose to the brown sugar, 1:2 reflects negatively on cereal bar appearance. This is due to the fact that the brown sugar cannot bind well with the syrup glucose and butter during the process of making the cereal bar. For the texture attributes, the high mean score for CB 5 and CB 6 probably due to the high content of honey. Since honey content of dextrose and levulose, therefore it binds well with the butter and other ingredients and improves the texture of the cereal bar (White and Doner, 1980).

The color attribute score for all formulations has no significant among each formulation; approximately range score from 4.50 to 5.04. This data indicates the color of these formulated cereal bars were similar to the color of commercial product. However, the CB4 and CB6 shows slightly higher value with other formulation which might be due to the absence of syrup glucose and high content of brown sugar.

The attribute of taste for the entire formulation, range score approximately 4.02 to 5.28, had shown no significant different among themselves. However, the taste attribute had lowest score for CB 3 and CB 4. It may be happened due to the high content of brown sugar which contributes to the less sweetness of cereal bar formulation (Figure 7).

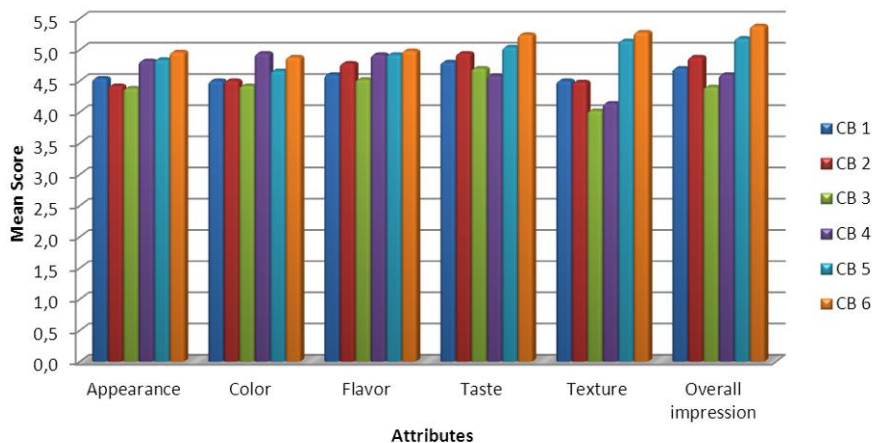


Figure 7. Sensory score of cereal bar

CONCLUSIONS

In conclusion, based on the texture analysis, the CB 1 formulation gives the most favorable result since the ratio of syrup glucose to honey 1:2 produces hardness and adhesiveness in the formulations without losing the shape of the cereal bar. In addition, CB 1 contain of high energy value in 100 g of cereal bar. However, during the sensory evaluation, the CB 6 formulation with the ratio of honey to brown sugar 3:3 had high preferable value of consumer acceptance. If comparing the CB 6 value for texture analysis, the value for hardness, adhesiveness and water activity is still within the acceptable range.

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REFERENCES

1. Palazzolo, G., 2003: Cereal bars Theyre not just for breakfast anymore. *Cereal Foods World*. 48(2): 70-72, Ch- II
2. Bower J. A. and R. Whitten. 2000. Sensory characteristics and consumer linking for cereal bar snack foods. *J. Sens. Stud.* 15:327-345.
3. Esteller, M. S., Yoshimoto, R. M. O., R. Amaral, R.L. and Lanne S. C. S. 2004. Use of sugars in baked products. *Food Sci. Tech.* 4:602-607.

4. Gray, C. 2011. Nutrition Facts for Glutinous Rice. Article Review. Available at <http://www.livestrong.com/article/332586-nutrition-facts-for-glutinous-rice/> on 12 December 2012.
5. Golomb, L. 1976. The origin, spread and persistence of glutinous rice as a staple crop in mainland Southeast Asia. *Journal Southeast, Asian Study*. 7:1–15.
6. USDA. 2007. Food Stamp Program. Exploring California Dried Fruit. California Department of Public Health. USDA. Nutrient Data Base 2012.
7. Moroshima, H., Sano, Y. and Oka, H.I. 1992. Evolutionary studies in cultivated rice and its wild relatives. *Oxford Surveys in Evolutionary Biology*. 8:135-184.
8. Ayres, N. M., M. A., McClung, P. D., Larkin, H. F., Bligh, J. and Jones, G.A. 1997. Microsatellites and a single-nucleotide polymorphism differentiate apparent Amylase classes in an extended pedigree of US rice germ plasma. *Theor. Appl. Genet.* 94:778-781.
9. E. R.Torres, , S. C.Everton, F. S.Roneval, C. C. Juliana, M. F. Cleideand S. L.Alvaro. Cereal Bar Development Using Exotic Fruit. Universidade Tiradentes, Aracaju Sergipe, Brasil.
10. Mathlouthi, M. 2001. Water content, water activity, water structure and the stability of food stuffs. *Food Control*. 12:409-417.
11. Official Methods of Analysis AOAC 2000. The Association of Official Analytical Chemists: Washington, DC, USA.
12. Nielsen, S. S. 2003. Food Analysis Laboratory Manual. Kluwer Academic/ Pelnum Publishers.
13. Stone, H.; Sidel, J.L. 1993. Sensory Evaluation Practices. 2nd ed. Academic Press: San Diego.
14. Villavicencio, A. L. C. H., M. M.Araujo, G. B.Fanaro, P. R.Rela and J.Mancini-Filho. 2007. Sensorial analysis evaluation in cereal bars preserved by ionizing radiation processing. *Radiation Physics and Chemistry*. 76:1875– 1877.
15. Olivia. 2011. Difference between white sugar and brown sugar. Article Review. Available at <http://www.differencebetween.com/difference-between-brown-sugar-and-vs-white-sugar/> on 15 December 2012.
16. Hull, P. 2010. Glucose Syrup: Technology and Applications. Wiley-Blackwell Publishing. 1:1-119.
17. R. B. Sharon, R. W. Brander, and E. C. Coleman. 2009. Cereal Bar and Method of their Manufacture. Krafts Foods Holdings Inc.
18. White, J. W. and Doner, L.W. 1980. Bee keeping in the United States Agriculture. Science and Education Administration, Eastern Regional Research Center Philadelphia. 335: 82-91.
19. Willet, B. 2011. Glucose Syrup Nutrition. Available at <http://www.livestrong.com/article/459049-glucose-syrup-nutrition> on 25 May 2012.
20. Wolf, N. 2011. The calories in One Tablespoon of Brown Sugar. Article Review. Available at <http://www.livestrong.com/article/306026-the-calories-in-one-tablespoon-of-brown-sugar/> on 28 December 2012.

EFFECT AND OPTIMIZATION OF CONCENTRATION OF DIFFERENT ENZYMES ON THE VISCOELASTICITY OF SUBSTANDARD WHEAT DOUGH

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ABSTRACT

The objective of this study was to analyze the individual and synergistic effects of three enzyme preparations (transglutaminase, lipase and xylanase) on the viscoelastic properties of wheat dough. The experiment was done by Box–Behnken design. A response surface method was used to evaluate the effects observed on the dynamic oscillatory parameters of in wheat dough system made from substandard quality flour.

Transglutaminase (TG) and lipase (LP) have a significantly effect on the strength of wheat dough. Lipase significantly decreased loss modulus (G'') of dough. Xylanase (Xyl) does not significantly affect on the G' , but with increasing concentration significantly increases the value of G'' .

Individual effects of transglutaminase, lipase and xylanase by their intensity and direction depend on the applied concentration. The interaction between TG and LP leads to improved handling of dough (significantly increases the storage modulus and also decreases its loss modulus, but not in the domain of statistical significance). Optimal levels of the tested enzymes were determined by the desirability function approach. It was found that the combined effect of 25.98 mg/kg transglutaminase, 25.59 mg/kg lipase and 34.06 mg/kg xylanase achieved a positive synergistic effect on wheat dough viscoelasticity made from substandard flour.

Keywords: transglutaminase, lipase, xylanase, storage modulus, loss modulus optimization, desirability function

INTRODUCTION

Climate change could strongly affect the wheat crop and quality. In recent years, a variation of technological quality of mercantile wheat has been expressed. This phenomenon is present not only in Serbia, but also in neighbouring countries; it can be said that the whole Pannonian Plain is included. Geneticists are constantly creating new wheat varieties resistant to antibiotic stress, however the usability of their potential is

restricted due to inadequate application of agro-techniques. All the aforementioned facts influence processing industry which has a problem in its production process due to great quantities of wheat flour of substandard quality. The processing industry is assigned the task to elevate the quality of wheat flour to the level optimal for processing [1-3]. In this context, the treatment of flours with functional additives must be considered. The use of enzymes is the best alternative to chemical compounds because they are generally recognized as safe and do not remain active after baking. Enzymes with the effect of strengthening the wheat gluten suitable for flour of substandard quality. One of the enzymes that can confer strength to dough is transglutaminase.

Transglutaminase (EC 2.3.2.13, protein-glutamine γ -glutamyltransferase) catalyses the reaction of transfer of acyl group between γ -carboxamide group from protein bound glutamine residue (acyl donors) and different primary amines (acyl acceptors). Acyl acceptors are most frequently ϵ -amino groups of lysine residues in a protein or peptide. As a result of this reaction, there occurs inter- or intramolecular cross-linking in covalent bonds. The formation of these bonds results in strengthening of gluten network [4].

Lipase (EC 3.1.1.3) is an enzyme which hydrolyses ester bonds primarily in the 1- and 3-position of fatty acids in triglycerides. Lipases hydrolyses triacylglycerols producing: monoacylglycerols, diacylglycerols, glycerol and free fatty acids. The surface active properties of the hydrolysis reaction products along with modifications on the interactions between lipids and gluten proteins caused good dough conditioning effects in terms of improved dough rheological properties, dough stability in case of over fermentation, increased oven spring and improved crumb structure of bread without added shortening [5].

Xylanase (EC 3.2.1.8) is an enzyme which degrades linear polysaccharide β -1.4-xylan into xylose. Cereal xylans contain large quantities of L-arabinose and are therefore often referred to as arabinoxylans. The most favorable xylanases for breadmaking are those that preferentially act on water insoluble arabinoxylan because they tear the insoluble arabinoxylans which interfere with the formation of the gluten network, giving rise to enhancing dough stability [5, 6].

In this paper, we applied the gluten cross-linking enzyme, lipid hydrolytic enzyme and non-starch degrading enzyme into wheat dough to optimize its rheological parameters: storage modulus (G') and loss modulus (G''). The usage of TG, LP and Xyl as dough improvers is well known and established [7-9]. The innovative character of the paper lies in the fact that so far there has been no research of the combined effects of these enzymes in flour of substandard quality. This is one of the papers which, in the experiment performed, applies low quality flour and defines optimum doses of these dough improvers in order to achieve the goals set. These goals are related to optimising of ratio of elastic and plastic properties of dough. Response surface methodology (RSM) and desirability function are statistical techniques that have been successfully applied in the development and optimization of cereal products [10-12]. The objective of the presented study was to evaluate the combined effects of transglutaminase, lipase and xylanase

addition on the dough viscoelastic properties, using small deformation mechanical testing and explore the possibility of improving behavior during handling the dough of substandard flour.

MATERIALS AND METHODS

Commercial refined soft wheat flour was used in this study. This flour was obtained from local soft wheat and has the following characteristics: protein content is 10.7% of dry weight, dough energy by extensograph is 33 cm², resistance by extensograph is 130 BU, extensibility by extensograph 157 mm and ratio number of the resistance through extensibility is 0.83. The sample of flour is of poor quality. Protein content of the tested wheat flour (N×6.25) was analysed using a Kjeldahl procedure [13]. Rheological properties were determined by the Brabender[®] extensograph [14]. Three commercial enzymes were used: transglutaminase (VERON[®] TG), xylanase (VERON[®] 191S) and lipase (VERON[®] Hyperbake-T), all of them from company AB Enzymes, Germany.

All the dough samples were mixed in a farinograph bowl till maximum peak development was adopted. Doughs prepared were placed in a plastic bag and immediately sealed and allowed to rest for 30 min at ambient temperature before rheological testing. Oscillatory test was performed in a HAAKE Mars rheometer (Thermo Scientific, Karlsruhe, Germany). The rheometer was equipped with a 35 mm parallel plate measuring geometry. A dough sample was placed on the lower plate, and the upper plate was lowered until the gap of 1.0 mm was reached. The excess dough was trimmed and the edges were sealed with a paraffin oil to prevent the dough from drying during measurements. All the experiments were performed at 30 °C.

The response surface model (RSM) used in this study involved three factors X_1 (transglutaminase (TG)), X_2 (lipase (LP)) and X_3 (xylanase (Xyl)). The *Box and Behnken* design contained a total of 15 experiments with three replicates at the central points (Table 1). The data were analysed using the Design Expert 7.0 software (Stat-Ease, Inc, Minneapolis, MN, USA). The relationships between the measured variables (G' and G'') and the three independent factors (TG, LP and Xyl) were estimated by fitting the data to a second-order polynomial (Eq. 1). In this polynomial Y is the response variable (e.g. storage modulus (G'), loss modulus (G'')), b_0 is a constant, b_1 and b_2 are the regression coefficients for the linear effects, b_{11} and b_{22} are the quadratic coefficients and b_{12} is the interaction coefficient.

$$Y = b_0 + b_1TG + b_2LP + b_3Xyl + b_{12}TG \cdot LP + b_{13}TG \cdot Xyl + b_{23}LP \cdot Xyl + b_{11}TG^2 + b_{22}LP^2 + b_{33}Xyl^2 \quad (1)$$

The G' and G'' were continuously monitored during dynamic rheological testing in the frequency range from 1 to 10 Hz and their value at 1 Hz was used as the response variable in the model. Coefficients of determination (R^2) were computed, and the

adequacy of models was tested by estimating the lack of fit. A stepwise deletion of non-significant terms was applied. Calculation of optimal enzyme concentrations for a defined set of criteria was performed using a desirability function.

RESULTS AND DISCUSSION

The results of the experimental design are presented in Table 1.

Table 1. Results of experiment design

Runs	TG mg/kg $X_1 (x_1)$	LP mg/kg $X_2 (x_2)$	Xyl mg/kg $X_3 (x_3)$	G' ($\times 10^3$) Pa	G'' ($\times 10^3$) Pa
Control	0	0	0	11.2	4.9
1	0 (-1)	0 (-1)	25 (0)	12.7	4.4
2	30 (1)	0 (-1)	25 (0)	12.8	4.8
3	0 (-1)	30 (1)	25 (0)	12.2	4.0
4	30 (1)	30 (1)	25 (0)	14.0	4.5
5	0 (-1)	15 (0)	0 (-1)	11.9	4.0
6	30 (1)	15 (0)	0 (-1)	13.2	4.3
7	0 (-1)	15 (0)	50 (1)	12.1	4.6
8	30 (1)	15 (0)	50 (1)	12.7	4.3
9	15 (0)	0 (-1)	0 (-1)	13.2	4.3
10	15 (0)	30 (1)	0 (-1)	12.5	4.5
11	15 (0)	0 (-1)	50 (1)	12.8	4.8
12	15 (0)	30 (1)	50 (1)	13.1	4.3
13	15 (0)	15 (0)	25 (0)	12.7	4.3
14	15 (0)	15 (0)	25 (0)	13.0	4.1
15	15 (0)	15 (0)	25 (0)	12.5	4.2

The effects of individual and combined action of the investigated enzymes on viscoelastic characteristics are presented by the regression models (Eq. 2 and 3).

$$G' = 12.573 + 249.4 \cdot TG + 259.4 \cdot LP + 887.5 \cdot TG \cdot LP \quad (2)$$

$$G'' = 40.22 - 148.4 \cdot LP + 166.1 \cdot LP^2 + 324.5 \cdot Xyl^2 \quad (3)$$

In order to achieve visual effects of the independent variables on the rheological parameters of dough, the perturbation graphs are shown for each parameter (Figure 1).

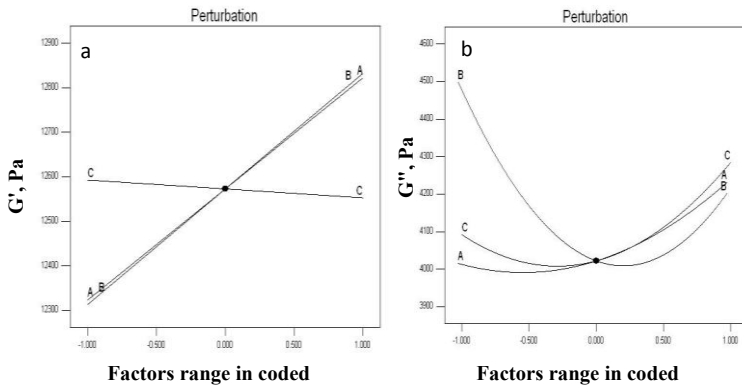


Figure 1. Perturbation graphs showing the effect of enzymes (TG-A; LP-B; Xyl-C) on a) storage modulus (G'); b) loss modulus (G'')

TG and LP have significant ($p \leq 0.1$) positive linear effect on the storage modulus. Their interactive effect ($TG \cdot LP$) is even higher and more significant ($p \leq 0.05$) and leads to the increase of dough elasticity. These results are achieved as a consequence of the effects of TG to networking of protein chains and strengthening of protein dough structure [15]. The effect of lipases manifests hydrolysis of wheat lipids, resulting in an *in situ* production of di- and monoglycerides which have been shown to have a much stronger tendency to binding to gluten proteins and to show emulsifying effect. Therefore, at initial concentrations of LP also has been registered a significant decrease of G'' values. With the increase of lipase concentration above its optimal level there occurs a more expressed hydrolytic effect which causes the increase of G'' values. Increased

concentrations of xylanase also caused a significant ($p \leq 0.1$) increase in G'' . This effect occurs due to the hydrolytic activity of xylanase to hydrolyze glycosidic bonds within the Xylan and results in a decrease of the degree of polymerisation [16]. The decrease in the degree of polymerisation leads to changes in the rheology of dough such as dough consistency [17]. Such alteration of consistency in fact represents the increased value of G'' . The intensity and direction of individual and interactive effects of the TG, LP and Xyl in the investigated range depend on the applied dosage, and primarily on specific enzymes [18]. By determination of optimal doses for all the three enzymes in the optimized mixture it is possible to reach maximal results in the direction of improving characteristics of dough made from substandard wheat flour. Desirability function approach was used to locate the optimal doses of these enzymes which produce adequate responses regarding the quality of dough made from substandard wheat flour. The proposition of the criteria necessary for the determination of optimal experimental conditions was based upon preliminary experiments and the literature data and is shown in Table 2.

Table 2. Optimization solutions of TG, LP and Xyl concentration for the set requirements of wheat dough made from substandard quality flour

Factors and responses	Criteria		Lower	Upper
	Goal	Relative importance	Limit	Limit
TG, mg/kg	is in range	3	0	30
LP, mg/kg	is in range	3	0	30
Xyl, mg/kg	is in range	3	0	50
G' , Pa	maximize	3	11920	14025
G'' , Pa	minimize	3	4016	4847.5
Optimal solutions for concentration			Prediction values	
TG, mg/kg	LP, mg/kg	Xyl, mg/kg	G'	G''
25.98	25.59	34.06	13409.2	4205.38

The solution of the proposed criteria in the optimization of the TG, LP and Xyl doses is also shown in Table 3. Using the set criteria, a solution having 66.0% desirability was selected. Therefore, this solution was subsequently analysed to compare the predicted responses with measured values in a new experiment with optimal concentration TG doses. (25.98 mg/kg), LP (25.59 mg/kg) and Xyl (34.06 mg/kg) The results of comparison are also given in Table 3. Predicted solution: 13409.2 Pa for G' and 4205.38 Pa for G'' ,

obtained under the optimum conditions were close to the obtained experimental results: 13410 Pa for G', 4200 Pa for G'', indicating that the models were adequate to predict these responses. The adequacy of the model is confirmed relatively high values of determination coefficient ($R^2 = 61.2$ for G'; $R^2 = 75.6$ for G'') and insignificant lack of fit ($p = 0.68$ for G' and $p = 0.80$ for G'').

Table 3. Verification of the optimal solution

Factors	Optimal concentration	Overall desirability			
TG, mg/kg	25.98				
LP, mg/kg	25.59	0,66			
Xyl, mg/kg	34.06				
Responses	Prediction values	Obtained values	Deviation, %	Control	Effect, %
G', Pa	13409.2	13410	0.02	11200	+19.7
G'', Pa	4205.38	4200	0.13	4900	-16.7

CONCLUSION

In this study the influence of three factors, concentration of transglutaminase, lipase and xylanase, on the rheological quality of wheat dough was measured on rheometer by the application of oscillatory test. Individual and interactive effects of the applied enzymes on viscoelastic characteristics of substandard wheat dough are determined by the help of regression coefficient values. Transglutaminase had a great effect on the strength of substandard wheat dough. TG and LP have significantly synergistic effect of increased storage modulus (G'). Inicial concentracion of LP decreased loss modulus (G''). With increasing levels of LP and Xyl significantly increases their hydrolytic effect, which contributes to the plasticity of the dough manifested through the values of loss modulus (G'').

Individual effects of transglutaminase, lipase and xylanase by their intensity and direction depend on the applied concentration. When incorporated into the formulation, the optimized levels of TG (25.98 mg/kg), LP (25.59 mg/kg) and Xyl (34.06 mg/kg) yielded good quality wheat dough made from substandard wheat flour.

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REFERENCES

1. Mikhaylenko, G. G., Czuchajowska, Z., Baik, B.-K., and Kidwell, K. K. 2000. Environmental influences on flour composition, dough rheology, and baking quality of spring wheat. *Cereal Chem.* 77:507-511.
2. Malešević, M., Jaćimović, G., Jevtić, R., and Aćin, V. 2011. Exploiting the genetic potential of wheat under conditions of abiotic stress, Page 3-14 in: Proceedings of 45th Conference on agriculture Serbian, Zlatibor, Serbia.
3. Balla, K., Rakszegi, M., Li, Z., Bekes, F., Bencze, S. and Veisz, O. 2011. Quality of winter wheat in relation to heat and drought shock after anthesis. *Czech J. Food Sci.* 29:117-128.
4. Larré, C., Denery-Papini, S., Popineau, Y., Deshayes, G., Desserme, C., and Lefebvre, J. 2000. Biochemical analysis and rheological properties of gluten modified by transglutaminase. *Cereal Chem.* 77:32-38.
5. Miguel, A. S. M., Martins-Meyer, T. S., Figueiredo, É. V. C., Lobo, B. W. P., and Dellamora-Ortiz, G. M. 2013. Enzymes in Bakery: Current and Future Trends, Food Industry, Dr. Innocenzo Muzzalupo (Ed.). Available from: <http://www.intechopen.com/books/food-industry/enzymes-in-bakery-current-and-future-trends>.
6. Butt, M. S., Tahir-Nadeem, M., Ahmad, Z., and Tauseef, M. S. 2008. Xylanases in baking industry. *Food Technol. Biotechnol.* 46:22-31.
7. Joye, I. J., Lagrain, B., and Delcour, J. A. 2009. Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking – A review. *J. Cereal Sci.* 50:11-21.
8. Valeri, D., Lopes, A. M. and Pessoa-Júnior, A. 2011. Evaluation of xylanases from *Aspergillus niger* and *Trichoderma* sp. on dough rheological properties *Afr. J. Biotechnol.* 10:9132-9136.
9. Colakoglu, A.S., and Özkaya, H. 2012. Potential use of exogenous lipases for DATEM replacement to modify the rheological and thermal properties of wheatflour dough. *J. Cereal Sci.* 55:397-404.
10. Gallagher, E., O'Brien, C. M., Scannell, A. G. M., and Arendt, E. K. 2003. Use of response surface methodology to produce functional short dough biscuits. *J. Food Eng.* 56:269-271.
11. Šimurina, O., Ikonić, B., Jevtić-Mučibabić, R., Belović, M., Koprivica, G., and Mišljenović, N. 2012. Application of response surface methodology in the development of specialty bread with sugar beet molasses, flax seed and vital wheat gluten. *Food and Feed Research.* 39:11-22.

12. Šimurina, O., Filipčev, B., Popov, S., Bodroža-Solarov, M., Brkljača, J., Grbić, J. and Nježić, Z. 2013a. Optimization of the strength of wheat dough made from substandard quality flour using enzyme preparations, *Journal on processing and energy in agriculture*. 17:29-33.
13. Method for Using the Brabender Extensograph. ICC Standard No. 114/1. Approved 1972. Revised 1992. The International association for cereal science and technology.
14. Determination of crude protein in cereals and cereals products for food and feed. ICC-Standard No 105/2. Approved 1980. Revised 1994. The International association for cereal science and technology.
15. Jong, G. A. H. and Koppelman, S. J. 2002. Transglutaminase catalyzed reactions: impact on food applications. *J. Food Sci.* 67:2798-2806.
16. Sunna, A. and Antranikian, G. 1997. Xylanolytic enzymes from fungi and bacteria. *Crc. Rev. Biotechn.* 17:39-67.
17. Sorensen, J. F. 2003. Novel tailor-made xylanases: their characterisation, performance in cereal processing and use as a tool to understand xylanase functionality in baking. Page 241-245 in: *Recent Advances in Enzymes in Grain Processing*, C.M. Courtin, W.S. Veraverbeke and J.A Delcour. (Eds.). Laboratory of Food Chemistry, Katholieke Universiteit Leuven, Leuven.
18. Šimurina, O. 2013b. Optimization of doses of organic acids and enzymatic preparations in biochemical matrix of substandard quality. PhD thesis, Faculty of technology, University of Novi Sad, Serbia.

DEVELOPMENT OF SPECIFIC PROFILE IN MIXOLAB FOR STARCH-PROTEIN CHARACTERIZATION OF WHEAT GENOTYPES IN GENETIC BREEDING PROGRAMS

UDC 664.231 : 532.135

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ABSTRACT

Wheat (*Triticum aestivum* L.) end-use quality evaluation is an important component of breeding programs. Small scale methods to characterize starch and proteins are needed for early generation selection, due to the grain quantities limitation in breeding. Mixolab is an option to evaluate simultaneously protein and starch features in advanced selection, but using relatively small sample amount. The traditional mixolab profile "Chopin+" uses fixed mixing time (8 min), but the development of gluten network is greatly dependent on wheat cultivars. The aim of this study was to set up specific profile for screening wheat quality attending to protein and starch behavior during mixing, heating and cooling. Four wheat flour samples were performed using both a variable and fix time of mixing to differentiate genotypes and results were compared. Only the sample with lower stability (<8 min) presented the same analysis time for both profiles. The most important mixolab parameters that presented significant differences were: stability, initial pasting temperature, gamma value, and cooking stability range. Overall results suggest that mixolab "breeding" profile can be used successfully to differentiate wheat in genetic breeding programs.

Keywords: wheat, breeding, rheological analysis, mixolab, mixing

INTRODUCTION

Wheat cultivars (*Triticum aestivum* L.) are characterized by several tests to predict functionality and to determine end-use quality, which is fundamental to assist the breeding programs in the selection of better genotypes for crossings.

Cereal laboratories involved with wheat breeding programs are always seeking for new procedures that could be applied to wheat quality selection. Such procedures must be simple, rapid, and reliable, and they must have high correlations with end-use

properties, and retain their predictive capacity independently of the location or year of growth [1].

The mixolab is an option to evaluate simultaneously protein and starch features, using relatively small sample amount. It has been successfully compared with traditional equipment/method for determining dough quality like mixograph [2], farinograph, extensograph, amylograph [3], falling number [4], rapid visco-analyser [5], alveograph and baking test [6], providing good predictors for all the parameters measured with these traditional methods.

The mixolab is an instrument that can be used to characterize the rheological behavior of dough subjected to a dual mixing and temperature constraint. It allows the measurement of important physical dough properties, like dough development time and stability, and pasting properties of starch in actual dough, all of these parameters being indicators of quality and potential end-use in products [7].

The traditional mixolab profile "Chopin+" uses fixed mixing time (8 min), but the development of gluten network is greatly dependent on wheat cultivars strength, and for some of them, it is necessary more than 8 minutes to develop de wheat flour dough.

Thus, the aim of this study was to set up specific profile for screening wheat quality attending to protein and starch behavior during mixing, heating and cooling and to compare with the traditional mixolab profile. In addition, possible correlations with the most used wheat quality validation parameters were determined.

MATERIALS AND METHODS

Raw material

Four wheat cultivars grain samples were milled on a Brabender (Model, Quadrumat Senior) laboratory mill after tempering, according to AACC method 26-10 [1].

Samples quality characterization

Wheat grains and flour quality characterization was performed according to AACC [8] methods: hardness index (method 55-31), falling number (method 56-81B), wet gluten and gluten index (method 38-12A), damaged starch (method 76-33A), alveograph parameters (method 54-30A), and farinograph parameters (method 54-21).

Mixing and pasting properties of wheat flour samples using the mixolab analyzer (Chopin Technologies, Villeneuve-la-Garenne Cedex, France) were studied. For each assay, 50 g of wheat flour in 14% basis (flour+water=75 g) were poured into the mixolab bowl and mixed with the necessary amount of water for reaching optimum dough development [9]. The "Chopin+" profile was used and, the "breeding" profile was developed, joined with Chopin, to differentiate flour end-use. In this profile, the first stage corresponds to mixing till 10% decrease of C1 torque, to assess high stability genotypes. Settings of the two mixolab profiles used are compared in Table 1.

Table 1. Mixolab established profile "Chopin+" used to wheat rheological characterization and the new one, "breeding", proposed to be used in breeding programs

PARAMETER	MIXOLAB PROFILE	
	"CHOPIN+"	"BREEDING"
Kneading speed (rpm)	80	80
Target torque, C1-target consistency (Nm)	1.05 a 1.15	1.05 a 1.15
Dough mass, 14% basis (g)	75	75
Tank temperature (°C)	30	30
Temperature first level (°C)	30	30
Duration first level (min)	8 (fixed)	variable (10% of C1)
First temperature gradient (15 min)	4 °C/min	4 °C/min
Temperature second level (°C)	90	90
Duration second level (min)	7	7
Second temperature gradient (10 min)	-4 °C/min	-4 °C/min
Temperature third level (°C)	50	50
Duration third level (min)	5	5
Total analysis time (min)	45	variable (according to flour strength)

Figure 1 shows a typical curve recorded in the mixolab device along the different stages (mixing, heating, and cooling) recorded in the mixolab plot.

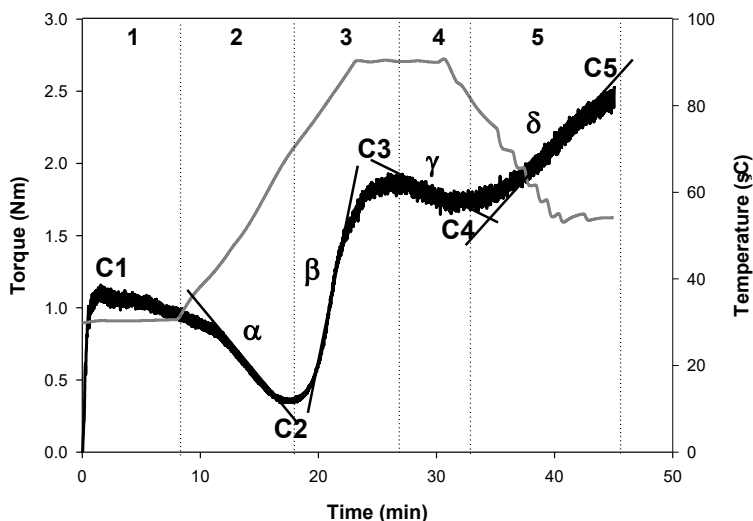


Figure 1. Typical curve recorded by the mixolab. Numbers show the different stages and parameters are displayed on the plot

Description of the physical changes that occurred along mixolab measurement was reported by Rosell et al. [10]. Parameters obtained from the recorded curve are summarized: the first part of the mixolab curve recorded the dough behavior during mixing and overmixing; during this stage, the torque increased until it reached a maximum (C1). At that point, the dough was able to resist the deformation for certain time, which determined the dough stability. The simultaneous mechanical shear stress and temperature constraint (second stage) decreased the torque until a minimum value (C2) was reached that could be related to the beginning of the protein structure destabilization or protein weakening. As the temperature increased, starch gelatinization took place (third stage) with a concomitant increase in the torque until a new maximum value (C3) was reached. A reduction in viscosity was observed in the fourth stage derived from the physical breakdown of the starch granules, leading to a minimum value of the torque (C4). The decrease in the temperature produced an enhancement in the dough consistency (fifth stage), resulting in a maximum torque (C5). In addition, the slopes defined along ascending and descending curves were calculated. According to Mixolab [11], α represents the slope of the curve between the end of the period of 30 °C and C2; gives indication about the rate of the proteins thermal weakening; β represents the slope of the curve between C2 and C3; gives indications about the gelatinization rate; and γ represents the slope of the curve between C3 and C4; gives indications about the rate of enzymatic hydrolysis.

Statistical Analyses

Analysis of variance (ANOVA) and multiple comparisons (Tukey's test) were used for the statistical analysis of mixolab data. The correlation analysis was performed among mixolab results ("Chopin+" and "breeding" profiles, separately) and other technological wheat quality parameters.

RESULTS AND DISCUSSION

Wheat flours were characterized assessing grain hardness, falling number, gluten, alveographic and farinographic parameters (Table 2).

Table 2. Characteristics of four refined (RF) samples obtained from wheat milling

QUALITY PARAMETER	SAMPLE			
	1 RF	2RF	3RF	4RF
Hardness index	86.38	81.70	88.58	80.09
Grain falling number (s)	565	274	572	390
Flour falling number (s)	428	314	386	336
Damage starch (%)	4.7	5.4	5.2	5.6
<i>. Gluten parameters</i>				
Gluten index	100	100	93	99
Wet gluten (%)	22.5	27.8	30.2	27.1
Dry gluten (%)	8.2	10.4	10.6	9.4
<i>. Alveographic parameters</i>				
Deformation energy, W (10 ⁻⁴ J)	347	356	295	366
Curve of deformation ratio (P/L)	0.60	1.50	1.40	4.00
Swelling index	25	19	20	15
Elasticity index (%)	64	72	52	59
<i>. Farinographic parameters</i>				
Water absorption (%)	56	62	63	67
Stability (min)	11	12	6	11
Dough development time (min)	9	21	6	24
Mixture tolerance index (FU)	24	15	28	21

It can be observed that the samples presented different quality characteristics. The farinographic results showed that samples 1RF, 2RF, and 3RF presented stability and dough development time higher than 8 min.

Flour rheological behavior determined with the traditional profile of the mixolab ("Chopin+" profile) did not show significant differences among the flours (Figure 2 A). Conversely, significant differences were observed among the plots obtained with the "breeding" profile (Figure 2 B), with the exception of sample 3RF, which presented a dough development time within eight minutes. Thus, wheat flour samples with high stabilities must not be analyzed by "Chopin+" profile, especially if the objective of analysis is to differentiate wheat genotypes characteristics.

Only the sample with lower stability (<8 min) presented the same analysis time for both profiles. In consequence, strong wheat flours that required longer development time to form the gluten network should be analyzed extending the assay time to their specific mixing requirements.

Mixolab parameters recorded from the plots are presented in Table 3. The mixolab parameters that presented significant differences between "Chopin+" and "breeding" protocols were: stability, initial pasting temperature, gamma value, and cooking stability range, which are always very important parameters to be considered in wheat quality evaluation to indicate end-use.

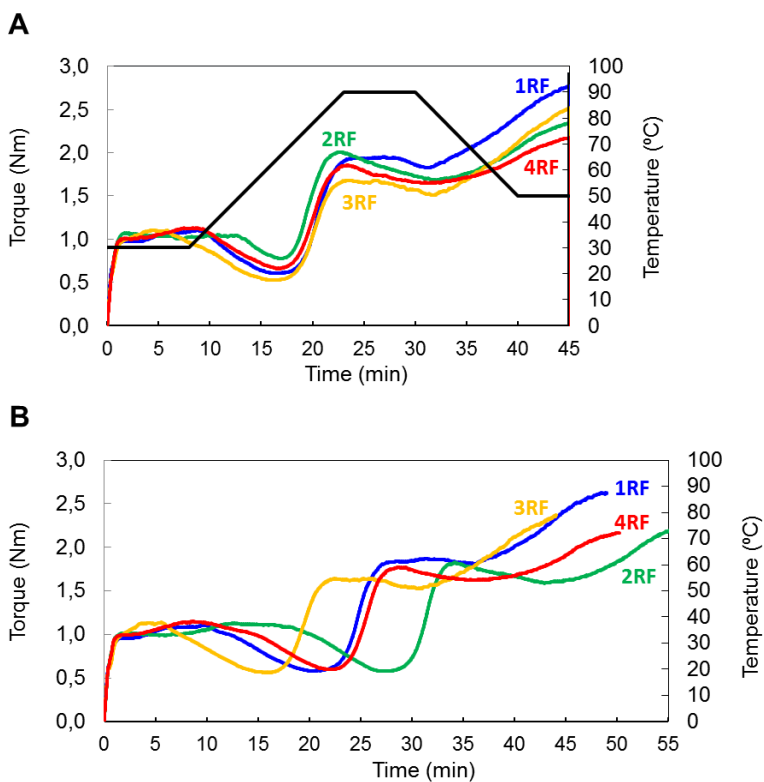


Figure 2. Mixolab curves performed by “Chopin+”(A) and “breeding”(B) profiles
Where: RF, refined flour; and 1, 2, 3, and 4: wheat refined flour samples.

Rosell et al. [12] observed that rheological analysis allowed identifying the role of the proteins and the relationship between the protein content and different primary and secondary parameters obtained from the recorded curves. The microstructure analysis revealed the changes that proteins and starch molecules underwent during mixing, heating, and cooling. Therefore, considering the parameters that showed significant differences depending on the profile used, proteins and starch functionalities were not fully developed when using the traditional mixolab profile.

Dhaka et al. [13] suggested that mixolab has potential to be used for selection of superior bread wheat, based on higher dough stability, C2 and development time (time to C1) values, which were determined using the Chopin Simulator profile. They concluded that Mixolab proved to be a simple and an effective instrument for evaluating flour and

gluten protein quality, thus enabling wheat breeders to assess wheat quality more effectively and timely in the breeding program.

To validate the mixolab profiles with the usual parameters used for wheat quality assessment, independent correlations were determined between traditional parameters and the mixolab profiles (Table 4).

Correlation analysis showed that only five mixolab parameters presented the same correlations in both profiles, those comprised: C5-C4 (gelling range) presented positive correlation with hardness index and negative with farinographic dough development time; C5 was correlated negatively with damage starch and positively with swelling index; and mixolab stability with farinographic mixing tolerance index.

Table 3. Mixing characteristics of wheat refined flour (RF) samples performed by "Chopin+"(A) and Mixolab "breeding"(B) profiles

MIXOLAB PARAMETER	SAMPLE / PROFILE*							
	1 RF		2RF		3RF		4RF	
	A	B	A	B	A	B	A	B
Time to C1, development time (min)	8.9 ^a	9.8 ^a	10.6 ^b	12.9 ^a	4.4 ^b	5.5 ^a	8.6 ^a	8.7 ^a
C1, maximum torque (Nm)	1.12 ^a	1.11 ^a	1.06 ^a	1.14 ^a	1.11 ^a	1.14 ^a	1.14 ^a	1.16 ^a
Mixolab stability (min)	9.4 ^a	10.8 ^a	13.2 ^b	19.2 ^a	6.6 ^a	5.9 ^b	10.0 ^b	13.3 ^a
Amplitude (Nm)	0.07 ^a	0.05 ^b	0.08 ^a	0.07 ^a	0.06 ^a	0.06 ^a	0.07 ^a	0.08 ^a
C2, minimum torque, protein weakening (Nm)	0.60 ^a	0.58 ^a	0.77 ^a	0.58 ^b	0.53 ^a	0.56 ^a	0.66 ^a	0.60 ^b
Temperature at C2 (°C)	53.7 ^a	51.0 ^a	55.0 ^a	49.2 ^b	52.6 ^a	53.5 ^a	54.1 ^a	53.5 ^a
Initial pasting temp (°C)	18.2 ^b	22.5 ^a	17.8 ^b	29.5 ^a	17.8 ^a	17.1 ^b	17.8 ^b	23.4 ^a
C3, starch gelatinization (Nm)	1.96 ^a	1.88 ^a	2.01 ^a	1.83 ^b	1.69 ^a	1.66 ^a	1.86 ^a	1.78 ^b
Temperature at C3 (°C)	82.80 ^a	84.85 ^a	76.10 ^a	74.55 ^a	81.75 ^a	80.80 ^a	79.55 ^a	78.50 ^a
C4, amylase activity (Nm)	1.82 ^a	1.81 ^a	1.68 ^a	1.59 ^b	1.50 ^a	1.52 ^a	1.65 ^a	1.62 ^a
C5, starch gelling (Nm)	2.77 ^a	2.64 ^a	2.36 ^a	2.25 ^a	2.52 ^a	2.37 ^a	2.18 ^a	2.17 ^a
Protein weakening range, C2-C1 (Nm)	-0.52 ^a	-0.53 ^a	-0.29 ^a	-0.56 ^b	-0.58 ^a	-0.58 ^a	-0.48 ^a	-0.56 ^b
Starch gelatinization range, C3-C2 (Nm)	1.36 ^a	1.30 ^a	1.24 ^a	1.26 ^a	1.17 ^a	1.10 ^b	1.20 ^a	1.18 ^a
Cooking stability range, C4-C3 (Nm)	-0.14 ^a	-0.06 ^a	-0.33 ^b	-0.25 ^a	-0.19 ^b	-0.14 ^a	-0.21 ^b	-0.16 ^a
Pasting temperature range (°C)	29.1 ^a	33.9 ^a	21.1 ^a	25.4 ^a	29.2 ^a	27.3 ^a	25.5 ^a	25.1 ^a
Gelling range (setback), C5-C4 (Nm)	0.95 ^a	0.83 ^b	0.68 ^a	0.67 ^a	1.02 ^a	0.86 ^b	0.54 ^a	0.56 ^a
α, protein weakening speed due to heat (Nm/min)	-0.085 ^b	-0.059 ^a	-0.063 ^a	-0.065 ^a	-0.063 ^a	-0.064 ^a	-0.064 ^a	-0.058 ^a
β, starching speed (Nm/min)	0.394 ^a	0.381 ^a	0.364 ^a	0.423 ^a	0.322 ^a	0.310 ^a	0.306 ^a	0.329 ^a
γ, enzymatic degradation speed (Nm/min)	-0.039 ^b	-0.014 ^a	-0.035 ^b	-0.026 ^a	-0.027 ^a	-0.023 ^a	-0.024 ^b	-0.021 ^a
δ, retrogradation speed (Nm/min)	0.070 ^a	0.075 ^a	0.060 ^a	0.069 ^a	0.084 ^a	0.072 ^a	0.070 ^a	0.052 ^a
Test time (min)	45.00 ^b	50.00 ^a	45.00 ^b	58.00 ^a	45.00 ^a	45.00 ^a	45.00 ^b	51.00 ^a

*Means with different superscript lowercase letters in the same row, by sample (1RF, 2RF, 3RF, and 4RF), are different at 5 % significance level ($P \leq 0.05$) according to Tukey's test.

Significant correlations at: * <0.05 ; ** <0.01 ; *** <0.001 of probability (P-value). *Black, "Chopin+" profile, *Gray, "breeding" profile. Where: GFN, grain falling number; FFN, flour falling number; GI, gluten index; WG, wet gluten; P/L, curve of deformation ratio;

SI, swelling index; EI, elasticity index; WA, water absorption; STB, stability; DDT, dough development time; and, MTI, mixture tolerance index.

From the correlation analysis can be displayed that using the “Chopin+” profile 18 significant correlations could be defined with traditional quality parameters, whereas 23 correlations were found with parameters recorded using “breeding” profile. Results indicated that “breeding” profile allowed complete developing of gluten network and even starch functionality was also affected.

Table 4. Significant correlation coefficients (r) between mixing characteristics performed by “Chopin+”(A) and “breeding”(B) mixolab profiles and other parameters for wheat quality evaluation

PARAMETER MIXOLAB	HARDNESS INDEX		F.NUMBER		DAMAGE STARCH		GLUTEN		ALVEOGRAPHY			FARINOGRAPHY				TEST TIME	
	A B		A B		A B		GI WG		PL SI EI			WA STB DDT MTI					
	A	B	A	B	A	B	A	B	B	A	B	A	B	A	B		A
TIME TO C1							0.96	*			**	0.99	*	0.98			*
C1					*		0.97			**	-0.99		**	1.00			
STABILITY						*			*							**	**
AMPLITUDE							0.97		*		-0.97					-0.99	-1.00
C2			*													**	**
TEMPERATURE AT C2																-1.00	*
INITIAL PAST. TEMP.												*					*
C3											0.95					-0.99	**
TEMPERATURE AT C3			*	*							0.96						**
C4			0.97	0.98				**									**
C5					**	**	-1.00	-1.00		**	**	1.00	0.98	*	-0.97		*
C2-C1					**	*			**		-0.99						*
PAST. TEMP. RANGE			**		0.99		-0.97		**								*
C5-C4	**	**														**	**
β	0.99	0.98														-0.99	-0.98
δ									*		0.98						*
TEST TIME									**	-0.99	-0.96						**
																	-0.99

CONCLUSIONS

Overall results suggest that mixolab “breeding” profile is better than “Chopin+” to differentiate wheat in genetic breeding programs, especially when the target is to find hard wheat genotypes (high stability >10 min). Parameters recorded from “breeding” profile showed very high significant correlations with farinographic and alveographic parameters, as well as wet gluten and damage starch.

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REFERENCES

1. Gras, P.W. and O'Brien, L. 1992. Application of a 2-Gram Mixograph to Early Generation Selection for Dough Strength. *Cereal Chem.* 69(3):254-257.
2. Peña, R.J. and Posadas-Romano, G. 2011. Comparing mixolab parameters obtained with conventional slow and accelerated testing protocols. Session III: Chapter 41. 662p. In: Chibbar, R.N., Dexter, J. (Ed.) *Wheat science dynamics challenges and opportunities*. Agrobios International, Jodhpur, India.
3. Dapčević, T., Hadnađev, M. and Pojić, M. 2009. Evaluation of the possibility to replace conventional rheological wheat flour quality control instruments with the new measurement tool – Mixolab. *Agric. Consp. Sci.* 74(3):169-174.
4. CodiNă, G.G., MiroNeaSa, S., Bordei, D., and Leahu, A. 2010. Mixolab versus alveograph and falling number. *Czech J. Food Sci.* 28(3):185-191.
5. Collar C., Bollain C., Rosell C.M. 2007. Rheological behaviour of formulated bread doughs during mixing and heating. *Food Sci. Tech. Int.*, 13:99-107.
6. Banu, I., Stoenesc, G., Ionesc, V. and Aprodu, I. 2011. Estimation of the baking quality of wheat flours based on rheological parameters of the mixolab curve. *Czech J. Food Sci.* 29(1):35-44.
7. Rosell, C. M., Collar, C., and Haros, M. 2007. Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the mixolab. *Food Hydrocoll.* 21:452-462.
8. *Approved Methods of the AACC.* 2000. 10th ed. The Association: St. Paul, MN.
9. *International Association for Cereal Science and Technology.* 2011. Standard methods. Vienna, Austria: ICC.
10. Rosell, C.M., Santos, E., and Collar, C. 2010. Physical characterization of fiber-enriched bread doughs by dual mixing and temperature constraint using the Mixolab. *Eur Food Res Technol.* 231:535-544.
11. *Mixolab applications handbook: rheological and enzymatic analysis.* 2006 (Update 2009). Villeneuve la Garenne, France: Chopin Applications Laboratory. 79p.
12. Rosell, C.M., Altamirano-Fortoul, R., Don, D., and Dubat, A. 2013. Thermomechanically induced protein aggregation and starch structural changes in wheat flour dough. *Cereal Chem.* 90(2):89-100.
13. Dhaka, V., Gulia, N., and Khatkar, B. S. 2012. Application of Mixolab to Assess the Bread Making Quality of Wheat Varieties. *Open Access Scientific Reports* 1(3): <http://dx.doi.org/10.4172/scientificreports>. 183.

STUDY OF OXIDATIVE IMPROVERS ON RHEOLOGICAL PROPERTIES OF WHEAT DOUGH

UDC 664.66 : 532.135

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ABSTRACT

Impact of oxidants on the rheological properties of bread dough is known, mechanisms of these effects are not yet fully described. L-ascorbic acid is the most widely used commercial bakery improver with oxidation effect. This paper presents influence of oxidizing improvers studied by empirical rheology. Investigations include the analysis of the impact of commercial pure L-ascorbic acid (0.005, 0,008, and 0.011%), hydrogen peroxid (0.001, 0.002 and 0.003%) and glucose oxidase (0,002, 0,004, 0,006%). The study includes, farinograph and extensograph analysis of dough with and without oxidative improvers (control sample). Effect of L-ascorbic acid as the most used oxidative improver is compared with glucose oxidase as an alternative oxidative improver and with hydrogen peroxid as a product of glucose oxidase reaction with an oxidative effect on dough.

Keywords: dough, improver, oxidation, rheological properties

INTRODUCTION

Selection of additives used in baking is performed on the basis of their effect on quality parameters of bakery products such as specific volume, product shape, bark thickness, elasticity, porosity, mid, mid hardness, taste and aroma [1].

Nowadays, it is common practice in the production of bakery products to use oxidative improvers to optimize the quality of wheat flour. Reduction of the number of free sulfhydryl groups (-SH group) by action of various oxidizing agents in the process of protein networking has been experimentally proven [2-5].

Action enables significant increase in volume of bread during baking and optimization of the characteristics that customers take into account for selection of products [6]. L ascorbic acid is the most widely applied oxidative improver.

The use of enzymes has become very attractive nowadays, since the enzymes are seen as the natural substances that are not toxic, enabling thus utilization enzymatic oxidative

improvers in baking [7-9]. It is believed that the interest in the application of enzymes in the baking industry in the future will only grow [10].

Glucose oxidase is an enzyme with oxidizing effect due to the release of hydrogen peroxide in catalyzed reactions. In the presence of oxygen, glucose oxidase catalyzes the oxidation of β -D-glucose to the α -D-glukonlaktone and hydrogen peroxide [11]. Due to these facts and release of hydrogen peroxide, glucose oxidase can be use in baking as oxidative improver, alone or in synergy with L-ascorbic acid.

Glucose oxidase, given its enzymatic nature, among consumers has the attribute of "naturalness" and therefore safety. Influence that glucose oxidase manifest upon the dough and the quality of the finished product should be quantified and compared with the most frequently used oxidizing improver, L-ascorbic acid. Since glucose oxidase catalyzes the reaction in which a hydrogen peroxide is generated and which exhibits oxidizing effect on dough hydrogen peroxide was used to examine differences in the activities of added hydrogen peroxide solution and adding glucose oxidase.

MATERIALS AND METHODS

Wheat flour used for investigations was characterized with the following chemical parameters: protein content: 12.6% on dry matter ; moisture: 13.2%, ash content: 0.518% on dry matter; water absorption: 64.95%, wet gluten: 32, 81%.

Oxidation improvers applied in the research are:

- *L-ascorbic acid* (Weifang Ensign Industry Co., Ltd., China), in the form of white crystalline powder, dosed after dilution in water. Three dosages were used (0.005%; 0.008%; 0.011% based on flour);
- *Glucose oxidasa* (Bakezyme® GO 1500 BG - Royal DSM, Netherlands), in the form of brown crystalline powder, dosed after dilution in water. Three dosages were used (0.002%; 0.004%; 0.006% based on flour);
- *Hydrogen peroxide* (Laboratory chemical), dosed after dilution in water. Three dosages were used (0.001%; 0.002%; 0.003% based on flour).

Other ingredients used in this work were: Salt (So product, Serbia), yeast (Kvas Ltd., Croatia), which were purchased at the local supermarket.

Determination of rheology indicators of wheat dough by mixing test on the Farinograph is conducted by modification of the standard Serbian procedure [12].

The modification is reflected in addition of solution of water and oxidizing improvers directly in the right bottom corner by the pipette after mixing of flour for 1 minute, then standard operation is resumed. Determination of rheology indicators of wheat dough using uniaxial test of extensibility on the Extensograph is conducted by standard procedure [12].

RESULTS AND DISCUSSION

Comparison of effects of oxidative improvers (L-ascorbic acid, glucose oxidase, hydrogen peroxide) on the rheological parameters, measured by empirical rheological methods is shown below. Results of farinograph examination with and without oxidizing improvers are shown in Table 1.

Table 1. Farinograph parameters of investigated oxidizing improvers and control sample

	Development time (min)	Stability (min)	Softening degree (FJ)	Quality number	Quality group	Water absorpt. capacity (%)
<i>Control</i>	4	3	65	68.5	b1	68.9
<i>L-ascorbic acid</i>						
0.005%	6	2.5	45	76.4	a2	68.5
0.008%	6	3	50	77.1	a2	68.2
0.011%	6,5	2.5	55	74	a2	67.9
<i>Hydrogen peroxide</i>						
0.001%	4	2.5	75	68.5	b1	69.2
0.002%	5	1.5	65	69.9	b1	68.7
0.003%	5	3	65	65.7	b2	69.0
<i>Glucose oxidase</i>						
0.002%	6	2	35	67.8	b1	68.6
0.004%	6	4.5	35	80.4	a2	67.4
0.006%	6.5	1.5	50	72.5	a2	68.2

Application of L-ascorbic acid leads to an increase of development time in all test samples. Stability of the samples treated with 0.005% and 0.011% of L-ascorbic acid is less for 0.5 minutes, compared to the control sample, while at doses of 0.08% it remains at the same value as the control sample and it is 3 minutes. The L-ascorbic acid dosed in all concentrations produce a decrease in the degree of softening of all samples. All samples are classified in a higher quality group. Addition of L-ascorbic acid produced no significant change in the water absorption.

Glucose oxidase has the same effect on the increase of dough development time while stability decreases to a greater extent than with the addition of L-ascorbic acid, except in

the case of treatment with 0.004% glucose oxidase. The results partially agree with results of Bonet and associates, they described the increase in dough stability with increase of added glucose oxidase [7]. The reduction of softening is most pronounced in samples treated with 0.002% and 0.004% of glucose oxidase and it has value of 35 FJ. Samples treated with 0.002% and 0.006% are classified in higher quality group compared to the control sample. Water absorption did not significantly change.

By the application of hydrogen peroxide as oxidative improver same change can be observed in the dough development time and dough stability as in samples of L-ascorbic acid. Degree of softening of samples treated with concentrations of 0.002% and 0.003% is same as in the control sample, while the degree of softening of the dough increased in sample treated with 0.001% of hydrogen peroxide. Dough development is the longest when 0.011% L- ascorbic acid is used and 0.006% glucose oxidase. The smallest stability have samples treated with 0.002% hydrogen peroxide and with 0.006% glucose oxidase and it was 1.5 minutes, while the sample treated with 0.004% glucose oxidase had the highest stability of 4.5 minutes.

Degree of dough softening is reduced in all samples by all investigated oxidative improvers, except for the sample treated with 0.001% hydrogen peroxide which has a degree of softening 75 FJ. Reducing the level of softening with retaining water absorption values approximate to the control sample, shows that the application of oxidizing improvers optimise dough technological characteristics.

Table 2. Extensograph parameters of investigated oxidizing improvers and control sample

	Energy (cm ²)	Resistance (EJ)	Extensibility (mm)	R/E
<i>Control</i>	55	310	174	1.08
<i>L-ascorbic acid</i>				
0.005%	88	542	132	2.68
0.008%	79	547	119	3.93
0.011%	88	632	120	4.64
<i>Hydrogen peroxide</i>				
0.001%	98	545	157	2.73
0.002%	97	557,5	151	2.79
0.003%	104	645	137	3.56
<i>Glucose oxidase</i>				
0.002%	81	470	132	2.87
0.004%	80	485	127	3.76
0.006%	91	555	134	3.44

Parameters obtain by extensograph (Table 2) reveal that all oxidative improvers strengthen the gluten of wheat flour, increase energy and resistance and decrease extensibility.

Increasing resistance and reduced extensibility of dough increases R/E ratio above optimal values for the production of wheat bread. Energy of the dough, compared to the control sample, is increased by all oxidative improvers. The minimum energy of 55 cm² is shown by control sample, while the maximum energy is 104 cm² was registered for the sample with the addition of 0.003% hydrogen peroxide. The highest values of energies of dough are found for samples with added hydrogen peroxide in general.

Resistance of dough is the smallest in the control sample 190 EJ and the highest in the sample with the addition of 0.003% hydrogen peroxide 645 EJ. Similar as with energy values, highest resistance are found for samples with added hydrogen peroxide.

Extensibility of wheat dough is the lowest in the sample treated with 0.008% L-ascorbic acid, 119 mm and highest in the control sample, 174 mm. The minimum value of the R/E ratio is found in control sample and the highest in the sample treated with the addition of 0.011% L-ascorbic acid.

CONCLUSIONS

Examination conducted with the addition of L-ascorbic acid (0.005%, 0.008% and 0.011%) showed improvement of the quality group, prolongs development time while stability remains at the approximate level as in the control sample. Addition of L-ascorbic acid increases the energy and the values of resistance of all test samples, while the value of the extensibility decreases. The above modifications lead to changes in the ratio of resistance and extensibility.

Examination of hydrogen peroxide as the oxidizing improvers (0.001%, 0.002% and 0.003%) shows no improvement of quality group while higher doses can produce negative effect and reduce quality group. Obtained wheat dough has high energy and resistance, indicating a pronounced strengthening of gluten. However, the same dough possesses low extensibility values and the ratio of resistance and extensibility changes in the negative direction. Effects of glucose oxidase (0.002%, 0.004% and 0.006%) leads to a prolongation of the dough development time as well as with L-ascorbic acid, dough stability, compared to the control sample have largest changes. Two higher concentrations of glucose oxidase causes increase of quality group. Increase in energy and reduction of extensibility is mutual characteristic for all samples. The use of glucose oxidase in higher concentrations (0.004% and 0.006%) leads to more intensive networking of gluten, which reduces elasticity and produce negative effects on the properties of wheat test.

Action between glucose oxidase and hydrogen peroxide differs, the effects of glucose oxidase are probably not associated only with hydrogen peroxide, and it is of great significance to further explore the mechanism of glucose oxidase action.

REFERENCES

1. Primo, M. C., De Beukelear, H., Hamer R. J., Van Vliet, T. 2008. Effect of ingredient modification. *J. Cereal Sci.* 48:604-612.
2. Sokol, H. A., Mecham, D. K., Pence, J. W. 1960. Sulfhydryl losses during mixing of dough: Comparasion of flours having varius mixing characteristics. *Cereal chem.* 39:739.
3. Hird, F.J.R., Yates, J.R. 1961. The oxidation of protein thiol groups by iodate, bromate and persulphate. *Biochemistry Jurnal.* 80: 612.
4. Bloksma, A. H. 1963. Oxsidation by molecular oxygen of thiol groups in unleavened doughs from normal and defatted wheat flours. *J. Sci. Food Agr.* 14:529.
5. Tsen, C. C., Hlynka, I. 1963. Flour lipids and oxidation of sulfhydryl groups in dough. *Cereal chem.* 40:145.
6. Selomylio, V. O., Zhou, W. 2007. Frozen bread dough: Effect of freezing storage and dough improvers. *J. Cereal Sci.* 45:1-17.
7. Bonet, A., Rosell, C. M., Caballero, P. A., Gomez, M., Perez-Manuera, I., Liuch, M. A. 2006. Glucose oxidase effect on dough rheology and bread quality: a study from macroscopic to molecular level. *Food Chem.* 99:408-415.
8. Selinheimo, E., Kruus, K., Buchert, J., Hopia, A., Autio, K. 2006. Effect of laccase, xylanase and their combination on rheological properties of wheat doughs. *J. Cereal Sci.* 43:152-159.
9. Stojceska, V., Ainsworth, P. (2008). The effect of different enzymes on the quality of high-fibre enriched brewer's spent grain breads. *Food Chem.* 110:865-872.
10. Shah, A. R., Shak, R. K., Madamwar, D. 2006. Improvement of the quality of whole wheat bread by supplemetation of xylanase from *Aspergillus foetidus*. *Bioresour. Technol.* 97:2047-2053.
11. Steffolani, E., Ribotta, P., Pérez, G., León, A. 2010. Effect of glucose oxidase, transglutaminase, and pentosanase on wheat proteins: Relationship with dough properties and bread-making quality. *J. Cereal Sci.* 51:366-373.
12. Kaluderski, G., Filipović, N. 1998. Metode ispitivanja kvaliteta žita, brašna i gotovih proizvoda. Tehnološki fakultet, Zavod za tehnologiju žita i brašna, Novi Sad.

INFLUENCE OF HYDROCOLLOIDS AND PROTEINS COMBINATION IN GLUTEN FREE BREAD CRUMBS ASSESSED BY DIGITAL IMAGE ANALYSIS

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664.236 : 004.932.2

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ABSTRACT

To develop a viscoelastic network in gluten free matrixes is still a challenge in food technology. Different structuring agents have been proposed for building a network with sufficient elasticity, like hydrocolloids and proteins and the quality of the final breads was usually determined by quantifying volume and texture. The objective of this research was to analyze the potential use of digital image analysis (DIA) for assessing the effect of hydrocolloids and proteins on the crumb structure of gluten free breads. Specifically, hydrocolloids -Hydroxypropylmethylcellulose (HPMC), UltraCel™ WF and a blend of xanthan gum and guar gum - and proteins -egg powder and pea protein- were included in a basic recipe of rice based bread. Breads with vegetal protein showed more open crumb structure than those containing animal proteins. The hydrocolloid blend of xanthan gum and guar gum presented higher values of cell/cm² and mean cell area. UltraCel™ and HPMC hydrocolloids led to greater heterogeneity in the cell crumb distribution. Therefore, hydrocolloids and proteins largely affected the crumb structure of the rice based gluten free breads, and DIA of the crumbs seems to be a potent tool for analyzing the quality of the gluten free breads regarding size and gas cells distribution.

Keywords: gluten free bread, hydrocolloids, protein, crumb, image digital analysis, rice

INTRODUCTION

Celiac disease is a permanent gluten intolerance characterized by an inflammatory reaction that occurs in genetically predisposed individuals. Although extensive research is still carrying on the unique effective treatment consists in a strict gluten free diet long life. Celiac disease affects both children and adults and the worldwide incidence is quoted in 1% of the population. The increasing detection of the celiac patients and the interest for the gluten free diet, which has been associated to healthiness in spite of the lack of scientific basis, has prompted a huge increase in the sales of gluten free breads.

To overcome the problems associated with the lack of viscoelasticity, structuring agents such as guar gum, xanthan gum, hydroxypropylmethylcellulose (HPMC), agar and corn starch, yucca or potato are often incorporated, in formulations of fermented gluten free products [1]. Among hydrocolloids, cellulose derivatives like HPMC are the most

suitable to improve the volume and texture of the formulations made with rice flour [2-4]. The addition of proteins may be also a good substitute for gluten, since they allow the incorporation of air and the maintenance of the structure during the fermentation. Moreover, added proteins increase the nutritional value of the gluten-free products, reducing the variability in relation to the protein composition compared to traditional wheat bread. For this type of formulation proteins derived from different sources such as, egg albumin, soy protein, pea protein and milk protein among others are added [5, 6].

In general, the quality of the gluten free breads is assigned by the same methods applied to gluten breads; particularly physical appearance and texture are two of the most used quality attributes. Crumb structure, associated with the structure of the walls forming the air cells in the bread crumbs, has been also proposed as a quality parameter of gluten breads that is greatly affected by recipes and breadmaking process [7, 8].

The main objective of this research was to analyze the potential use of digital image analysis (DIA) for assessing the effect of hydrocolloids and proteins on the crumb structure of gluten free breads. Specifically, hydrocolloids - Hydroxypropylmethylcellulose (HPMC), UltraCel™ WF and a blend of xanthan gum and guar gum - and proteins -egg powder and pea protein- were included in a basic recipe of rice based bread.

MATERIALS AND METHODS

Commercial rice flour (Harinera Belenguer, Valencia, Spain), corn starch (Huici Leidan, Pamplona, Navarra), pea protein (Roquette Frères, Lestrem, France), egg powder (EPSA, Valencia, Spain), HPMC (Methocel K4M, Dow Chemical, Pittsburg, USA), xanthan gum (Jungbunzlauer, Ladenburg, Germany), guar gum (Quimidroga, Barcelona, Spain), and UltraCel™ (Watson, West Haven, USA) were of food grade. Vegetal seed oil, dried yeast, sugar and salt were purchased from a local market.

Breadmaking process

Dry ingredients were firstly added to a mixer bowl followed by fluid ingredients. Dough was mixed in a Hobart N50 for 10 minutes (4 min at slow speed, and 6 min at fast speed). The dough (800 g) was placed into a mould (20x6x9 cm). Fermentation was carried at 30 °C for 50 minutes in a proofing cabinet at 90% relative humidity. Baking was carried in an electric oven (Salva LT4, Gipuzkoa, Spain) for 90 min at 160 °C. After baking, loaves were kept at room temperature for cooling down for one hour and then put into polypropylene pouches till further analysis after 24 hours.

Slice images analysis

Bread was sliced using an automatic slicer. Images of the gluten-free bread slice (10-mm thick) were captured using a flatbed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP scanjet 4400C, Hewlett– Packard, USA). The images were scanned at

600 pixels per inch and analysed in levels of grey (eight bits) and captured in jpeg format for each measurement. In the captured images of the slices width (cm), height (cm), area (cm²) and perimeter (cm) were measured by Image J software (National Institutes of Health, Bethesda, MD, USA).

Crumb cell analysis

A 30x30 mm square field of view was evaluated for each image. This square field captured the majority of the crumb area of each slice. Images were analyzed by Image J software (National Institutes of Health, Bethesda, MD, USA) using the Otsu's algorithm (Figure 1) for assessing the threshold according to Gonzales-Barron and Butler [9]. Data derived from the crumb structure analysis included: number of cells or alveoli, average cells area and cell circularity, and were used for comparing purposes among different samples. Circularity was calculated using the following equation (1):

$$\text{Circularity} = 4 \cdot \pi \cdot \text{area} / (\text{perimeter})^2 \quad (1)$$

A value of 1.0 indicates a perfect circle.

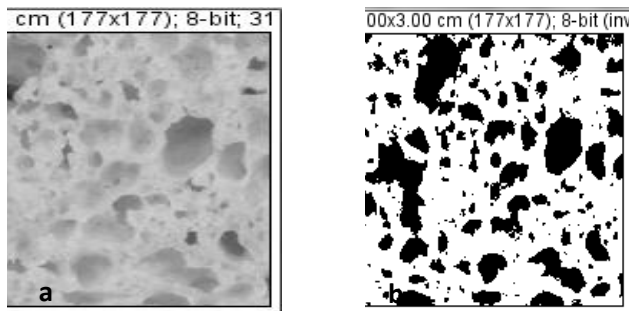


Figure 1. Bread crumb before (a) and after using Otsu threshold (b)

Statistical analysis

The results were expressed as mean values. For each parameter, a one-way analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Fisher's least (LSD) test was used to assess significant differences ($P < 0.05$) among samples that might allow discrimination among them.

RESULTS AND DISCUSSION

To determine the possible effect of hydrocolloids and proteins on the crumb structure of gluten free breads, six formulations were selected combining proteins of different origin with each of hydrocolloids (Table 1)

Table 1. Combinations selected for the study

Sample	HPMC	Blend xanthan gum + guar gum	Ultrace1™	Pea protein	Egg powder
S1	x			x	
S2		x		x	
S3			x	x	
S4	x				x
S5		x			x
S6			x		x

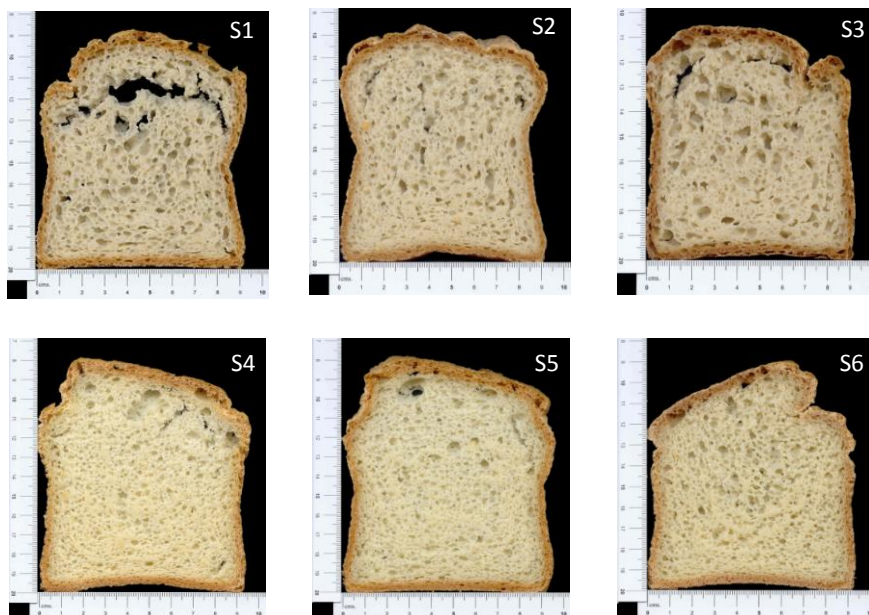


Figure 2. Digital images of gluten-free bread slices obtained from different formulations.

The captured images of the slices are shown in Figure 2. The crumb of the sample S1 showed a broken structure in the upper part of the slice, which was due to the structure was unable to hold the gas released during fermentation. In addition samples S1, S2 and S3, containing protein of vegetable origin in their formulation, showed larger and uneven gas in their crumbs. On the contrary, it was observed that crumbs from formulations S4, S5 and S6 showed significant differences on the structure, specifically they presented crumbs with cells distributed homogeneously, which resembled those obtained in wheat bread. This may be due to the presence of egg protein, which is well known for its emulsifying properties.

Results obtained from the slice analysis (Table 2) showed significant differences in the width, height and perimeter of the slice. Samples S3 and S6 showed the lowest area values, both samples contained Ultracel™ as hydrocolloid. However, samples S1 and S4 containing HPMC as hydrocolloid presented higher area. These results agree with the findings of Barcenas and Rosell [10], who reported that the inclusion of HPMC in wheat breads led to an increase of volume. The effect of HPMC could be attributed to its ability to retain water and the formation of a network gel, which increases the viscosity and allows expand the cells dough producing a gas retention during baking [11].

Table 2. Analyses of slices gluten free loaves

Sample codes	Width (cm)	Height (cm)	Area (cm ²)	Perimeter (cm)
S1	9.26ab	10.61c	85.89c	37.57b
S2	9.33ab	10.13ab	81.84abc	37.02ab
S3	9.10a	10.09ab	80.05ab	35.83a
S4	9.65c	10.31bc	84.97bc	37.54b
S5	9.35b	10.28abc	83.57abc	36.18ab
S6	9.28ab	9.92a	79.61a	35.76a

For each parameter, values followed by the same letter are not significantly different at $P \leq 0.05$

Parameters from the image analysis of the gluten-free bread showed a large variability among crumb bread structures (Table 3). Sample S5, with animal protein, and S2 containing vegetal protein, presented greater values of cell/cm², both samples contained a blend of xanthan gum and guar gum as hydrocolloid.

Significant differences were observed for average cell area showing values between 0.60 and 2.19 mm². Gluten-free crumbs had circularity values ranging from 0.78 to 0.84, indicating their divergence from the perfect circle (Figure 2), although there were not statistically significant differences among the mean circularity at the 95.0% confidence

level. Besides, cell (air) total area of bread crumbs showed significant differences among gluten-free breads.

Table 3. Crumb microstructure analysis of different gluten free breads

Sample codes	Number of cell/cm ²	Average cell area (mm ²)	Total cell area (cm ²)	Circularity
S1	9a	2.19d	1.93b	0.79ab
S2	14ab	1.48bc	1.83b	0.84b
S3	9a	1.66c	1.39a	0.83ab
S4	19bc	1.12ab	1.94b	0.82ab
S5	24c	0.98ab	2.11b	0.82ab
S6	13a	1.53bc	1.76ab	0.78a

For each parameter, values followed by the same letter are not significantly different at $P \leq 0.05$

CONCLUSIONS

Digital image analysis of the crumbs seems to be a potent tool for analysing the quality of the gluten free breads regarding size and gas cells distribution. Hydrocolloids and proteins largely affected the crumb structure of the rice based gluten free breads. Vegetable protein generated more open and heterogeneous crumbs while animal proteins generated the most closed and homogeneous crumbs.

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REFERENCES

1. Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., and Biliaderis, C. G. 2007. Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *J. Food Eng.* 79:1033-1047.
2. Sivaramakrishnan, H., Senge, B., and Chattopadhyay, P. 2004. Rheological properties of rice dough for making rice bread. *J. Food Eng.* 62:37-45.
3. Gujral, H. S., and Rosell, C. M. 2004. Improvement of the breadmaking quality of rice flour by glucose oxidase. *Food Res Int.* 37:75–81.
4. Gujral, H. S., and Rosell, C. M. 2004. Functionality of rice flour modified with a microbial transglutaminase. *Food Res Int.* 39:225–230.
5. Gallagher, E., Kunkel, A., Gormley, T. R., and Arendt, E. K. 2003. The effect of dairy and rice powder addition on loaf and crumb characteristics, and on shelf life (intermediate and long-term) of gluten-free breads stored in a modified atmosphere. *Eur. Food Res. Technol.* 218:44–48.
6. Marco, C., and Rosell, C.M. 2008. Effect of different protein isolates and transglutaminase on rice flour properties. *J. Food Eng.* 84:132-139.
7. Gellynck, X., Kühne, B., Van Bockstaele, F., Van de Walle, D., and Dewettinck, K. 2009. Consumer perception of bread quality. *Appetite.* 53:16-23.
8. Scanlon, M., and Zghal, M. 2001. Bread properties and crumb structure. *Food Res Int.* 34:841-864.
9. Gonzales-Barron, U., and Butler, F. 2006. A comparison of seven thresholding techniques with the k-means clustering algorithm for measurement of bread-crumbs features by digital image analysis. *J Food Eng.* 74:268–278.
10. Barcenas, M. E., and Rosell, C. M. 2005. Effect of HPMC addition on the microstructure, quality and aging of wheat bread. *Food Hydrocoll.* 19/6:1037-1043.
11. Bell, D. A. 1990. Methylcellulose as a structure enhancer in bread baking. *Cereal Foods World.* 35:1001–1006.

PHYSICOCHEMICAL PROPERTIES OF TAGLIATELLE ENRICHED WITH BUCKWHEAT FLOUR

UDC 664.694 : 664.64.016

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ABSTRACT

In order to find a new nutritionally perspective pasta recipe, the replacement of wholegrain wheat flour with wholegrain buckwheat flour in pasta formulation was studied with the aim to determine the effect of the addition on pasta cooking quality. The wholegrain buckwheat flour was added at the substitution levels of 10, 20% and 30%. The buckwheat enrichments had no significant ($P<0.05$) effect on volume increase (VI). There was significantly decrease of optimal cooking time (OCT) and increase of cooking loss (CL) in comparison with the control sample. The results were also showed that buckwheat pasta samples were significantly less firm and adhesive than the control sample. Correlation analysis revealed very strong significant correlation ($P<0.05$) between hardness and CL, and strong but not significant correlation between adhesiveness and CL.

Keywords: wholegrain buckwheat flour, cooked pasta, cooking quality, textural properties

INTRODUCTION

In recent years, consumers are increasingly become aware of health impact of food. Numerous foods are already considered as health promoting, but for traditional durum pasta that cannot be said. Namely, durum pasta is low in protein content and essential amino acids, as well as some vitamins and minerals [1]. So far, many studies have attempted to improve the nutritional and functional properties of pasta by substituting durum wheat semolina with different flours, such as amaranth, buckwheat, lupin [2, 3], barley [4], split pea, faba bean [5], chickpea [6, 7], and Mexican common bean flour [8].

Among potential pseudocereals, buckwheat is one of the best plant sources of proteins, minerals, antioxidants and dietary fibres [9] which make it a good choice for incorporation into the pasta formulation. Antioxidants components in buckwheat, such as flavonoids, phenolic acids, tannins, phytosterols and tocopherols play important role as anti-inflammatory and anticarcinogenic agents [10, 11]. Furthermore, another

functionality of buckwheat comes from its gluten-free characteristics making buckwheat suitable food for celiac disease patients [9].

Changes in traditional pasta formulation in order to improve nutritional and functional values cause changes in texture which is considered to be one of the most important quality properties of pasta. According to Jambrec et al. [12], light buckwheat flour addition led to an increase in sensory quality of pasta in regard to wholegrain wheat flour pasta.

The aims of this research were to examine textural and cooking quality of three wholegrain buckwheat enhanced pasta formulations in comparison with wholegrain wheat pasta formulation and to find which of these formulations is the most sustainable for the cooking pasta quality.

MATERIAL AND METHODS

Pasta production

Four types of tagliatelle were produced on an industrial scale using Ital past Mac 60 (Parma, Italy). The control sample was made of commercial wholegrain wheat flour. Buckwheat pasta was produced by substitution of wholegrain wheat flour with wholegrain buckwheat flour at the substitution levels of 10, 20, and 30%. Flour of certain formulation was hydrated with deionised water in order to achieve proper dough consistency for extrusion.

Pasta cooking quality evaluation

Optimal cooking time (OCT) was defined as the time required for the white core in the centre of the sample to disappear. Cooking loss (CL) was defined as the amount of material leached out of the pasta strip during cooking. Taking a correction factor for the added salt into account the results are expressed as cooking loss (%). Volume increase (VI) was defined as the coefficient which represents the increase of pasta volume during cooking. VI was calculated as the ratio of the volumes of cooked and uncooked pasta samples.

All measurements were carried out three times according to AACC method [13].

Textural properties of cooked pasta

Hardness and adhesiveness of cooked tagliatelle samples were measured by using a TA.XT Plus Texture Analyzer (Exponent Stable Micro System, UK), equipped with a 5 kg load cell. The samples were tested in time up to 15 min after cooking and at room temperature (~22 °C). The compression test was performed by a flat cylinder probe (P/35) on two strips of each tagliatelle sample. The instrumental setting was taken from the sample projects of the software package (Texture Exponent TEE32 6.1.1.0, Stable Micro System, UK). Data reported are means of ten measurements of each sample.

Textural properties of tagliatelle samples were expressed as force-time curve where height of the peak represents hardness while the area under the curve corresponding to adhesiveness.

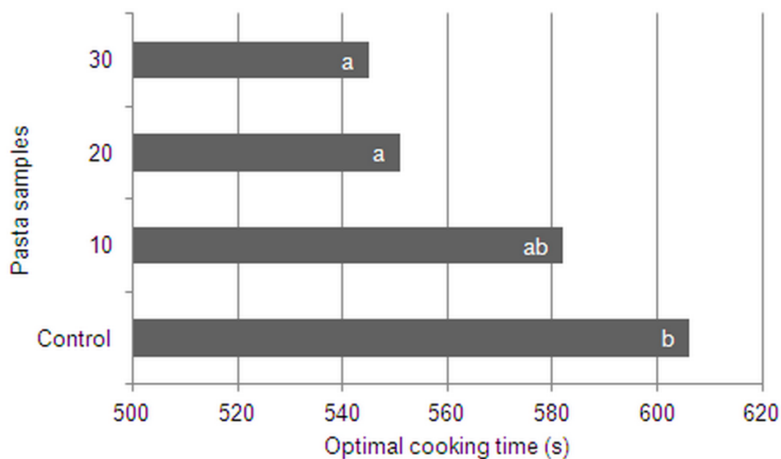
Statistical analysis

Results were expressed as the mean of replications with the standard deviation for all measurements. ANOVA and Fisher LSD test were used to compare means at 5 % significance level. Pearson's correlation coefficients were calculated between textural and cooking properties, and values were compared at $P < 0.05$. Data of all measurements were analysed using the Software XLSTAT, version (2012.2.02) (<http://www.xlstat.com/>).

RESULTS AND DISCUSSION

Pasta cooking quality

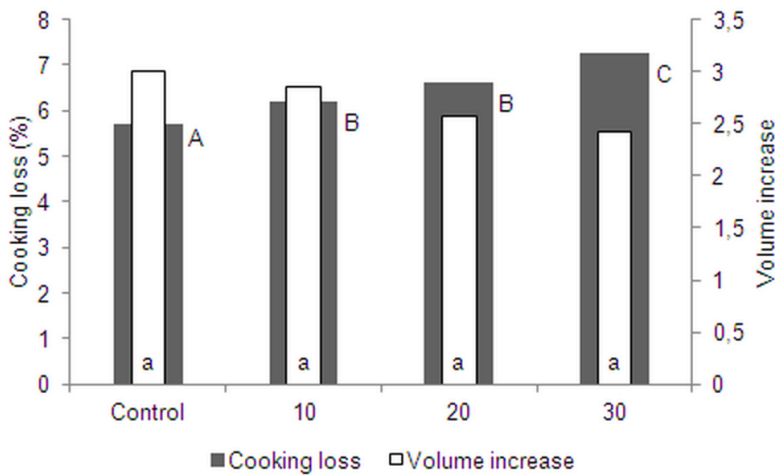
Parameters of cooking pasta quality are presented in Figure 1 and Figure 2. The increasing amount of wholegrain buckwheat flour caused a significant ($P < 0.05$) reduction in optimal cooking time (Fig.1) and enhancement of the cooking loss (Fig. 2). In addition, there were no significant changes in relation to volume increase of the cooked samples.



Lowercase letters (a, b) within the bars indicate significant differences ($P < 0.05$)

Figure 1. Optimal cooking time

In terms of volume increase, the obtained results were no in agreement with the statement by many authors [14, 15], which pointed the effect of buckwheat flour addition. Furthermore, the results of the other two cooking parameters were in agreement with other authors [2, 3, 16, 17, 18]. Namely, some parts of grain such as bran and germ, lead to disruption of protein network, thus allowing a reduction in its density. At the same time, easier absorption of water through the weakened network affects reducing cooking time of pasta. Moreover, the incorporation of gluten-free flours into the pasta formulation, such as wholegrain buckwheat, additionally weakens protein network and allows starch granules to leach out easily during cooking.

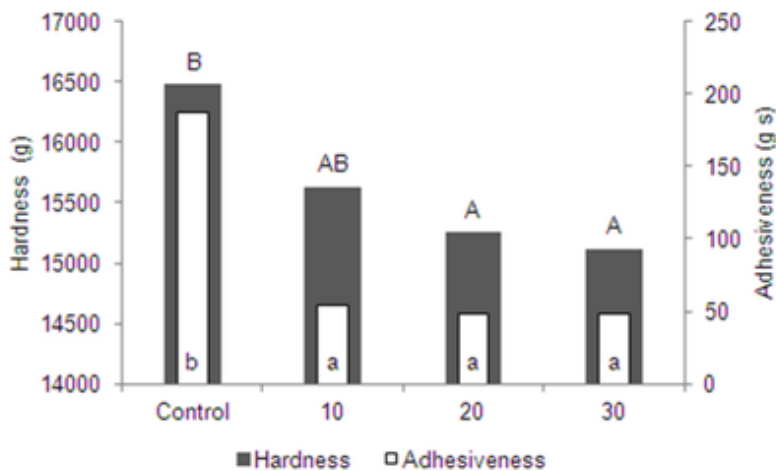


Capital letters (A, B) next to the bars indicate significant differences for pasta cooking loss ($P < 0.05$). Lowercase letter (a) within the bars indicates significant differences for pasta volume increase ($P < 0.05$).

Figure 2. Parameters of pasta cooking quality

Textural properties of cooked pasta.

It is generally accepted that the texture is the main criterion for assessing overall quality of cooked pasta [19]. The good quality of pasta provides its good textural properties such as better firmness, elasticity and less stickiness of cooked pasta products [20]. All those properties are in direct relationship with protein content of pasta and more importantly, with the nature of its gluten network [21-23]. The results of hardness and adhesiveness of the cooked tagliatelle samples measured instrumentally are showed in Figure 3.



Bars are means of 10 replications. Capital letters (A, B) above the bars indicate significant differences ($P < 0.05$) for pasta hardness. Lowercase letters (a, b) within the bars indicate significant differences ($P < 0.05$) for pasta adhesiveness.

Figure 3. Textural properties of cooked pasta samples

The protein content in the pasta samples with the addition of wholegrain buckwheat flour was higher than the control sample made of wholegrain wheat flour (data not shown). It was mentioned on the importance of gluten and its responsibility of the rapid development of protein network that enables organic matter to leach out from pasta into the cooking water. Based on all above, it can be concluded that the substitution levels of wholegrain buckwheat flour significantly ($P < 0.05$) contributed to the changes of hardness and adhesiveness of the cooked tagliatelle samples.

Relations between pasta cooking quality and textural properties

In order to examine the empirical relationship between pasta cooking and textural properties a scatter plots between them was applied. Based on the calculated values it could be conclude whether there was a linear relationship between observed variables or not. Furthermore, value of correlation coefficients (r) enabled to measure the degree of linear relationship between variables. On the other hand, the value of coefficient of determination (r^2) provided an insight into how the data are fitted by a linear line and also indicated the predictive ability of the obtained regression equation. The obtained values of all above mentioned statistical parameters are showed in Table 1.

Table 1. Correlation coefficients between pasta cooking quality and textural properties

	Parameters		
	r	r ²	E
<i>Hardness</i>			
OCT	0.9690*	0.9380	y = 3682.13+20.91*OCT
CL	-0.9177	0.8422	y = 21126.21-853.77*CL
VI	0.9277	0.8605	y = 9781.80+2148.92*VI
<i>Adhesiveness</i>			
OCT	0.8444	0.7130	y = - 076.13+2.03*OCT
CL	-0.7687	0.5908	y = 599.24 - 79.78*CL
VI	0.7633	0.5826	y = -451.19 + 197.26*VI

*correlation is significant at $P<0.05$;

OCT - Optimal cooking time; CL - Cooking loss; VI - Volume increase;

r - correlation coefficient, r² - coefficient of determination, E - linear regression equation

Results indicate that there was very strong correlation ($r>0.9$) between hardness and all observed cooking quality properties while there was no so strong correlation between them and adhesiveness. It should be noticed that the cooking time significantly ($P<0.05$) affected the hardness of cooked tagliatelle samples.

CONCLUSION

Incorporation of wholegrain buckwheat flour into the pasta formulation had a significant effect on both pasta cooking and textural properties. Specific structure components of wholegrain buckwheat flour caused disruption of the gluten network and allowed organic matter to leach into the cooking water. It led to decrease of optimal cooking time and significant increase of cooking loss of buckwheat pasta in comparison with the control pasta sample. Buckwheat tagliatelle samples possessed less strength, since the substitution levels of wholegrain flour significantly reduced the hardness. A correlation analyses indicated very strong linear relationship between hardness and all cooking quality parameters and not so strong relationship between these parameters and adhesiveness.

Regardless of the fact that the certain amount of incorporated wholegrain buckwheat flour had the impact on the overall quality of tagliatelle, the results of this paper could be relevant for the production of suitable food for the general population of consumers. The externally quality of tagliatelle showed that wholegrain buckwheat flour may be

used in pasta formulation without adversely affecting the physicochemical properties of pasta samples.

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REFERENCES

1. Heger J., and Frydrich, Z. 1987. Optimum amino acid supplementation of a high-protein wheat: sulfur-containing amino acids and tryptophan. *Nutr. Rep. Int.* 36:1105-112.
2. Rayas-Duarte, P., Mock, C. M., and Satterlee, L. D. 1996. Quality of spaghetti containing buckwheat, amaranth, and lupin flours. *Cereal Chem.* 73:381-387.
3. Chillo, S., Laverse, J., Falcone, P. M., Protopapa, A., and Del Nobile, M. A. 2008. Influence of the addition of buckwheat flour and durum wheat bran on spaghetti quality. *J. Cereal Sci.* 47:144-152.
4. Lamacchia, C., Baiano, A., Lamparelli, S., Terracone, C., Trani, A., and Luccia, A. D. 2011. Polymeric protein formulation during pasta-making with barley and semolina mixtures, and prediction of its effect on cooking behaviour and acceptability. *Food Chem.* 129:319-328.
5. Petitot, M., Boyer, L., Minier, C., and Micard, V. 2010. Fortification of pasta with split pea and faba bean flours: Pasta processing and quality evaluation. *Food Res. Int.* 43:634-641.
6. Wood, J. A. 2009. Texture, processing and organoleptic properties of chickpea-fortified spaghetti with insights to the underlying mechanisms of traditional durum pasta quality. *J. Cereal Sci.* 49:128-133.
7. Fares, C., and Menga, V. 2012. Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L.) flour added to durum wheat pasta. *Food Chem.* 131:1140-1148.
8. Gallegos-Infante, J. A., Rocha-Guzman, N. E., Gonzales-Laredo, R. F., Ochoa-Martínez, L. A., Corzo, N., Bello-Perez, L. A., Medina-Torres, L., and Peralta-Alvarez, L. E. 2010. Quality spaghetti pasta containing Mexican common bean flour (*Phaseolus vulgaris* L.). *Food Chem.* 119:1544-1549.
9. Sedej, I., Sakač, M., Mandić, A., Mišan, A., Pestorić, M., Šimurina, O., and Čanadanović-Brunet, J. 2011. Quality assessment of gluten-free crackers based on buckwheat flour. *LWT-Food Sci. Technol.* 44:694-699.
10. Kreft, I., Fabjan, N., and Yasumoto, K. 2006. Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chem.* 98:508-512.

11. Lin, L. Y., Liu, H. M., Yu, Y. W., Lin, S. D., and Mau, J. L. 2009. Quality and antioxidant property of buckwheat enhanced wheat bread. *Food Chem.* 112:987-991.
12. Jambrec, D., Pestorić, M., Psodorov, Đ., Sakač, M., Nedeljković, N., Mandić, A., and Sedej, I. 2011. Instrumental and sensory properties of buckwheat flour pasta. *Food and Feed Research.* 38:45-50.
13. Approved Methods of the AACC.1995. 9th ed. The Association: St. Paul, MN.
14. Alamprese, C., Casiraghi, E., and Pagani, M. A. 2007. Development of gluten-free fresh egg pasta analogues containing buckwheat. *Eur. Food Res. Technol.* 225:205-213.
15. Bilgiçli, N. 2009. Effect of buckwheat flour on chemical and functional properties of tarhana. *LWT-Food Sci. Technol.* 42:514-518.
16. West, R., Seetharaman, K., and Duizer, L. M. 2013. Effect of drying profile and whole grain content on flavor and texture of pasta. *J. Cereal Sci.* 58:82-88.
17. Lucisano, M., Cappa, C., Fongaro, L., and Mariotti, M. 2012. Characterisation of gluten-free pasta through conventional and innovative methods: Evaluation of the cooking behaviour. *J. Cereal Sci.* 56:667-675.
18. Martinez, C. S., Ribotta, P. D., León, A. E., and Añón, M. C. 2007. Physical, sensory and chemical evaluation of cooked spaghetti. *Texture Stud.* 38:666-683.
19. Altan, A., and Maskan, M. 2004. Microwave assisted drying of short-cut (ditalini) macaroni: cooking process and textural properties. *Food Sci. Technol. Int.* 10:187-196.
20. D'Egidio, M. G., Mariani, B. M., and Novaro, P. 1993. Influence of raw material characteristics and drying technologies on pasta cooking quality: A review of our results. *Italian Food and Beverage Technology.* 1:29-32.
21. Troccoli, A., Borrelli, G. M., De Vita, P., Fares, C., and Di Fonzo, N. 2000. Durum wheat quality: A multidisciplinary Concept. Mini review. *J. Cereal Sci.* 32:99-113.
22. Dexter, J. E., and Matsuo, R. R. 1977. Influence of protein content on some durum wheat quality parameters. *Can. J. Plant Sci.* 57:717-727.
23. Matsuo, R. R., Bradley, J. W., and Irvine, G. N. 1972. Effect of protein content on the cooking quality of spaghetti. *Cereal Chem.* 49:707-711.

TEXTURAL AND RHEOLOGICAL CHARACTERISTICS OF DOUGH FOR COOKIES WITH CHESTNUT FLOUR

UDC 664.641.2 : 664.681

532.135

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ABSTRACT

Chestnut and chestnut-based food has beneficial nutritional and organoleptic qualities. The biochemical composition of chestnut flour is close to that of many cereals and it can be used instead of wheat flour for people who suffer from celiac disease. The aim of this research is to characterise rheological and textural properties of dough for cookies in function of the amount of chestnut flour (20, 40 and 60%) and moisture content (20, 22 and 24%). The addition of chestnut flour leads to less flexibility of dough and worse ability to recover because of bad connection and brittle consistency of the dough, which is a consequence of reduced gluten content. The gradual substitution of wheat flour with chestnut flour contributes to the elasticity of the dough, due to chestnut starch granules ability to bind water. Extensibility and resistance to extension decrease with increasing addition of chestnut flour as a result of reduction gluten content. The increase in the share of chestnut flour from 20 to 60% resulted in an increase of hardness of the dough.

Keywords: chestnut flour, cookies, rheological properties, textural properties

INTRODUCTION

The technology of cookies is based on the characteristics of flour as the main raw material, which with fat, sugar, water and other additives forms the dough appropriate rheological characteristics and gives the product optimal physical and sensory properties [1]. Today, in food industry much attention is given to the healthier nutritional products which contain innovative, natural and cost effective ingredients.

Chestnut and chestnut-based food has beneficial nutritional and organoleptic qualities. Chestnut flour (CF) is obtained by grinding the dried fruit chestnut with a stone or hammer mill in two stages. The CF can be stored for several months at room temperature or several years at 4 °C. Despite great nutritional value is characterized by

the absence of cholesterol and low protein content [2]. The biochemical composition of CF is close to that of many cereals [3]. Starch is the main component and the protein and lipid contents, mineral salts, dietary fibres and vitamins (B1, E and C) make CF a very good substitute for people on a gluten-free diet. Indeed, CF can be consumed by people who suffer from celiac disease, i.e. permanent intolerance to gluten [4].

The aim of this work was to investigate physical (extensibility and resistance to extension and hardness) and rheological (viscous and elastic moduli, creep and recovery) characteristic of the dough for cookies. Wheat flour in the formulation of cookies was partially replaced with chestnut flour on the levels of 20, 40 and 60% and the dough samples made with different water content (20, 22 and 24%).

MATERIALS AND METHODS

Materials

Wheat flour for cookies and biscuits (T-500 "Jaffa", Crvenka) and commercial chestnut flour ("Mollino Rossetto", Padova, Italija) were used for preparation of dough for cookies. Wheat flour showed (% dry basis) moisture contents of 12.29, starch of 73.75, reducing sugars of 1.83, protein of 9.7, ash of 0.45 and fat of 0.84. CF showed (% dry basis) moisture contents of 5.71, starch of 62.4, reducing sugars of 21.10, protein of 5.54, ash of 2.06 and fat of 3.19. Chemical composition data of these flours were obtained according to standard AOAC methods [1]. Vegetable fat "Vitalina" was obtained from "Dijamant", Zrenjanin. Salt, sodium bicarbonate and ammonium bicarbonate were obtained from "Centrohem", Stara Pazova. Powdered sugar ("Centroproizvod", Beograd) was purchased in a local food store.

Dough preparation

Control samples were prepared only with wheat flour and in other samples 20%, 40% and 60% of wheat flour was substituted with chestnut flour. Cookies dough was prepared according to the following formula: flour (i.e. flour blend) 199.39 g, vegetable fat 41.88 g, sugar 69.79 g, NaHCO_3 0.6 g, NH_4HCO_3 0.4 g and NaCl 1.09 g. The amount of water was calculated in relation to the water content of the flour blends in order to obtain dough samples with 20%, 22% and 24% moisture content. The measured amount of flour was mixed in a mixer for 0.5 min, and after the addition of the total amount of fat and powdered sugar, the mixing was continued for 5.5 min at low speed (60 min^{-1}). All other components dissolved in distilled water were added into the mixer, the mixer closed and the dough mixed for 15 min.

Dough viscoelastic measurement

Oscillatory and creep-recovery tests were carried out with a Haake Rheo Stress 600 (Karlsruhe, Germany) by plate-plate sensor geometry (60 mm in diameter with a 1-mm gap) at 25 °C. The excess of dough was removed and Vaseline oil was applied to cover

the exposed sample surfaces. Before the measurement, the dough was rested for 15 min to allow relaxation.

Stress sweep tests (1 Hz at 25 °C, modulating shear stress values from 1 to 100 Pa) were carried out to determine the linear viscoelastic region of the samples. On the basis of determined LVE regime measuring for frequency sweep conditions were defined [5]. Dynamic oscillatory measurements (frequency sweep parameters) were observed modulating the ω -value from 6.28 to 62.8 rad/s (frequency 1–10 Hz) in a constant value of shear stress (30 Pa). The storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta$) were observed.

For creep and recovery test constant stress applied on the dough at creep phase was 30 Pa ($\sigma=30$ Pa). This phase lasted 150 s. After that, the dough was recovering in 300s. The final data were expressed as creep compliance ($J(t)$ (Pa^{-1})= γ/σ , where γ is shear deformation) over time (t) [6].

Dough texture analysis

Textural properties of dough were determined by Texture Analyser TA. XT Plus (Stable Micro Systems, Surrey, U.K.). Dough hardness was determined using the penetration test by application of measuring accessories P/6 (cylinder diameter of 2 mm) which penetrate to a depth of 2 mm in the dough set to a solid metal platform. The maximum force registered at the depth of 2 mm represents hardness. Measurements were performed in five replicates at 25 °C using load cells of 5 kg and following operating parameters: pre-test speed: 1 mm/s; test speed: 2 mm/s; post-test speed: 10 mm/s; distance: 2 mm.

To define extensibility and resistance to extension of dough, micro-method Kiffer Extensibility Rig [7] was used. Measurements were performed in five replicates at 25 °C using load cells of 5 kg and following operating parameters: pre-test speed: 2.0 mm/s; test speed: 3.3 mm/s; post-test speed: 10 mm/s; distance: 75-mm; trigger force:auto-5 g.

RESULTS AND DISCUSSION

Viscoelastic properties of the dough

Viscoelastic properties are very important because they affect the characteristics of the dough during the production process and the quality of the finished product. Figure 1a, b and c show storage or elastic (G') and loss or viscous (G'') module values with frequency for control and samples. Figure 1a show viscoelastic properties of dough for control and samples with 20% moisture content, figure 1b with 22% moisture content and Figure 1c with 24% moisture content.

In dough samples containing 20% of water the substitution 60% of wheat flour with chestnut flour resulted in the increase of both dynamic moduli compared with control sample, while difference between control sample and samples with 20% and 40% substitution of wheat flour were not significant. The highest dosage of CF (60%) in the

dough sample causes an increase in the elasticity of the dough, which is reflected in the increase of its hardness. Similar results were observed by Moreira et al. [8] who pointed out that the addition of CF increases elasticity of the dough as a result of a higher degree absorption and retention of water by chestnut starch granules.

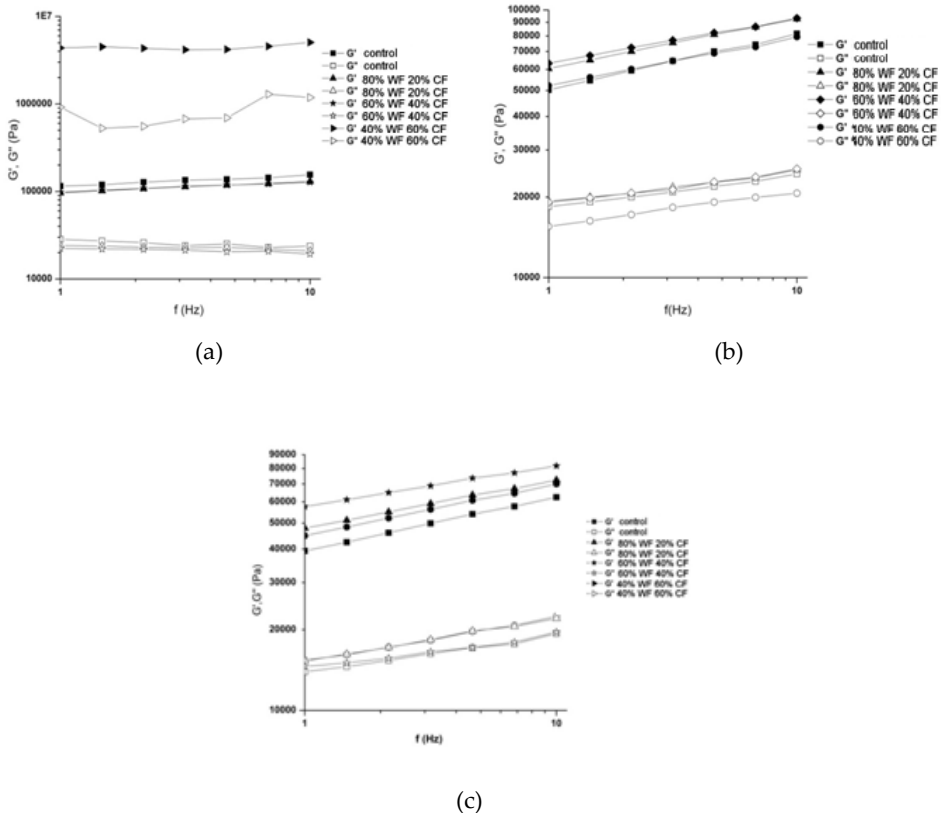


Figure 1. elastic (G') and viscous (G'') moduli values with frequency for control and samples (a-dough with 20% moisture, b-dough with 22% moisture, c-dough with 24% moisture)

With an increase in moisture content from 20% to 22% do not detect significant changes in the values of G' and G'' in the samples with CF compared to the control sample. Increase the moisture content in the dough compensates increase in the elasticity caused

by the introduction of CF in formulation. As water content of the dough increases, both moduli decrease [9]. Values of these modules are significantly lower than in the dough containing 20% of water.

Based on shown changes of moduli of samples with 24% water and different amount of chestnut flour, can be seen that there is reducing in difference between elastic and viscous moduli. This means that we have the dough samples which are less hard then samples containing 20% and 22% of water. Also there is increasing of elastic moduli values with an increase of CF amount.

For all samples dominated elastic moduli over the viscous. Values of $\tan \delta < 1$ (results are not shown), which is characteristic for dough as viscoelastic system with the dominant elastic component. With the increasing water content, the hardness of the dough decreases and the ratio of moduli (values of $\tan \delta$) increases. Also, in the samples with the same water content, the increasing of CF content causes decrease of $\tan \delta$ values.

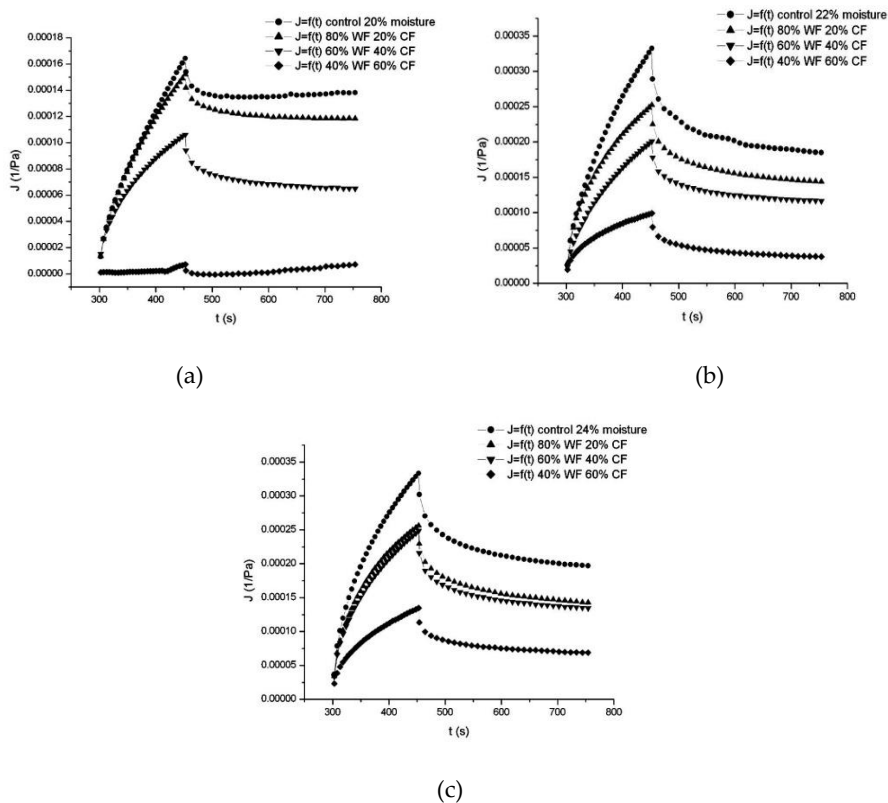


Figure 2. Creep and recovery curves of dough samples

Creep and recovery curves (Figure 2 a, b and c) show the characteristic viscoelastic behaviour of the dough, with some ability to recovery and partial return to initial state after stress exposure. From the Figure 2a (dough samples containing 20% of water), can be seen that sample with 20% CF has the most similar consistency to the control sample. Creep and recovery curves of these samples with 40% and 60% of CF are significantly lower, which means that these samples have less flexible structure to the stress influence.

The influence of CF on viscoelastic properties of the dough samples containing 22% of water is shown at Figure 2b. The flexibility of the dough decreases with increasing levels of CF. Also, it is evident that there is smaller difference between sample with 60% CF and control sample. Creep and recovery curves of the dough samples containing 24% of water are shown at Figure 2c. As with previous samples it is evident that the control sample has the most flexible consistency, which is a consequence of the largest amounts of the gluten in the dough. Increasing the amount of chestnut flour in the dough contributes to lower connection and brittle consistency. As water content of the dough increase, the resistance that dough provides to the application of force decreases and possibility of recovery increase.

Textural characteristics of Dough

The results of extensibility and resistance to extension and hardness of the dough samples with the addition of CF and different content of water are given in Table 1. At all samples with increasing content of CF extensibility of the dough decreases, which is a consequence of reduced gluten content. Resistance to extension decreases, too. As water content in the dough increases, values of these parameters increase. It is because extensibility and resistance to extension depend on the gluten content and quality and on the content of starch in the dough. Starch granules bind a portion of water, which contributed to the increase dough resistance with increasing its content. The effects of substitution wheat flour with chestnut flour on the physical characteristics of the dough are expressed at samples with high content of chestnut flour (40% and 60%) which resulted in the impossibility of registering the characteristic curves of extensibility. Absence of measurement for these samples can be consequence of reducing gluten content in the dough and higher content of saccharose in the CF which intensifies interaction saccharose-starch.

The increasing water content in dough samples, as expected, contributes to the decreasing of dough hardness. The hardness of the dough samples without CF is higher compared to samples with CF addition at all water content. CF affects on reducing dough connection, since substitution wheat flour with CF contributes to reducing gluten content. Starch granules need a sufficient amount of water for hydrating, which is not case at the dough samples containing 20 % of water, so the hardness of these samples increases. Higher hardness value has the dough sample containing 60% of CF and 20% of water, which is consistent with published data. Demirkesen et. al. [10] indicated that chestnut flour has the higher ability of water absorption due to the presence of dietary

fibre, which hydroxyl groups bind water molecules and reducing the amount of free water in the dough.

Table 1. Textural characteristics of dough samples

Sample	Extensibility (mm)	Resistance to extension (g)	Hardness (g)
<i>Dough with 20% moisture</i>			
Control	3.51±0.36	15.67±0.89	236.05±20.23
80% WF 20% CF	2.84±0.41	13.94±1.34	171.55±8.34
60% WF 40% CF	2.62±0.41	12.32±0.92	181.35±12.6
40% WF 60% CF	n.d.	n.d.	229.85±8.94
<i>Dough with 22% moisture</i>			
Control	8.01±0.72	15.58±0.53	130.21±5.05
80% WF 20% CF	6.22±1.1	14.32±1.55	106.55±4.32
60% WF 40% CF	4.15±1.07	10.05±1.34	86.65±4.05
40% WF 60% CF	n.d.	n.d.	90.33±6.02
<i>Dough with 22% moisture</i>			
Control	11.38±0.78	19.16±0.54	86.52±4.92
80% WF 20% CF	7.83±0.6	14.02±0.71	76.69±2.99
60% WF 40% CF	7.82±8.88	10.41±3.91	75.92±2.0
40% WF 60% CF	n.d.	n.d.	69.02±4.38

CONCLUSION

For all samples, as expected, dominated elastic module over the viscous, which is characteristic for dough as viscoelastic system with the dominant elastic component. The addition of CF increases elasticity of the dough as a result of a higher degree absorption and retention of water by chestnut starch granules. Creep and recovery curves show that the control samples have the more flexible consistency compared with the samples with CF addition. Increasing the amount of CF in the dough contributes to lower connection and brittle consistency. As water content of the dough increase, the resistance that dough provides to the application of force decreases and possibility of recovery increase. The increasing amount of CF contributes to decreasing the dough extensibility and resistance to extension, which is a consequence of reduced gluten content. The hardness of the dough increases as CF content increases.

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REFERENCES

1. Pajin, B. 2009. Praktikum iz tehnologije konditorskih proizvoda. Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad.
2. Alary, K., Buissonade, C., Joudrier, P., Gautier, M. F. 2007. Detection and discrimination of cereals and leguminous species in chestnut flour by PCR. *Eur. Food Res. Technol.* 225:427-343.
3. Mottin, Demiante, I., Oetterer, M., Wosiacki, G. 2001. Characterization of Chestnut (*Castanea sativa* Mill) Starch for Industrial Utilization. *Brazilian archives of biology and technology.* 44:69-78.
4. Demirkesen, I., Sumnu, G., Sahin, S. 2010. Utilization of chestnut flour in gluten-free bread formulations. *J. Food Eng.* 101:329-336.
5. Mezger, T. G. 2006. *Rheology Handbook*, 2nd Ed., Vinsentz Verlag, Hannover, Germany.
6. Steffe, J.F. 1996. *Rheological Methods in Food Bioprocess Engineering*. Freeman Press, East Lansing, MI.
7. Kiffer, R., Garnreiter, F. and Belitz, H. D. 1981. Beurteilung von Teigeigenschaften durch Zugversuche im Mikromastab. *Z. Lebensm. Unters. Forsch.* 172:193-194.
8. Moreira, R., Chenlo, F., Torres, M. D. Prieto, D. M. 2011. Technological assessment of chestnut flour doughs regarding to doughs from other commercial flours and formulations. *Food Bioprocess Technol.* DOI 10.1007/s11497-011-0524-8.
9. Amemiya, J. I. Menjivar, J. A. 1992. Comparison of small and large deformation measurements to characterize the rheology of wheat-flour doughs. *J. Food Eng.* 16:91-108.
10. Demirkesen, I. Sumnu, G., Sahin, S. 2011. Utilization of chestnut flour in gluten-free cakes. *Proceedings of the 6th CIGR Section VI International Symposium "Towards a Sustainable Food Chain"* Food Process. Bioprocessing and Food Quality Management Nantes. France.

RHEOLOGICAL AND TEXTURAL PROPERTIES OF DOUGH FOR COOKIES WITH DIETARY FIBERS

UDC 664.681 : 532.135

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ABSTRACT

Alternative methods of dietary fibers application are within components for processed food enriched with dietary fibers. Bakery product, cookies and crackers, different kind of snack products, can be observed for this purpose.

The objective of this work was to determine the influence of three different dietary fibers on rheological and texture properties of dough for cookies. The part of main component of the dough, wheat flour, was replaced by 10% of oat fibers, potato fibers and wheat fibers, calculated on total weight of the flour. The dough moisture was 22%.

Alkaline water retention capacity (AWRC) analysis of wheat flour and the mixtures of the flour and 10% of different dietary fibers showed that each kind of dietary fibers increased hydration ability of the mixture, compared to AWRC value of wheat flour. That was reflected to rheological properties of obtained dough. Disrupted gluten matrix caused reduced viscoelastic properties of the dough with dietary fibers. Recovery ability decreased compared to control dough without fibers. Hardness of the dough with dietary fibers increased and extensibility decreased, thus the texture measurement confirmed the rheological results. The type of applied dietary fibers was significant. According to obtained results the influence of potato and oat fibers was more pronounced than the influence of wheat fibers.

Keywords: dietary fibers, dough for cookies, rheology and texture

INTRODUCTION

The term functional food appeared for the first time in the 1980 in Japan, the first country that recognized the functional food and defined it as an alternative to medication. The concept of functional food refers to food which continuous and long application can affect preventive or therapeutic on some aspects of human health. Dietary fibers are beneficial for human health and they act as prevention of many

diseases that are caused by improper diet, like obesity, cardiovascular disease, hypertension, osteoporosis, diabetes type II and some forms of cancer) [1,2]. Dietary fibers come from a wide variety of foods such as whole grain cereals, legumes, fruits and vegetables. Fiber-rich processed foods are also with high levels of dietary fibers, and they are more available and convenient for application. Physico-chemical properties of dietary fibers play a fundamental role in their functionality in food products and they limit the use of dietary fibers as food technological agents. Fibres can modify the consistency, texture, rheological properties and sensory characteristics of the fibre supplemented food products. Organoleptic and processing challenges are frequently encountered during formulating food products high in dietary fiber. In bakery products, the addition of fibres modifies the breadmaking performance of wheat dough, affecting mixing properties, rheological behaviour and viscometric pattern due to their interaction with the large polymers (starch and proteins) present in the system [3]. Important technological properties of dietary fiber that determine their application are water holding capacity (WHC, related to the length and density of fibers), fat binding capacity (future dependent on the porosity of the fibers), viscosity (application as thickener agents), gel formation ability (important feature in using fiber as fat replacers), chelating capacity and texture modification [2]. Water holding capacity, particle size distribution and apparent viscosity are repeatedly described as crucial fibre properties with a significant influence in food technology [3]. Fibres can be incorporated in wide variety of foods, like dairy, meat or fish, but bakery products are the preferred food enriched with dietary fibers [4]. Fine bakery product, cookies and crackers, different kind of snack products, can be observed for this purpose.

The objective of this work was to determine the influence of three different dietary fibers on rheological and texture properties of dough for cookies. The part of main component of the dough, wheat flour, was replaced by 10% of oat fibers, potato fibers and wheat fibers, calculated on total weight of the flour.

MATERIALS AND METHODS

Basic materials for cookies dough were wheat flour (moisture 12.29% and ash content 0.49% on dry basis), vegetable fat, powdered sugar, chemicals (NH_4HCO_3 , NaHCO_3 and NaCl) and water. Used dietary fibers were wheat fibers VITACEL® WF 200, oat fibers VITACEL® HF 200 and potato fibers VITACEL® KF 200, all produced by J.Rettenmaier & Söhne, Rosenberg, Germany.

Specific characteristics of the wheat flour and the mixtures of 90% of wheat flour and 10% of different fibers were determined by analysis of AWRC values (alkaline water retention capacity of the flour and the mixtures). It represents the ability of the flour sample to retain water in alkaline environment [5].

The samples of the dough were prepared by method of baking test. Control dough sample was with 100% of wheat flour and samples with fibers contained the mixtures of 90% of wheat flour and 10% of different fibers (wheat fibers, oat fibers or potato fibers).

Ingredients of cookies dough were wheat flour or mixture of the flour and fibers (189.62 g), powdered sugar (66.37 g), vegetable fat (39.83 g), NaCl (1.04 g), NaHCO₃ (0.57 g), NH₄HCO₃ (0.38 g) and calculated amount of water in the aim to achieve 22% of dough moisture. Required amount of water was calculated according to wheat flour moisture and AWRC values of the flour and the mixtures with fibers [5].

The characteristics of the different samples of dough were determined by rheological and textural measurements. Rheological measurements were performed by rotational viscometer HAAKE RheoStress RS600 (Thermo Electron Corporation, Karlsruhe, Germany) with plate-plate sensor PP60 Ti (plate diameter was 60 mm and gap 1 mm). The measurements were done at 25±0.1 °C. Storage modulus (G') and loss modulus (G'') were defined by dynamic oscillatory measurements in the range of linear viscoelastic regime (LVE). The moduli were observed during increased frequency from 1 to 10 Hz and at constant shear stress of 30 Pa. The results were expressed as value $\tan \delta = G''/G'$.

Viscoelastic response of the samples at constant stress, as well as their behaviour after removing the stress, were determined by creep and recovery test. The test was performed in the LVE regime in which the deformation amplitude was proportional to applied stress amplitude. The creep time with constant stress ($\sigma=30$ Pa) was 150 s and the recovery period after removing the stress was 300 s. Creep data, collected under constant stress (σ) over time (t), can be described by a creep compliance (J) function, in terms of shear deformation (γ), using equation $J(t)=\gamma(t)/\sigma$. The creep data were analyzed by Burger's model presented by equation $J(t)=J_0+J_1 \cdot (1-\exp(-t/\lambda))+t/\eta_0$. For the recovery phase the equation of the Burger's model is $J(t)=J_{\max}-J_0-J_1 \cdot (1-\exp(-t/\lambda))$. The value J_0 is the instantaneous compliance, J_1 is retarded (viscoelastic) compliance, J_{\max} is maximum compliance, λ is mean retardation and η_0 is Newtonian viscosity [6,7,8].

RESULTS AND DISCUSSION

Specific characteristics of flour and the mixtures with dietary fibers

The results of the AWRC analysis represent specific characteristics of the flour samples. Alkaline water retention capacity (AWRC values) of the wheat flour and flour mixtures with dietary fibers are presented in Table 1.

Table 1. AWRC values of wheat flour and of mixtures with 10% of different fibers

Samples of flour and the mixtures	AWRC (%)
Wheat flour	65.90
90% of wheat flour + 10% of oat fibers	74.33
90% of wheat flour + 10% of wheat fibers	76.11
90% of wheat flour + 10% of potato fibers	83.67

Obtained results of AWRC analysis showed that each kind of dietary fibers increased hydration ability of the mixtures compared to AWRC value of wheat flour. The most evident influence on AWRC value had the potato fibers. Hydration ability and ability to retain water are usually associated to particle size distribution of the flour or fibres. A reduction in the particle size might expose large surface area, and simultaneously more polar groups with water binding sites, to the surrounding water [3].

Rheological characteristics of the dough samples

Rheological properties of the dough for cookies include the viscoelastic properties. These properties are very important because of their significant influence on dough behaviour during production of cookies, as well as on the quality of the finished product. Dough is a complex real system with elastic and viscous properties in a variety of their ratios and combinations. Thus, under the stress this system can be deformed, and the deformations are partly reversible and partly irreversible. One part of the reversible deformation can be instantaneous and independent of time (ideally elastic), and the second part can be slow and it takes place during some shorter or longer time. The constant stress leads to certain structural changes of the dough, which affect the internal structure, and thus the internal resistance of the system and the degree of irreversible deformation. Reversibility of deformation is a function of time also, thus enough long stress can cause completely irreversible deformation. The specificity of viscoelastic systems is manifested in fact that the structural changes cause the appearance of relaxation and spontaneous decrease of resistance and the formation of new linkages released during stress [9].

Results of dynamic oscillatory measurements are presented in Table 2, as values $\tan \delta = G''/G'$ and they pointed to contribution and ratio of viscous and elastic components of dough structure. Elastic modulus G' was dominated over viscous modulus G'' for all dough samples, thus the values $\tan \sigma$ were <1 , which is typical for viscoelastic dough structure.

The values of $\tan \sigma$ decreased compared to control sample thus the application of dietary fibers increased the hardness of the dough structure. The contribution of elastic component is the largest for dough sample with potato fiber.

Table 2. The values of $\tan \sigma$ for dough samples

Dough samples	$\tan \delta = G''/G'$
Control sample	0.451
Sample with 10 % of oat fibers	0.394
Sample with 10% of wheat fibers	0.273
Sample with 10% of potato fibers	0.219

Creep and recovery curves indicate the typical viscoelastic behavior of the dough, with certain ability of recovering and partial reconstruction after applying the stress. Creep and recovery curves of the dough samples are shown at Figure 1.

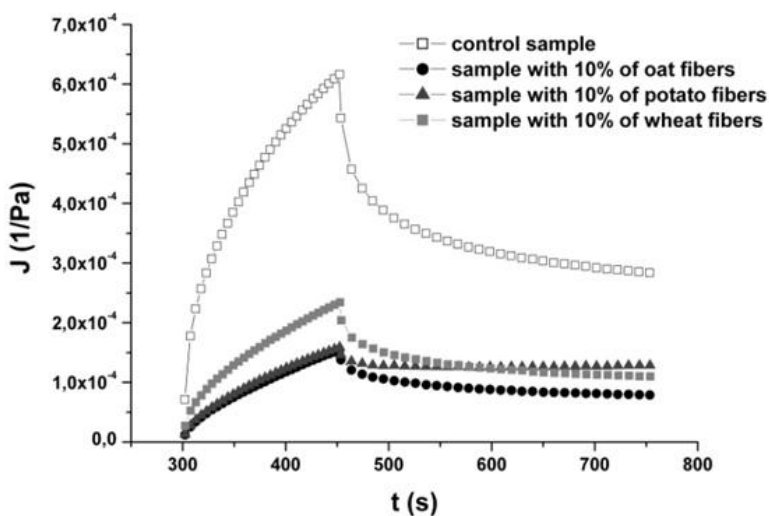


Figure 1. Creep and recovery curves of dough samples

The influence of introduction of dietary fibers in the structure on dough compliance is obvious (Figure 1). Creep compliance values are mainly associated with softness. High creep values indicate the weak structures whereas low values are representative of strong or stiffer structure [7]. The compliance of the dough samples with dietary fibers decreased compared to control sample. The viscoelastic properties of the dough with dietary fibers were reduced. The values of maximum compliance, J_{max} , decreased and Newtonian viscosity η increased. The amount of viscous J_v and elastic J_e part of J_{max} is generally evenly distributed, but recovery ability of the dough sample with potato fiber is drastically reduced, because of its very stiff and inflexible consistency. The parameters of creep and recovery curves are collected in Table 3.

Table 3. The parameters of creep and recovery curves

Dough samples	$J_{\max} \cdot 10^{-4}$ (1/Pa)	$\eta \cdot 10^6$ (Pas)	J_v/J_{\max} (%)	J_e/J_{\max} (%)
Control sample	6.164	0.734	46.06	53.94
+ 10% of oat fibers	1.544	2.929	51.11	48.89
+ 10% of wheat fibers	2.343	1.932	46.95	53.05
+ 10% of potato fibers	1.590	2.845	80.82	19.18

Textural characteristics of the dough samples

Textural properties of the dough samples are presented by textural parameters of the dough samples, hardness, resistance to extension and extensibility of dough (Table 4).

Table 4. Textural parameters of the dough samples

Dough samples	Hardness \pm SD (g)	Resistance \pm SD (g)	Extensibility \pm SD (mm)
Control sample	84.89 \pm 3.59	10.03 \pm 0.53	5.12 \pm 0.31
+ 10% of oat fibers	310.57 \pm 11.63	17.56 \pm 2.07	3.36 \pm 0.74
+ 10% of wheat fibers	224.22 \pm 14.67	28.76 \pm 7.26	1.61 \pm 0.60
+ 10% of potato fibers	312.19 \pm 11.78	27.72 \pm 4.29	4.75 \pm 0.81

Hardness of the dough with dietary fibers significantly increased compared to control sample with wheat flour. Same trend of changes was characteristic for resistance to extension. Extensibility of the dough was reduced by application of dietary fibers. These changes of textural parameters pointed that addition of dietary fibers contributed to more firm and less extensible consistency of the dough. All textural parameters were more affected by application of potato and oat fibers than by wheat fibers. The results of textural measurements confirmed the rheological results.

CONCLUSIONS

Oat, wheat and potato fibers had pronounced hydration ability. Incorporation of dietary fibers into the dough structure significantly affected to rheological properties of obtained dough compared to control sample without fibers. Formation of gluten matrix was disturbed with dietary fibers thus viscoelastic properties were reduced. The consistency of obtained dough was more firm, less flexible and less recoverable than control sample. The type of applied dietary fibers was significant. According to obtained results the influence of potato and oat fibers was more pronounced than the influence of wheat fibers.

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REFERENCES

1. Kay, R. M. 1982. Dietary fiber, *Journal of Lipid Research*. 23: 221-234.
2. Borderias, A.J., Sanchez-Alonso, I., Perez-Mateos, M. 2005. New Applications of Fibres in Foods: Addition to Fishery products, *Trends in Food Science & Tehnology*. 458-465.
3. Rosell, C.M., Santos, E., Collar, C. 2009. Physico-chemical properties of commercial fibres from different sources: A comparative approach, *Food Research International*. 42:176-184.
4. Dreher, M. 1999. Food Sources and Uses of Dietary Fiber. Page 327-373. in: *Complex Carbohydrates in Food*, S.S Cho, L. Prosky & M. Dreher (Ed.), Marcel Dekker, NY.
5. Pajin, B. 2009. Praktikum iz tehnologije konditorskih proizvoda, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad.
6. Mezger, T. 2002. *The Rheology Handbook: For users of rotational and oscillation rheometers*. Hannover: Vincentz Verlag.
7. Nesli, S. 2009. Rheological properties of rice pasta dough supplemented with proteins and gums, *Food Hydrocolloids*. 23:849-855.
8. Steffe, F. J. (1996). *Rheological methods in food process engineering*, (2nd ed), Freeman Press, Still Valley.
9. Đaković, Lj. 1990. *Koloidna hemija*, Tehnološki fakultet, Novi Sad.

INVESTIGATION OF STRESS RELAXATION PARAMETERS DURING THE STORAGE OF GINGERBREAD COOKIES MADE FROM SPELT WHEAT

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ABSTRACT

Relaxation test in uniaxial compression mode was performed on gingerbread cookies made from spelt wheat. Relaxation data were modeled by Peleg and Normand. The aim was to calculate stress relaxation parameters (percent stress relaxation, k_1 (initial rate of relaxation), k_2 (extent of relaxation) and relaxation time) for this type of product and to observe the changes in these parameters during their storage. At 10-80% strains tested, the measured stress relaxation parameters (k_1 , k_2 , % SR, RT) varied little up to 60% strain. In general, gingerbread cookies had little elastic character. During storage, solid character of the samples increased in all samples. Relaxation time was the parameter that was unable to observe changes in the textural properties of the cookies during storage at high relative humidity whereas in the dried out sample, all parameters changed significantly.

Keywords: gingerbread cookie, spelt, stress relaxation, Peleg-Normand model

INTRODUCTION

Gingerbread is a honey-based baked product with the longest tradition dating back to several centuries BC. In Europe, gingerbread was brought in the 8th century. In Medieval times, gingerbread was thought to have medicinal properties and was highly appreciated as a cure for indigestion. For centuries, it was only available in monasteries and pharmacies. In the 19th century started the industrial production of gingerbread cookies. Today, in spite of dominating industrial production, artisan production of gingerbread cookies has still been very popular.

In contrast to other cookie type products, gingerbread cookies are characterized with higher moisture content. The minimal moisture content required is 7%. Good quality gingerbread cookie should have soft, elastic and soggy crumb. Because of high moisture content, gingerbread type products have high moisture gradient and are prone to excessive drying resulting in a hard, non-elastic and crumbly product, especially when kept at low relative humidity. At high relative humidity, gingerbread gain moisture and gradually soften its structure. After baking, home-made gingerbread cookies are usually kept in cold room or metal box for 30-40 days to soften before consumption.

Stress relaxation technique has a strong potential for texture characterization of food products and has been employed as one of the principal means for measuring viscoelastic behaviour of food. In this test, an instantaneous strain is applied and the force required to maintain the deformation is observed as a function of time. From the force-time curves, using appropriate mathematical models, the proportion of elastic component of the matrix can be studied. Peleg and Normand suggested a relative simple and quick model to analyse stress relaxation data [1]. The analysis is based on normalization of force data (Eq. 1) and subsequent linearization of the normalized values (Eq. 2)

$$Y(t) = \frac{F_0 - F_t}{F_0} \dots\dots (Eq. 1)$$

$$\frac{t}{Y(t)} = k_1 + k_2 * t \dots\dots(Eq. 2)$$

$Y(t)$ is the decaying force, F_0 is the initial force, F_t is the force at time t , k_1 and k_2 are constants.

To our best knowledge, no data on the stress relaxation properties of gingerbread type cookies exist in the literature. Therefore this study was aimed to preliminary investigate the stress relaxation parameters of gingerbread cookies made from spelt wheat and their changes during storage.

MATERIALS AND METHODS

Gingerbead cookies were prepared according to artisan recipe which basic form and preparation procedure is described in detail elsewhere [2 -4]. After baking samples were kept at 15 °C and 70% relative humidity. One set of cookies was kept at room conditions (22 °C and 35% relative humidity) for comparison. The storage period was 45 days and the dynamic of data reading was at 0, 15, 35 and 45 day of storage.

Stress relaxation parameters of gingerbread cookies were measured using a Texture Analyzer (TA-XTplus, Stable Micro Systems, Surrey, UK) equipped with a 30 kg load cell. Before analysis, 19 mm diameter cylinders were cut out from the centre of cookie with an apple-corer knife. The cylinders were deformed with a 36 mm cylindrical probe in compression to a constant strain of 15% at test speed of 0.5 mm/s. Coefficients from Peleg's model and other parameters extracted from the relaxation curves were subjected to one-way ANOVA and to Tukey's test.

RESULTS AND DISCUSSION

Effect of strain

Figure 1 shows the relaxation curves of gingerbread cookies made from spelt wheat. After application of constant strain, a decrease in force value necessary to maintain the

deformation was observed which is characteristic for viscoelastic materials. At 10–60% strains, the relaxation curves were not distinct but mostly overlapped showing no particular pattern except that relative residual stress values at 10-40% strains were somewhat higher in comparison to those at 50-60% strains showing that the cookies had more solid like behaviour at smaller strains. At 70% and 80% strains, relative residual stress values increased substantially which is in contrast to bread in which, at higher strains, elasticity decreases as found by Nussinovitch et al. [5]. But, it should be noted that at 80% strain, some permanent deformations occurred in gingerbread cookies.

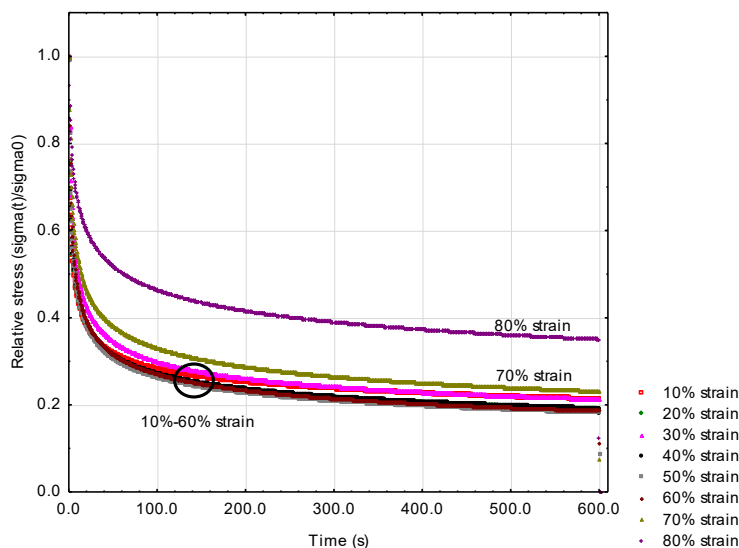


Figure 1. Relaxation curves of gingerbread cookies at different strains

Figure 2 depicts the effect of strain on the stress-relaxation parameters of spelt gingerbread cookies. The parameter k_1 gives information on the rate at which mechanical stress decay. Actually, the reciprocal value of k_1 is the initial decay rate. Higher k_1 values indicate low decay rates which are characteristic for elastic behaviour. The parameter k_1 is suggested as an indicator of viscous nature of food but its interpretation is not unequivocal. This parameter is usually found unsuitable to depict the changes in texture as it might be affected by shape or measurement errors. In gingerbread cookies k_1 reached lower values which is an indicator of quick relaxation i.e. less-elastic and more viscous behaviour.

The parameter k_2 represents the residual stress in the sample. It shows the extent of sample relaxation. In food, it is suggested as a representative of elastic nature [6] (Singh et al.) and a more reliable parameter to describe texture changes than k_1 [1, 6, 7]. The

values for k_2 range from 1 for ideal liquid and infinity for ideal elastic material. Low values of k_2 in gingerbread cookies also indicated low-elastic nature.

Percent stress relaxation (% SR) is considered a meaningful parameter to understand viscoelastic properties of food. It is obtained directly from the stress relaxation curve using an equation 3:

$$\%SR = \frac{\sigma_0 - \sigma_{(t)}}{\sigma_0} * 100\% \dots\dots (\text{Eq. 3})$$

where σ_0 is the initial stress (Pa) during constant deformation of sample and $\sigma_{(t)}$ is the stress at arbitrary time after the initial strain was reached. For the ideal solid, % SR is 0% (does not relax at all) and for the ideal liquid, % SR is 100%. For spelt gingerbread type cookies % SR ranged from 76.75% to 81.25% at 10-70% strains. At 80% strain, significantly lower magnitude of % SR value was achieved (64.95%) therefore the stress relaxation measurements should be performed in the former range. Relatively high values of % SR (around 80%) indicate that spelt gingerbread cookies are more viscous-like with only 20% of elastic component.

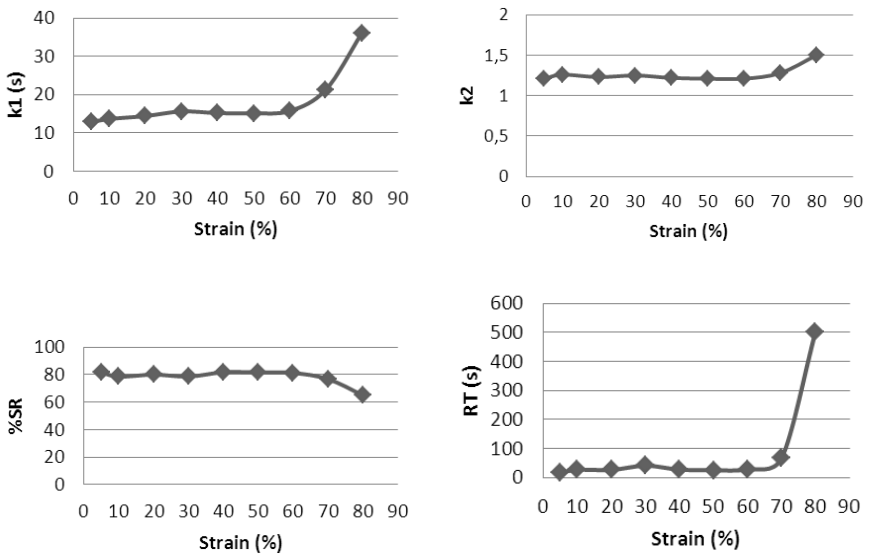


Figure 2. Effect of strain on the stress relaxation parameters of spelt gingerbread cookies

Relaxation time (RT) is defined as the time needed for relaxation force to reach 36.8% of initial value. Higher values of RT means that material needs more time to relax which is

the case when material has a more elastic nature. The relaxation times for spelt gingerbread type cookies ranged between 24.48 and 41.2 s within 10-60% strains and increased substantially at higher strains. Short relaxation times obtained in this study also indicates low elastic and more viscous structure. Singh et al. [6] reported similar relaxation times for poor quality flour doughs.

From Figure 1, it can be concluded that the measured stress relaxation parameters (k_1 , k_2 , % SR, RT) varied little in the 10–60% strain range. At higher strains, the spelt gingerbread cookies showed a more pronounced elastic response. Wu et al. [8] suggested that the suitable range of strain magnitudes for collection of stress relaxation parameters is the range in which these parameters do not significantly vary with strain. Therefore, suitable range for gingerbread cookies is up to 60% strain. Since at lower strains (10-30%), material shows more elastic behaviour, strains between 10–20% seems suitable for gingerbread testing.

Effect of storage

Table 1 shows the changes in relaxation parameters of spelt gingerbread cookies during a 42-days storage period. During storage, the gingerbread cookies exposed to higher humidity gradually became softer as seen from decreasing magnitude of initial force F_0 . Softness development was significant for the last period of storage. In contrast, the sample kept under low humidity conditions developed significant hardness after 42 days. Parameters k_1 and k_2 increased during storage in all samples, showing an increase in elastic behaviour. Percent stress relaxation decreased significantly during storage, confirming the increase in elastic i.e. solid character. Relaxation time did not show significant differences during the tested period among the samples stored at 70% relative humidity but significantly increased in the excessively dried out sample. The dried out sample was characterized with high values of initial force, k_1 , k_2 and relaxation time and lowest percent stress relaxation.

Table 1. Stress relaxation parameters of spelt gingerbread cookies during storage

Storage period	F_0 (g)	k_1	k_2	%SR	RT (s)
I	2053.4±310.2b	10.1±0.8a	1.2±0.02a	82.0±1.1d	43.2±8.9a
II	1827.2±280.6b	10.5±0.8a,b	1.2±0.01b	78.6±1.1c	46.9±11.0a
III	1672.6±180.0b	10.7±0.9a,b	1.3±0.01b	77.5±0.9c	30.3±5.3a
IV	1100.5±4.5a	11.7±0.6b,c	1.3±0.03c	74.1±1.6b	44.8±10.3a
Dried out sample	22740.6±452.2c	12.2±0.9c	1.5±0.03d	63.22±1.0a	297.1±13.5b

Values followed by the same letter are not significantly different at $P \leq 0.05$

CONCLUSIONS

Gingerbread cookies made from spelt in general present little elastic character. During storage, the majority of calculated parameters pointed to an increase in solid character of the samples. However, initial force decreased with storage indicating less rigid texture in samples exposed to higher humidity. Only relaxation time was the parameter that was unable to observe changes in the textural properties within cookies stored at humid conditions. However, when compared to the dried out samples, all parameters were significantly different.

The texture of gingerbread type cookies is very complex and depends much on the moisture migration. Gingerbread cookies are increasingly prone to drying out with storage. At high relative humidity, gingerbread cookies gain moisture at the edge, softening firstly the outer layers. Further examinations of stress-relaxation parameters and their correlation with moisture migration are necessary to better understand the changes in the textural and micro-structural characteristics of gingerbread cookies during storage. In order to elucidate the final ability of stress relaxation technique for texture characterization of gingerbread type cookies, additional data on the correlation with empirical textural characteristics are necessary.

ACKNOWLEDGEMENT

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REFERENCES

1. Peleg, M. 1979. Characterization of the stress relaxation curves of solid foods. *J. Food Sci.* 44:277-281.
2. Filipčev, B., Šimurina, O., Sakač, M., Sedej, I., Jovanov, P., Pestorić, M., Bodroža-Solarov, M. 2011. Feasibility of use of buckwheat flour as an ingredient in ginger nut biscuit formulation. *Food Chem.* 125:164-170.
3. Filipčev, B., Bodroža-Solarov, M., Šimurina, O., Cvetković, B. 2012. Use of sugar beet molasses in processing of gingerbread type biscuits: effect on quality characteristics, nutritional profile, and bioavailability of calcium and iron. *Acta Alim.* 41:494-505.
4. Filipčev, B., Šimurina, O., Bodroža-Solarov, M. 2012. Quality of gingernut type biscuits as affected by varying fat content and partial replacement of honey with molasses. *J. Food Sci. Technol.* (doi: 10.1007/s13197-012-0805-x), (published on-line 29.08.2012.).
5. Nussinovitch, A., Steffens, M., Chinachoti, P. and Peleg, M. 1992. Effect of strain level and storage time on the recoverable work of compressed bread crumb. *J. Texture Stud.* 23:13-24.

6. Singh, H., Rockall, A., Martin, C.R., Chung, O.K., and Lookhart, G.L. 2006. The analysis of stress relaxation data of some viscoelastic foods using a texture analyzer. *J. Texture Stud.* 37:383-392.
7. Buňka, F., Pachlová, V., Pernická, L., Burešová, I., Kráčmar, S. and Lošák, T. (2013). The dependence of Peleg's coefficients on selected conditions of a relaxation test in model samples of Edam cheese. *J. Texture Stud.* (in press).
8. Wu, M-Y., Chang, Y-H., Shiau, S-Y., Chen, C-C. 2012. Rheology of fiber-enriched steamed bread: stress relaxation and texture profile analysis. *J. Food Drug Anal.* 20:133-142.

CONSUMER ACCEPTANCE OF THE COOKIES ENRICHED WITH A DIGESTION STIMULATING MEDICINAL PLANT MIXTURE

UDC 664.681 : 543.92
664.681 : 613.2

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ABSTRACT

The objective of this study was to evaluate consumers' acceptance of cookies made of 'Vitalplant' digestion stimulating mixture, which was added in the form of powder and extract. Cookies were evaluated by 64 consumers and ANOVA was performed on scores using the categories of each demographic factor. Comparing the obtained results of enriched samples, the differences were more pronounced in the case of the extract enriched cookies. Men and women rated extract enriched samples higher scores than the control. The old group reacted positively only to colour changes, while they were totally indifferent to the changes in colour, mouthfeel, and taste of the enriched cookies. Regarding the results of both concepts, consumers with higher education degree seem to be more positive towards the functional ingredients in the cookies. The obtained results showed that cookie presents suitable carrier for the development of a digestion stimulating functional product by mixture addition in the both forms.

Keywords: consumer acceptance, cookies, plant mixture, plant extract, sensory properties

INTRODUCTION

Consumers' awareness of a relationship between diet and health has led to the increased acceptance of functional foods [1]. Popularity of added value confectionary products has increased dramatically over the past decade. However, consumers' acceptance of functional foods does not depend only on trustworthiness of health claims, but on sensory properties as well. Several authors [2-8] reported that consumers are hardly willing to compromise on the taste of functional foods for eventual health benefits. In addition, some demographic variables, such as gender, age, and education cannot be ignored when testing the acceptance of functional foods [9]. For this reason, consumer's research related to foods that claim specific health benefits has concentrated on profiling consumers who accept the specific enriched foods in terms of their socio-demographics [10]. It is known that the carrier product, relative to the type of enrichment, has large effect on consumers' acceptance and willingness to try different functional food

concepts. Referring to the fact that they are widely consumed food products, appreciated for their versatility, convenience, conservation, and especially for attractive sensory properties, short dough cookies can be considered to be suitable carrier products for functional bakery formulations [11, 12]. Furthermore, medicinal plants are not only recognized to be safe by the consumers, but also attractive to many of them who question the safety of synthetic food additives [13]. Regarding confectionery industry, medical herbs have been considered to be the most useful resources for the development of functional foods because of a relatively high antioxidant activity, which was explained by synergistic effects of the components. [14-16].

The aim of this research was to investigate consumers' acceptance of the cookies enriched with a digestion stimulating medical plant mixture.

MATERIALS AND METHODS

Materials

The commercial plant mixture 'Vitalplant' (*Frangulae cortex* 35%, *Petroselinii fructus* 25%, *Menthae pip. folium* 20%, and *Carvi fructus* 20%) was in the form of powder with granulation of up to 3 mm. Commercially available refined wheat flour (13.3% water content, 0.39% db ash content, and 10.5% db protein content), vegetable fat, salt, powdered sugar, and baking powder were purchased in a local food store.

Preparation of plant extracts for the formulation of cookies

Crude plant extracts were obtained by maceration with ethanol/water mixture (80:20, v/v), with the ratio of raw materials to ethanol solution of 1:10, for 24 h at room temperature, and subsequently extracted in an ultrasonic bath at room temperature in 10 min. After filtration through a filter paper (Whatman, Grade 4 Chr, UK), vacuum-evaporation of the solvent at 40 °C, the extracts were stored at -4 °C until further use.

Preparation of cookies

Bake trials were performed on laboratory-scale equipment. The control cookie dough formulation contained 100 g of flour, 40 g of fat, 30 g of sugar, 1 g of salt, 1 g of baking powder, and a required amount of water (~5 g). The dough was sheeted to a thickness of 3.5 mm, cut into a circular shapes, baked at 180 °C in 10 min, and cooled. Enriched cookies were made by adding 2, 4, and 6 g of 'Vitalplant' mixture to the control formulation. The powdered mixture was blended with fat, and used for the dough preparation. Enriched extract cookies were also prepared to provide the same three levels of enrichment. Extract was previously suspended in water and blended with fat to prepare the dough.

Sensory evaluation

Sensory evaluation was carried out 24 after baking by using a panel of 64 consumers, whose demographic characteristics are shown in Table 1. Consumers were selected on the basis of their availability for the assessment, interest to participate in the study, absence of aversions, allergies or intolerances, normal perception abilities, and moderate preference towards this kind of product.

Table 1. Demographic characteristics

	Gender		Age			Education		
	Men	Women	< 30	30-50	> 50	P/H*	BSc	MSc/PhD
Total, n	28	36	21	22	21	21	21	22
%	43.75	56.25	32.81	34.38	32.81	32.81	32.81	34.38

*P - Primary school; H - High school

Consumer test was conducted in 5 hourly session between 10 am and 15 pm on two days. Samples were coded with three-digit random numbers and served at ambient temperature. Four samples were presented to each consumer per session (control and three samples of different powder (session 1), and extract (session 2) enrichment. Consumers were given a printed response sheet with written instructions for the test during the session. They were requested to indicate their perception of colour, odour, taste, and mouthfeel, using a three-box scale labelled on the left with 'dislike very much' (1 score), 'indifferent' in the middle (3 scores), and on the right with 'like very much' (5 scores).

Statistical analysis

For evaluating differences between demographic characteristics ANOVA was performed on date using the categories of each demographic factor as variation factor. Duncan's multiple range test was used to compare means at 5% significance level using statistical data analysis software system STATISTICA (StatSoft, Inc. (2008), version 10.0 (www.statsoft.com)).

RESULTS AND DISCUSSION

Different acceptance patterns of the gender groups for the cookies enriched with 'Vitalplant' in the form of powder and extract are presented in Table 2. In the case of colour, women showed only significantly higher affinity ($P < 0.05$) for the cookies enriched with 2% powder. Contrary to them, men preferred colour and odour of all

powder enriched samples versus the control. Regarding the results related to the difference in acceptance pattern between genders (Table 2, row II_a), just the odour of the control sample was more acceptable for women than men.

Table 2. Effect of gender on acceptance of the enriched cookies

Property	Sample	GENDER						EXTRACT					
		POWDER			EXTRACT			POWDER			EXTRACT		
		Female		Male	Female		Male	Female		Male	Female		Male
	Mean ± SD	I	II	Mean ± SD	I	II	Mean ± SD	I	II	Mean ± SD	I	II	
Colour	Control	3.26 ± 1.67	A	a	2.44 ± 1.50	A	a	2.52 ± 1.53	A	a	2.00 ± 1.24	A	a
	2%	3.97 ± 1.17	B	a	3.44 ± 1.46	B	a	3.61 ± 1.18	B	a	3.11 ± 1.08	B	a
	4%	3.78 ± 1.15	AB	a	4.00 ± 1.03	B	a	4.52 ± 0.86	C	b	3.89 ± 1.21	B	a
	6%	3.35 ± 1.42	A	a	3.89 ± 1.23	B	a	3.65 ± 1.20	B	a	3.78 ± 1.40	B	a
Odour	Control	3.83 ± 1.50	A	b	2.44 ± 1.34	A	a	2.91 ± 1.58	A	a	2.67 ± 1.57	A	a
	2%	3.65 ± 1.34	A	a	3.44 ± 1.29	B	a	3.39 ± 1.37	AB	a	3.00 ± 1.19	AB	a
	4%	3.61 ± 1.32	A	a	3.78 ± 1.22	B	a	3.96 ± 1.38	B	a	3.89 ± 1.00	B	a
	6%	3.39 ± 1.67	A	a	4.00 ± 1.24	B	a	3.57 ± 1.56	B	a	3.56 ± 1.50	AB	a
Taste	Control	3.74 ± 1.36	A	a	3.33 ± 1.41	A	a	3.26 ± 1.56	A	a	2.78 ± 1.35	A	a
	2%	3.78 ± 1.36	A	a	3.78 ± 1.40	A	a	3.61 ± 1.32	AB	a	3.22 ± 0.94	A	a
	4%	4.00 ± 1.25	A	a	4.00 ± 1.41	A	a	4.17 ± 1.16	B	a	4.44 ± 1.21	B	a
	6%	3.57 ± 1.50	A	a	3.89 ± 1.23	A	a	4.00 ± 1.32	B	a	4.11 ± 1.02	B	a
Mouthfeel	Control	3.70 ± 1.53	A	a	3.22 ± 1.52	A	a	3.43 ± 1.33	A	a	3.22 ± 1.80	A	a
	2%	3.57 ± 1.24	A	a	3.33 ± 1.41	A	a	3.26 ± 1.50	A	a	3.56 ± 1.34	AB	a
	4%	3.65 ± 1.40	A	a	4.00 ± 1.03	A	a	4.09 ± 1.09	B	a	4.33 ± 0.97	B	a
	6%	3.87 ± 1.38	A	a	3.57 ± 1.50	A	a	3.78 ± 1.23	AB	a	4.22 ± 1.22	B	a

Mean values within one column (I) and row (II) followed by different letters are significantly different ($P < 0.05$).

The results of the second session (Table 2, column I_b) revealed quite similar acceptance patterns between the genders for the extract enriched cookies. There were significant differences in all observed properties, with the highest acceptability among women was

in favour of the enrichment of 4%, especially for colour and mouthfeel. In general, men showed significantly greater acceptability to higher % enrichments compared to the control. Besides, the colour of the sample enriched with 4% extract only was significantly more acceptable for women than men (Table 2, row II_b).

Table 3. Effect of age on acceptance of the powder enriched cookies

Property	Sample	AGE										
		Young				Middle-aged				Old		
		Mean ± SD	I	II		Mean ± SD	I	II		Mean ± SD	I	II
Colour	Control	2.76 ± 1.56	A	a		3.76 ± 1.48	A	b		2.56 ± 1.76	A	a
	2%	3.64 ± 1.38	BC	a		4.14 ± 1.20	A	a		3.67 ± 1.19	B	a
	4%	4.20 ± 1.15	C	b		3.86 ± 1.20	A	ab		3.33 ± 0.77	AB	a
	6%	3.16 ± 1.52	AB	a		3.76 ± 1.34	A	a		3.67 ± 1.19	B	a
	Control	3.40 ± 1.73	A	a		3.67 ± 1.46	A	a		3.22 ± 1.52	A	a
	2%	3.80 ± 1.29	A	a		3.67 ± 1.46	A	a		3.22 ± 1.17	A	a
Odour	4%	3.72 ± 1.14	A	a		3.76 ± 1.48	A	a		3.44 ± 1.29	A	a
	6%	3.64 ± 1.70	A	a		3.67 ± 1.59	A	a		3.33 ± 1.41	A	a
	Control	3.64 ± 1.50	A	a		3.95 ± 1.02	AB	a		3.22 ± 1.52	A	a
	2%	4.28 ± 0.98	A	b		3.57 ± 1.57	A	ab		3.33 ± 1.41	A	a
	4%	3.88 ± 1.30	A	a		4.43 ± 1.12	B	a		3.67 ± 1.37	A	a
	6%	3.64 ± 1.60	A	a		3.86 ± 1.35	AB	a		3.44 ± 1.29	A	a
Taste	Control	3.16 ± 1.62	A	a		4.24 ± 1.18	A	b		3.33 ± 1.57	A	ab
	2%	3.64 ± 1.25	A	a		3.48 ± 1.40	A	a		3.33 ± 1.24	A	a
	4%	3.56 ± 1.36	A	a		4.05 ± 1.36	A	a		3.67 ± 1.19	A	a
	6%	3.96 ± 1.31	A	a		3.86 ± 1.62	A	a		3.44 ± 1.29	A	a
	Control	3.16 ± 1.62	A	a		4.24 ± 1.18	A	b		3.33 ± 1.57	A	ab
	2%	3.64 ± 1.25	A	a		3.48 ± 1.40	A	a		3.33 ± 1.24	A	a
Mouthfeel	4%	3.56 ± 1.36	A	a		4.05 ± 1.36	A	a		3.67 ± 1.19	A	a
	6%	3.96 ± 1.31	A	a		3.86 ± 1.62	A	a		3.44 ± 1.29	A	a
	Control	3.16 ± 1.62	A	a		4.24 ± 1.18	A	b		3.33 ± 1.57	A	ab
	2%	3.64 ± 1.25	A	a		3.48 ± 1.40	A	a		3.33 ± 1.24	A	a
	4%	3.56 ± 1.36	A	a		4.05 ± 1.36	A	a		3.67 ± 1.19	A	a
	6%	3.96 ± 1.31	A	a		3.86 ± 1.62	A	a		3.44 ± 1.29	A	a

Mean values within one column (I) and row (II) followed by different letters are significantly different ($P < 0.05$).

Comparing the obtained results, it can be seen that the differences between the samples are more pronounced in the case of extract enrichment. Both genders rated the extract enriched samples higher scores than the control. It should be noted that for both genders, the powder enriched cookies were on the same level of acceptability as the control. These results were partially consistent with the previous studies that have shown that females are mostly open to functional foods [11, 17-20].

Table 4. Effect of age on acceptance of the extract enriched cookies

Property	Sample	AGE												
		Young					Middle-aged					Old		
		Mean ± SD	I	II	Mean ± SD	I	II	Mean ± SD	I	II	Mean ± SD	I	II	
Colour	Control	2.04 ± 1.17	A	a	3.00 ± 1.67	A	b	2.11 ± 1.41	A	a				
	2%	3.32 ± 1.25	B	a	3.95 ± 1.02	BC	a	3.11 ± 1.08	B	a				
	4%	4.68 ± 0.75	C	b	4.62 ± 0.80	C	b	3.56 ± 1.34	B	a				
	6%	4.12 ± 1.01	C	b	3.76 ± 1.18	B	b	3.00 ± 1.37	B	a				
Odour	Control	2.60 ± 1.63	A	a	2.90 ± 1.34	A	a	3.11 ± 1.75	A	a				
	2%	3.32 ± 1.38	AB	a	3.29 ± 1.31	AB	a	3.22 ± 1.35	A	a				
	4%	4.28 ± 1.14	C	b	4.05 ± 1.20	B	ab	3.33 ± 1.41	A	a				
	6%	3.88 ± 1.54	BC	a	3.57 ± 1.57	AB	a	3.11 ± 1.45	A	a				
Taste	Control	3.00 ± 1.41	A	a	3.19 ± 1.54	A	a	3.22 ± 1.66	A	a				
	2%	3.64 ± 1.25	A	a	3.48 ± 1.25	A	a	3.33 ± 1.24	A	a				
	4%	4.36 ± 1.11	B	b	4.62 ± 0.80	B	b	3.67 ± 1.19	A	a				
	6%	3.64 ± 1.25	AB	a	4.81 ± 0.60	B	b	3.67 ± 1.37	A	a				
Mouthfeel	Control	3.08 ± 1.35	A	a	3.95 ± 1.36	B	a	3.11 ± 1.60	A	a				
	2%	3.80 ± 1.41	B	a	3.10 ± 1.48	A	a	3.00 ± 1.37	A	a				
	4%	4.40 ± 0.92	B	b	4.43 ± 0.93	B	b	3.44 ± 1.10	A	a				
	6%	3.96 ± 1.02	B	ab	4.33 ± 1.15	B	b	3.33 ± 1.41	A	a				

Mean values within one column (I) and row (II) followed by different letters are significantly different ($P < 0.05$).

Perceived properties of the powder enriched cookies, sorted by consumers' age are presented in Table 3. There were small significant differences between the samples within each age category almost for all observed properties, except colour and taste (Table 3, column I). Young group seemed to have more positive and significant affinity only towards the colour, middle-aged towards the taste of the cookies enriched with 4% powder. Old group was only in colour was more open to cookies enriched with 2 and 6% powder. Regarding the results related to the difference in acceptance pattern between ages (Table 2), middle-aged category showed the highest preference towards the colour and the mouthfeel of the control sample, while the young group was significantly favoured colour of the cookies with 4% powder enrichment.

Table 5. Effect of education on acceptance of the powder enriched cookies

Property	Sample	EDUCATION											
		P/H				BSc				MSc/PhD			
		Mean ± SD	I	II		Mean ± SD	I	II		Mean ± SD	I	II	
Colour	Control	3.27 ± 1.55	A	a		3.19 ± 1.66	A	a		2.62 ± 1.75	A	a	
	2%	3.82 ± 1.18	A	a		3.48 ± 1.40	A	a		4.14 ± 1.20	B	a	
	4%	4.00 ± 1.02	A	a		3.86 ± 1.20	A	a		3.67 ± 1.15	B	a	
	6%	3.91 ± 1.02	A	a		3.10 ± 1.61	A	a		3.48 ± 1.40	B	a	
	Control	3.73 ± 1.70	B	a		3.29 ± 1.71	A	a		3.29 ± 1.31	A	a	
	2%	2.73 ± 1.28	A	a		3.95 ± 1.20	A	b		4.14 ± 1.01	B	b	
Odour	4%	3.64 ± 1.29	B	a		3.95 ± 1.20	A	b		3.38 ± 1.36	AB	a	
	6%	3.91 ± 1.48	B	a		3.57 ± 1.69	A	a		3.19 ± 1.54	A	a	
	Control	3.91 ± 1.34	A	a		3.38 ± 1.50	A	a		3.57 ± 1.29	A	a	
	2%	3.91 ± 1.48	A	a		3.67 ± 1.15	A	a		3.76 ± 1.48	A	a	
	4%	4.09 ± 1.19	A	a		4.24 ± 1.18	A	a		3.67 ± 1.46	A	a	
	6%	3.82 ± 1.18	A	ab		4.05 ± 1.36	A	b		3.10 ± 1.61	A	a	
Taste	Control	3.82 ± 1.33	A	a		3.57 ± 1.43	AB	a		3.29 ± 1.82	A	a	
	2%	3.83 ± 1.18	A	a		3.29 ± 1.31	A	a		3.38 ± 1.36	A	a	
	4%	4.00 ± 1.35	A	a		3.95 ± 1.02	AB	a		3.29 ± 1.45	A	a	
	6%	3.55 ± 1.53	A	a		4.33 ± 1.32	B	a		3.48 ± 1.25	A	a	
	Control	3.82 ± 1.33	A	a		3.57 ± 1.43	AB	a		3.29 ± 1.82	A	a	
	2%	3.83 ± 1.18	A	a		3.29 ± 1.31	A	a		3.38 ± 1.36	A	a	

Mean values within one column (I) and row (II) followed by different letters are significantly different ($P < 0.05$).

Referring to young and middle-age category, significant differences were found between the samples for all observed properties. These groups were more positive to higher levels of enrichment. Concerning the old group, significant differences between samples existed only in the case of colour. In general, young and middle-age group reacted positively to extract enrichments. Ares and Gámbaro [21] pointed that different age groups react differently in terms of perceived healthiness of different carrier products. According to many authors [21, 22], the most positive group towards functional foods are middle-aged or especially elderly consumers, whose interest arises from health care concerns.

Table 6. Effect of education on acceptance of the extract enriched cookies

Property	Sample	EDUCATION											
		P/H				BSc				MSc/PhD			
		Mean ± SD	I	II	a	Mean ± SD	I	II	a	Mean ± SD	I	II	a
Colour	Control	2.73 ± 1.55	A	a	a	2.43 ± 1.57	A	a	a	1.95 ± 1.20	A	a	a
	2%	3.73 ± 1.16	B	a	a	3.38 ± 1.02	B	a	a	3.29 ± 1.31	B	a	a
	4%	4.45 ± 0.91	B	a	a	4.52 ± 0.87	C	a	a	4.05 ± 1.36	B	a	a
	6%	3.82 ± 1.01	B	a	a	3.48 ± 1.40	B	a	a	3.76 ± 1.34	B	a	a
Odour	Control	3.18 ± 1.37	A	a	a	2.62 ± 1.63	A	a	a	2.71 ± 1.71	A	a	a
	2%	3.36 ± 1.59	A	a	a	3.29 ± 1.31	AB	a	a	3.19 ± 1.08	AB	a	a
	4%	3.82 ± 1.47	A	a	a	4.14 ± 1.01	B	a	a	3.86 ± 1.35	B	a	a
	6%	3.73 ± 1.70	A	a	a	3.48 ± 1.40	AB	a	a	3.48 ± 1.54	AB	a	a
Taste	Control	3.55 ± 1.65	A	a	a	2.81 ± 1.40	A	a	a	3.00 ± 1.41	A	a	a
	2%	3.64 ± 1.14	A	a	a	3.19 ± 1.54	A	a	a	3.67 ± 0.97	AB	a	a
	4%	4.00 ± 1.20	A	a	a	4.43 ± 0.93	B	a	a	4.33 ± 1.15	B	a	a
	6%	4.18 ± 1.33	A	a	a	4.05 ± 1.20	B	a	a	3.86 ± 1.20	B	a	a
Mouthfeel	Control	3.36 ± 1.47	A	a	a	3.38 ± 1.36	AB	a	a	3.38 ± 1.63	AB	a	a
	2%	3.64 ± 1.29	A	a	a	3.19 ± 1.66	A	a	a	3.19 ± 1.40	A	a	a
	4%	4.00 ± 1.20	A	a	a	4.24 ± 1.00	B	a	a	4.24 ± 1.00	B	a	a
	6%	4.09 ± 1.19	A	a	a	3.95 ± 1.20	AB	a	a	3.67 ± 1.32	AB	a	a

Mean values within one column (I) and row (II) followed by different letters are significantly different ($P < 0.05$).

Manu previous studies [7, 18-20] confirmed that different levels of education have been associated with positive attitudes towards functional foods. In this paper, the obtained results showed (Table 5, column I) that the odour of the cookies enriched with 2% plant mixture was less attractive to the consumers with the lowest level of education (P/H). Quite opposite to P/H group, MSc/PhD group showed that the colour of the powder enriched cookies, regardless of all levels of enrichment more attractive than the control sample. BSc group liked the mouthfeel of the cookies enriched with 6% powder the most. Regarding the results related to the difference in acceptance pattern between education (Table 5, row II), the odour of the cookies enriched with lower % enrichment was more acceptable for higher education. In the case of odour and BSc group of education, acceptability was the significant for the highest % enrichment.

Referring to liking of the cookies enriched with the extract (Table 6, column I), BSc and MSc/PhD group expressed the preferences similarly to each other by giving the samples enriched with 4% extract the highest scores for almost all observed properties. P/H group expressed the preferences towards the colour of the enriched samples. Generally, regarding the results of both concepts, consumers with higher education degree seem to be more positive towards the functional ingredient in the cookies.

CONCLUSION

Concepts, powder and extract addition can be applied, meaning that the cookies present suitable carrier product for the development of digestion stimulating functional product by 'Vitalplant' mixture addition. Due to the fact that the extract supplemented samples were significantly more attractive than the control, it can be concluded that these kinds of cookies may seem more acceptable by the consumers. Apart from antioxidant efficiency, costs of extract production go in favour of powder potential usage. Further research needs to be done, clinical studies at first place in order to provide the information about the optimum dosage of the functional ingredient.

ACKNOWLEDGMENTS

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REFERENCES

1. Jones, P., and Jew, S. 2007. Functional food development: concept to reality. *Trends Food Sci. Technol.* 18:387-390.
2. Drewnowski, A., and Gómez-Carneros, C. 2000. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.* 72:1424-1435.
3. Westenhoefer, J., and Pudiel, V. 1993. Pleasure from food: Importance for food choice and consequence of deliberate restriction. *Appetite.* 20:246-249.
4. Wennström, P. 2000. Functional foods and the consumer's perception of health claims. *Scand J Food Nutr.* 44:30-33.
5. Tuorila, H., and Cardello, A.V. 2002. Consumer responses to an off-flavor in juice in the presence of specific health claims. *Food. Qual. Prefer.* 13:561-569.
6. Augustin, M. 2003. Functional foods: an adventure in food formulation. *Food Australia.* 53:428-432
7. Verbeke, W. 2006. Functional foods: consumer willingness to compromise on taste for health? *Food. Qual. Prefer.* 17:126-131.
8. Ares, G., Giménez, A., and Gámbaro, A. 2008. Does information about the source of functional ingredients influence consumer perception of functional milk desserts? *J. Sci. Food Agric.* 88:2061-2068.
9. Dagevos, H. 2005. Consumers as four-faced creatures. Looking at food consumption from the perspective of contemporary consumers. *Appetite.* 45:32-39.
10. Van Kleef, E., van Trijp, H. C. M., and Luning, P. 2005. Functional foods: health claim-food product compatibility and the impact of health claim framing on consumer evaluation. *Appetite.* 44:299-308.
11. Verbeke, W., Scholderer, J., and Lähteenmääki, L. 2009. Consumer appeal of nutrition and health claims in three existing product concepts. *Appetite.* 52:84-692.
12. Krutulyte, R., Grunert, K. G., Scolderer, J., Lähteenmääki, L., Hagemann, K. S., Elgaard, P., Neilsen, B., and Graverholt, J. P. 2011. Perceived fit of different combinations of carriers and functional ingredients and its effect on purchase intention. *Food. Qual. Prefer.* 22:11-16.
13. Lee, S., Xiao, C., and Pei, S. 2008. Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. *J. Ethnopharmacol.* 117:362-377.
14. Šimurina, O. Filipčev, B., Psodosrov, Đ., Sakač, M., Bodroža Solarov, M., Pestorić, M., and Novaković, A. 2008. Bread supplemented with herbal blend VITALPLANT. *Food Processing, Quality & Safety.* 35:113-117.
15. Mišan, A. 2010. Antioxidant properties of food supplemented with medicinal plants. *Food & Feed Research.* 37:81-85.
16. Mišan, A., Mimica-Dukić, N., Sakač, M., Mandić, A., Sedej, I., Šimurina, O., and Tumbas V. 2011. Antioxidant activity of medicinal plant extracts in cookies. *J. Food Sci.* 76:C1239-C1244.
17. Urala, N., Arvola, A., and Lähteenmääki, L. 2003. Strength of health-related claims and their perceived advantage. *Int. J. Food Sci. Technol.* 38:815-826.

18. Urala, N., and Lähteenmääki, L. 2007. Consumers' changing attitudes towards functional foods. *Food. Qual. Prefer.* 18:1-12.
19. De Jong, N., Ocke, M. C., Branderhorst, and H. A. C., Friele, R. 2003. Demographic and lifestyle characteristics of functional food consumers and dietary supplement users. *Br. J. Nutr.* 89:273-281.
20. Herath, D., Cranfield, J., and Henson, S. 2008. Who consumes functional foods and nutraceuticals in Canada? Results of cluster analysis of the 2006 survey of Canadians' Demand for Food Products Supporting Health and Wellness. *Appetite.* 51:256-265.
21. Ares, G., and Gámbaro, A. 2007. Influence of gender, age and motives underlying food choice on perceived healthiness and willingness to try functional foods. *Appetite.* 49:48-158.
22. Landström, E., Hursti, U-K K., and Magnusson, M. 2009. Functional foods compensate for an unhealthy lifestyle: Some Swedish consumers' impressions and perceived need of functional foods. *Appetite.* 53:34-43.

QUALITY INSPECTION OF COOKIES USING COMPUTER VISION

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664.681 : 004.932.2

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ABSTRACT

Product appearance plays a vital role in making a purchase decision. Colour, size and shape are significant factors that influence purchase decision of bakery products. Food shape is often closely regarded as its quality. Broken or deformed product may taste the same as an undamaged ones, but customers are reluctant to buy seemingly damaged goods. Due to the demands of high quality foods, automated food shape inspection has become an important need for the food industry. Computer vision provides one alternative for an automated, non-destructive and cost-effective technique to accomplish these requirements. Features such as the internal and external appearance contribute to the overall impression of the products quality. Consequently such characteristics have been evaluated by computer vision. The objective of this paper is to summarize and evaluate shape descriptors which can be used as indicators for quality of final product, and also as additional parameter for estimating end of baking process.

Keywords: computer vision, food quality, shape descriptors

INTRODUCTION

Computer vision systems are being used increasingly in the food industry for quality assurance purposes. Essentially, such systems replace human inspectors for the evaluation of a variety of quality attributes of raw and prepared foods. In food industry computer vision can be use for defect detection, dimensional measurement, and orientation detection, grading, sorting and counting 1,2.

Generally, computer vision system are comprised of image acquisition device with lighting system, storage system and digital computer with appropriate software for image segmentation, feature extraction and analysis. Features are parts of image with specific information about product and are described by colour, shape, size and texture¹. Shape of baked cookies is important parameter of quality as consumers expects that all cookies have uniform shape and size. There are many shape descriptors available in literature, they are mainly categorised in two categories: contour-based shape descriptors and region-based shape descriptors 3,4. Overview and classification of shape descriptors can be visualised as displayed in Figure 1.

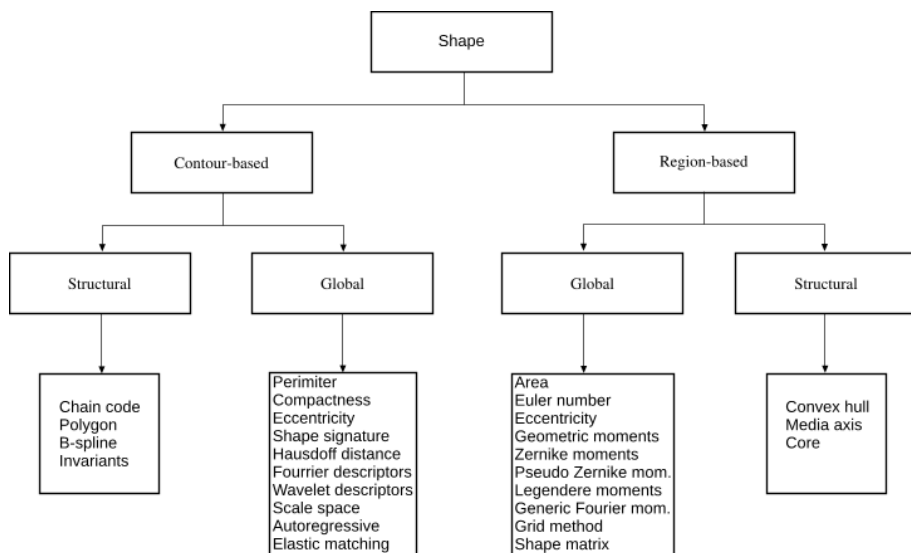


Figure 1. Classification and overview of shape descriptors

Two kinds of information are contained in each pixel, i.e. brightness value and locations in the coordinates that are assigned to the images. Brightness value is the colour feature while information of locations of pixel is known as size or shape features. Three commonly used features for size measurement of an object can be found for food quality evaluation: area, perimeter, and length and width. Compared with other features such as colour and texture, shape is easier to measure using image processing techniques.

MATERIAL AND METHODS

The cookies were prepared according to standard AACC (American Association of Cereal Chemists) formulation. It was used standard flour for production of cookies with three different moisture content (standard, S, with 16 g water/225 g of flour, dry, D, with 12 g water/225 g of flour and wet, W, with 20 g water/225 g of flour). Investigation was conducted on 33 samples during 10 minutes of baking. Cookies were baked at 205 °C and temperature was measured in the middle of samples which were thick 60 mm and high 7 mm.

Computer vision system includes image capturing device (EPSON™ Perfection V500 Photo), personal computer with EPSON software. Image processing has been done in ImageJ5 and Python with some libraries (Numpy, SciPy, OpenCV) which are all open

source programs. Acquired images first were thresholded in ImageJ and converted to 8-bit binary images (black and white). Using macro option, measurements of area, perimeter, Feret's diameter was determined for all samples (images). Python script has been written for measurements of sample height at specific points from sample centre axis.

RESULTS AND DISCUSSION

Result of analysis conducted with ImageJ, determination of classical image descriptors (area, perimeter, Feret's diameter,...), showed that during baking, first there is increase in cookie size (height and width) which results in increase of area and therefore perimeter (Figure 1), After that, there is slight decrease in observed quantities which is in compliance with baking dynamics (heat and mass transfer). Observed oscillations in Figure 1 can be explained through cookies deformation during baking and manipulation.

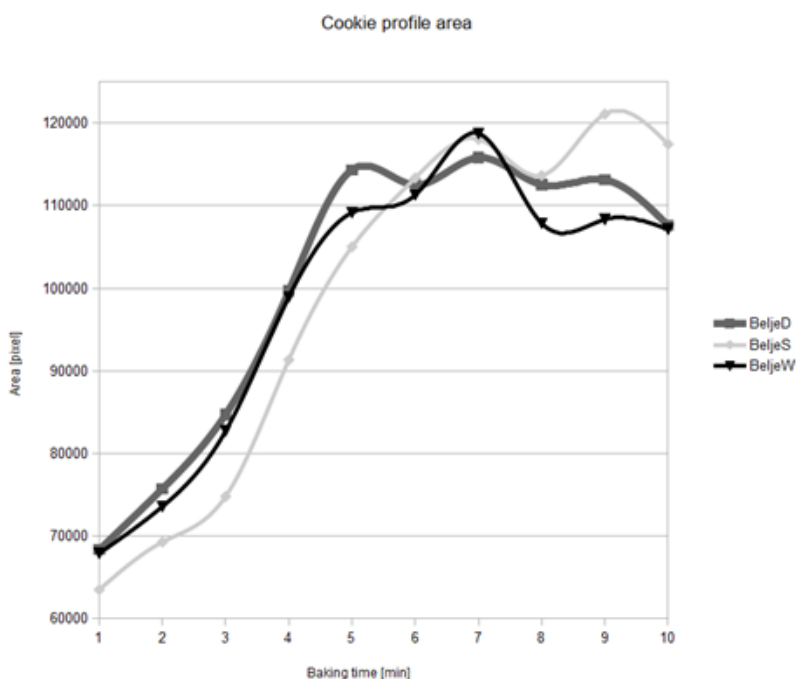


Figure 2. Cookie cross section area in pixels during baking

Feret's diameters provided information about maximum width and maximum height of cookies, which is useful for cookie shape description. Other parameters do not provide easy understandable information about cookies shape. More usable information is obtained from height profiles during baking (Figure 3, Figure 4) which contains information about cookies symmetry around vertical centre axis which is at 425 pixels.

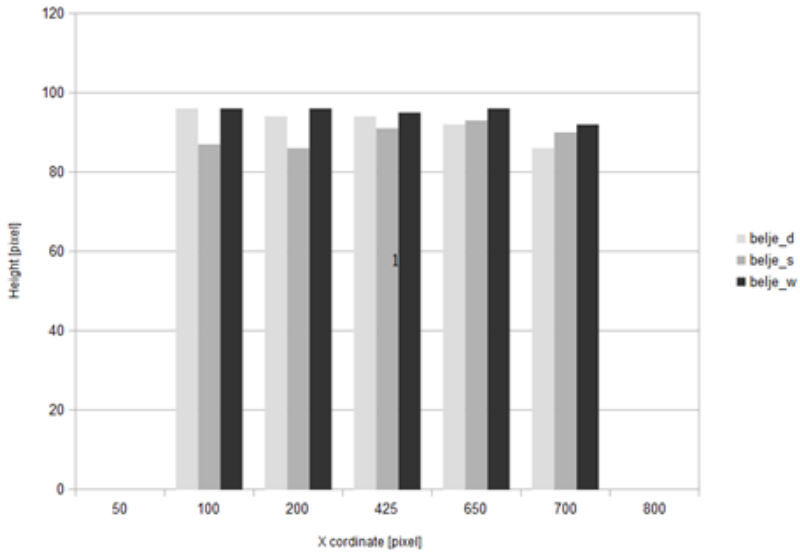


Figure 3. Cookies height profiles after 1 minute of baking

Profile is obtained by measuring heights at equal distances from centre axes to left and right. Distances have been chosen with respect to image size (850*200 pixels), and were: 0, 100, 200, 425 (middle), 650, 750, 800 pixels. Symmetry can be easily calculated as difference of equally distant heights from cookie centre.

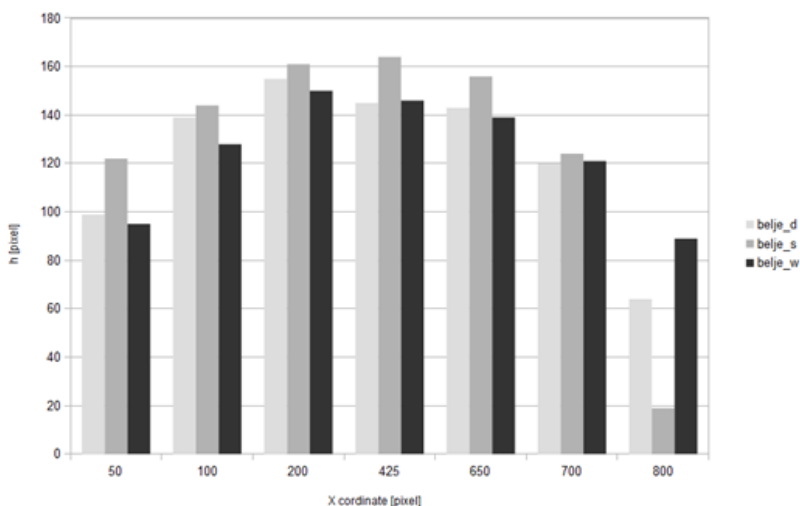


Figure 4. Cookies height profiles after 10 minute of baking

CONCLUSIONS

According to results of size (width and height) and shape deformation of cookies during baking process, computer vision can be applied to inspect shape of final product and in combination with other process parameters better define the end of baking process. We believe that the proposed framework opens up new possibilities for dynamic visual inspection of cookies during baking (i.e. inspection texture and colour) with aim of better process control.

REFERENCES

1. Chaoxin Z., Da-Wen S., Liyun Z., 2006. Recent developments and applications of image features for food quality evaluation and inspection – a review. *Trends Food Sci. & Tech.* 17:642-655.
2. Tadhg B., Da-Wen S. 2004. Improving quality inspection of food products by computer vision - a review. *J. Food Eng.* 61:3-16.
3. Amanatiadis, A; Kaburlasos, V. G.; Gasteratos, A., 2011. Evaluation of shape descriptors for shape-based image retrieval. *IET Image Process.* 5:493-499.
4. Cheng-Jin D., Da-Wen S. 2006. Learning techniques used in computer vision for food. *J. Food Eng.* 72:39-55.
5. Abramoff, M.D., Magalhaes, P.J. Ram, S.J. 2004. Image Processing with ImageJ. *J. Biofotonics.* 7:36-42.

THE USE OF FACE READING TECHNOLOGY TO PREDICT CONSUMER ACCEPTANCE OF BAKED GOODS

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ABSTRACT

Traditional sensory and consumer tests predict long term consumer acceptance of new products rather poorly, as evidenced by the high their failure rates in the market. These tests typical reflect conscious processes whereas consumer acceptance may also be based on unconscious processes. The measurement of emotions can provide a common lexicon for sensory and marketing to communicate and for product development that meet a marketing need.

The aims of this work were to examine whether emotional facial expressions measured with the Noldus FaceReader technology are a sufficiently accurate measure for differentiating between between various types of wheat bread, and to investigate whether facial reactions are able to explain liking ratings on hedonic scales.

Naive consumers (mean age 22 years) were recruited at the Kaunas University of Technology. They were asked to rate the sample with an intentional facial expression, which was recorded and then characterized by FaceReader 5 software (Noldus Information Technology, Wageningen, The Netherlands). The measurements showed significant differences between facial expressions elicited by the different bread samples and reflected the introspective liking ratings well. The positive correlations of "happy", and negative correlations of "sad" expression intensity against self-reported liking ratings suggest that these may be the most valuable descriptors for explaining the self-reported hedonic quality of baked goods. It can be concluded that Noldus FaceReader technology is sufficiently accurate to detect significant differences in facial expressions elicited by different samples of baked goods and can deliver additional information to conventional acceptance tests.

Keywords: wheat bread, facial expressions, FaceReader, hedonic liking

INTRODUCTION

Around the world bread is a staple food in almost every single diet. Breads come in many forms and types and are consumed with every meal of the day, therefore accurate measure of consumer acceptance is very important. However, “standard” sensory and consumer tests, which typically include sensory analytical profiling and liking tests, have a low predictive validity with respect to general product performance. Possibly, consumer food choice outside the laboratory may be less based on cognitive information processing and rational reasoning, and more on unarticulated/unconscious motives and associations [1, 2]. Reasons for likes or dislikes of specific foods are typically difficult to articulate but may determine much of our food choice. Certain foods are more attractive than other foods because for some reason they make us feel good – i.e. they trigger positive emotions.

For some time, sensory practitioners within the commercial sector have looked for better means to connect with marketing. The measurement of emotions might help in the further connection of sensory science and marketing. The measurement of emotions also serves as a further tool to support product development. Measurement of emotions allows us to compare existing products, and measure the emotional response to product prototypes. In these ways, the measurement of emotions can provide a common lexicon for sensory and marketing to communicate and for product development that meet a marketing need. Emotions can be the common language to bring these areas together.

There are many studies showing that tastes and odors elicit different emotions and facial reactions in neonates [3] children [4, 5] and adults [6, 7]. In most of these studies quite intense stimuli were used, like for example, concentrated basic taste solutions [7] or odours ranging from fruity to fecal [4]. The study of emotions in relation to food choice has recently advanced by work of King and Meiselman [8] and De Smet and Schiffertein [9]. Their results suggest that most emotions related to food are mildly positive, are only partly related to liking, and improve the predictions of food choice.

Facial expressions can be analyzed with the anatomically based Facial Action Coding System (FACS). These FACS analyses are very time-consuming and require trained observers. To overcome these difficulties, different automated facial expression recognition systems like Nviso (nViso SA, Lausanne, Switzerland), Affdex (Affectiva Inc., Waltham, USA) and FaceReader (Noldus Information Technology, Wageningen, The Netherlands) have been developed. These systems are capable of analyzing facial expression patterns from video data. Currently, these systems are used mainly for research in the fields of psychology, education, market research and consumer behavior. De Wijk et al. [10] analyzed the facial expressions elicited by the prospect of tasting or smelling liked or disliked food with FaceReader. The first sight of disliked foods compared to liked foods resulted in increased facial expressions of sadness, disgust, and anger. However up until now, little work has been published about the measurement of facial expressions elicited directly by the actual tasting of food products using facial expression recognition technology. Danner et al. [11] examined consumers' facial reactions elicited by the flavor of orange juice products using FaceReader

technology in implicit and an explicit measurement approach. Both, implicit and explicit measurements showed significant differences between facial expressions elicited by the different samples. The explicit measurement reflected the introspective liking ratings well. Especially “happy” and “disgusted” showed a high correlation with liking and were good indicators for liked and disliked samples, respectively. To minimize artifacts, caused by eating and drinking, which can be easily misinterpreted by the FaceReader software as emotion, they used liquid samples (juice) which need less processing in the mouth than solid samples.

The aims of this work were to examine whether facial expressions measured with the Noldus FaceReader technology are a sufficiently accurate measure for differentiating between solid samples – various types of wheat bread, and to investigate whether facial reactions are able to explain bread liking ratings on hedonic scales.

MATERIALS AND METHODS

Samples and sample preparation

Six different types of wheat bread were tested in the experiment. Each set of tested breads was made from 3 to 4 samples with significant differences in flavor or texture (Table 1). All samples were presented in a sequential way, at room temperature (20-22 °C), randomized and coded. Water was provided to rinse the mouth before and between tasting the samples. Some of bread samples were produced in the Bread Laboratory at Center of Excellence for Food Science and Technology, Kaunas University of Technology, others were commercial bread delivered by Lithuanian bakeries.

Table 1. Wheat bread (WB) samples

Wheat bread (WB) description	Significant difference
WB with barley	Barley products: groats, flakes, chopped grains
WB with buckwheat	Buckwheat products: groats, flakes, flour
WB with oats	Oat products: groats, flakes, flour
WB with potatoes	Amount of potatoes (15% and 30%)
WB with sugar	Amount of sugar (2%, 4% and 7%)
Wheat buns with felling	Fellings (pear, raspberry, apricot)

Participants and measurements

The number of participants in testing of each type bread samples varied from 12 to 20. All participants were students of the Department of Food Technology, Kaunas University of Technology, with an average age of 22 years.

The experiments took place in the Sensory Laboratory at Kaunas University of Technology. At first, participants were introduced to the procedure for tasting. They were asked to taste the whole presented bread sample (10 g) at once, take some seconds to think about an impression of it, then give a signal with their right hand and visualize the taste experience of the sample with a facial expression best representing their liking of the sample (explicit measurement). Afterwards, they rated their liking or disliking of the sample on a 7-point hedonic scale, ranging from 1 (dislike extremely) to 7 (like extremely).

The whole procedure was filmed with a Microsoft LifeCam Studio webcam, mounted on the laptop facing the participants, using Media Recorder (Noldus Information Technology, Wageningen, The Netherlands) software. The recordings with a resolution of 640×480 at 25 frames per second were saved as AVI files and analyzed frame by frame with FaceReader 5 software (Noldus Information Technology, Wageningen, The Netherlands), scaling the 6 basic emotion patterns (“angry”, “happy”, “disgusted”, “sad”, “scared”, “surprised”) and “neutral” from 0 (not present at all) to 1 (maximum intensity of the fitted model). For each sample, the section of intentional facial expression (exactly from the point when the subject had finished raising their hand to give the signal until the subject started lowering the hand again) was extracted and used for the statistical analysis. FaceReader contains an image quality bar, which gives a good indication of how well the program is able to model the face depicted in the image. For the best image quality, the main attention was focused on camera position and illumination. For this reason, participants were asked to sit and look frontally into the camera. All of the participants agreed to the use of their data in the context of this experiment.

Statistical analysis

For the statistical analysis, the maximum values of the facial expression patterns (“angry”, “disgusted”, “happy”, “neutral”, “sad”, “scared”, “surprised”) of the respective section were used. To examine the correlation between facial expressions and the hedonic liking, a Multiple Linear Regression was performed. All analyses were performed with STATISTICA V10 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Results presented in Fig. 1-3 showed that there were significant differences in facial expressions between tasted types of bread as well as between the samples of the same bread type. Tested types of bread differ significantly concerning the emotion patterns

“happy”, “sad” and “angry” (Fig.1). The similar tendencies were found during the analysis of the samples of the same bread type (Fig. 2 and Fig. 3). No significant differences in other facial expressions between tested bread samples were observed.

Tests showed that the wheat bread with sugar elicited significantly more intense facial reactions of “happy” than all other breads. Bread with buckwheat elicited the strongest “sad” and “angry” facial expressions, significantly differing from all others. The statistical analyses of the self-reported liking ratings identified the wheat buns with feeling as the most liked sample and the bread with buckwheat as the least liked. The data collected suggest that emotional intensity sometimes tracks with acceptance, and sometimes differs. For example, the acceptances of wheat buns with apricot and pear 2 fellings do not track with the emotion profile (Fig.2). Thus emotions might help to explain acceptance data and why acceptance data might not always predict market success.

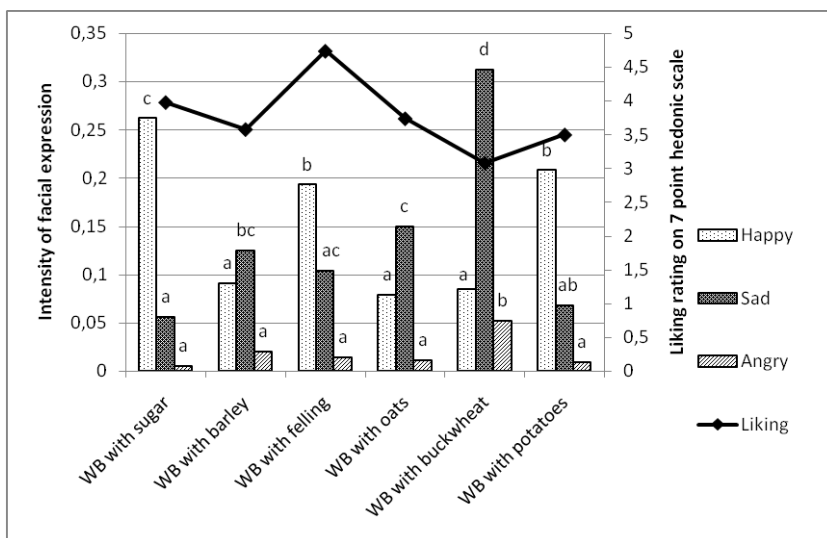


Figure 1. Intensity of facial expressions and self-reported liking of different types of wheat bread (WB)

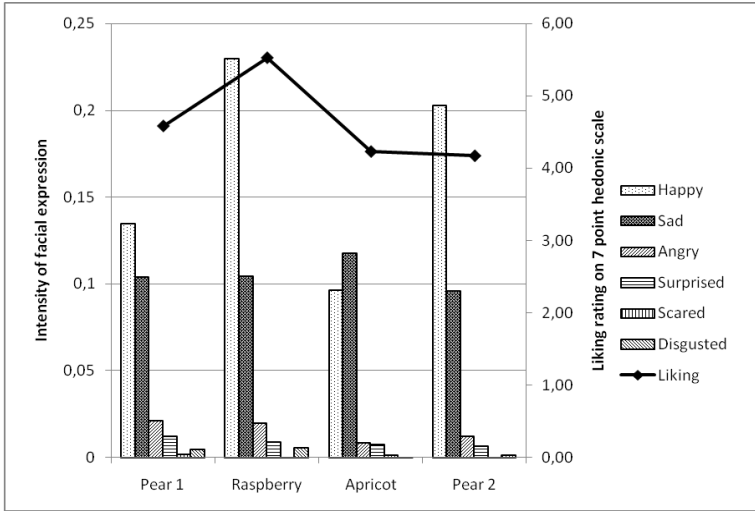


Figure 2. Intensity of facial expressions and self-reported liking of wheat buns with different fillings

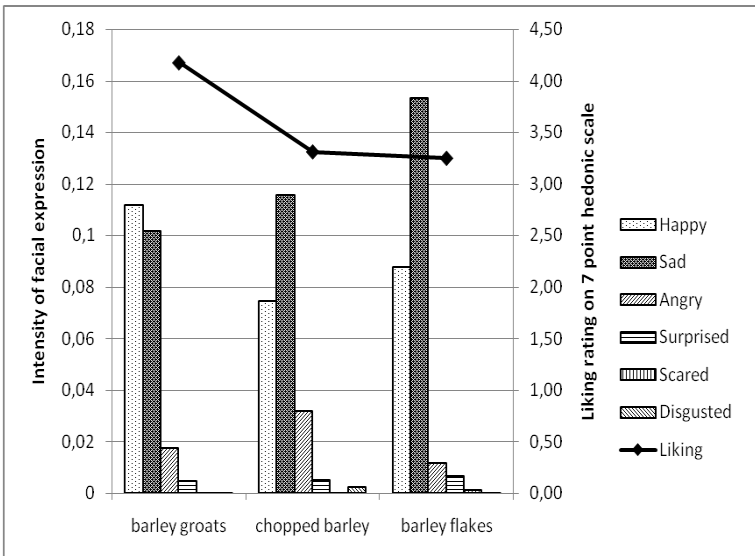


Figure 3. Intensity of facial expressions and self-reported liking of wheat bread with different barley products

While the measurement of emotions gives new information beyond acceptance, it is nevertheless interesting to relate emotions and acceptance. Linear regressions of the mean values of the facial expressions showed positive correlations of “happy”, and negative correlations of “sad” (Table 2). This indicates that liked samples elicited more intense facial expressions of “happy” than disliked samples.

Table 2. Regression of facial expression intensity against self-reported liking of different wheat bread (WB) samples

WB type	Emotions						
	N	H	Sa	A	Su	Sc	D
WB with barley	-0,90	0,68	-0,43	-0,19	-0,59	-0,84	-0,43
WB with buckwheat	0,09	0,31	-0,36	0,77	0,86	0,24	-0,46
WB with oats	-0,62	0,29	-0,21	-0,51	-0,29	-0,42	-0,95
WB with potatoes	0,62	0,98	-0,99	-0,99	-0,94	0,95	-0,94
WB with sugar	-0,29	0,41	-0,82	-0,70	0,97	-0,07	0,93
Wheat buns with felling	-0,98	0,75	-0,16	0,68	0,36	0,02	0,84

N – neutral; H – happy; Sa – sad; A – angry; Su – surprised; Sc – scared; D –disgusted.

This study shows that measuring facial expressions using Noldus FaceReader 5 is a sufficiently accurate method to differentiate between various wheat bread samples. A discrimination between liked and disliked samples was possible on the basis of the intensity of elicited “happy” and “sad” facial expressions. This supports the findings of Danner et al. [11] who used the FaceReader technology for study of orange juice and found high correlation of facial reactions “happy” and “disgusted” with liking.

It should also be mentioned that major differences in the intensity of facial reactions between participants were observed. The participants could be divided into two groups, the ones who showed clearly visible facial reactions when tasting the samples and those who had a “poker face” showing only little to almost no facial reactions. This can partially be attributed to the sensory laboratory test setup, where the participants are facing an unfamiliar environment and may feel stressed to a certain degree, or are very concentrated on the task. Further examinations in a more natural environment, also if possible without directly asking the participants to rate the products, could be interesting.

It is also important to point out the limitations and requirements of FaceReader technology. It does not work with children below the age of three. Pose, movement and rotation of the test person are limited. The test person needs to face the camera head on (angle <40°). The face must not be partially obscured by hair or when handling samples.

Motor artifacts, caused by eating and drinking, can be misinterpreted by the FaceReader software as emotion. In more complex tasting situations, like full meals that involve longer and potentially overlapping oral processing actions, motor artifacts can compromise the measurement of facial expressions to a higher degree.

CONCLUSIONS

It could be concluded that Noldus FaceReader technology is sufficiently accurate to detect significant differences in facial expressions elicited by different samples of wheat bread and can deliver additional information to conventional acceptance tests. However, more research is needed to see how this technology performs in more complex testing procedures, simulated or "real life" environments.

The positive correlations of "happy", and negative correlations of "sad" expression intensity against self-reported liking ratings suggest that these may be the most valuable descriptors for explaining the self-reported hedonic quality of baked goods.

ACKNOWLEDGEMENTS

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REFERENCES

1. Dijksterhuis, A., and Smith, P. K. 2005. What do we do unconsciously? And how? *J. Consum. Psychol.* 15:225–229.
2. Köster, E. P. 2009. Diversity in the determinants of food choice. A psychological perspective. *Food Qual. Prefer.* 20:70–82.
3. Soussignan, R., Schaal, B., Marlier, L., and Jiang, T. 1997. Facial and autonomic responses to biological and artificial olfactory stimuli in human neonates: Reexamining early hedonic discrimination of odors. *Physiol. Behav.* 62:745–758.
4. Soussignan, R. 1996. Children's facial responsiveness to odors: Influences of hedonic valence of odor, gender, age, and social presence. *Dev. Psychol.* 32:367–379.
5. Zeinstra, G. G., Koelen, M. A., Colindres, D., Kok, F. J., and De Graaf, C. 2009. Facial expressions in school-aged children are a good indicator of "dislikes", but not of "likes". *Food Qual. Prefer.* 20:620–624.

6. Greimel, E., Macht, M., Krumhuber, E., and Ellgring, H. 2006. Facial and affective reactions to tastes and their modulation by sadness and joy. *Physiol. Behav.* 89:261–269.
7. Wendin, K., Allesen-Holm, B. H., and Bredie, W. L. P. 2011. Do facial reactions add new dimensions to measuring sensory responses to basic tastes? *Food Qual. Prefer.* 22:346–354.
8. King, S. C., and Meiselman, H. L. 2010. Development of a method to measure consumer emotions associated with foods. *Food Qual. Prefer.* 21:168–177.
9. De Smet, P. M. A., and Schiffertein, H. N. J. 2008. Sources of positive and negative emotions in food experience. *Appetite.* 50:290–301.
10. De Wijk, R. A., Kooijman, V., Verhoeven, R. H. G., Holthuysen, N. T. E., and De Graaf, C. 2012. Autonomic nervous system responses on and facial expressions to the sight, smell, and taste of liked and disliked foods. *Food Qual. Prefer.* 26: 196–203.
11. Danner, L., Sidorkina, L., Joechl, M., and Duerrschmid, K. 2013. Make a face! Implicit and explicit measurement of facial expressions elicited by orange juices using face reading technology. *Food Qual Prefer.* <http://dx.doi.org/10.1016/j.foodqual.2013.01.004>.

WHAT KIND OF CEREALS STUDENTS FROM UNIVERSITY OF ZAGREB EAT FOR BREAKFAST?

UDC 664.696-057.875(497.521.2)

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ABSTRACT

Breakfast, as a first meal of the day has shown to improve health and the cognitive abilities of individuals who consume it, especially when it is composed of high-quality nutrients. The aim of this study was to determine what kind of cereals students eat for breakfast and how this affects their micronutrient intake. The subjects were 84 female undergraduates of the Nutrition studies aged from 18 to 24. Questionnaire was used to determine usual breakfast pattern. The results show that all subject consume breakfast at least 2 days a week, yet only 54% of them eat breakfast every day. Usually, 56% of subjects eat breakfast cereals (ready-to-eat or cooked cereals), 21% eat whole wheat bread, 15% eat white bread and 4% eat pastry. Average energy intake from breakfast was 395±157 kcal. Subjects who eat breakfast cereals (ready-to-eat or cooked) had higher dietary fiber, thiamine, folate, calcium and iron intake than those who eat bread or pastry ($p<0.05$). Since breakfast cereals are often fortified, having them for breakfast may be a good strategy to reduce risk of dietary inadequacy of certain micronutrients.

Keywords: breakfast, cereals, micronutrients

INTRODUCTION

Breakfast consumption is considered an important determinant of a healthy dietary behaviour [1]. As a first meal of the day breakfast provides energy, as well as macro and micronutrients after an overnight fast. Studies have shown that regular consumption of breakfast improves cognitive function related to academic performance [2, 3], while skipping breakfast is associated with higher body mass index [4], higher body adiposity and abdominal obesity [5]. However, some researchers found that not only breakfast consumption itself is associated with body mass index but that the type of food eaten for breakfast is crucial [6]. Grains are the most popular food eaten for breakfast all over the world, however the type of grains, such as whole grains, eaten for breakfast make a big difference. Whole grains are good source of vitamins, minerals, fibre and bioactive

phytochemical and may reduce risk for chronic disease. Consumption of breakfast cereals is inversely associated with weight gain and body mass index [7] and it had been shown that consumption of cereals for breakfast has positive effect on overall nutrient intake [8] and lower serum cholesterol level [9]. The aim of this study was to determine what kind of cereals students eat for breakfast and how this affects their micronutrient intake.

SUBJECTS AND METHODS

Subjects were 84 female undergraduates of the Nutrition studies from University of Zagreb aged from 18 to 24. Questionnaire was used to determine usual breakfast pattern and breakfast eating frequency.

The subjects were asked to record detailed information regarding the types and the amounts of all foods and beverages consumed as usual breakfast pattern. This included the brand name of food (where appropriate) and preparation method. When portion size was unknown it was estimated by cups or tablespoons. Nutritional value of breakfast was calculated for each participant using food composition table [10], while for breakfast cereals were taken from food label. Breakfasts were divided into 5 different categories depending on the type of cereals which are consumed: "white bread" (white and corn bread); "whole grain bread" (breads made from germ and bran wheat, oat, barley or rye flour); "breakfast cereal" (ready-to-eat or cooked cereals); "pastry" (doughnuts, croissants, cakes and biscuits); "other" (non-cereal products).

Results are presented as mean \pm SD, or percentage. Differences between categories were assessed using ANOVA followed by post-hoc Tukey test. All statistical analyses were performed with SPSS software v.17 (Chicago, IL, USA).

RESULTS AND DISCUSSION

The results of this study show that none of the subjects skip breakfast on weekly basis, however the lowest frequency of breakfast consumption is two days a week. Also, only 54% of the subjects consume breakfast every day. These results are in accordance with the previous research on Croatian university students [11] where it was concluded that regardless of gender, breakfast is the most frequently skipped meal of the day. Studies in other countries also showed that breakfast is frequently skipped by students [12]. Possible reasons for skipping breakfast could be the lack of time, being late to class, getting up late, and not being hungry in the morning [12, 13].

Distribution of subjects according to breakfast category (Figure 1) showed that 96% of the subjects consume some kind of cereal for breakfast what is in accordance with previous research conducted among Croatian students [14].

The highest number of subjects (56%) consume breakfast cereals (cooked or ready-to-eat). Nutritionally, breakfast cereals are high in carbohydrate, low in fat and if fortified contain significant amount of vitamins and minerals. Since they are usually consumed

with dairy products, such as milk or yogurt, they enhance calcium intake and therefore may reduce risk for osteoporosis and poor nutritional status [15].

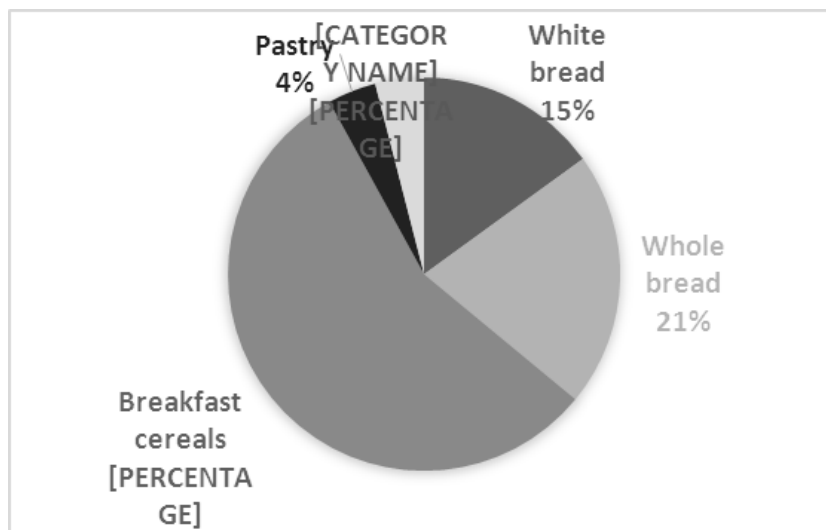


Figure 1. Distribution of subjects according to breakfast category (n=84)

Second most frequent cereal consumed for breakfast is bread. Whole grain bread is consumed by 21% of the subjects, while 15% consume white bread (Figure 1). Due to high concentration of fiber, minerals, vitamins, lignans and phenolic compounds in whole grains, studies have shown that consumption of whole grains is associated with decreased risk of cardiovascular disease, diabetes mellitus, cancer and obesity [16,17]. Most of these nutrients are eliminated during milling process for white flour production, therefore whole grain bread is more desirable for consumption than white bread.

Additionally, the results showed that only 4% of the subjects consume pastry for breakfast. However, the consumption was on a daily basis, which was not expected since the subjects are nutrition students and should be aware that pastry breakfast represents a high calorie, high-fat and high-carbohydrate meal.

Average energy intake from breakfast was 395 ± 157 kcal. Breakfast based on pastry had as expected the highest caloric value (512 ± 162 kcal), while breakfast categorized as Other, which include non-cereal food item such as fruits or dairy products, had the lowest caloric value (63 ± 14 kcal). No statistical difference were observe between caloric value of breads and cereals categories (ANOVA, $p > 0.05$).

Average intake of fibre and micronutrients is shown in Table 1. Thiamine, folate, calcium, and iron intake was higher among Cereal breakfast eaters ($p < 0.001$) than in other breakfast categories.

Table 1. Average nutrients intake at breakfast according to breakfast category

Nutrients	Breakfast category					P*
	White bread	Whole bread	Breakfast cereals	Pastry	Other	
Dietary fibre (g)	3.0±0.8a	5.5±2.8b	4.5±2.6b	5.8±1.3b	2.3±1.2a	<0.001
Thiamine (mg)	0.2±0.1a	0.4±0.2	0.4±0.3b	0.1±0.1	0.0±0.0	0.006
Riboflavin (mg)	0.4±0.2a	0.6±0.4b	0.5±0.2	0.3±0.1	0.0±0.0	0.001
Folate (µg)	63.7±21.5a	70.9±40.8b	82.0±46.6d	37.6±21.5c	60.4±67.6a	0.018
Calcium (mg)	262.0±195.3	316.0±115.4	377.1±288.4a	196.3±141.9	16.6±12.9b	0.028
Iron (mg)	1.3±0.8a	2.5±1.3b	3.0±1.2c	1.5±0.8	0.1±0.2	<0.001

Values are expressed as means ± S.D.

Means with different letters were significantly different (* $P < 0.05$, ANOVA, Tukey-HSD).

Ready-to-eat cereal eaten by subject are fortified with several nutrient including folic acid, riboflavin, niacin, vitamin B6, and iron. Study conducted among Irish adults population shown that fortified breakfast cereals contributed significantly to mean daily intakes of iron, thiamine, riboflavin, niacin, vitamin B6, total folate and vitamin D [18].

Higher consumption of breakfast cereal was been associated with better vitamin status and lower total and low-density lipoprotein cholesterol [19]. The importance of daily breakfast consumption as well as healthy breakfast choices, such as breakfast cereals, should be encourage among these population.

CONCLUSION

Only 54% of the subjects consume breakfast every day. 96% of the subjects consume some kind of cereal for breakfast. The highest number of subjects (56%) consume breakfast cereals (cooked or ready-to-eat).

Subjects who eat cereals (ready-to-eat or cooked) had higher dietary fiber, thiamine, folate, calcium and iron intake than those who eat bread or pastry ($p<0.05$). Since breakfast cereals are often fortified, having them for breakfast may be a good strategy to reduce risk of dietary inadequacy of certain micronutrients.

REFERENCES

1. Affenito, S.G. 2005. Breakfast: a missed opportunity. *J. Am. Diet. Assoc.* 107:565-569.
2. Rampersaud, G. C., Pereira, M. A., Girard, B. L., Adams, J., and Metz, J. D. 2005. Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *J. Am. Diet. Assoc.* 105:743-760.
3. Taras, H. 2005. Nutrition and student performance at school. *J Sch Health.* 75:199-213.
4. Gibson, S.A. and O'Sullivan, K.R. 1995. Breakfast cereal consumption patterns and nutrient intakes of British schoolchildren. *J. R. Soc. Health* 115:336-370.
5. Nurul-Fadhilah, A., Teo, P.S., Huybrechts, I. and Foo, L.H. 2013. Infrequent Breakfast Consumption Is Associated with Higher Body Adiposity and Abdominal Obesity in Malaysian School-Aged Adolescents. *PLoS ONE* 8(3): e59297. doi:10.1371/journal.pone.0059297.
6. Cho, S., Dietrich, M., Brown, C.J., Clark, C.A. and Block, G. 2003. The Effect of Breakfast Type on Total Daily Energy Intake and Body Mass Index: Results from the Third National Health and Nutrition Examination Survey (NHANES III). *J. Am. Coll. Nutr.* 22:296-302.
7. Bazzano, L.A., Song, Y., Bubes, V., Good, C.K., Manson, J.E. and Liu, S. 2005. Dietary Intake of Whole and Refined Grain Breakfast Cereals and Weight Gain in Men. *Obes. Res.* 13:1952-1960.
8. Barton, B.A., Eldridge, A.L., Thompson, D., Affenito, S.G., Striegel-Moore, R.H., Franko, D.L., Albertson, A.M. and Crockett, S.J. 2005. The relationship of breakfast and cereal consumption to nutrient intake and body mass index: the National Heart, Lung, and Blood Institute Growth and Health Study. *J. Am. Diet. Assoc.* 105:1383-9.
9. Stanton, J.L. and Keast, D.R. 1989. Serum cholesterol, fat intake, and breakfast consumption in the United States adult population. *J. Am. Coll. Nutr.* 8:226-229.

10. Möller, A., Saxholt E., Christensen, A. T., Hartkopp, H. B. and Hess Ygil, K. 2005. Danish Food Composition Databank, revision 6.0. Food Informatics, Department of Nutrition, Danish Institute for Food and Veterinary Research.
11. Colić Barić, I., Šatalić, Z. and Lukešić, Ž. 2003. Nutritive value of meals, dietary habits and nutritive status in Croatian university students according to gender. *Int. J. Food Sci. Nutr.* 54:473-484.
12. Ozdogan, Y., Ozcelik, A.O. and Surucuoglu, M.S. 2010. The Breakfast Habits of Female University Students. *Pak. J. Nutr.* 9:882-886.
13. Shaw, M.E. 1998. Adolescent breakfast skipping: an Australian study. *Adolescence* 33:851-861.
14. Krešić, G., Kendel Jovanović, G., Pavičić Žeželj, S. and Magdić, I. 2007. The association among food eaten for breakfast, daily energy and cereals intake in student population. Page 372 in Proceedings of 4th international congress FLOUR-BREAD '07, 6th Croatian congress of cereal technologists, Opatija, Croatia.
15. Song, W.O. Chun, O.K, Kerver, J., Cho, S., Chung, C.E. and Chung S.J. 2006. Ready-to-eat breakfast cereal consumption enhances milk and calcium intake in the US population. *J. Am. Diet. Assoc.* 106:1783-1789.
16. Slavin, J. 2004. Whole grains and human health. *Nutr. Res. Rev.* 17:99-110.
17. Jacobs, D.R, Meyer, H.E. and Solvoll, K. 2001. Reduced mortality among whole grain bread eaters in men and women in the Norwegian County Study. *Eur. J. Clin. Nutr.* 55:137-143.
18. Galvin, M.A., Kiely, M. and Flynn, A. 2003. Impact of ready-to-eat breakfast cereal (RTEBC) consumption on adequacy of micronutrient intakes and compliance with dietary recommendations in Irish adults. *Public Health Nutr.* 6:351-363.
19. Gibson, S. 2003. Micronutrient intakes, micronutrient status and lipid profiles among young people consuming different amounts of breakfast cereals: further analysis of data from the National Diet and Nutrition Survey of Young People aged 4 to 18 years. *Public Health Nutr.* 6:815-820.

ULTRASOUND ASSISTED EXTRACTION OF SOLUBLE DIETARY FIBER FROM CEREAL BRAN

UDC 633.16: 543.5

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ABSTRACT

The major component of soluble dietary fiber from cereal grains especially from barley are composed of mixed linkage (1→3) (1→4) β -glucan. In this research, barley bran was used as a source for β -glucan extraction. For this purpose two different technique of extraction were used: mechanical-water and ultrasound bath assisted extraction. The effect of temperature, extraction time and amplitude has been studied for ultrasound bath assisted extraction. Water assisted extraction took 2 hours with ethanol pretreatment (inactivation of β -glucosidase) while application of ultrasonic device (37 kHz) extraction took between 7-23 minutes without ethanol pretreatment.

Conventional mechanical-water extraction resulted with lowest amount of extracted β -glucan (BGE=0.4200 g/100g) and extraction yield (Y1=27.11%). Extraction yield and extracted β -glucan for ultrasound assisted extraction was in the range of 48.70-98.52% and 0.7546-1.5267 g/100g, respectively. For ultrasound assisted extraction temperature and amplitude showed a strong influence on extraction process. The best extraction yield (98.52% and 98.14%) were observed on 45 °C by application of 60 and 100% of amplitude. Also, all studied cases with application of ultrasound assisted extraction resulted with 1.80-3.63 time higher efficiency of extraction (Y2) than conventional mechanical water extraction.

Keywords: ultrasound, extraction, barley, β -glucan

INTRODUCTION

Soluble dietary fiber from cereal grains are mainly composed of mixed linked (1→3) (1→4) β -glucan. The major influence on concentration of β -glucan in barley are connected to thickness of the cereal cell wall and activity of β -glucanase [1]. β -glucan from barley positively influence human health by lowering plasma cholesterol, improving lipid metabolism and reducing glycemic index [2-4]. Conventional extraction methods (wet techniques) for β -glucan extraction include inactivation of β -glucanase and extraction with water or alkali solution. Extraction yield and efficiency of extraction are depending of temperature, extraction time, solvent:flour ratio, pH, particle size, stirring rate and solvent [5]. To avoid gelatinization of starches, temperature of extraction should not be higher than 55 °C [6]. In order to improve extraction yield and

to reduce time of treatment, novel technology (ultrasound probe assisted extraction and pressurized hot water extraction) have been applied in recent time [7, 8]. Application of those types of extraction have been resulted with higher extraction yield and with shorter time of extraction.

In the present study, ultrasound bath assisted extraction of β -glucan from barley bran was investigated. This is an inexpensive, simple and efficient method for use in extraction process. By using this kind of extraction, increasing of contact surface area between solid and liquid phase are present and at the same time greater penetration of solvent into the barley matrix are allowed [9]. Influence of operation variables (temperature, time and amplitude) on extracted β -glucan, extraction yield and efficiency of extraction from barley bran using ultrasound bath assisted extraction was investigated, statistically analyzed and optimized using response surface methodology (RSM), by application of three-level, three-variable central composite design in order to obtain the optimal condition for the extraction of β -glucan from barley bran.

MATERIALS AND METHODS

Barley bran was provided by BiVita (Božjakovina d.d., Croatia). Chemical composition of barley grain was determined: 8.74% of moisture content, 12.43% of proteins, 3.79% of fats, 6.18% of ash, 48.14% of total dietary fiber (45.5% non-soluble and 2.64% soluble) and 1.5495 g/100g of β -glucan (BGE_{TOTAL}). Barley bran was milled in laboratory mill (Labinka) and particle size distributions were determined. For the purpose of the research particular size $\leq 455 \mu m$ was used. Moisture content was determined according to ISO 712:2010 method, ashes content according to ISO 2171:2010 method, fat content according to ISO 6492:2001, protein content according to ISO 1871:1999 (ISO 1871:1975 (E) ICC 105/2). Total dietary fiber and the amount of β -glucan was determined using assay kit "Integrated total dietary fiber" and "Mixed-linkage β -glucan" from Megazyme International Ltd., respectively.

Extraction of β -glucan from barley bran was performed with two different techniques. First one was mechanical water extraction (conventional extraction process). For this purpose the milled barley bran was suspended in water (10% suspension) and extraction was performed in bioreactor (Chemap AG) on 55 °C for 3 hour. Stirring rate was set on 600 rpm. Extracted barley bran was centrifuges (10 min/5000 rpm) and dried overnight in drying oven and solid phase was used for determined β -glucan content. Pretreatment was also performed in order to inactivate β -glucanase (milled barley bran was suspended in ethanol (80% v/v) under reflux for 2 hour). After pretreatment centrifugation was applied (10 min/5000 rpm), barley bran was dried overnight and once again mill (partical size $\leq 455 \mu m$). Ultrasound bath assisted extraction (Elmasonic P, 380 W) was performed at working frequency of 37 kHz and this extraction represents the second technique that was used in this work. The same suspension (10%) of barley bran in water was treated on different temperature (28-62 °C), time (7-23 min) and amplitude (60-100%). Solid-liquid mixture was centrifuged (10 min/5000 rpm), dried overnight and solid phase was used for the determination of β -glucan content.

For the experimental design central composite design was used (Table 1). Three factors at three levels were obtained: temperature (28-62 °C), time (7-23 min) and amplitude (60-100%). The upper and lower limits of these levels were selected based on preliminary testing. The experiment of ultrasound bath assisted extraction was set to 20 run, with 6 repetition of the central point of the experimental design. Three response (depending) variables: extracted β -glucan (BGE; g/100g), extraction yield (Y_1 ; %) [eq.1] and efficiency of extraction ($Y_2 = \text{BGE}/\text{BGE}_{\text{mech.extraction}}$), were considered in the optimization process. Response surface methodology (RSM) was used to determined mathematical model of significant variable (temperature, time and amplitude) on effectiveness ultrasonic extraction. The response of each investigated parameters was analyzed by fitting quadratic models with interaction of investigated variables to the data with backward elimination regression in order to identify significant ($p < 0.01$) effects of the variations of parameters on the responses. Significance of the lack-of-fit error term, R2 value, coefficient of variation and model significance were used to judge adequacy of model fit (F-test has to show significant effects ($p < 0.05$) and lack-of-fit insignificant ($p > 0.05$). At the same time the multiple regression coefficient R2 (in the range 0-1) represents the power of fit and is a measure of how well the regression model fits the raw data (if 1 it is a perfect model). Base on this, the best solution was chosen. For optimization of BGE, Y_1 and Y_2 , a multiple response method was applied with maximize of those three variable.

$$Y_1 = \frac{\text{BGE}_{\text{TOTAL}} - \text{nonextracted}\beta - \text{glucan}}{\text{BGE}_{\text{TOTAL}}} * 100 \quad (1)$$

Table 1. Experimental conditions (temperature (T), time (t) and amplitude (A)) for the ultrasound bath assisted extractions (Run 1-20)

Run	T (°C)	t (min)	A (%)	Run	T (°C)	t (min)	A (%)
1	28	15	80	11	45	7	80
2	35	10	90	12	45	23	80
3	35	20	70	13	45	15	80
4	35	10	70	14	45	15	80
5	35	20	90	15	45	15	60
6	45	15	80	16	55	20	90
7	45	15	80	17	55	20	70
8	45	15	80	18	55	10	70
9	45	15	100	19	55	10	90
10	45	15	80	20	62	15	80

The software Design Expert Version 7 (Stat-Ease, Inc., Minneapolis, U.S.A.) was used for statistical analysis. With the results of compositional analysis, analysis of Variance (ANOVA) and Fisher test was performed to determine significant differences between efficiency of β -glucan extraction and optimization of process was carried out.

RESULTS AND DISCUSSION

Raw barley grains are composed of 48.14 % of total dietary fiber (45.5% non-soluble and 2.64% soluble). Barley bran that was used in this work contains floral shell and this was a reason why a non-soluble dietary fiber are higher than usually (45.5%). As it is known, β -glucan are soluble dietary fiber, so 57.7% of total soluble dietary fiber (2.64%) are composed of β -glucan ($BGE_{TOTAL}=1.5495$ g/100g). In Table 2 are summarized results for mechanical and ultrasound bath assisted extraction of β -glucan from barley bran.

Table 2. Experimental conditions (temperature (T), time (t) and amplitude (A)) and experimental results (extracted β -glucan (BGE), extraction yield (Y_1) and efficiency of extraction (Y_2) for mechanical water extraction (Run 0) and for the ultrasound bath assisted extractions (Run 1-20).

Run	T (°C)	t (min)	A (%)	BGE (g/100 g)	Y_1 (%)	Y_2 (-)
0	55	120		0.4200	27.11	
1	28	15	80	0.7546	48.70	1.80
2	35	10	90	1.2670	81.76	3.02
3	35	20	70	1.3648	88.08	3.25
4	35	10	70	1.1995	77.41	2.86
5	35	20	90	1.3208	85.24	3.14
6	45	15	80	1.2684	81.86	3.02
7	45	15	80	1.4909	96.22	3.55
8	45	15	80	1.3461	86.87	3.21
9	45	15	100	1.5208	98.14	3.62
10	45	15	80	1.4141	91.26	3.37
11	45	7	80	1.3823	89.21	3.29
12	45	23	80	1.3339	86.08	3.18
13	45	15	80	1.3798	89.05	3.29
14	45	15	80	1.3798	89.05	3.29
15	45	15	60	1.5267	98.52	3.63
16	55	20	90	1.2988	83.82	3.09
17	55	20	70	1.3049	84.21	3.11
18	55	10	70	1.2545	80.96	2.99
19	55	10	90	1.2475	80.51	2.97
20	62	15	80	0.9850	63.57	2.35

Mechanical water extraction (convention method) was performed in bioreactor (Chemap AG) on 55 °C for 3 hour, with stirring rate of 600 rpm. Total extracted β -glucan from barley bran with mechanical water extraction was 0.4200 g/100g with extraction yield of 27.11%. Mechanical water extraction that was reported earlier [7] resulted with extraction yield of 41.5%. In this resource the average particle size was $240 \pm 4 \mu\text{m}$ and stirring rate was set to 1000 rpm, so lower extraction yield (27.11%) in this work can be caused by different process parameter (600 rpm) and larger particle size ($\leq 455 \mu\text{m}$). The same authors in earlier work [5] have been conducted β -glucan extraction with convention water extraction from different barley cultivation and obtained extraction yields were in the range from 38.2-72.4%.

Result for the ultrasound bath assisted extractions for BGE, Y_1 and Y_2 are showed in Table 1. (Run 1-20). In all cases extraction yield (Y_1) was higher (48.70-98.14%) then extraction yield (27.11%) obtained with convention water-mechanical extraction. For all performed extractions efficiency of extraction (Y_2) was in the range from 1.80-3.62 and this is also higher than when conventional extraction is performed.

Analysis of response variables (T, t and A) on extracted β -glucan (BGE), yield extraction (Y_1) and efficiency of extraction (Y_2) was performed according to response surface methodology (RSM) by using a quadratic model with interaction of investigated variables. After the calculation was done for all coefficient of the model, the analysis of significant of the model was done with application of t-test. All insignificant coefficients were rejected from equation and without them a new significant coefficients was calculated. Mathematical models for extracted BGE (eq.2), extraction yields (eq.3) and efficient of extraction (eq.4) were tested with Fischer test ($p < 0.01$). Results of applied statistical analysis can be found in Table 2.

$$Y_{BGE} = 1,04908 + 0,15967 * T - 0,082645 * A - 1,74626 * 10^{-3} * T^2 + 5,16548 * 10^{-4} * A^2 \quad (2)$$

$$Y_{Y_1} = 67,54392 + 10,30414 * T - 5,32946 * A - 0,11269 * T^2 + 0,03331 * A^2 \quad (3)$$

$$Y_{Y_2} = 3,47679 + 0,36779 * T - 0,21497 * A - 4,02007 * 10^{-3} * T^2 + 1,34326 * 10^{-3} * A^2 \quad (4)$$

Influence of temperature (T) and amplitude (A) on extracted β -glucan (BGE) are presented on Figure 1. The highest content of extracted β -glucan was observed when ultrasound bath assistant extraction was performed on 45 °C by application of 60% and 100% of amplitude (Figure 1., Table 2.) and this two variables (T and A) showed a strong influence when ultrasound bath assistant extraction was performed. According to these results it is possible to extract almost all available β -glucan (BGE) from barley bran with

application of lower amplitude (60%) and in the same time it is possible to use less power for efficient extraction. Also, with application of ultrasound bath assistant extraction it is possible to extract more β -glucan (BGE) than with convention mechanical water extraction. In the same time this kind of extraction is much easier and faster (15 min compared to 3 hours) and there is no need to applied pretreatment of inactivation of β -glucanase with ethanol. Mathematical models (eq. 2-4) that was described earlier can be very useful and accurate in prediction of ultrasound bath assisted extraction of β -glucan from barley barn.

Table 3. Results of statistical analysis of investigated influence of ultrasound bath assisted extraction of β -glucan from barley bran

	R ²	Adj R ²	Pred R ²	F	p
BGE [g/100g]	0.9076	0.8830	0.7725	36.84	<0.0001
Y ₁ [%]	0.9076	0.8829	0.7724	36.84	<0.0001
Y ₂ [-]	0.8840	0.8530	0.7368	28.57	<0.0001

Design-Expert® Software

BGE
1.5267
0.7546

X1 = A: T
X2 = C: A

Actual Factor
B: t = 15.00

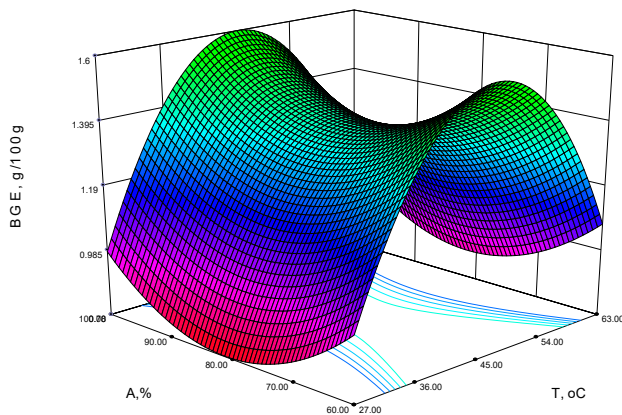


Figure 1. Influence of temperature (T) and amplitude (A) on β -glucan extraction (BGE) from barley bran

Optimization of mathematical model (Figure 2) showed different solution for efficient extraction of β -glucan. Optimization was performed with maximize value for BGE, Y_1 and Y_2 , while temperature and amplitude was in the range of 28–62 °C and 60–100%, respectively. 45 different combination were find to maximize value for BGE (1.5 g/100 g), Y_1 (99.54%) and Y_2 (3.6755)

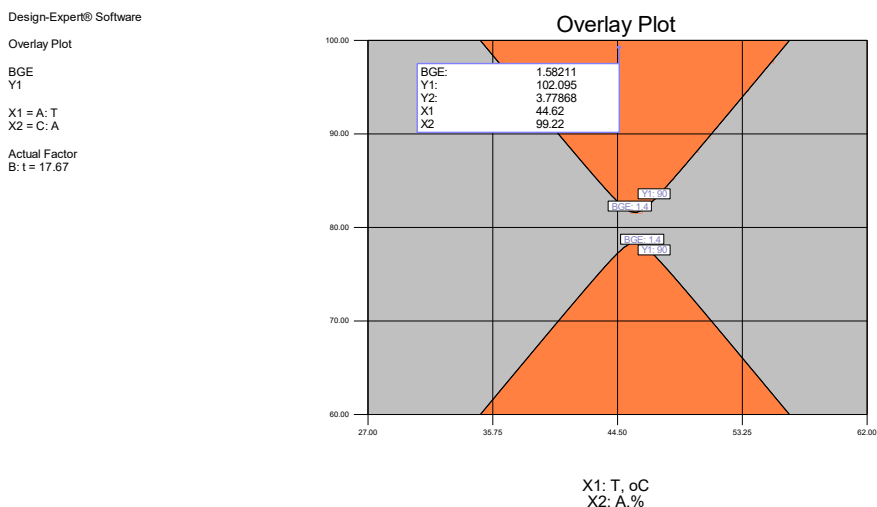


Figure 2. Model optimization and prediction of ultrasound bath assisted extraction of β -glucan from barley bran

Extraction of β -glucan from barley bran with ultrasound bath assisted extraction has showed to be more effective than ultrasound-assisted extraction with Ultrasonic processor (400 W, 24 kHz) equipped with 22 mm titanium probe [7]. In this work, extraction yield was in the range 40.5–66.1% ($T=55$ °C, $t=3-17$ min, $A=60-100\%$ and cycle=0.8–1%) and pretreatment with ethanol was applied.

Research with application of ultrasound bath assistant extraction has showed that application of this technology has a promising future. Also, it is important to applied modern analytical analysis, like HPLC, in future research, in order to investigate the effect of ultrasound on the molecular weight of β -glucan.

CONCLUSION

β -glucan extracted from the barley bran is value product. Ultrasound bath assisted extraction of β -glucan from barley bran has showed as very efficient method. In all cases when this type of extraction was performed extraction yield was higher (48.70–98.14%)

then extraction yield (27.11%) obtained with convention water-mechanical extraction. Also, efficiency of extraction was in the range 1.80-3.62 (Y₂). With application of this technology it is possible to extract almost all available β -glucan from barley bran without performing of pretreatment with ethanol deactivation of β -glucanase. The highest content of extracted β -glucan was observed when ultrasound bath assistant extraction was performed on 45 °C by application of 60% and 100% of amplitude. This two variables (T and A) showed a strong influence when ultrasound bath assistant extraction was performed. Application of this technology is much easier and faster (15 min compared to 3 hours) then convention methods of extraction. Finally, application of this technology has a promising future, but further research should include modern analytical analysis, like HPLC, in order to investigate the effect of ultrasound on the molecular weight of β -glucan.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bhatti, R. S. 1997. Milling of regular and waxy starch hull-less barleys for the production of bran and flour. *Cereal Chem.* 74(6):693–699.
2. Cavallero, A., Empilli, S., Brighenti, F., Stanca, A.M. 2002. High (1 \rightarrow 3) (1 \rightarrow 4)- β - D-glucan fractions in bread making and their effect on human glycemic response. *J. Cereal Sci.* 36(1): 59–66.
3. Keenan, J.M., Goulson, M., Shamliyam, T., Knutson, N., Kolberg, L., Curry, L. 2007. The effects of concentrated barley β -glucan on blood lipids in a population of hypercholesterolaemic men and women. *Brit. J. Nutr.* 97:1162–1168.
4. Wood, P.J. 2007. Cereal β -glucans in diet and health. *J. Cereal. Sci.* 46:230-238.
5. Benito-Román, Ó., Alonso, E., Lucas, S. 2011. Optimization of the β -glucan extraction conditions from different waxy barley cultivars. *J. Cereal. Sci.* 53:271-276.
6. Fastrought, C.E., Berglung, P.T., Holm, E.T., Fox, G.T. 1996. Genetic and environmental variation in beta-glucan content and quality parameters for barley for food. *Crop Sci* 36:941-946.
7. Benito-Román, Ó., Alonso, E., Cocero, M.J. 2013. Ultrasound-assisted extraction of β -glucans from barley. *LWT-Food Sci. Technol.* 50:57-63.
8. Benito-Román, Ó., Alonso, E., Cocero, M.J. 2013. Pressurized hot water extraction of β -glucans from waxy barley. *J. of Supercritical Fluids* 73:120–125.
9. Rostagno, M.A., Palma, M., Barroso, C.G. 2003. Ultrasound assisted extraction of soy isoflavones. *J. Chromatogr. A* 1012:119–128.

COMPLIANCE OF THE BAKERY AND CONFECTIONERY PRODUCTS WITH THE CONTEMPORARY FOOD CONSUMPTION HABITS

UDC 664.66 : 664.64.016

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ABSTRACT

The bakery and confectionery products are in a constant focus of the massive population in accordance to their nutritional value, while the production and sale of this type of products are in permanent growth. The contemporary food consumption habits affect the whole food industry, thus the bakery and confectionery products are not an exception. The contemporary consumer requirements are basically related to a healthy aspect, the convenience, the sustainability and the authenticity of food products. The dark chocolate cakes, the cranberry muffins, a pumpkin pie, an apple pie and the cherry cakes, are some of the products which may satisfy this kind of market requirements. A new product acceptance by consumers' depends on their preferences. Therefore, it is important to record a consumers' reaction on the product. The aim of this work is to present a model for choosing ingredients for bakery and confectionery products in accordance to customers' preferences. The model is in complying with the quality management and continual improving process.

Keywords: bakery products, confectionery products, contemporary trends, quality management

INTRODUCTION

Bakery and confectionery products belong to the essential food group intended for the mass population. Moreover, the grain products are an important part of human food consumption history. It's not far from the truth, when some indicate that the beginnings of grain cultivation affected the contemporary civilization establishment. Hardly anyone, whatever is young or old, could resist the fantastic taste of chocolate cake or fresh pastry. However, circumstances change together with the habits of people, leading us to a situation where some of the world population starving, while some others eat too much.

From the time of the earliest civilizations, until the end of the twentieth century, white bread and cakes were reserved for the rich people. Recently, a health and diet experts encourage public to eat "peasant" bread from the history [1]. Contemporary habits of

consumers' are the result of increased awareness of the food related diseases by the educated public, and the fact that consumption of the specific food may lead individuals to the balanced diet and the healthy lifestyle.

The bakery and confectionery response to the contemporary trends are healthy, or less harmful: dark chocolate cake, different kinds of muffins with berry fruit, a pumpkin pie, carrot cake, non gluten bread and pastry, an apple pie, cherry pies and cakes etc. Any of these products is designed to satisfy upper mentioned criteria, although the sensory attributes of these products are not their weak spot.

The market of bakery and confectionery products is stable from the global site. However, innovations and the new products belong to the integral process of the quality management that covers the whole food sector. Some of the consumers highly appreciate top quality ingredients in purchased products. Thus, the price of the product is not a limitation in the food acceptance, especially when the customer is satisfied with the rich flavor and taste. A consideration of consumer preferences about bakery and confectionery products with a different ingredients and functional properties is a necessary step that should be taken.

The aim of this work is to present a new approach in the selection of bakery and confectionery products manufacture, taking care of the permanently growing consumer demands. Furthermore, a possible production model of bakery and confectionery products in accordance to customers' preferences is also suggested.

The contemporary food consumption trends

There are certain contemporary demands, which influence a consumers' choice of food and are mostly related to: advertising and marketing, lifestyle, nutritional knowledge, health, environmental issues etc. [2]. According to the Grunert et al. (2012) report [3], in an analysis that covered five continents (not including Africa), four new major trends of consumer food acceptances are: health, convenience, authenticity and sustainability. Close to that, the consumers choose socially and environmentally responsible brands, which are connected with organic food and drinks, healthy living, fair-trade, downsizing and localism [4]. Among the major factors that affect the consumers' choice of food, are a health factor an enhanced with constantly growing awareness of dietary related disease's such as heart disease, obesity, diabetes etc. [5].

The health awareness of the food products is one of the major consumer demands, which leads food manufacturers in a very difficult situation. The consumers' require a healthy food, assuming that taste and aroma are at high level. Bioactive components from food sources may play important role in the prevention of lifestyle related diseases [6].

The authenticity of food products mainly refers to the natural constitutes products, absence from additives, or local production. Subconsciously, consumers' associate good characteristics of food with their natural, local or traditional origin [3]. The authenticity of food products could be easily connected with a health demand, thus most of the

people assumes that carefully grown ingredients necessary for the bakery products are healthy and therefore desirable.

Emphasis on authentic, local and pure ingredients is increasingly seen as a sign of quality [3]. Particular super-market managers, knowing that bread is a primary necessity, offer a cheap bakery product made from low quality ingredients. However, producing the cheapest bread and other bakery products does not have the appropriate effect on quality [7].

Bakery and confectionery products in accordance to the contemporary food consumption trends

The consumers' needs, beside the conventional products, are also connected with the non-gluten, artisan and additive free bakery products, while the global market is in deficiency with a health related products [2]. Therefore a production of bakery and confectionery products with functional properties is a step forward in the understanding of consumers' demands.

The bakery and confectionery products possess some less desirable nutrients, such as sugar and fats. Regardless, these food products could create a different health benefits with the presumption that contain a bioactive ingredients. The addition of a fresh fruit in confectionery products, may lower the use of a high energy nutrients as sucrose, thus present a growing trend of the food industry [8]. A daily consuming of 50-80 g of honey could offer a health benefits to adult individuals, moreover application of honey in bakery and confectionery products improves a texture and sensory characteristics, holding the water etc. [9]. There is a wide range of different food components that could maintain healthy and balanced psycho-physical lifestyle (Table 1.).

Bakery and confectionery products are an appreciative substrate for functional ingredients incorporation. Recommendable products for functional ingredients incorporation are: special bread and pastry, cakes, muffins, pies and raised dough products. Some of already prove combinations are: the dark chocolate with red pepper, the cranberry muffins with ginger, a pumpkin pie with cinnamon, an apple pie and the cherry cakes with the dark chocolate.

Beside the healthy aspect of the products, it is important to produce quality bakery and confectionery products with verified dark chocolate, using spices instead of aromas, and without sparing on the fruits. It is a consumers' choice rather or rather not the new product will be accepted. This means that one and only criteria for a product quality check is a product acceptance by the consumers, which depends on their preferences.

Table 1. Applicable functional foods for bakery and confectionery products [10]

Class/ Component	Food Source	Potential Benefit
α -carotene	Pumpkin, Carrot	Neutralizes free radicals
β -carotene	Apricot, Pumpkin	Neutralizes free radicals
Zeaxanthin	Eggs, citrus, corn	Contributes to the maintenance of healthy vision
Beta glucan	Oats	Reduces risk of cardiovascular disease
Whole Grains	Cereal grains	Reduces risk of cardiovascular disease
Anthocyanidins	Blackberries, blackcurrant, plums	Anti-oxidant, anti-microbial and anti-inflammatory activities
Catechins	Green tea, Black tea	Anti-oxidant, anti-microbial and anti-inflammatory activities
Lactobacillus	Yogurt, other dairy	LDL Oxidation
Proanthocyanidins	Cranberries, cocoa, chocolate	Reduce Urinary tract infection
Tocopherols	Soy Oil, fats, rice	Inhibit lipid peroxidation (LDL), anticancer apoptic activities

A model for food production according to the consumers preferences

Food quality management is an integral part of agro-food sector along with HACCP system. Food quality management consists of several important segments such as:

quality strategy, quality design, quality control, quality improvement and quality assurance [11].

The continual improvement represents a major driving force of the quality management, which leads to a customer satisfaction, and the final result is an external quality improvement [12]. One of the most widely used quality management tools for continuous improvement is a four-step quality model, the plan-do-check-act (PDCA) cycle. A brief explanation of PDCA cycle implies: plan - identify an opportunity and plan; do- implement the change; check- use data to analyze the results of the change and determine whether it made a difference; act: If the change was successful, implement it. In the case of failure, begin the cycle again [13].

A consumers' demands are sometimes in opposite from their preferences. The best explanation of this problem is an example where the consumer insists on healthy bread from the whole grain, and after trying just one bite, realizes that doesn't prefer the taste. In accordance with that fact, an investigation of consumers' preferences should be conducted. Monitoring of the changing consumers' preferences is an important issue of food quality management, along with a right time reactions on the market. Thus, it's important to record a consumers' reaction to the new products.

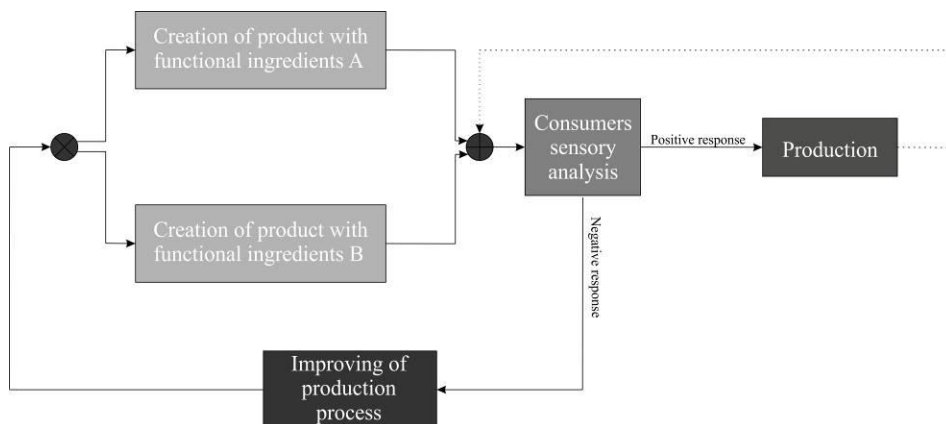


Figure 1. A food production model in accordance to consumers' preferences

The cycles' starting point is a creation of the same products (fixed factor) using the different functional ingredients (various factors). For example, a dark chocolate sponge cake is a fixed factor, and blackberries, apricot and carrots as additional ingredients are the various factors. The total number of products may be two, three or more. After the

creation of probe products, they should be presented to the costumers' trough the sensory evaluation. The consumers' sensory evaluation of bakery and confectionery products may be conducted in accordance to the hedonic scale test [14]. The consumer preferences are indicated with the assistance of the test results. The product that satisfies a consumer demands and preferences is ready for the production. Furthermore, the sensory analysis provides a perspective to reconsideration a production process of lower evaluated products, because costumers' advices about quality and quantity of ingredients should be taken seriously. In that case, next step should be improving of the production process with new ingredients, different quantities etc. Creation of new improved products indicates the end of the cycle, and in complies with the PDCA continual improvement, after implementation of the new process or procedure, the new monitoring start right away to confirm the actions taken. A permanent monitoring and sensory evaluation by the customers of already confirmed products are necessary according to continual improvement and quality management.

CONCLUSIONS

Even though the contemporary trends create a new consumption patterns, a human habit to have a sweet dessert after the meal is still present. Nevertheless, the composition and functionality of a modern bakery and confectionery product, now plays a huge role in the product acceptance by the consumer. Consumers' demands are in a permanent growth due to better education of the population and the technology progress. Beside the conventional requests for bakery and confectionery products, some contemporary demands such as: health, convenience, authenticity and sustainability, took place in a food product acceptance. Bakery and confectionery products in accordance with contemporary consumption trends are: dark chocolate cake with red pepper, a pumpkin or apple pie with cinnamon, the cranberry muffins with ginger, the cherry cakes with a dark chocolate etc. However, consumers' demands are frequently in opposite from their preferences, therefore it is important to investigate the potential market. A constant monitoring of consumer preferences and market reactions are in comply with the continual improvement of the company, which finally results with a consumer satisfaction.

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REFERENCES

1. Cauvain, S.P., Young, L.S. 2005. Baked Products: Science, Technology and Practice, Blackwell Publishing Ltd, Oxford, UK ISBN-10: 1-4051-2702-3 (hardback : alk. paper ISBN-13: 978-1-4051-2702-8.
2. Learning and Teaching Scotland 2005. Home Economics Health and Food Technology Consumer Studies, Learning and Teaching Scotland ISBN 184399089X.
3. Grunert, K.G., Tudoran, A.A., Chrysochou, P. Migkou, T 2012. Trends in new product launches in the food industry, Report prepared for: Natur- og Landbrugskommissionen. MAPP Centre for Research on Customer Relations in the Food Sector, Aarhus University <http://www.naturoglandbrug.dk/Admin/Public/DWSDownload.aspx?File=%2FFiles%2Ffiler%2FNLKOM%2FPublikationer%2F2012-11-15++BILAG+2+c++Report+summary.pdf>.
4. Kleanthous, A., Peck, J. 2005. Let them eat cake – Satisfying the new consumer appetite for responsible brands, abridged version, WWF http://www.wwf.org.uk/filelibrary/pdf/let_them_eat_cake_abridged.pdf.
5. Market indicator report 2011. Bakery Product in Wester Europe, Agriculture and Agri-Food Canada, International Markets Bureau.
6. Wang, L., Bohn, T. 2012. Health-Promoting Food Ingredients and Functional Food Processing. In: Nutrition, Well-Being and Health, Bouayed, J. (Ed.), InTech ISBN: 978-953-51-0125-3 http://www.researchgate.net/publication/221925407_Health-Promoting_Food_Ingredients_and_Functional_Food_Processing/file/9fcfd509bf049e9d3e.pdf.
7. Edwards W. P. 2007. The Science of Bakery Products, Edited by Bardfield Consultants, Braintree, Essex, UK: The Royal Society of Chemistry, Cambridge, UK.
8. Abate, G., Peterson C. 2005. Rapid Opportunity Assessment: Fruit Sector, The Strategic Marketing Institute. Product Center for Agricultural and Natural Resources, East Lansing, MI.
9. Bogdanov, S. 2012. Honey as Nutrient and Functional Food. Bee Product Science www.bee-hexagon.net.
10. Raghuvveer C., Tandon R.V. 2009. Consumption of functional food and our health concerns. Pak J Physiol 1:76-83.
11. Van der Spiegel, M., Luning, P.A., Ziggers, G.W., Jongen, W.M.F., 2003. Towards a conceptual model to measure effectiveness of food quality systems. Trends in Food Science and Technology 14 (10):424-431.
12. Dahlgaard, J.J., Kristensen, K., Kanji, G.K. 1994. The quality journey; A journey without an end, Carfax Publishing Company, Arbingdon.
13. BIR, 2011. Tools for quality management—for an ISO compliant quality management system that includes "end of waste" procedures. Bureau of International Recycling(BIR), Brussels <http://www.bir.org/assets/Documents/Public/BIR-Tools-for-Quality-Management-EN.pdf>
14. Muresan, C., Stan, L., Man, S., Scrob, S., Muste, S. 2012. Sensory evaluation of bakery products and its role in determining of the consumer preferences, Journal of Agroalimentary Processes and Technologies 18(4):304-306.

USE OF MAIZE, SOY AND RICE BREADCRUMBS IN THE FORMULATION OF THE GLUTEN FREE MEATBALLS

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The effect of replacing the wheat with the maize, soy and rice flour and breadcrumbs in the formulation of the gluten-free coating mixture for fried food products was investigated. Six different coating butters were prepared by mixing water and egg white with wheat (control), maize, soy and rice flour, as well as with the mixture of maize and soy flour (50:50) and maize and rice flour (50:50), respectively. Dry breadcrumbs from the previously baked wheat, maize, soy and rice bread were also prepared. Turkey nuggets, made from minced turkey breasts, were dipped in the prepared wet coating batters and breadcrumbs and deep-fried in the palm oil for 3 minutes. Sensory evaluation, colour determination, oil uptake and texture profile analysis (hardness, springiness cohesiveness, chewiness and resilience) of turkey nuggets were performed. In addition to that, textural properties of fried coating that was removed from nuggets were also determined. Results showed that only the addition of soy flour decreased the acceptability of nuggets. Replacing the wheat flour and breadcrumbs with that of maize and rice did not deteriorate the fried nuggets characteristics and some of the properties (colour, appearance and total sensory score) were even better when maize and maize/rice mixture were used. In conclusion, in order to benefit consumers with celiac disease and gluten intolerance, maize and rice flour could be used as an excellent replacement for wheat which is traditionally used in the formulation of the coating mixture for fried food products.

Key words: gluten-free meatballs, maize breadcrumbs, rice breadcrumbs, texture profile analysis, sensory evaluation

INTRODUCTION

Traditional Croatian meatballs are prepared from minced pork and beef, bread crumbs, and a mixture of various herbs and spices, including onion and black pepper.

The preparation of value-added meat products enables incorporation of various binders/extenders. These processes not only bring down the cost of production but also improve palatability and yield of products. Majority of the consumers prefer slight to

medium extended meat products compared to full-meat products. Fillers like starches, flours and potato increases the juiciness of the product due to their high water absorption [1]. Different flour types other than wheat can also be used as an optional ingredient. Further, enrobing of meat products provides advantages such as preserving the nutritive value, preventing moisture and weight loss, improving tenderness and juiciness. Breading on the fried product enhances texture, flavour and appearance of the product [2].

Flour functionality in batter and breading systems depends largely on the two major constituents of all flours, starch and protein. It is known that the main microstructural changes produced during frying includes starch gelatinization and protein denaturation [3]. Therefore, different flours with variable composition of starch and protein will have different influence on microstructure and their combination may also provide special effects [4, 5].

Rice flour is often used in batter systems since it is known to be a healthier alternative providing fewer calories [6]. It reduces oil absorption better than wheat flour although it is less effective as a thickening agent. Legumes are important ingredients of a balanced human diet in many parts of the world, due to their high protein contents. Soy flour based batters provides lower moisture loss and fat absorption compared to rice flour based batters [7, 8]. It was reported that replacing part of wheat flour with rice flour and soy flour enhanced the nutritive value due to the complementary nature of the amino acids in these raw materials [9, 10].

Since traditional meatballs in their composition contain wheat bread crumbs, there are not suitable for patients who suffer from celiac disease. Celiac disease is genetically based autoimmune condition that is characterized by intolerance to gluten, a component in wheat, barley, and rye which affects approximately 1% Americans and from 1 in 130 and 1 in 300 Europeans [11]. Because of the need for gluten-free products to meet market needs, this experiment was designed to determine the formulation of meatballs with satisfactory properties without wheat. The effect of replacing the wheat with the maize, soy and rice breadcrumbs in the formulation of the gluten-free meatballs were investigated. Four different formulations of meatballs were prepared by mixing the minced meat (pork and beef, 50 : 50) with 20% of wheat (control), maize, soy and rice breadcrumbs, eggs and spices, respectively.

MATERIALS AND METHODS

Material

Meatballs were prepared from minced lean pork and beef (50 : 50) with addition of 6% of egg, 6% of onion, 2.4% of salt, 0.2% of black pepper and with 20% of wheat (control), maize, soy and rice breadcrumbs. Dry breadcrumbs from the previously baked wheat, maize, soy and rice bread were prepared. The wheat, maize, soy and rice flours were purchased from local supermarket. Weight of each sample was checked to have a uniform range of 60 ± 1 g. Meatballs 80 mm in diameter and 25 mm thick were fried in

the pan on the sunflower oil for 5 minutes on both sides. Samples were fried at 180 °C in commercial bench-top deep-fat fryer (Moulinex, Bagnolet, France) containing 2.5 l of palm oil. Six nuggets were deposited in the frying oil each time. Samples were fried for 3 min.

Methods

Textural analysis (TPA). Texture profile analysis (TPA) tests were performed using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, England). TPA test included double compression of whole meatball to 60% of their thickness with a cylindrical probe P 75.

For TPA tests force-time curves were recorded at across-head speed of 1 mms⁻¹ and the recording speed was also 1 mms⁻¹. The following parameters were quantified [12]: hardness (g), the maximum force required to compress the sample, springiness (mm), the ability of the sample to recover its original form after the deforming force was removed and cohesiveness, the extent to which the sample could be deformed prior to rupture, chewiness (g.mm) which is calculated as gumminess x springiness and resilience which is the ratio of work returned by the sample as compressive strain is removed. TPA test were conducted one hour after frying.

Determination of colour. Colour measurements (L^* , a^* , and b^* values) were taken using a Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA). The Hunter L^* , a^* , and b^* values correspond to brightness (0-100), greenness (-a) or redness (+a), and blueness (-b) or yellowness (+b), respectively. The colour measurements were performed at room temperature (20±2 °C).

Sensory analysis. A random 1-digit number was incorporated to identify the samples, and each panellist evaluated 6 treatment samples (1 nugget per treatment). The samples were evaluated using a 5- point hedonic scale for four properties (Appearance, Odour, Consistency and Taste). The category definitions were defined as 1 = dislike, 2= dislike moderately, 3 = neither like nor dislike, 4 = like moderately, 5 = like very much.

Data analysis. Three determinations for texture and six for colour parameters were measured from each sample. Experimental data were analyzed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD), with significance defined at $p < 0.05$. Statistical analysis was carried out with Statistica ver. 7.0 StatSoft Inc. Tulsa, OK, USA.

RESULTS AND DISCUSSION

Table 1. shows the effect of different breadcrumbs on textural properties of meatballs. There were not statistically significant differences between textural properties among the meatballs prepared with wheat, maize, soy and rice breadcrumbs except in springiness, cohesiveness and resilience of meatballs with rice breadcrumbs that had significantly lower values. The highest value of hardness had a sample with maize breadcrumbs.

These results were similar with those of Altunakar *et al.* [13].

Table 1. Texture profile analysis of meatballs with different breadcrumbs

Sample	Hardness(g)	Springiness	Cohesiveness	Chewiness	Resilience
Wheat	16228.3a ± 345.3	0.92ab± 0.01	0.89a ± 0.07	13374.3a ± 117.7	0.46abc ± 0.0
Maize	215717.4a± 375.7	0.93a ± 0.0	0.88ab ± 0.10	12774.9a ± 378.4	0.53a ± 0.03
Soy	16458.6a ± 524.3	0.92a ± 0.05	0.83b ± 0.02	12570.5a ± 222.8	0.48b± 0.05
Rice	18129.1a ± 990.9	0.88b ± 0.03	0.75c ± 0.05	119156a ± 398.5	0.43c ± 0.03

Values are means ± SD of three measurements. Values in the same column with different superscripts (a-c) are significantly different ($p < 0.05$).

The effect of breadcrumb types on the colour of deep-fat fried meatballs was examined in terms of L^* , a^* and b^* values (Table 2). Soy breadcrumbs was found to provide the darkest and most yellow colored meatballs which is in agreement with the results of Dogan *et al.* [14], who investigated the influence of soy batters on colour properties of chicken nuggets. This could be related to the high amount of protein in soy flour undergoing Maillard reactions. Relatively high L^* and b^* values for the sample with maize breadcrumbs could be related to higher carotenid content in maize flour. The wheat and the rice breadcrumb formulations gave a lighter and less yellow product.

Table 2. Instrumental colour measurement of meatballs with different breadcrumbs

Sample	L^*	a^*	b^*
Wheat	54.28c ± 2.34	4.85 ± 0.91	18.96b ± 0.75
Maize	59.33b ± 1.43	4.65a ± 1.12	19.51b ± 1.93
Soy	61.18a ± 2.81	4.73a ± 1.94	20.44a ± 3.91
Rice	58.22b ± 2.42	4.48a ± 0.62	19.16b ± 2.03

Values are means ± SD of six measurements.

Values in the same column with different superscripts (a-c) are significantly different ($p < 0.05$).

Table 3. Sensory analysis of meatballs with different breadcrumbs

Sample	Appearance	Odour	Consistency	Taste	Total score
Wheat	4.80a ± 0.63	5.00a ± 0.48	4.80a ± 0.58	4.40a ± 0.58	4.75a ± 0.38
Maize	4.60a ± 0.00	5.00a ± 0.58	4.40a ± 0.00	4.60a ± 0.58	4.65a ± 0.29
Soy	3.40b ± 0.85	4.60b ± 0.85	4.20a ± 0.00	3.00b ± 0.82	3.80b ± 0.38
Rice	4.60a ± 0.58	5.00a ± 0.96	4.40b ± 0.58	4.20a ± 1.15	4.55a ± 0.69

Values are means ± SD of three measurements. Values in the same column with different superscripts (a-c) are significantly different ($p < 0.05$).

There were not statistically significant differences between sensory properties among the meatballs prepared with wheat, maize and rice breadcrumbs (Table 3). Samples with soy breadcrumbs had the lowest score for all examined sensory parameters.

CONCLUSIONS

The textural properties of examined meatballs were quite similar. Sensory analysis showed that the panellists gave the highest score for overall quality to meatballs with addition of wheat, maize and rice breadcrumbs. Only the addition of soy breadcrumbs decreased the acceptability of meatballs. In order to benefit consumers with celiac disease and gluten intolerance, maize and rice breadcrumbs could be used as an excellent replacement for wheat that is traditionally used in the formulation of the meatballs.

REFERENCES

1. Chetana, P., Yogesh, K., Pant Chetana, Anita, Bharti S. K., Tanwar V. K. (2014). Effect of Incorporation of Potato on the Quality of Chicken Cutlets. *IOSR Journal of Agriculture and Veterinary Science*, 7, 12-15.
2. Rao, VNM, Delaney RAM. (1995). An engineering perspective on deep fat frying of breaded chicken pieces. *Food Technolgy*, 49, 138-141.
3. Llorca, E., Hernando, I., Perez-Munuera, I., Fiszman, S.M., Lluch, M.A. (2001). Effect of frying on the microstructure of frozen battered squid rings. *European Food Research and Technology*, 213, 448-455.

4. Barutcu, I., Sahin, S., Sumnu, G. (2009). Effects of microwave frying and different flour types addition on the microstructure of batter coatings. *Journal of Food Engineering*, 95, 684–692.
5. Suderman D.R. (1983). Use of batters and breadings on food products: A review. In F. C. D.R.Suderman (Ed.), *Batter and breading technology*, Westport: Avi Publishing Company, 2.
6. Yokoyama, W. (2004). Nutritional properties of rice and rice bran. In: *Rice: Chemistry and Technology*. American Association of Cereal Chemists, St. Paul, Minnesota, 595–609.
7. Dogan, S.F., Sahin, S., Sumnu, G. (2005). Effects of soy and rice flour addition on batter rheology and quality of deep-fat fried chicken nuggets. *Journal of Food Engineering*, 71, 127–132.
8. Shih, F.F., Daigle, K.W. (1999). Oil uptake properties of fried batters from rice flour. *Journal of Agricultural and Food Chemistry*, 47, 1611–1615.
9. Bakar, J., Hin, Y.S. (1985). High-protein rice-soya breakfast cereal. *Journal of Food Processing and Preservation*, 8, 163–174.
10. Gonzalez-Galan, A., Wang, S.H., Sgarbieri, V.C., Moraes, M.A.C., 1991. Sensory and nutritional properties of cookies based on wheat–rice–soybean flours baked in a microwave-oven. *Journal of Food Science*, 56, 1699–1701.
11. Thompson, T. (2005). National Institutes of Health consensus statement on Celiac disease. *Journal of the American Dietetic Association*, 105, 194–195.
12. Bourne, M. (2003). Texture in food, vol 1, Semi-solid foods. *Journal of Texture Studies*, 34, 575–576.
13. Altunakar, B., Sahin, S., & Sumnu, G. (2004). Functionality of batters containing different starch types for deep-fat frying of chicken nuggets. *European Food Research and Technology*, 218, 318–322.
14. Dogan, S. F., Sahin, S., & Sumnu, G. (2005). Effects of soy and rice flour addition on batter rheology and quality of deep-fat fried chicken nuggets. *Journal of Food Engineering*, 71, 127–132.

MOLDS PRESENT IN WHOLEGRAIN FLOURS USED IN GLUTEN-FREE PRODUCTS

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ABSTRACT

The approach in the creation and production of gluten-free products implies the use of gluten-free mixtures containing wholegrain buckwheat, corn and rice flour. According to the nutritive characteristics, the wholegrain flour is a high quality product, due to its high vitamin, mineral, and dietary fibers content. However, the cereal grains are susceptible to the series of contamination during the ripening, harvesting, processing and storage. On a grain surface, beside the impurities, numerous colonies of microorganisms can be developed and consequently affect the safety of a grain and the final product. The aim of this work was to determine molds presence in buckwheat, corn and rice flour. The total number and determination of isolated genera and species of molds were subject of this research.

All samples were contaminated by the molds. The total number of molds ranged between $10\text{-}3.7 \times 10^4$ cfu/g for the rice and the buckwheat flour, respectively. From the analyzed samples, 12 genera were isolated. The highest number of mold species was isolated from the *Aspergillus* and *Penicillium* genera. The most common species was *Aspergillus flavus*.

The results pointing out a necessity of the grain surface treatment, preceding the milling in wholegrain buckwheat, corn and rice flour production.

Keywords: wholegrain buckwheat flour, corn flour, rice flour, molds

INTRODUCTION

Wheat and flour derived food products plays an important role in human nutrition. Wheat products are rich in carbohydrates, proteins, minerals, vitamins, and other nutritious materials, moreover they are well known as a highly nutritious food. Different kinds of wheat participate in a human diet. The most important wheat, intended for the milling process and for human consumption are: wheat, rye, corn, rice, buckwheat etc.

Lately, consumption habits of consumers are increasingly limited by the various metabolic diseases, such as gluten enteropathy (celiac disease). Celiac disease is a hereditary disease, manifests as allergic reaction of small intestine to gliadin (a fundamental component in the protein complex of flour). A new approach in non-gluten products manufacture implies an application of new-creative non-gluten blends (mixes). The base of these blends is composed of: buckwheat flour, rice flour, corn flour, soy flour, Amaranth flour, and quinoa flour, with the addition of potato or corn starch.

According to the nutritive characteristics, whole grain flour is a high-quality flour, because of the high content of vitamins, minerals and especially dietary fibers. Whole grain flour contains all the parts of a cleaned and milled grain with the inclusion of bran and germ. Therefore, is rich with dietary fibers, vitamins of B group, vitamin E, minerals (Na, K, Ca, Fe), further, has a high protein content in the comparison with white flour. The consumption of whole grain and their flour products carries a many different technological, dietary, and health problems. The wheat grain can be the subject of a numerous contaminations during the growing, harvest, storing and processing period. For that reason, the colonies of numerous microorganisms could appear on the surface, next to the various impurities, whose actions may jeopardize a health aspect of the grain. Molds are among the most visible microorganisms of the wheat grain, along with the bacterias and yeasts. The main characteristic of molds is adaptability to the external conditions of moisture and temperature [1].

A healthy grain has his own natural protection, but the milled grain products are a completely exhibit to the microorganism growth [2]. According to the legislation, a maximal moisture content of merchandise in trade is 15%. Those flours permit a mold growth, first of all in xerophile forms, however many forms are a toxigenic molds [2-4]. By examining the microscopically structure of the grain, it was found that contamination of the grain starts in the grain bran ruptures, the protective layer of the germ ruptures, and especially germ itself, because that is the most vulnerable protective layer of the grain. Flour in comparison with the wheat is susceptible to the microorganism growth, because the lack of protective coat. The mold grow affects the nutritive value and the technological quality of flour. The mold growth occurs when the relative humidity exceeds 79% [5, 6].

The most frequently isolated species of molds from the flour, belongs to the following genera: *Aspergillus*, *Penicillium*, *Eurotium*, *Alternaria*, *Cladosporium*, *Rhizopus*, *Mucor*, *Emericella* and *Fusarium*. Species of the genera *Aspergillus*, *Penicillium* and *Eurotium*, are a "storage molds", and their growth starts at water activity (a_w) of 0.85 or lower. Further, species of the genera *Fusarium* and *Alternaria* are a "field molds", whereby the higher content of substrate moist and the lower temperature is necessary for their growth. These species will be mostly found in/on the wheat grain and related products [3, 7, 8].

The filamentous molds could produce a huge number of enzymes during grow on/in the food, such as: lipases, proteases and carbohydrogenases. These enzymes may continue their activities independently from destruction or removal of molds micelles. An enzymatic activity could affect taste, odor, and colour of food [8].

In the last 50 years, the presence of molds in the food products has attracted a lot of attention, due to its ability to produce mycotoxins. Toxicogenic molds could produce the toxins in a certain concentration, which depends on a particular surrounding factor (temperature, relative humidity, a_w value, the quantity of nutrients) [9-11].

Buckwheat (*Fagopyrum esculentum* and *Fagopyrum tataricum*) is a traditional crop, whose origin is from China. A specific chemical composition classifies the buckwheat as a high value ingredient, primarily because of the proteins that are rich with essential amino acids, vitamins, minerals, and dietary fibers. Hulled buckwheat grain contains 55% of the starch, 12% proteins, 4% lipids, 2% soluble carbohydrates, 7% of the total dietary fibers, 2% ash, and 18% of other components (organic acids, polyphenolic compounds, tannins, nucleotides and nucleic acids). Furthermore, the buckwheat contains micro and macro elements such as: K, Mg, P, Fe, Ca, Cu, Zn, Se, Ba, B, I, Pt and Co. These elements are concentrated in an external layer of the grain and in the bran of the buckwheat [12-14]. In contrast to many wheats, the buckwheat does not contain gluten, therefore it is appropriate for a diet of celiac disease patients. The primary cultivation of the buckwheat is for milling purposes of the flour. Two methods are applied in the milling process of buckwheat, and the choice of the method depends on the type of flour, the following methods are: complete milling of the grain which results in whole grain flour, and the milling process in which the separation of grain parts takes place. The usual procedure in the production process of whole grain buckwheat flour, assumes the milling of whole buckwheat grain on the water mill, and the sieving process on the $\varnothing 1100 \mu\text{m}$ mesh size of the sieve, which results in a 6-8% of the bran in whole grain buckwheat flour. Lately, buckwheat flour has increased application in non-gluten flour blends, because buckwheat flour enhances the quality and quantity of the final products' proteins. Presence of buckwheat flour in non-gluten products increases the total content of dietary fibers [15].

Corn (*Zea mays*) belongs to the group of millets. Together with wheat, corn presents the most important wheat culture in accordance with the cultivation area, trade, and commercial significance. A corn grain consists of an endosperm, the bran, and the germ. The bran represents approximately 16-19%, and the germ represents 8-11% of the whole grain. The corn grain on average contains 14% of the water, 69.5% of carbohydrates, approximately 10% of proteins, 4.8% oils, and 1.4% of mineral compounds. The whole range of products with a different use is obtained from a dry processing of corn. Characteristic products are: grits, semolina, corn flour, flour for animal feed and the corn germ. These products are in different sizes and shapes of particles, also as the chemical structure. The corn germ should be removed during the milling process, for the reason of its relatively high oil content. Therefore, by removing the germ, a longer freshness of the corn products could be achieved. From a nutritive aspect, whole grain corn flour represents a concentrated source of energy, essential fatty acids and amino acids, proteins, vitamins A, B and E, β -carotene, mineral substances (primary K and Mg). Corn flour does not contain gluten, therefore it possesses a crucial role in the production of food for patients with celiac disease [1].

Rice (*Oryza sativa*), also as a corn, belongs to the millets. A rice grain, similarly to the other wheat grains, consists of the three basic anatomic layers: the endosperm, the bran and the germ. Every rice species contains the carbohydrates, proteins, vitamins and minerals, however does not contain the fats. The protein content is approximately 4%, thus is in deficiency in comparison with other wheats. The rice bran contains vitamins B₁ and B₂, also as minerals, therefore whole grain rice is a nutritive important in comparison with the white rice.

Many products could be obtained through the rice processing, which depends from a processing method or a milling grade. Thereby, hulled rice is divided into the three group. Also, rice flour with the mesh size of the sieve lesser than 0.18 mm, could be achieved [1, 6].

MATERIAL AND METHODS

Three types of whole grain flour were investigated: buckwheat flour, corn flour and rice flour. The research involved five samples of each flour. Mycological research covered a total number of molds in 1g of sample, also as a determination of molds.

Isolation and determination of the total number of molds

The total number of molds was determinate by the Koch method. For the preparation of dilution, 0.1% sterile dilution of peptone water was used.

Isolation and the determination of total number of molds, were performed on two substrates:

1. DG18 was applied in isolation of a xerotolerant molds (*Penicillium* spp., *Aspergillus* spp., *Eurotium* spp.) which growth occurs at $a_w < 0.90$
2. MY50G was applied in isolation of an extremely xerophilic molds which growth occurs at $a_w < 0.70$

Seeded substrates were incubated on the temperature of 25 °C. The results were read after five and seven days. The researches were performed in triplets.

Identification of molds

A mold sieving process (monocultivation) on identification substrates was performed, after determination of the total number of molds. Conidia's and fragments of hyphae's from a colonies of molds were transferred on a CYA-Czapek yeast extract agar or on a MEA-Malt extract agar [16]. The colonies for which existed an assumption, according to the macromorphologically properties, that originated from following genera: *Penicillium*, *Aspergillus*, *Eurotium* and *Emericella*, were sieved on CYA, and the others were sieved on MEA. Sieved substrates were seven days incubated on the temperature of 25 °C.

Criteria's described by Samson et al. (2004) [16], Samson & Frisvad (2004) [17] and Pitt & Hocking (2009) [11], were applied for identification of species from *Penicillium* genera, while *Aspergillus*, *Eurotium* and *Emericella* species were determined by Samsonu et al. (2004) [16] and Pittu & Hockingu (2009) [11]. Cultivated molds were stored at DG18 on the temperature of +4 °C.

RESULTS AND DISCUSSION

As could be seen in Table 1, all the samples from buckwheat, corn or rice flour, were contaminated at different level by molds. Their total amount varied from 10 cfu/g in rice flour, to 3.7×10^4 cfu/g in buckwheat flour. The most contaminated samples were from the buckwheat flour (from 4.0×10^2 to 3.7×10^4 cfu/g).

Table 1. The total number of molds in flour samples*

Sample	The total number of molds in 1g	
	DG18 (cfu/g)	MY50G(cfu/g)
Buckwheat flour		
1	3.7×10^4	3.0×10^4
2	6.3×10^2	2.5×10^2
3	4.0×10^2	1.8×10^2
4	1.3×10^3	5.3×10^2
5	5.0×10^2	2.3×10^2
Corn flour		
1	2.0×10^2	90.0
2	50.0	20.0
3	4.7×10^2	2.7×10^2
4	1.1×10^3	4.7×10^2
5	1.3×10^3	8.3×10^2
Rice flour		
1	10.0	-
2	10.0	-
3	1.7×10^2	1.0×10^2
4	6.3×10^2	3.7×10^2
5	20.0	60.0

*the result presents an average value of the three repetition;
 - not found

A numerous molds were isolated from the samples, and classified after a detailed review in 12 genera and 32 species. *Aspergillus* and *Penicillium* genera were represented with the most different species, ten species of both genera. Observing the flour samples contamination, could be noticed that the most species were isolated from the buckwheat flour. The results weren't surprising, considering that flour is obtained from the buckwheat whole grain, apropos a fragment presence of the grain bran. In accordance with the appearance and structure of grain, buckwheat represents a suitable environment for microorganisms surfaces populating, which could be subsequently found in flour [18]. The reason of increased number of microorganisms on the buckwheat grain surface, is the presence of a fine hairs or "villus", whose function is the retention of a dust and sand particles. For that reason is necessary to conduct a surface treatment of grain with different types of "scrubs", brushes and other equipment for the surface treatment.

Table 2. Mold species isolated from flour samples

Molds species	Sample		
	Buckwheat flour	Corn flour	Rice flour
<i>Absidia corymbifera</i>	+ (3)*		
<i>Alternaria alternata</i>		+ (1)	
<i>Aspergillus niger</i>	+ (1)	+ (2)	+ (1)
<i>A. candidus</i>	+ (1)	+ (1)	+ (3)
<i>A. flavus</i>	+ (5)	+ (4)	+ (3)
<i>Acremonium strictum</i>		+ (2)	
<i>Cladosporium cladosporioides</i>		+ (1)	
<i>Eurotium chevalieri</i>	+ (3)	+ (2)	
<i>E. herbariorum</i>	+ (1)	+ (1)	+ (2)
<i>Emericella nidulans</i>	+ (1)		
<i>Fraseriella bisporus</i>	+ (1)		
<i>Mucor circinelloides</i>	+ (1)		
<i>Penicillium expansum</i>	+ (3)	+ (3)	+ (1)
<i>P. aurantiogriseum</i>	+ (1)	+ (1)	+ (2)
<i>Paecilomyces fulvus</i>	+ (1)	+ (4)	+ (2)
<i>Rhizopus oryzae</i>	+ (2)	+ (2)	

+ - presence of mold species in sample; (N) – the number of contaminated samples

Corn and rice flour contains a lesser amount of molds in 1g, because there is a difference in mechanical protection of grain (cellulose bran from corn and protective chaff as the rice grain bran). Corn grain contamination problems could occur if grain is mechanically

damaged during milling, transport, drying, or a particular physical treatment. Rice grain also passes through the mechanical hulling process, and if there is a need, the transport and drying process.

Mold that appears as a contaminant of flour; mostly originate from the wheat grain. The milling of grain could only partially remove molds, especially if penetrates with filaments in a grain tissue. Considering that flour may contain 15% of moisture, which is a suitable environment for the xerophilic molds development. These species are mostly present in *Aspergillus*, *Eurotium* and *Penicillium* genera, however they are also present in other genera. The water activity (a_w value) of wheat grain and flour is in the most cases between 0.86 and 0.70, which enables an optimal growth of xerophilic molds.

As Table 2 shows, a dominant role in micropopulation of the examined samples has following genera: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus candidus*, *Eurotium herbariorum*, *Penicillium expansum*, *Penicillium aurantiogriseum*. That is probably a consequence of the ability to grow on substrates with low a_w values (between 0.74 for *E. herbariorum* to 0.84 for *P. aurantiogriseum*) [3].

The presence of these molds on wheats and derived products is not desirable, considering their characteristics of mycotoxicity. *A. flavus* is the most famous producer of aflatoxins, beside, other species could also synthesize a series of toxic metabolites [8, 19, 20].

Food protection from a mycotoxin and mold contaminations, first of all includes a prevention of toxigenic species growth on ingredients and products.

CONCLUSIONS

- All the samples of buckwheat, corn and rice flour, were contaminated by molds;
- total number of molds varied from 10 cfu/g in rice flour, to $3,7 \times 10^4$ cfu/g in buckwheat flour;
- the most contaminated samples were from the buckwheat flour (from $4,0 \times 10^2$ cfu/g to $3,7 \times 10^4$ cfu/g);
- isolated molds were classified in 12 genera and 32 species;
- the most frequent species were from *Aspergillus* and *Penicillium* genera;
- the high presence of molds indicates the necessity of the mold grow prevention on/in wheats and mill products, which implies the surface treatment of grain in the procedure of whole buckwheat, corn and rice grain flour production.

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REFERENCES

1. Žeželj, M. 1995. Tehnologija žita i brašna. Tehnološki fakultet Novi Sad.
2. Kljusurić, S. 2000. Uvod u tehnologiju mljevenja pšenice. Prehrambeno tehnološki fakultet Sveučilišta Josip Juraj Strossmayer Osijek.
3. Škrinjar, M., Kolarević, I., Dimić, G., and Jovanović, O. 2000. Occurrence of moulds and some mycotoxins in raw materials used in confectionary. *Žito hleb.* 19-14.
4. Škrinjar, M., and Tešanović, D. 2007. Hrana u ugostiteljstvu i njeno čuvanje. Prirodno-matematički fakultet, Univerzitet u Novom Sadu.
5. Plavšić, D., Sakač, M., Čabarkapa, I., Šarić, Lj., Psodorov, Đ. 2007. Mikrobiološka ispravnost pšeničnog brašna. *Žito-hleb.* 34(5-6):83-90.
6. Stojanović, T. and Psodorov, Đ. 2007. Savremena tehnologija žita, brašna i hleba. Page 46-118, Prokuplje, Srbija.
7. Maletić, Ž. 2005. Kserofilne mikopopulacije i proizvođači mikotoksina u musliju i komponentama. Specijalistički rad, Tehnološki fakultet, Univerzitet u Novom Sadu.
8. Kocić-Tanackov, S., and Dimić, G. 2013. Gljive i mikotoksini-kontaminanti hrane. *Hem.ind.* 67(4):639-653.
9. Muntanjola-Cvetković, M. 1990. Opšta mikologija. Naučna knjiga Beograd.
10. Škrinjar, M., Vengušt, S., and Kocić-Tanackov, S. 2004. Mycotoxins in food and feed – sampling, detection, regulations. *Tehnologija mesa.* 45(5-6):163-169.
11. Pitt, J. I., and Hocking, A. D. 2009. *Fungi and Food Spoilage.* Springer Science – Business Media, New York, USA.
12. Li, S., Zhang, H. 2001. Advances in the development of functional foods from buckwheat. *Crit. Rev. Food Sci. Nutr.* 41: 451-464.
13. Steadman, K. J., Burgoon, M. S., Lewis, B. A., Edwardson, S. E., Obendorf, R. L. 2001b. Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *J. Sci. Food Agric.* 81: 1094-1100.
14. Bonafaccia, G., Gambelli, L., Fabjan, N., Kreft, I. 2003b. Trace elements in flour and bran from common and tartary buckwheat. *Food Chem.* 83:1-5.
15. Sedeji, I. 2011. Funkcionalna i antioksidativna svojstva novih proizvoda od heljde. Doktorska disertacija. Tehnološki fakultet, Univerzitet u Novom Sadu.
16. Samson, R. A., Hoekstra, E. S. and Frisvad, J. C. 2004. Introduction to food-airborne fungi. Centaalbureau voor Schimmelcultures, P.O. Box 85167, 3508 Ad utrecht, The Netherlands.
17. Samson, R. A., and Frisvad, J. C. 2004. Penicillium subgenus Penicillium: new taxonomic schemes, mycotoxins and other extrolites. Centaalbureau voor Schimmelcultures, P.O. Box 85167, 3508 Ad utrecht, The Netherlands.

18. Plavšić, D., Psodorov, Đ., Filipčev, B., Šimurina, O., Šarić, LJ., Čabarkapa, I., and Košutić M. 2012. Microbiological safety of buckwheat products. Page 384 in Proceedings, 6th International Congress Flour – Bread '11 8th Croatian Congress of Cereal Technologists, Opatija, Croatia.
19. Kocsube, S., Varga, J., Szigeti, G., Baranyi, N., Suri, K., Toth, B., Toldi, E., Bartok, T. and Mesterhazy, A. 2013. *Aspergillus* species as mycotoxin producers in agricultural products in central Europe. *Matica srpska. Journal for natural science.* 124:13-25.
20. Krnjaja, V., Lević, J., Stanković, S., Petrović, T. and Lukić, M. 2013. Molds and mycotoxins in freshly harvested maize. *Matica srpska. Journal for natural science.* 124:111-120.

INVESTIGATION OF QUANTITY, QUALITY AND USAGE OF LEFTOVER BREAD IN HUMAN AND ANIMAL NUTRITION

UDC 338.439.053.22:613.2

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ABSTRACT

Lack of food for the continually growing population is becoming an increasing problem in the world and in our country as well. One of the solutions to increase food and feed supplies may be the use of novel technologies in bioindustry. On the basis of numerous inquiries made in small bakeries, large industrial bakeries and supermarket chains, restaurants, and municipal service companies, there is evidence for large quantities of leftover bread. Bread is a dominant cereal product and a staple food in numerous countries. It is made from flour and water as basic ingredients and some minor ingredients; by mixing, shaping, fermentation and baking. In our population, it has been a habit to use only fresh bread; bread one day post baking is considered aged due to staling and usually discarded. This paper focuses on several issues relating to leftover bread: quantity, quality and safety issues of old bread in light of elaborating possibilities for further uses.

Keywords: leftover bread, safe food

INTRODUCTION

Products based on wheat flour have a dominant place in the nutrition of people especially in developing countries. In some communities, these products provide more than 60% of energy intake [1]. This percentage is estimated to be even higher for low socio-economic status groups. Bread is the most frequent product made from cereals and also a staple in many countries. Considering the high average intake of bread in our diet (300 g/day per capita), bread represents the basic energy and nutrient source for majority of Serbian population, too. Bread is made by mixing flour and water and certain secondary materials, which after fermentation, shaping and baking becomes a final product. After leaving the oven, loaves have to be cooled and over the time they become drier, and their quality changes. [2]

Average Serbian inhabitant annually consumes around 109 kg of bread which is much higher relative to European average of 30-40 kg. Bread consumption is affected not only by standard of life but numerous other factors, in the first place by local dietary habits [3]. One of the habits our population has is to discard bread which is not consumed in a

single day. On the basis of numerous inquiries made in small bakeries, large industrial bakeries and supermarket chains, restaurants, and municipal service companies, there is evidence for large quantities of leftover bread. This raises the question of quantity and quality of leftover bread and its safety for further use. Mycotoxins in discarded bread pose serious ecological and health threat for the increasing number of Romanies and other destitute population.

Depending on the safety status of leftover bread and the presence of harmful substances we can obtain a very attractive raw material or a major problem.

RESULTS AND DISCUSSION

In our country there are no official statistics on the quantities of leftover and discarded bread. The percentage of leftover bread compared to the amount of produced in the EU countries is about 1% and in England the percentage is about 0.1%. According to the survey carried out in January - March 2012 by the Institute for Food Technology in Novi Sad (Province of Vojvodina, Serbia) the amount of leftover bread ranges from 5-10% of the produced quantity. It is concluded that from one million loaves weighting 500 g produced in Vojvodina, a minimum of 50 000 loaves are discarded which accounts for a total of 25 tons of bread per day. [4]

Several factors are anticipated as possible reasons for such high percentage of leftover and discarded bread:

- Bread is still a social category;
- Relatively low price of bread;
- Relatively low level of bread quality;
- Habits of the consumers - tendency of buying much more bread than one usually consumes;
- Serbian people do not have a habit to reuse leftover bread in their diet.

According to the survey, significant amounts of leftover bread originate from:

1. From households (it is thrown away together with other waste or in separate bags); bread is usually 1 to 5-day stale and of questionable microbiological safety;
2. From large bakeries - undelivered products or products returned from supermarkets;
3. From city dumps where large quantities of bread end up mixed with and/or get contaminated by other waste.

Small bakeries quite rationally and economically organize their production. Leftover bread is not a problem for them according to the survey carried out with small bakeries.

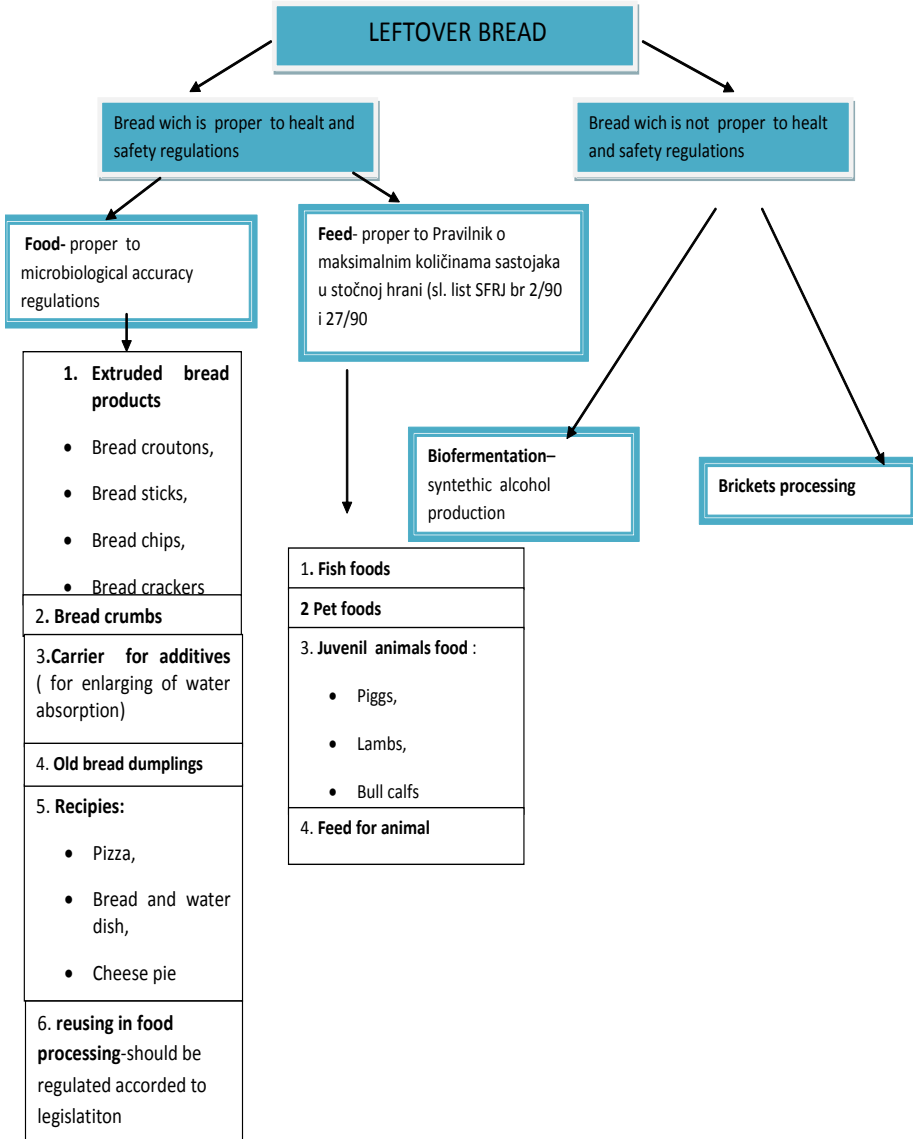


Figure 1. Possibilities of leftover bread processing and refining

However, there is no organized way of leftover bread collection and distribution for possible further processing. As a result, there is evident increase of the environment pollution, and created problems in the food chain. In other hands, depending on sanitary quality, leftover bread can be used in feed production, production of pellets and briquettes to be used as bioenergetic fuel, as a raw material for biofermentation in production of biogas, etc. If leftover bread is not contaminated with mycotoxins or if bacterial and mold contamination is not at a level to be quantitatively determined, it can be processed by extrusion into valuable protein-energetic feed for juvenile animals, fish feed and pets. Figure 1 shows the possibilities of leftover bread processing and refining [5].

CONCLUSION

In Serbia, it is evident the existence of significant amounts of leftover and discarded bread which pose serious environmental, health and safety risks threatening to burden the food chain. On the other hand, if sanitary appropriate, there are multiple possibilities for reuse of leftover bread. Introduction of HACCP standard would undoubtedly improve the situation on the market. In practice, the most important thing is to strictly implement the principle of Good Sanitary Practice which is obligatory for all bakeries.

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REFERENCES

1. Žeželj, M. 2005. Tehnologija žita i brašna, knjiga II, Beograd.
2. Marić A., Arsovski S., and Mastilović J. 2009 Contribution to the improvement of products quality in baking industry, Page 21-28 in Proceedings of 3rd international quality conference, Kragujevac, Serbia.
3. Živković, J., Mastilović, J., Pestorić, M., and Radusin, T. 2007 Vrednovanje stavova i zadovoljstva potrošača hleba kao masovnog proizvoda. *Žito-hleb*. 34(5-6):109-113.
4. Nježić, Z., Psodorov, Đ., Živković, J., Filipović, J., and Cvetković, B. 2010 Leftover bread as safe food on a territory of Vojvodina, Page 305-311 in Proceedings of XIV International ECO-conference 2010, Ecological Movement of the City of Novi, Serbia.
5. Technological proposals for the implementation of practical solutions are given by the team of researchers (FINS), which has extensive practical experience in industrial conditions in the production of food for animals, plants design and design of technological procedures for pelleting and extrusion, mill and bakery plant design, as well as solving effluents problem in the mass production of food.

INFLUENCE OF ANTIOXIDANTS ADDITION ON THE OXIDATIVE STABILITY OF A MIXTURE OF SUNFLOWER AND CORN OILS

UDC 665.3 : 66.094.3

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ABSTRACT

Oxidative stability is an important parameter in evaluation the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative deterioration. It is generally accepted that natural antioxidants are more potent, more efficient and safer than synthetic antioxidants. The aim of this study was to investigate the oxidative stability of sunflower oil, corn germ oil, and mixtures there of (50:50), and the effect of addition of rosemary extract (StabilEnhance® OSR and OxyLess® CS of 0.1% and 0.3%), and propyl gallate (0.01%) on the extension oxidative stability of the oil mixture (50:50). A determination of oxidation stability of oils and their combination, and the effect of antioxidants (rosemary extract and propyl gallate) was conducted by a test of rapid oxidation of oil (Schaal oven and Rancimat test). The result of oil oxidation was expressed by the induction period (IP), a protective factor (PF) and by antioxidant activity of antioxidants (AA). The stability of a mixture of oil is proportional to the induction period. From these results it can be concluded that an addition of corn germ oil (50%) in sunflower oil led to changes in the oxidation stability of oil blends expressed within the induction period. The addition of corn germ oil in sunflower oil prolongs the stability of blends of oil degradation by oxidation. The natural antioxidant rosemary extract OxyLess®CS significantly increases the oxidative stability of a mixture of sunflower and corn oils compared to StabilEnhance®OSR and propyl gallate.

Key words: vegetable oils, oxidative stability, rosemary extract, propyl gallate

INTRODUCTION

Knowledge of vegetable oils oxidation stability is important to determine in advance time to keep of oil from stronger oxidation, without significant changes in quality and to define the shelf life of oil. Oxidative deterioration rate of vegetable oils depends on the composition of oil, storage conditions, the presence of substances that speed up or slow down the oxidation [1]. The composition of oleic, linoleic and linolenic acids in oil affects the oxidative stability [2, 3]. Secondary products, created from the auto-oxidation

process of oils (aldehydes, ketones) in small quantities affect to the sensory properties of oil [4, 5]. Methods for determination of the oxidation stability of vegetable oils were based on the accelerated oxidation of oils, these are Rancimat test, Schaal oven test and AOM test [6-8]. Farhoosha et al. (2008) were showed that the kinetic parameters of the oxidation of vegetable oils using the Rancimat test affected to the viability of the oil [9]. Vegetable oils stability may be improved by addition of antioxidants, substances that slow down the oxidative decay. Synthetic and natural antioxidants were applied for the vegetable oils stabilization [10, 11, 23]. Synthetic antioxidants are cheaper than natural, but some natural antioxidants are more effective and health safer. Bera et al. (2006.) were nvestigated the thermal stabilisation of synthetic antioxidants (BHT, TBHQ, EQ) and natural antioxidant extract ajowan, used for stabilization of linseed oil [12]. They found that TBHQ has a higher thermal stability, but natural antioxidants are often used due to the attractive spice flavors and fragrances. Today, they are used various extracts of herbs for protection of oxidative deterioration of peanut oil, high-oleic sunflower oil and other vegetable oils [13, 14]. Greedy et al., (2006) report that green tea ethanol extract has a higher antioxidant activity, measured as induction period, in relation to the activity of BHT and black tea extract in sunflower oil [15]. Corn germ oil has good oxidation stability, which increases the added antioxidants.

The aim of this study was to investigate the oxidative stability of sunflower oil (linoleic type), corn germ oil and their mixtures 50:50, and the influence of added natural antioxidants rosemary extract type Oxy'Less ® CS, type StabilEnhance ® OSR and synthetic antioxidants propyl gallate to change the oxidative stability of the oil mixture. Addition of corn germ oil (50%) in sunflower oil tends to be obtained the mixture of oils that will provide the greater oxidative stability compared to the stability of the pure sunflower oil.

MATERIALS AND METHODS

Examination of oxidation stability will be carried with refined sunflower oil (linoleic type) and rapeseed oil. Examined refined oils were purchased in the store. The share of the major fatty acids of the oils is: sunflower oil (21-23% oleic, linoleic 62-67%); corn germ oil (24-32% oleic, linoleic 55-62%); [16]. Mixture of these two types of oil in a ratio of 50:50 (100 mL) was prepared by mechanical mixing. The study of the impact of individual antioxidants addition on the stability of these oil mixtures was carried out with synthetic antioxidant propyl gallate (0.01%), natural antioxidants rosemary extract type Oxy'Less ® CS and type StabilEnhance ® OSR in shares of 0.1% and 0.3%.

Oxy'Less ® CS - powdered rosemary extract, obtained from *Rosmarinus officinalis* L., carnosol acid 18-22%, dry extract 92-98%, Naturex, France. StabilEnhance ® OSR - liquid rosemary extract, obtained from *Rosmarinus officinalis* L., carnosol acid min. 5%, Naturex, France. Propyl gallate -synthetic antioxidant, Danisco, Denmark.

Each antioxidant is added directly to the oil mixture and homogenized mechanically at a temperature of 70 °C for 30 minutes. Examination of the initial chemical characteristics (quality parameters) of vegetable oils was carried out using standard methods.

Determination of free fatty acids

Free fatty acids (FFA) were determined by the standard method (ISO 660:1996) which is based on the principle of the titration with sodium hydroxide solution $c(\text{NaOH})=0.1 \text{ mol/L}$. The result is expressed as a percentage (%) of free fatty acid (FFA), as oleic acid.

Determination of peroxide value

Peroxide value (PV) is an indicator of the degree of oxidative deterioration of vegetable oils. Peroxide value of oil is determined by the standard method (ISO 3960:1998). The result is expressed as mmol of active oxygen derived from the resulting peroxide present in 1 kg of oil (mmol/kg).

Determination of anisidine value

The calculated value of anisidine (AV) gives us an insight into the share of non-volatile carbonyl compounds, which are secondary products of oxidation of vegetable oils that have a negative impact on sensory properties and oxidative stability. It is believed that a good quality vegetable oil has the anisidine value less than 10 (no limitation in legislation). Anisidine value is determined by the standard method (ISO 6885). The determination is based on the reaction of p-anisidine with higher unsaturated aldehydes (2, 4-dienal and 2-enal) in acidic medium, to form Schiff bases.

Determination of the number of Totox

Peroxide number in combination with anisidine value is used to determine the total value of the oxidation of vegetable oils or Totox number (TV) (ISO 6885:2006). Totox number is calculated according to the formula: $\text{Totox number} = 2 \text{ PV} + \text{AV}$ Totox number is considered to be a very useful indicator of the quality and oxidative stability of the oil; from anisidine value we can get the information about history oil oxidation, and from the peroxide value about current oil oxidation state.

Oxidative stability of oil

Schaal oven test

Schaal oven test is one of the oldest tests for examining oxidation stability of vegetable oils. It is based on the accelerated oxidation of oils influenced by of heat that accelerated this process. To perform this test, the samples of examined vegetable oils were heated in

a thermostat at a constant temperature of 63 °C and monitored changes of peroxide number or sensory changes caused by oxidative deterioration of the oil at regular intervals. The result of the oxidation stability of examined plant oils, using of this test, is shown as the value of peroxide number after a certain time of the implementation of the test (for 4 days).

Rancimat test

Oxidation stability of examined vegetable oils, their mixtures (50:50) with and without added antioxidants is determined by Rancimat test of accelerated oils oxidation (ISO 6886:1996). The test is based on the rapid spoilage of oil at constant temperature and constant air intake, and determines the induction period (IP). For oxidation stability determination was used the Rancimat device, model 743 (Metrohm, Switzerland). The effect of antioxidants (natural and synthetic) on the extension of the oil mixture oxidative stability was determined by the protection factor (PF) [10] according to the formula: $PF = IP_{inh} / IP_0$. IP_{inh} - induction period of the oil sample with antioxidants addition (h), induction period IP_0 -oil samples without added antioxidant (h). The protection factor indicates how many times increase the stability or sustainability of vegetable oil by adding of antioxidants. Determination of the oil samples oxidation stability was performed in duplicate, and showed the average value of the induction period. Longer induction period indicates on greater activity of added antioxidants and protection factor higher than 1.0 indicates on better protection from oil oxidative deterioration [15].

RESULTS AND DISCUSSION

Initial chemical characteristics (free fatty acids, peroxide, anisidine value, Totox number) of the examined vegetable oils are shown in Table 1.

Table 1. Initial chemical characteristics of vegetable oils

Vegetable oils	FFA (% olein)	PV (mmol /kg)	AV	TV
Sunflower oil	0.12±0.03	0.43±0.11	6.15±0.09	7.01
Corn germ oil	0.22±0.02	0.89±0.08	3.84±0.02	5.62

FFA - free fatty acid, % oleic acid; PV- peroxide value, mmol /kg; AV- anisidine value;
TV- Totox value

Values, obtained for free fatty acid (FFA) and peroxide value (PV) have shown that the examined vegetable oils had good quality and in accordance with the Rules of edible oils

and fats [17]. Calculate anisidine value (AV) and Totox number (TV) also point to the satisfactory quality of oil.

Oxidative stability of sunflower oil and corn oil was determined with the Schaal oven test (63 °C) in period of 4 days by peroxide number every 12 hours (Table 2.). Changes caused by oil oxidation method of accelerated deterioration from heat resulted in the formation of primary oxidation products expressed as peroxide value (PV). The results in the table were showed that sunflower oil had a lower stability, after 4 days of the testing was achieved higher PV value of 11.29 mmol/kg of oil. This oil, after 60 hours of the testing, was exceeded the max. PV according to the Regulations. The results were showed that corn seed oil had a better stability, greater resistance to the oxidative spoilage and reached a low value of PV 2.38 mmol/kg after 4 days of the test. Good oxidation stability of this oil was attributed to the high content of total tocopherols, 70-80% in the form of γ -tocopherol and ubiquinone (200 mg/kg).

Table 2. Oxidative stability of vegetable oils determined by the Schaal oven test during 4 days follow of peroxide values each 12 hours

Sample	PV (mmol /kg)								
	0	12	24	36	48	60	72	84	96
Sunflower oil	0.43	0.68	1.48	1.93	3.33	5.81	6.62	8.79	11.29
Corn germ oil	0.89	1.14	1.22	1.67	1.72	1.79	1.84	1.89	2.38

Table 3. Oxidative stability of vegetable oils determined by the Rancimat test

Sample	IP (h)	Increase IP (%)
Sunflower oil	2.38	--
Corn germ oil	4.96	--
Sunflower oil + corn germ oil (50:50)	3.19	34.03

IP – induction period, (h); Increase IP compared to sunflower oil, (%)

Oxidative stability of the oils and their mixtures (50:50) was determined and expressed by Rancimat test induction period (IP) showed that corn germ oil had a greater stability and resistance to oxidative decay (Table 3). The result of this test was showed that

sunflower oil had IP of 2.38 (h), and corn germ oil 4.96 (h). The addition of maize germ oil (50%) in sunflower oil, we wanted to increase the resistance of sunflower oil to oxidative spoilage. In this mixture the oil reached the composition of fatty acids and natural antioxidants that extended induction period of 19.3 (h) and a higher stability compared to pure sunflower oil.

Research results of oxidation stability mixture of sunflower oil and maize germ oil (50:50) and the impact of the addition of synthetic antioxidants propyl gallate (0.01%), natural antioxidant extracts of rosemary type Oxy'Less ® CS and type StabilEnhance ® OSR, in 0.1% and 0.3% at longer oxidation stability were shown in Tables 4-5.

Control sample is a mixture of sunflower oil and maize germ (50:50) with no added antioxidant was showed the value of the induction period of 3.19 (h). The value of the induction period (IP) showed the resistance of oil mixtures to oxidation spoilage, higher IP means better oxidation stability. Addition of natural antioxidant extracts of rosemary type OSR StabilEnhance ® (0.1%) in this oil mixture slightly increased the stability of the oil to 17.87%. Greater increase in stability of the oil is observed by the addition of synthetic antioxidants propyl gallate (0.01%), it was increased stability of 45.14% (IP is 4.63 h). Addition of rosemary extract type Oxy'Less ® CS (0.1%) significantly increased the time of the induction period (5.81 h) of oils mixture. Stability of the oil mixture was increased to 82.13% in relation to the stability of the control sample (Table 4). Chu and Hsu (1999) were investigated the effect of rosemary extract, ascorbyl palmitate and tocopherols on the stability of peanut oil by OSI test [18]. They pointed out that all of three added antioxidants were increased the value of the OSI index, the greatest impact on increasing the sustainability of oil had rosemary extract. Frankel et al. (1996) were founded that the additions of rosemary extract, carnosol and rosmarinic acid effectively protected corn germ oil from oxidative deterioration in compared to the application of carnosol [19].

Table 4. Induction period (IP) and protection factors (PF) determined by the Rancimat test

Antioxidant	Share (%)	sunflower oil + corn germ oil (50:50)			
		IP (h)	Increase IP (%)	PF	AA
Control sample	0	3.19	--	1.00	--
PG	0.01%	4.63	45.14	1.45	1.00
StabilEnhance OSR	0.1%	3.76	17.87	1.18	0.39
OxyLess CS	0.1%	5.81	82.13	1.83	1.80

Control sample: mixture of sunflower oil + corn germ oil (50:50)

PG-propyl gallate; StabilEnhance®OSR-rosemary extract; Oxy'Less®CS- rosemary extract; AA - antioxidant activity compared to propyl gallate

Merrillet et al. (2008) reported about the oxidation stability of conventional and high oleic vegetable oils and impact of the antioxidants addition (rosemary extract, ascorbyl palmitate, TBHQ and mixture of tocopherols) on the oil stability by OSI test. They noted that corn germ oil showed the good stability; added antioxidants successfully increased resistance to oxidative spoilage.

By increasing the content of added natural antioxidant rosemary extract (Oxy'Less ® CS and StabilEnhance ® OSR) from 0.1% to 0.3%, it significantly prolong the induction period (IP) in oil mixture compared to the control (Table 5). Addition of 0.3% StabilEnhance ® OSR was achieved the increase in IP 14.5 (h); with the addition of a 0.3% of Oxy'Less ® CS IP is considerably higher 2.9 (h). It is evident from the obtained results that two types of rosemary extracts extend the stability of oil mixture in 61.13% and 182.76%.

Table 5. Induction period (IP) and protection factors (PF) determined by the Rancimat test

Antioxidant	Share (%)	sunflower oil + corn germ oil (50:50)			
		IP (h)	Increase IP (%)	PF	AA
Control sample	0	3.19	--	1.00	--
PG	0.01%	4.63	45.14	1.45	1.00
StabilEnhance OSR	0.3%	5.14	61.13	1.61	1.35
OxyLess CS	0.3%	9.02	182.76	2.83	4.05

Control sample: mixture of sunflower oil + corn germ oil (50:50)

PG-propyl gallate; StabilEnhance®OSR-rosemary extract; Oxy'Less®CS- rosemary extract; AA - antioxidant activity compared to propyl gallate

The calculated values of the protective factor (PF) in all tested samples showed that oil rosemary extract Oxy'Less ® CS (0.1% and 0.3%) had a better efficiency of oil mixture protection from oxidative deterioration because of the higher antioxidant activity. Oxy'Less ® CS was increased the stability of the mixture of sunflower and corn oil; protection factors were 1.83 and 2.83. Erkan et al. (2008) were reported that rosemary extract had a high antioxidant activity because of the high proportion of phenolic compounds [20]. Martinez-Tome et al. (2001) were founded that rosemary extract was more effective in refined olive oil protection in comparison to the synthetic antioxidants (propyl gallate, BHA, BHT) [21]. Synthetic antioxidant, propyl gallate, was showed a greater protection of tested oil mixture from oxidation (PF 1.45) compared to rosemary extract type OSR StabilEnhance ® (0.1%) wherein the PF 1.18. However, the addition of

0.3% StabilEnhance ® OSR was increased protection of oil mixture from PG. Silva et al. (2001) were reported that application of propyl gallate was more efficient in protecting from oxidation of refined sunflower oil in relation to the application of natural antioxidant tocopherol [22].

CONCLUSIONS

Based on results of oxidation stability of a sunflower oil and corn oil mixture (50:50), without the addition of antioxidants, may be performed the following conclusions:

- The corn germ oil, due to a larger share of oleic acid has a greater stability to oxidative spoilage compared to sunflower oil (Schaal oven test and the Rancimat test).
- Addition of corn germ oil (50%) in sunflower oil increases the stability to oxidative spoilage.
- Application of examined antioxidants increases the oxidative stability of sunflower oil and corn oil mixture (50:50).
- Addition of rosemary extract type Oxy'Less ® CS (0.1% and 0.3%) efficiently protects examined oil mixture (50:50) in relation to the use of rosemary extract type OSR StabilEnhance ® (0.1% and 0.3%) and propyl gallate (0, 01%).
- Use of StabilEnhance ® OSR (0.1%) rosemary extract slightly increases the stability of oil mixture compared to oil sample without the antioxidants addition.
- StabilEnhance ® OSR (0.3%) gives a better protection of oil mixture compared to the propyl gallate.

REFERENCES

1. Martin-Polvillo, M., Marquez-Ruiz, G., Dobarganes, M. C. 2004. Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature. *J. Am. Oil Chem. Soc.* 81:577-583.
2. Min, D. B., and Boff, J. F. 2001. Lipid oxidation of edible oil. Page. 335–363. In C. Akoh& D. B. Min (Eds.), *Food lipids* New York: Marcel Dekker.
3. Nawar, W. W. 1996. Lipids. Page 225–319 In O. R. Fennema (Ed.), *Food chemistry* (3rd ed.),. New York: Marcel Dekker.
4. Gray, J.I. 1978. Measurement of lipid oxidation: a review. *J. Am. Oil Chem. Soc.* 55:539-546.
5. Broadbent, C. J., Pike, O. A. 2003. Oil stability indeks correlated with sensory determination of oxidative stability in canola oil. *J. Am. Oil Chem. Soc.* 80:59-63.

6. Shahidi, F. 2005. Bailey's Industrial Oil & Fat Products (Sixth edition). Volume 1, Edible Oil & Fat Products: Chemistry. Properties and Health Effects, Page 269-513 Wiley-Interscience publication.
7. Suja, K.P., Abraham, J.T., Thamizh, S.N., Jayalekshmy, A., Arumughan, C. 2004. Antioxidant efficacy of sesame cake extract in vegetable oil protection. *Food Chem.* 84:393-400.
8. Abramović, H., Abram, H. 2006. Effect of added rosemary extract on oxidative stability of *Camelina sativa* oil. *Acta agriculturae Slovenica.* 87(2):255-261.
9. Farhoosh, R., Niazmand, R., Rezaei, M., Sarabi, M. 2008. Kinetic parameter determination of vegetable oil oxidation under Rancimat test conditions. *Eur. J. Lipid Sci. Technol.* 110 (6):587-592.
10. Yanishlieva, Nedyalka V., Marinova, Emma M. 2001. Stabilisation of edible oils with natural antioxidants. *Eur. J. Lipid Sci. Technol.* 103:752-767.
11. Merrill, L.L., Pike, O.A., Ogden, L.V. 2008. Oxidative Stability of Conventional and High-Oleic Vegetable Oils with Added Antioxidants. *J. Am. Oil Chem. Soc.* 85:771-776.
12. Bera, D., Lahiri, D., Nag, A. 2006. Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants. *J. Food Eng.* 74:542-545.
13. Pan, Y., Zhang, X., Wang, H., Liang, Y., Zhu, J., Li, H., Zhang, Z., Wu, Q. 2007. Antioxidant potential of ethanolic extract of *Polygonum cuspidatum* and application in peanut oil. *Food Chem.* 105:1518-1524.
14. Ahn, J-H., Kim, Y-P., Seo, E-M., Choi, Y-K., Kim, H-S. 2008. Antioxidant effect of natural plant extracts on the microencapsulated high oleic sunflower oil. *J. Food Eng.* 84:327-334.
15. Gramza, A., Khokhar, S., Yoko, S., Gliszczynska-Swiglo, A., Hes, M., Korczak, J. 2006. Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur. J. Lipid Sci. Technol.* 108:351-362.
16. Dimić, E. 2005. Hladno ceđena ulja. Page 99-102. Tehnološki fakultet Novi Sad.
17. Pravilnik o jestivim uljim i mastima, *Narodne novine*, 41, 2012.
18. Chu, Y.H., Hsu, H.F. 1999. Effects of antioxidants on peanut oil stability. *Food Chem.* 66 (1):29-34.
19. Frankel, E.N., Huang, S-W., Aeschbach, R., Prior, E. 1996. Antioxidant activity of rosemary extract and its constituents carnosic acid, carnosol and rosmarinic acid in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem.* 44:131-135.
20. Erkan, N., Ayranci, G., Ayranci, E. 2008. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chem.* 110:76-82.
21. Martinez-Tome, M., Jimenez, A.M., Ruggieri, S., Frega, N., Strabbioli, R., Murcia, M.A. 2001. Antioxidant properties of Mediterranean spices compared with common food additives. *J. Food Prot.* 64(9):1412-1419.
22. Silva, F.A.M., Borges, F., Ferreira, M.A. 2001. Effects of phenolic propyl esters on the oxidative stability of refined sunflower oil. *J. Agric. Food Chem.* 49(8):3936-3941.
23. Warner, K. 2005. Effects on the flavor and oxidative stability of stripped soybean and sunflower oils with added pure tocopherols. *J. Agric. Food Chem.* 53:9906-9910.

RELATIONSHIP OF BREAD INTAKE AND BLOOD LIPID PROFILE IN WOMEN

UDC 664.661 : 613.2-055.2

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ABSTRACT

Few researches suggest that bread, as important ingredient of human daily diet, could have possible protective effect to metabolic syndrome and could improve cardiovascular health. Our aim was to determine the relationship of bread intake and serum lipid profile in women of all ages (25-93 years).

Bread intake was assessed in 229 participants using four 24-hour dietary recalls. Body height (BH), body weight (BW), % body fat (% BF) and waist circumference (WC) were measured. From venous blood samples serum triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol were measured.

There was significant difference in triglycerides level ($t(227)=-3.11$, $p<0.01$), HDL cholesterol level ($t(227)=2.93$, $p<0.01$), % BF ($t(218)=-2.31$, $p<0.05$) and WC ($t(227)=-2.01$, $p<0.05$) between women with higher (>13.41% kJ/day) and lower (<13.41% kJ/day) daily energy intake from bread. The magnitude of difference was small; 1-4% of the variance of above mentioned parameters is explained by daily energy intake from bread. When participants were grouped according to median daily intake of bread (72 g/day), there was no significant difference in observed lipid profile parameters.

The results suggest that contribution of bread intake to total daily energy intake could have greater influence on blood lipid profile than bread intake itself.

Key words: bread intake, serum triglycerides, serum HDL, 24-hour dietary recall

INTRODUCTION

Bread – essential part of food pyramid base but also popular staple food in Croatia due to frequency and quantity of consumption present a very important part of a diet. Generally, wheat bread is considered to be a good source of energy and irreplaceable nutrients for the human body. This is especially true for the products made from wholegrain. Bread prepared from refined flour is nutritionally much poorer and does not adequately meet the requirements for many macro- or micro-nutrients [1]. Having in

mind everyday consumption of bread and the increasing consumer demands for healthful food products, different improved formulations of breads becomes more important, especially if carrying health benefits.

Recently as a part of PREDIMED study in Spain research revealed that daily bread consumption, especially wholemeal bread, can prevent cardiovascular disease (CVD) [2]. According to mentioned study, people who daily consumed bread, white or wholemeal, showed a healthier lipid profile (lower levels of LDL cholesterol and higher levels of HDL cholesterol) than the people who consume it sporadically or don't consume it. However, most epidemiological studies pointed more specifically to beneficial effect of whole-grain cereal foods in prevention of CVD [3, 4], metabolic syndrome [5] and much other food-related disease.

Therefore, the aim of our study was to determine the relationship of bread intake and serum lipid profile in women of all ages (25-93 years).

SUBJECTS AND METHODS

Study participants were part of the research on the scientific project "Diet, homocysteine and bone quality" (funded by The Ministry of Science, Education and Sport of the Republic of Croatia). All the participants were volunteers and gave written consent to the study which was approved by the ethics committee of Institute for Medical Research and Occupational Health. Two-hundred-twenty-nine participants who satisfied gender (women) and diet type (omnivores) criteria were selected for study.

Anthropometric data (body height, weight, fat percentage (% BF) and waist circumference (WC)) were collected at the time of their visit for blood withdraws by trained personnel. Overnight fasting blood samples were drawn in order to measure several serum biochemical parameters. Homocysteine was determined using Abbott AxSYM systems (Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA), according to the manufacturer's instructions. HDL cholesterol, total cholesterol, and serum triglycerides, were determined enzymatically (Olympus System Reagent) with a clinical chemistry analyser Olympus AU400 (Olympus Life and Material Science Europa GmbH, Lismeehan, Ireland). LDL-cholesterol was calculated by using Friedewald formula: $LDL\text{-cholesterol} = \text{total cholesterol} - \text{HDL-cholesterol} - \text{triglycerides} / 2.2$ (mmol/L).

For each participant four 24-hour dietary recalls were collected and average energy intake was calculated using food composition tables [6, 7]. Bread intake was analysed as well as daily share of energy from bread. Quantity of consumed food and drinks was estimated using description of the units of serving (piece, cup, glass, teaspoon, tablespoon, etc.) and portions food photographs [8, 9]. The first recall was obtained by trained personnel face-to-face and the remaining recalls were obtained by unannounced telephone calls.

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 17.0, Chicago, USA) (SPSS, 2008). Beside descriptive statistics Student's t-test for independent samples was used to check for differences between groups for quantitative

variables. For the effect size eta squared values were calculated for t-test. The selected significance level was 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Blood lipid profile, anthropometric and some of the dietary characteristics of participants are shown in Table 1. Mean age of the fertile women was 40.00 ± 7.83 years, while in the group of postmenopausal women mean age was 71.18 ± 11.36 years. All blood lipid parameters of fertile women differed significantly from postmenopausal women ($p < 0.05$). These results were expected because menopause have been reported to be associated with increased serum levels of total cholesterol, LDL-cholesterol, triglycerides and decreased levels HDL-cholesterol [10-15]. The shift toward upper body fat distribution after menopause has been mainly explained by hypo estrogenic effects [16-20].

Average bread intake for all participants was 79.62 ± 41.64 g/day while household budget survey data obtained by The Croatian National Statistics Bureau estimated availability of bread and bakery products 202.5 g per person/day [21]. Average daily energy intake from bread in all participants was 216.52 ± 114.19 kcal or $14.98 \pm 7.66\%$ kJ/day.

Table 1. Blood lipid profile, anthropometric and dietary characteristics of participants

Parameter	Women						Sig. ¹
	Total		Postmenopausal (N=134)		Fertile (N=95)		
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
Homocysteine ($\mu\text{mol/L}$)	12.12	4.61	13.90	5.07	9.60	2.06	0.000*
Triglycerides (mmol/L)	1.35	0.79	1.56	0.83	1.6	0.63	0.000*
Total cholesterol (mmol/L)	5.54	1.11	5.77	1.18	5.21	0.91	0.000*
HDL cholesterol (mmol/L)	1.32	0.39	1.19	0.38	1.49	0.32	0.000*
LDL cholesterol (mmol/L)	3.63	1.01	3.91	1.05	3.23	0.81	0.000*
Body fat (%)	36.12	8.68	41.14	5.42	29.51	7.68	0.000*
Waist circumference (cm)	88.68	13.69	93.92	12.52	81.29	11.77	0.000*
BMI (kg/m^2)	27.19	5.07	28.44	4.65	25.43	5.14	0.000*
Bread intake (g/day)	79.62	41.64	83.15	42.12	74.63	40.66	0.13
Energy intake of bread (%kJ/day)	14.98	7.66	16.22	7.86	13.24	7.04	0.004*
Dietary fiber intake from bread (g)	2.46	1.37	2.51	1.37	2.39	1.38	0.52
Dietary fiber intake from bread (% total daily fiber intake)	18.14	10.75	18.84	11.02	17.14	10.34	0.24

¹ t-test between groups of fertile and postmenopausal women

* $p < 0.05$

After dividing participants in two groups according to median daily energy intake of bread there was significant difference in triglycerides level ($t(227) = -3.11$, $p < 0.01$), HDL

cholesterol level ($t(227)=2.93, p<0.01$), % BF ($t(218)=-2.31, p<0.05$) and WC ($t(227)=-2.01, p<0.05$) between women with higher ($>13.41\%$ kJ/day) and lower ($<13.41\%$ kJ/day) daily energy intake from bread (Table 2). The magnitude of difference was small; 1–4% of the variance of above mentioned parameters is explained by daily energy intake from bread. The group of researchers from the Barcelona University have shown that people who daily consumed bread, white or wholemeal, showed a healthier lipid profile (lower levels of LDL cholesterol and higher levels of HDL cholesterol) than the people who consume it sporadically or don't consume it [2]. The results of the study of the Mennen et al. suggest that a high consumption of bread may be related to reducing the risk of the metabolic syndrome [22]. However, as mentioned already above, most of the epidemiological studies attribute beneficial effects to wholegrain cereals: on lipid profile, but also on the body weight regulation [23].

In our study, the data obtained from 24-hour dietary recalls were lacking the bread type in some participants, so for that participants we presumed consumption of bread made of refined grains because we believed that otherwise participants would have reported the type of bread. The data about consumption of the whole grain or rye bread was shown at figure 3. More participants from the postmenopausal group of women consume refined or rye bread than younger, fertile participants. The reason could be in the recommendation for postmenopausal women to consume more wholegrain cereals because of the protective role of phytochemicals, dietary fibres and antioxidants [24].

Table 2. Blood lipid profile and anthropometric parameters according to daily energy intake of bread

Parameter	Daily energy intake of bread				Sig.
	$\leq 13.41\%$ kJ/day (N=112)		$> 13.41\%$ kJ/day (N=117)		
	Mean	Std. Deviation	Mean	Std. Deviation	
Homocysteine ($\mu\text{mol/L}$)	11.63	4.12	12.58	5.02	0.118
Triglycerides (mmol/L)	1.19	0.73	1.51	0.81	0.002*
Total cholesterol (mmol/L)	5.46	1.00	5.61	1.21	0.278
HDL cholesterol (mmol/L)	1.39	0.38	1.25	0.39	0.004*
LDL cholesterol (mmol/L)	3.57	0.89	3.69	1.12	0.380
Body fat (%)	34.74	8.63	37.42	8.56	0.022*
Waist circumference (cm)	86.84	12.79	90.44	14.34	0.046*
BMI (kg/m^2)	26.90	5.09	27.46	5.06	0.404

After dividing participants in two groups according to median daily energy intake of bread there was significant difference in triglycerides level ($t(227)=-3.11, p<0.01$), HDL cholesterol level ($t(227)=2.93, p<0.01$), % BF ($t(218)=-2.31, p<0.05$) and WC ($t(227)=-2.01, p<0.05$) between women with higher ($>13.41\%$ kJ/day) and lower ($<13.41\%$ kJ/day) daily energy intake from bread (Table 2). The magnitude of difference was small; 1-4% of the variance of above mentioned parameters is explained by daily energy intake from bread.

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In our study, the data obtained from 24-hour dietary recalls were lacking the bread type in some participants, so for that participants we presumed consumption of bread made of refined grains because we believed that otherwise participants would have reported the type of bread. The data about consumption of the whole grain or rye bread was shown at figure 3. More participants from the postmenopausal group of women consume refined or rye bread than younger, fertile participants. The reason could be in the recommendation for postmenopausal women to consume more wholegrain cereals because of the protective role of phytochemicals, dietary fibres and antioxidants [24].

Table 2. Blood lipid profile and anthropometric parameters according to daily energy intake of bread

Parameter	Daily energy intake of bread				Sig.
	≤13.41 % kJ/day (N=112)		>13.41 % kJ/day (N=117)		
	Mean	Std. Deviation	Mean	Std. Deviation	
Homocysteine (μmol/L)	11.63	4.12	12.58	5.02	0.118
Triglycerides (mmol/L)	1.19	0.73	1.51	0.81	0.002*
Total cholesterol (mmol/L)	5.46	1.00	5.61	1.21	0.278
HDL cholesterol (mmol/L)	1.39	0.38	1.25	0.39	0.004*
LDL cholesterol (mmol/L)	3.57	0.89	3.69	1.12	0.380
Body fat (%)	34.74	8.63	37.42	8.56	0.022*
Waist circumference (cm)	86.84	12.79	90.44	14.34	0.046*
BMI (kg/m ²)	26.90	5.09	27.46	5.06	0.404

* p < 0.05

When participants were grouped according to median daily intake of bread (72 g/day), there was no significant difference in observed lipid profile parameters or anthropometric characteristic (Table 3).

Table 3. Blood lipid profile and anthropometric parameters according to daily intake of bread

Parameter	Daily bread intake				Sig.
	≤72 g/day (N=112)		>72 g/day (N=117)		
	Mean	Std. Deviation	Mean	Std. Deviation	
Homocysteine (μmol/L)	11.65	3.71	12.56	5.31	0.136
Triglycerides (mmol/L)	1.31	0.81	1.40	0.77	0.368
Total cholesterol (mmol/L)	5.60	1.14	5.47	1.08	0.365
HDL cholesterol (mmol/L)	1.35	0.37	1.29	0.40	0.237
LDL cholesterol (mmol/L)	3.71	1.01	3.55	1.01	0.232
Body fat (%)	35.41	8.59	36.80	8.75	0.236
Waist circumference (cm)	87.61	13.96	89.70	13.41	0.250
BMI (kg/m ²)	27.13	5.18	27.24	4.99	0.871

* p < 0.05

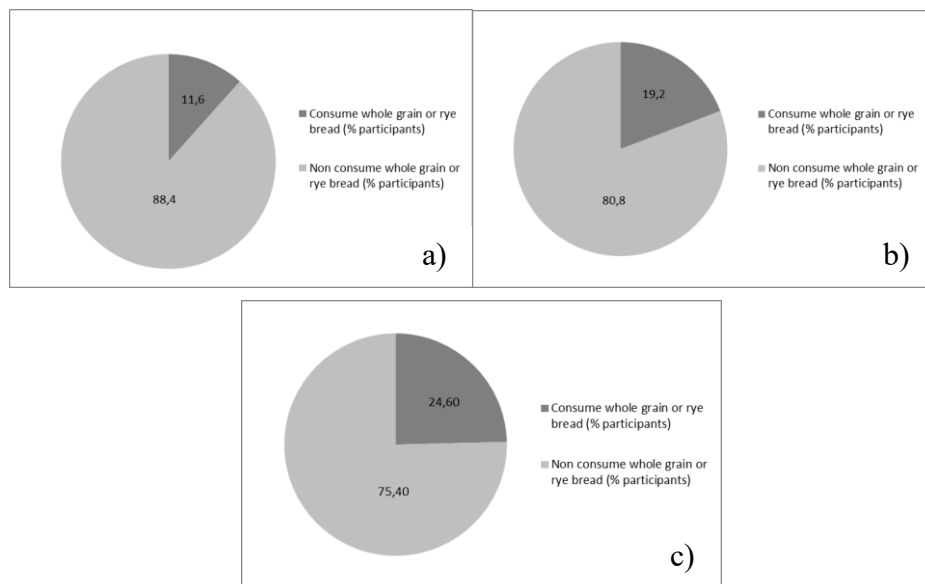


Figure 1. Consumption of whole grain and rye bread (% participants) in: a) total sample, b) fertile women and c) postmenopausal women

CONCLUSIONS

The results suggest that contribution of bread intake to total daily energy intake could have greater influence on blood lipid profile than bread intake itself, especially if bread includes both whole-grain and refined bread. The influence of certain types of bread should be observed in researches with larger number of the participants.

REFERENCES

1. Škrbić, B., Filipčev, B. 2008. Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed. *Food Chem.* 108:119–129.
2. A study explains how bread consumption can improve cardiovascular health.
http://www.ub.edu/web/ub/en/menu_eines/noticies/2012/11/066.html; Accessed 1 October, 2013.
3. McKeown, N. M., Meigs, J. B., Liu, S., et al. 2002. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *Am. J. Clin. Nutr.* 76:390–398.
4. Mellen P. B., Walsh T. F., Herrington D. M. 2008. Whole grain intake and cardiovascular disease: a meta-analysis. *Nutr. Metab. Cardiovasc. Dis.* 18:283–290.
5. Sahyoun, N. R., Jacques, P. F., Zhang, X. L., et al. 2006. Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. *Am. J. Clin. Nutr.* 83:124–131.
6. Kaic-Rak, A., and Antonic K. 1990. Tables of food and drink contents. Zagreb, Croatia: Croatian National Institute of Public Health.
7. US Department of Agriculture ARS. 2011. USDA nutrient database for standard reference. Release 24. Nutrient data laboratory home page. Available online at: <http://www.nal.usda.gov/fnic/foodcomp/Data/SR24/Sr24.html>. Accessed 24 February, 2012.
8. Senta A., Pucarín-Cvetković, J., Doko Jelinić, J. 2004. Kvantitativni modeli namirnica i obroka. Zagreb: Medicinska naklada.
9. Hess, M. A. 1997. Portion Photos of Popular Foods. American Dietetic Association, Chicago, IL.
10. Campos, H., McNamara, J. R., Wilson, P. W. F., Ordovas, J. M., and Schaefer, E. J. 1988. Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. *J. Clin. Endocrinol. Metab.* 67:30–35.
11. Brown, S. A., Hutchinson, R., Morrisett, J., Boerwinkle, E., Davis C. E., Gotto A. M., et al. 1993. Plasma lipid, lipoprotein cholesterol and apoprotein distributions in selected US communities: the atherosclerosis risk in communities (ARIC) study. *Arterioscler. Thromb.* 13:1139–1158.
12. Stevenson, J. C., Crook, D., and Godsland, I. F. 1993. Influence of age and menopause and serum lipid and lipoproteins in healthy women. *Atherosclerosis* 98:83–90.

13. Lyu, L. C., Shieh, M. J., Ordovas, J. M., Lichtenstein, A. H., Wilson, P. W., Schaefer, E. J. 1993. Plasma lipoprotein and apolipoprotein level in Taipei and Framingham. *Arterioscler. Thromb.*13:1429-1440.
14. Matthews, K. A., Meilahn, E., Kuller, L. H., Kelsey, S. F., Caggiula, A. W., Wing, R. R. 1989. Menopause and risk factors for coronary heart disease. *New Engl. J. Med.* 321:641-646.
15. Ushiroyama, T., Okamoto, Y., Sugimoto, O. 1993. Plasma lipid and lipoprotein levels in perimenopausal women. *Acta Obstet. Gynecol. Scand.* 72:428-433.
16. Pasquali, R., Casimirri, F., Labate, A.M., Tortelli, O., Pascal, G., Ancontetani, B., et al. 1994. Body weight, fat distribution and the menopausalstatus in women. *Int. J. Obes.* 18: 614-621.
17. Kaye, S. A., Folsom, A. R., Prineas, R. J., Potter, J. D., Gapstur, S. M. 1990. The association of body fat distribution with life style and reproductive factors in a population study of post- menopausal women. *Int. J. Obes.* 14:583-591.
18. Reubinoff, B. E., Wurtman, J., Rojansky, N., Adler, D., Stein, P., Schenker, J. G., et al. 1995 Effects of hormone replacement therapy on weight, body composition, fat distribution, and food intake in early postmenopausal women: a prospective study. *Fertil. Steril.* 64:963-968.
19. Ijuin, H., Douchi, T., Oki, T., Maruta, K., Nagata, Y. 1999. The contribution of menopause to changes in body-fat distribution. *J. Obstet. Gynaecol.* 25:367-372.
20. Tremollieres, F. A., Poilles, J. M., Ribot, C. A. 1996. Relative influence of age and menopause on total and regional body composition changes in postmenopausal women. *Am. J. Obstet. Gynecol.* 175:1594-1600.
21. The Croatian National Statistics Bureau. 2012. Statistical Yearbook of the Republic of Croatia. Zagreb:The Croatian National Statistics Bureau.
22. Mennen, L. I., Lafay, L., Feskens, E. J. M., Novak, M., Lépinay, P., Balkau, B. 2000. Possible protective effect of bread and dairy products on the risk of the metabolic syndrome. *Nutrition Res.* 20:335-347.
23. Karl, J. P., Saltzman, E. 2012. The Role of the whole grains in Body weight regulation. *Adv. Nutr.* 3:697-707.
24. Jacobs, D. R., Meyer, K. A., Kushi, L. H., Folsom, A. R. 1998. Whole-grain intake may reduce the risk od ischemic heart disease death in postmenopausal women: the Iowa Women's Health Study. *Am. J. Clin. Nutr.* 68:248-257.

ANTIOXIDANT AND SENSORY PROPERTIES OF RICE-BUCKWHEAT GLUTEN-FREE COOKIES

UDC 664.681 : 543.92
664.641.4 : 543.92

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ABSTRACT

Rice-buckwheat gluten-free cookies were produced using light buckwheat flour (LBF) to substitute rice flour (RF) in gluten-free cookies' formulation at the level of 10, 20% and 30%. Rice-buckwheat gluten-free cookies exhibited significantly higher ($P<0.05$) total phenolic and rutin content, antiradical activity on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH), antioxidant activity (AOA) and reducing power than the rice cookies which were used as the control. LBF in the gluten-free cookies' formulation improved chelating activity on Fe^{2+} of rice-buckwheat gluten-free cookies, but there were no significant differences ($P<0.05$) regarding the substitution levels. Cookies enriched with LBF at the level of 30% (30% RF/LBF cookies) possessed the highest antioxidant capacity. Concerning all evaluated sensory properties, cookies enriched with LBF at the level of 20% (20% RF/LBF) had the most acceptable sensory properties.

Keywords: gluten-free cookies, antioxidant activity, sensory properties, rice flour, buckwheat flour

INTRODUCTION

Celiac disease is one of the most common chronic autoimmune disorders. It is related to the incompetence to consume proteins of gluten complex present in many common cereals such as wheat, rye, barley and oat. The symptoms of celiac disease can only be avoided by being on a strict long-life gluten-free diet.

A number of studies indicate that gluten-free diet is unbalanced in carbohydrates, proteins and fat and deficient in certain essential nutrients [1]. Due to the limitation of some nutrients, the fortification of basic gluten-free formulations is recommended to develop added value products.

Cookies are widely used bakery products due to their long shelf-life and strong consumer preference. The fortification of gluten-free biscuit and cookie formulations with the aim to achieve better nutritional profile with acceptable sensory properties is already known [2, 3].

Rice and buckwheat flours are recommended as the safe components for celiac patients since they possess no gluten and can be used in the production of bread [4] and cookies [3]. Rice flour is known as the most suitable component for gluten-free formulations due to its mild taste, colorlessness, hypoallergenic properties and easily digestible carbohydrates [5]. Buckwheat flour is characterized as a well-balanced amino acid gluten-free component rich in polyphenols [6, 7]. The dominant polyphenol compound in buckwheat flour is rutin [8], which was evidenced as a potent antioxidant [9].

Schoenlechner, Linsberger, Kaczyc, and Berghofer [10] incorporated pseudocereal flours (amaranth, quinoa and buckwheat) in a gluten-free biscuit formulation. Cookies based on rice and light buckwheat flour were initially characterized by Torbica, Hadnađev, and Dapčević Hadnađev [3] from the aspect of their physicochemical and sensory characteristics and compared to the control which was based on wheat flour. According to the authors, the best overall quality was achieved for rice gluten-free cookies enriched with 20% of LBF.

The objective of this paper was to investigate the antioxidant capacity and sensory properties of the cookies based on rice-light buckwheat flour mixtures in three different ratios and to compare them with the control sample based on rice flour.

MATERIALS AND METHODS

Materials

Rice flour – RF (moisture: 11.67%, protein (Nx5.7) 7.96%, fat 0.27%, ash 0.25%, reducing sugars 1.37%, and starch 88.58%) and light buckwheat flour – LBF (moisture 11.24%, protein (Nx5.7) 8.68%, fat 1.47, ash 1.08%, reducing sugars 1.77%, and starch 85.38%) were obtained from Hemija Komerc, Novi Sad, Serbia. Vegetable fat A.P 34-36 originated from refined palm and sunflower oil was obtained from Puratos, Belgium. Sodium hydrogen carbonate – NaHCO₃ (≥99.5%, p.a) was purchased from Carl Roth, Germany, carboxymethyl cellulose sodium salt – CMC from Alfa Aesar, Germany, diacetyl tartaric acid esters of distilled monoglycerides – DATEM (Pantex DW90) from InCoPa, Germany, while the other ingredients (salt, sugar and honey) were purchased at the local market.

Preparation of cookies

The formulation of rice-buckwheat gluten-free cookies was made according to Torbica, Hadnađev, and Dapčević-Hadnađev [3]. Mixtures of RF and LBF were prepared. The proportion of RF to LBF was 90:10, 80:20, and 70:30, respectively. Rice flour was used for the preparation of control cookies. Dough mixing, processing and baking were performed on laboratory-scale equipment. The ingredients were weighed as follows: flour (300.0 g of RF for the control cookies or 270.0 g of RF+30.0 g of LBF for 10% RF/LBF cookies or 240.0 g of RF+60.0 g of LBF f for 20% RF/LBF cookies or 210.0 g of RF+90.0 g of

LBF for 30% RF/LBF cookies), deionized water 75.0 g, vegetable fat 85.0 g, granulated sugar 70.0 g, honey 45.0 g, NaHCO₃ 9.0 g, DATEM 9.0 g, CMC 4.5 g, and salt 2.1 g.

Proximate composition

Proximate composition of cookies including protein, fat, reducing sugar, total dietary fiber, ash and water contents were determined by the methods of AOAC [11]. Starch content was determined by hydrochloric acid dissolution according to the ICC Standard [12].

Preparation of ethanolic extracts

Cookie powder (5 g) was extracted with 50 mL of ethanol/water (80/20, v/v) by shaking the mixture at room temperature for 1 h. The procedure was repeated twice, and combined extracts were dried using vacuum-evaporator. The dried extract was redissolved in ethanol/water (80/20, v/v) to 10 mL volume and used for further investigation of antioxidant activity.

Total phenolic content

Total phenolic content of rice-buckwheat gluten-free cookie extracts was determined spectrophotometrically by using Folin-Ciocalteu's reagent [13]. Gallic acid was used as a standard reference and results were expressed as gallic acid equivalents (GAE) ($\mu\text{g GAE/g}$ of sample on dry mass basis).

Antioxidant activity (AOA) by β -carotene bleaching method

Oxidative loss of β -carotene in a β -carotene/linoleic acid emulsion was used to assess the antioxidant activity of the examined extracts [14]. The IC₅₀ value (mg/mL) was defined as effective concentration at which the AOA was 50% under the experimental conditions.

Reducing power

Reducing power of the ethanolic extracts was measured according to the method of Oyaizu [15]. The IC₅₀ value (mg/mL) was defined as an effective concentration of extract at which the absorbance of reaction mixture reach 0.5 for reducing power.

DPPH radical scavenging activity

Effect of the examined extracts on the content of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) was estimated according to the modified method of Hatano, Kagawa, Yasuhara, and Okuda [16]. The IC₅₀ value (mg/mL) was defined as the concentration of an

antioxidant extract which was required to quench 50% of the initial amount of DPPH under the experimental conditions given.

Chelating activity on Fe²⁺

Chelating activity of the ethanolic extracts on Fe²⁺ was measured according to the method of Decker and Welch [17]. The IC₅₀ value (mg/mL) was defined as the concentration of antioxidant extract which chelates 50% of present Fe²⁺ under the experimental conditions.

HPLC determination of rutin

Cookie powder (5 g) was extracted with 20 mL of boiling methanol/water (80/20, v/v) for 10 min, ultrasonicated for 10 min and filtered through 0.45 µm pore size nylon filter (Rotilabo-Spritzenfilter 13 mm, Roth, Karlsruhe, Germany) before injection into the HPLC system.

Liquid chromatograph (Agilent 1200 series), equipped with a DAD detector and an Eclipse XDB-C18, 1.8 µm, 4.6×50 mm column (Agilent) was used for quantification of rutin in cookie extracts. A single rapid resolution HPLC method reported by Mišan, Mimica-Dukić, Mandić, Sakač, Milovanović, and Sedej [18] was used.

Sensory evaluation

Sensory evaluation was conducted 24 h after baking by eight experienced panellists (seven females and 1 male, at the age of 30-43 years). The panellists were selected according to ISO 8586-1 [19]. Prior to sensory analysis, sensory profile of gluten-free cookies was established by a multidimensional approach. The established sensory profile included 10 descriptors with their definitions and evaluation techniques [20]. To express intensity of each perceived descriptor the intensity scale was applied, from 0 – absence of perception to 5 – strong perception/maximal intensity [21, 22]. The samples were evaluated on three occasions. Drinking water was provided for palate cleansing after each sample.

Statistical analysis

Results were expressed as the mean of replications ± standard deviation. ANOVA and Fisher's multiple range tests were used. P values ≤ 0.05 were regarded as significant. All analyses were made using the Software XLSTAT, version (2012.2.02) (<http://www.xlstat.com/>).

RESULTS AND DISCUSSION

The series of rice-buckwheat gluten-free cookies were produced with the proximate composition presented in Table 1.

Table 1. Proximate composition (% dry basis) of cookies

Component (g/ 100 g)	Control sample	10% RF/LBF cookies	20% RF/LBF cookies	30% RF/LBF cookies
Protein	4.41±0.13 ^a	4.48±0.02 ^{ab}	4.60±0.06 ^b	4.86±0.12 ^c
Fat	19.6±0.05 ^a	19.6±0.05 ^a	19.8±0.24 ^a	19.9±0.30 ^a
Starch	53.2±0.51 ^a	52.6±0.74 ^a	52.3±0.10 ^a	52.3±0.04 ^a
Reducing sugars	15.3±0.12 ^c	15.1±0.09 ^{ab}	15.2±0.05 ^b	15.0±0.03 ^a
Ash	2.32±0.02 ^a	2.41±0.02 ^b	2.41±0.02 ^b	2.46±0.01 ^c
Total dietary fiber	1.93±0.03 ^a	2.26±0.09 ^b	2.55±0.05 ^c	2.94±0.04 ^d

Values are means of three determinations ± standard deviation.

Values of the same row with the same superscript are not statistically different ($P<0.05$).

RF – rice flour; LBF – light buckwheat flour

Control sample – RF cookies; 10% RF/LBF cookies – RF cookies enriched with LBF at the level of 10%; 20% RF/LBF cookies – RF cookies enriched with LBF at the level of 20%; 30% RF/LBF cookies – RF cookies enriched with LBF at the level of 30%.

Due to the higher total phenolic content of LBF than of RF [4], the significant increase ($P<0.05$) in total phenolic content of rice-buckwheat gluten-free cookies was found in comparison with the rice ones (Table 2). Buckwheat was previously used for upgrading of gluten-free products [23], especially as a component for gluten-free bread [4] and cookies [3].

Rutin, a potent antioxidant [24], which is dominant in LBF [25], is not presented in RF, which contains ferulic and p-coumaric acids as the main polyphenols [26]. Therefore, incorporation of LBF in gluten-free cookies' formulation resulted in increased concentration of rutin in the following order: 30 % RF/LBF cookies >20% RF/LBF cookies >10% RF/LBF cookies (Table 2).

The antioxidant activity of buckwheat flour is superior to RF, due to its higher total phenolic content [4], especially rutin [25]. Therefore, its incorporation in gluten-free cookies' formulation was expected to cause increased antioxidant activity of enriched gluten-free cookies, which was proven by the results of four applied tests for the determination of antioxidant activity (Table 2). The increasing proportion of LBF in the

flour mixtures from 10% to 30% in gluten-free cookies' formulation resulted in cookies with increased antioxidant properties, expressed as IC₅₀.

Table 2. Phenolic content and antioxidant activities of rice cookies (RF cookies) and rice-buckwheat gluten-free cookies (10% RF/LBF cookies, 20% RF/LBF cookies and 30% RF/LBF cookies)

Extracts	Total phenolic content* (µg GAE/g)	Rutin* (µg/g)	AOA, IC ₅₀ (mg/mL)	Reducing activity, IC ₅₀ (mg/mL)	DPPH scavenging activity, IC ₅₀ (mg/mL)	Chelating activity on Fe ²⁺ , IC ₅₀ (mg/mL)
Control	955±46.2a	n.d.	25.0±0.21a	37.7±0.97a	23.2±0.69a	17.6±0.65a
10% RF/LBF	1038±43.9b	25.1±0.54a	21.9±2.59b	32.3±0.63b	22.9±1.37a	4.79±0.73b
20% RF/LBF	1248±13.7c	33.3±0.23b	18.7±1.70c	31.3±0.77b	16.8±0.75b	4.76±1.01b
30% RF/LBF	1349±16.9d	40.1±0.58c	11.4±0.97d	29.0±1.15c	14.7±0.93c	4.64±0.52b

*Results are presented on dry mass basis.

Values are means of three determinations ± standard deviation.

Values of the same row with the same superscript are not statistically different ($P < 0.05$).

Abbreviations are the same as in Table 1.

AOA negatively correlated with the total phenolic content ($R = -0.951$) suggesting that phenolic compounds presented in produced cookies mainly contributed to their overall antioxidant properties. Furthermore, thermal processing of cereals, such as baking, also results in the formation of substances with antioxidant properties, namely Maillard reaction products [27], which contribute to the overall antioxidant activity of bakery products.

Most of the sensory properties were not significantly influenced by the LBF addition compared to the control sample (Table 3). However, the enrichment of control gluten-free cookies' formulation with 20% LBF resulted in significantly distinct intensity ($P < 0.05$) of colour and odour. These results are in agreement with previously published findings of Luthar [28], who found that the presence of aromatic compounds in buckwheat flour improved the pleasant odour and taste compared to the bland and neutral rice flour [29]. More intensive colour was found to exist in 20% RF/LBF and 30% RF/LBF than in other two samples, due to the differences in amino acids profile [30] and the content of reducing sugars between buckwheat and rice flour, which further caused the differences in nonenzymatic browning reaction during baking process [31].

Better textural properties, such as hardness and crumbliness, of RF cookies could be addressed to their better quality of protein structure compared to the RF/LBF cookies (Table 3).

Generally, the addition of LBF in the gluten-free cookies' formulation did not have a negative influence on their sensory quality. In comparison with other investigated samples, 20% RF/LBF was found to have the most acceptable sensory properties. Our findings are in accordance with the previous findings of Torbica, Hadnađev and Dapčević [3], who applied different sensory method of evaluation.

Table 3. Sensory scores of cookies

Property	Control sample	10% RF/LBF cookies	20% RF/LBF cookies	30% RF/LBF cookies
Colour	3.08±0.51 ^a	3.33±0.49 ^{ab}	3.92±0.67 ^c	3.67±0.78 ^{bc}
Odour	3.08±0.67 ^a	3.17±0.39 ^a	3.67±0.65 ^b	3.25±0.62 ^{ab}
Fatness	3.25±0.75 ^a	3.33±0.49 ^a	3.50±0.52 ^a	3.58±0.79 ^a
Hardness	4.08±0.51 ^b	3.25±0.75 ^a	3.33±0.65 ^a	3.42±0.67 ^a
Crumbliness	1.67±0.78 ^a	1.75±0.87 ^{ab}	2.33±0.49 ^b	2.08±0.79 ^{ab}
Sharpness	3.17±0.39 ^a	2.75±0.75 ^a	2.83±0.83 ^a	2.83±0.72 ^a
Fracturability	3.17±1.11 ^a	3.67±0.89 ^a	3.83±0.94 ^a	3.50±0.80 ^a
Adhesiveness	2.58±0.90 ^a	2.33±0.89 ^a	2.08±0.90 ^a	2.25±0.62 ^a
Particle size/shape	3.25±0.62 ^a	3.42±0.67 ^a	3.50±0.67 ^a	3.67±0.78 ^a
Taste	3.07±0.58 ^a	3.42±0.67 ^b	3.53±0.49 ^b	3.58±0.51 ^b

Scores are means ± standard deviation.

Values of the same row with the same superscript are not statistically different (P<0.05).

Abbreviations are the same as in Table 1.

CONCLUSIONS

Light buckwheat flour (LBF) was used to enhance antioxidant capacity of rice-buckwheat gluten-free cookies compared to the rice cookies that were used as the control.

The substitution of rice flour in gluten-free cookies' formulation with LBF at the level of 10-30% resulted in significantly higher ($P < 0.05$) total phenolic content and rutin content, antiradical activity on DPPH, AOA and reducing power than in the control cookies.

Comparing all evaluated sensory properties, cookies enriched with LBF at the level of 20% (20% RF/LBF) expressed the most acceptable sensory properties.

These results indicate the benefit of using LBF in the production of gluten-free cookies, i.e. its contribution to their functionality.

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REFERENCES

1. Thompson, T., Dennis, M., Higgins, L. A., Lee, A. R., and Sharrett, M. K. 2005. Gluten-free diet survey: are Americans with celiac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *J. Hum. Nutr. Diet.* 18:163-169.
2. Rodrigues, F. T., Fanaro, G. B., Duarte, R. C., Koike, A. C., and Villavicencio, A. L. C. H. 2012. A sensory evaluation of irradiated cookies made from flaxseed meal. *Radiat. Phys. Chem.* 81:1157-1159.
3. Torbica, A., Hadnađev, M., and Dapčević Hadnađev, T. 2012. Rice and buckwheat flour characterization and its relation to cookie quality. *Food Res. Int.* 48:277-283.
4. Sakač, M., Torbica, A., Sedej, I., and Hadnađev, M. 2011. Influence of breadmaking on antioxidant capacity of gluten free breads based on rice and buckwheat flours. *Food Res. Int.* 44:2806-2813.
5. Gujral, H. S., and Rosell, C. M. 2004. Functionality of rice flour modified with a microbial transglutaminase. *J. Cereal Sci.* 39:225-230.
6. Alvarez-Jubete, L., Wijngaard, H., Arendt, E. K., and Gallagher, E. 2010. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chem.* 119:770-778.
7. Wronkowska, M., Zielińska, D., Szawara-Nowak, D., Troszyńska, A., and Soral-Śmietana, M. 2010. Antioxidative and reducing capacity, macroelements content and sensorial properties of buckwheat-enriched gluten-free bread. *Int. J. Food Sci. Tech.* 45:1993-2000.
8. Oomah, B. D., and Mazza, G. 1996. Flavonoids and antioxidative activities in buckwheat. *J. Agr. Food Chem.* 44:1746-1750.
9. Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J. A., and Briggs, C. J. 2007. Rutin and flavonoid in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*,

- and F. homotropicum and their protective effects against lipid peroxidation. *Food Res. Int.* 40:356-364.
10. Schonlechner, R., Linsberger, G., Kaczyk, L., and Berghofer, E. 2006. Production of gluten-free short dough biscuits from the pseudocereals amaranth, quinoa and buckwheat with common bean. *Ernahrung/Nutrition*, 30:101-107.
 11. Official Methods of Analysis AOAC 1984. 14th ed. The Association of Official Analytical Chemists: Washington, DC, USA.
 12. International Cereal Standard ICC Standard No. 123/1. 1994. Determination of starch content by hydrochloric acid dissolution. The International Association for Cereal Science and Technology.
 13. Singleton, V. L., Orthofer, R., and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 299:152-178.
 14. Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., Núñez, M. J., and Parajó, J. C. 2001. Natural antioxidants from residual sources. *Food Chem.* 72:145-171.
 15. Oyaizu, M. 1986. Studies on products of browning reaction – antioxidant activities of products of browning reaction prepared from glucoamine. *Jpn. J. Nutr.* 44:307-315.
 16. Hatano, T., Kagawa, H., Yasuhara, T., and Okuda, T. 1988. Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.* 36:2090-2097.
 17. Decker, E. A., and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agr. Food Chem.* 38:674-677.
 18. Mišan, A., Mimica-Dukić, N., Mandić, A., Sakač, M., Milovanović, I., and Sedej, I. 2011. Development of a rapid resolution HPLC method for the separation and determination of 17 phenolic compounds in crude plant extracts. *Cent. Eur. J. Chem.* 9:133-142.
 19. ISO 8586-1. 2012. Sensory analysis: General guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors. International Organization for Standardization: Geneva, Switzerland.
 20. Jambrec, D., Pestorić, M., and Žigon, U. 2012. Development and application of descriptors for establishing sensory profile of gluten-free cookies by a multidimensional approach. *Food Feed Res.* 39:41-49.
 21. ISO 11035 1994. Sensory analysis – Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach. International Organization for Standardization: Geneva, Switzerland.
 22. ISO 4121 2003. Sensory analysis – Guidelines for the use of quantitative response scales. International Organization for Standardization: Geneva, Switzerland.
 23. Alvarez-Jubete, L., Arendt, E. K., and Gallagher, E. 2010. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends Food Sci. Tech.* 21:106-113.
 24. Yang, J., Guo, J., and Yuan, J. 2008. In vitro antioxidant properties of rutin. *LWT-Food Sci. Technol.* 41:1060-1066.

25. Holasova, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, L., and Vavreinova, S. 2002. Buckwheat – the source of antioxidant activity in functional foods. *Food Res. Int.* 35:207-211.
26. Harukaze, A., Murata, M., and Homma, S. 1999. Analyses of free and bound phenolics in rice. *Food Sci. Technol. Res.* 5:74-79.
27. Lindenmeier, M., and Hofmann, T. 2004. Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *J. Agr. Food Chem.* 52:350-354.
28. Luthar, Z. 1992. Polyphenol classification and tannin content of buckwheat seeds (*Fagopyrum esculentum* Moench). *Fagopyrum.* 12:36-42.
29. Kadan, R. S., Bryant, R. J., and Miller, J. A. 2008. Effects of milling on functional properties of rice flour. *J. Food Sci.* 73:E151-E154.
30. Edwardson, S. 1996. Buckwheat: Pseudocereal and Nutraceutical. Page 195-207 in: *Progress in New Crops*, J. Janick (Ed.), Alexandria, VA: ASHS Press.
31. Zucco, F., Borsuk, Y., and Arntfield, S. D. 2011. Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. *LWT-Food Sci. Technol.* 44:2070-2076.

CEREALS AND CEREAL PRODUCTS CONSUMPTION RELATED TO FOLATE INTAKE IN OLDER WOMEN ACCORDING TO DENTURE WEARING

UDC 664.696 : 613.2-055.2

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ABSTRACT

One of the factors that are related to dietary selection of older individuals is oral health status. Food choices change significantly when a patient begins using dentures. Denture wearers tend to eat fewer vegetables, fruits, and whole grains, which results in less than desirable intake of certain vitamins such as folate. The aim of this study was to determine whether there is an impact of denture wearing on cereals and cereal products intake, and also on folate intake in older women.

The study included 57 women (mean age 63.5 years). Validated quantitative food frequency questionnaire was used.

Denture wearers (n=20) had higher average daily intake of cereals and cereal products than non-denture wearers (n=37) (155.9 g vs. 145.3 g), but the difference was not significant. Folate intake was not significantly different according to denture wearing. Higher number of denture wearers avoided hard-to-chew foods, such as crusty, dry breads (25.0% vs. 8.6%), and sticky foods, such as soft, doughy breads (75.0% vs. 45.7%) compared to non-denture wearers.

The results showed no significant difference in the amount of cereals and cereal products consumed and in the average folate intake in older women according to denture wearing.

Keywords: cereals, folate, older women, denture wearing

INTRODUCTION

One of the factors that are related to dietary selection of older individuals is oral health status [1]. The number and distribution of the remaining natural teeth plus the quantity and quality of saliva production influence oral function in older individuals [2].

There is some evidence that impaired dentition can affect individuals by causing dietary restrictions via difficulty in chewing [3]. This may be because people who cannot chew or bite comfortably are less likely to consume high-fiber foods such as bread, fruit, and vegetables, thereby risking reducing their intake of essential nutrients [4].

Food choices change significantly when a patient begins using dentures [2]. Eating abilities with dentures are not equivalent to natural teeth; the majority of published researches [5, 6] have demonstrated poor nutrient composition and eating-related quality of life in individuals with dentures as compared to those with natural teeth. The potential for these deficits to result in malnutrition, particularly among elderly people, who represent the largest cohort of denture wearers, is high [7]. Denture wearers tend to eat fewer vegetables, fruits, and whole grains, which results in less than desirable intake of certain vitamins such as vitamin C, vitamin E and folate [2].

The aim of this study was to determine whether there is an impact of denture wearing on cereals and cereal products intake, and also on folate intake in older women.

MATERIALS AND METHODS

The study included 57 women aged 55-80 years (mean age 63.5 years) from Zagreb. Participants were recruited via various advertisements and included colleagues, friends or acquaintances of researchers. Participants were divided in two groups according denture wearing; one group was women with dentures (n=20) and the other group was women with natural teeth (n=37).

Participants underwent a dental examination by a dentist. They were then asked to specify certain foods that they had difficulty to eat.

Validated quantitative food frequency questionnaire (FFQ) [8] was used to determine an average folate intake and also to determine intake of some typical cereal products such as pasta, rice, ready-to-eat cereals and different kind of breads. The FFQ was designed to measure folate intake in μg of dietary folate equivalent (μg DFE). The FFQ is a 39-item questionnaire that uses the previous month as a reference period with the following consumption frequencies: never, 1 time/month, 2-3 times/month, 1 time/week, 2-3 times/week, 4-6 times/week and every day. The subjects received the FFQ in the form of a booklet with incorporated food photographs [9]. Each photograph showed small, medium, and large portion sizes, or portion size was described with a kitchen utensils. Folate intake was calculated using national food composition tables [10].

RESULTS AND DISCUSSION

Denture wearers had higher average daily intake of cereals and cereal products (pasta, rice, bread, ready-to-eat cereals) than non-denture wearers (155.9 g vs. 145.3 g), but the difference was not significant. Non-denture wearers consumed more whole-grain bread in average than denture wearers (40.8 g vs. 37.1 g), and denture wearers consumed more

bread in total than non-denture wearers (60.3 g vs. 58.5 g), but the differences were also not significant (Table 1).

Table 1. Average cereals and cereal products intake according to denture wearing

Food groups (g)	Denture wearers (n=20)	Non-denture wearers (n=37)	P
Pasta	37.6 ± 35.0	37.5 ± 30.2	0.99
Rice	28.9 ± 19.9	28.3 ± 23.9	0.92
Whole-grain bread	37.1 ± 49.5	40.8 ± 38.0	0.76
Bread	60.3 ± 43.7	58.5 ± 32.5	0.86
Ready-to-eat cereals	19.1 ± 30.4	18.3 ± 26.1	0.91
Pasta, rice, bread, ready-to-eat cereals	155.9 ± 104.3	145.3 ± 62.1	0.68

Values are the means ± SD

Statistical significance at $p < 0.05$

Average daily folate intake by cereals and cereal products and total folate intake are presented in Table 2. Folate intake was not significantly different according to denture wearing. Total folate intake was higher in a group of denture wearers (211.1 µg DFE) than in a group of non-denture wearers (184.8 µg DFE), but it was not significantly different. In a study by Ranta et al., elderly individuals wearing complete dentures consumed lower levels of proteins and dietary fiber, as well as micronutrients such as thiamin, iron and folate [11]. Statistical differences were observed between the two groups of participants in a study with French elderly people, the study group (complete denture wearers) showing mean intakes lower than the control group (fully dentate) for many micronutrients, i.e. magnesium, calcium, iron, riboflavin, and folate. Mean folate intake was 231.0 µg in a group of complete denture wearers, and 301.2 µg in a fully dentate group [12], what was opposite of what we determined in this study. Participants in a group with impaired dentition had lower dietary intake levels of vitamin A, carotene, folic acid, and vitamin C in older American adults [13]. Non-denture wearers had higher intake of folate by ready-to-eat cereals (13.6 µg DFE vs. 6.5 µg DFE) than denture wearers (Table 2), because they consumed more ready-to-eat cereals enriched with folic acid.

Table 2. Average daily folate intake by cereals and cereal products according to denture wearing

Folate intake ($\mu\text{g DFE}$) from:	Denture wearers (n=20)	Non-denture wearers (n=37)	p
Pasta	0.8 \pm 0.7	0.8 \pm 0.6	0.99
Rice	1.7 \pm 1.2	1.7 \pm 1.4	0.92
Whole-grain bread	13.8 \pm 18.1	15.1 \pm 13.8	0.77
Bread	21.6 \pm 15.3	21.9 \pm 12.0	0.94
Ready-to-eat cereals	6.5 \pm 12.7	13.6 \pm 30.3	0.22
Pasta, rice, bread, ready-to-eat cereals	34.1 \pm 29.9	37.9 \pm 32.7	0.67
Folate total ($\mu\text{g DFE}$)	211.1 \pm 86.6	184.8 \pm 101.1	0.33
Folate total (% RDA)	52.8 \pm 21.7	46.2 \pm 25.3	0.33

Values are the means \pm SD

Statistical significance at $p < 0.05$

DFE=dietary folate equivalent

Higher number of non-denture wearers consumed bread every day (67.6%) than denture wearers (60.0%), but higher number of denture wearers consumed bread in average 5 and 2.5 times per week (Figure 1).

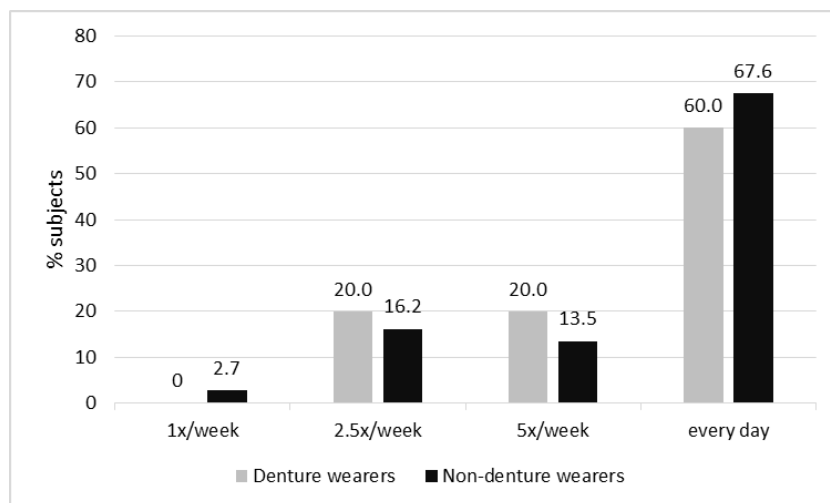


Figure 1. Frequency of bread consumption according to denture wearing (% subjects)

Higher number of non-denture wearers (37.8%) consumed exclusively whole-grain bread than denture wearers (30.0%). Higher number of denture wearers (40.0%) consumed all three types of bread equally than non-denture wearers (24.3%) (Figure 2).

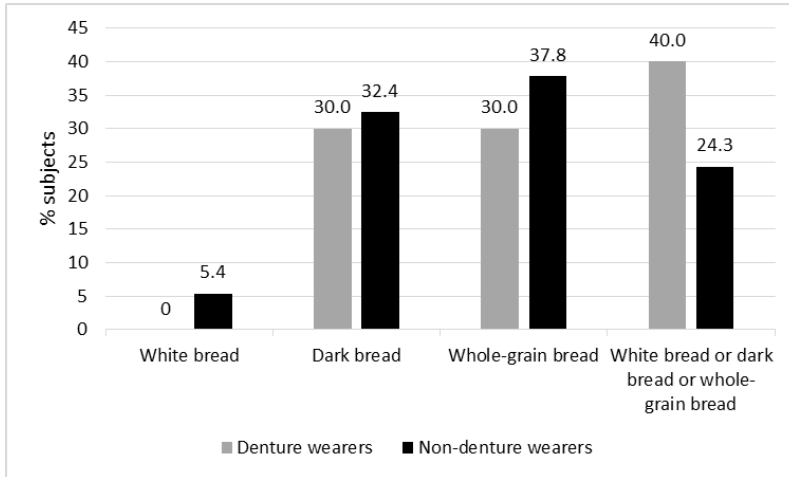


Figure 2. Types of bread the most often consumed according to denture wearing (% subjects)

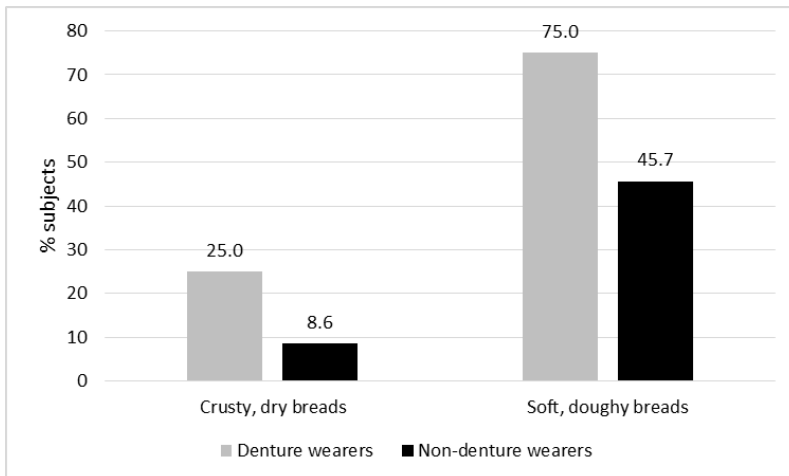


Figure 3. Types of bread that are usually avoided according to denture wearing (% subjects)

Poor oral health status is often associated with the avoidance of hard-to-chew foods [1]. These foods can be of the sticky/gummy, hard/chewy, or small-particle varieties and present difficulties for denture patients [2]. Elderly persons wearing complete dentures tended to have a lower intake of difficult-to-chew food items, such as vegetables, fruits, meat and whole grains, than those with natural dentition [14, 15]. In this study higher number of denture wearers avoided hard-to-chew foods, such as crusty, dry breads (25.0 vs. 8.6%), and sticky foods, such as soft, doughy breads (75.0% vs. 45.7%) compared to non-denture wearers (Figure 3).

The number of natural teeth significantly affected the ability to eat some foods in older people in British National Diet and Nutrition Survey [16]. All people with 21 or more teeth could eat sliced bread and crusty bread easily compared with people with fewer teeth, who had some difficulty eating them.

Impaired dental status and chewing difficulties did not affect the frequency of eating "difficult to chew" food, but it was related to more frequent consumption of softer food (fish, grains, and dairy products) in older Greeks. They did not exclude any food type from their diet, because of dental impairment. To overcome chewing problems they used various preparing methods to consume their favorite food [17].

CONCLUSIONS

The results showed no significant difference in the amount of cereals and cereal products consumed and in the average folate intake in older women according to denture wearing.

Higher number of denture wearers avoided some types of cereal products that are hard-to-chew or doughy compared to non-denture wearers.

REFERENCES

1. Budtz-Jorgensen, E., Chung, J-P., and Rapin, C-H. 2001. Nutrition and oral health. *Best Pract. Res. Clin. Gastroenterol.* 15:885-896.
2. Mobley C. C. 2005. Nutrition issues for denture patients. *Quintessence Int.* 36:627-631
3. Marcenes, W., Steele, J. G., Sheiham, A., and Gilmour Walls, A. W. 2003. The relationship between dental status, food selection, nutrient intake, nutritional status, and body mass index in older people. *Cad. Saude. Publica.* 19:809-816.
4. Brodeur, J. M., Laurin, D., Vallee, R., and Lachapelle, D. 1993. Nutrient intake and gastrointestinal disorders related to masticatory performance in the edentulous elderly. *J. Prosthet. Dent.* 70:468-473.

5. Witter, J., Tekamp, F. A., Slagter, A. P., Kreulen, C. M., Cregers, N. 2011. Swallowing threshold parameters of subjects with complete dentures and overdentures. *Open J. Stomatol.* 1:69-74.
6. Awad, M. A., Morais, J. A., Wollin, S., Khalil, A., Gray-Donald, K., Feine, J. S. 2012. Implant overdentures and nutrition: A randomized controlled trial. *J. Dent. Res.* 91:39-46.
7. Position of the Academy of Nutrition and Dietetics. 2013. *Oral Health and Nutrition. J. Acad. Nutr. Diet.* 113:693-701.
8. Colić Barić, I., Štalić, Z., Keser, I., Cecić, I., Sučić, M. 2009. Validation of folate food frequency questionnaire with serum and erythrocyte folate and plasma homocysteine. *Int J. Food Sci. Nutr.* 60 (Suppl 5):10-18.
9. Senta, A., Pucarin-Cvetković, J., Doko Jelinić, J. 2004. Kvantitativni modeli namirnica i obroka. *Medicinska naklada, Zagreb.*
10. Kaić-Rak, A., Antonić, K. 1990. *Tablice o sastavu namirnica i pića.* Zavod za zaštitu zdravlja SR Hrvatske, Zagreb.
11. Ranta, K., Tuominen, R., Paunio, I., and Seppanen, R. 1988. Dental status and intake of food items among an adult Finnish population. *Gerodontology.* 4:32-35.
12. Cousson, P. Y., Bessadet, M., Nicolas, E., Veyrune, J-L., Lesourd, B., and Lassauzay, C. 2012. Nutritional status, dietary intake and oral quality of life in elderly complete denture wearers. *Gerodontology.* 29:e685–e692.
13. Sahyoun, N. R., Lin, C-L., and Krall, E. 2003. Nutritional status of the older adult is associated with dentition status. *J Am Diet Assoc.* 103:61-66.
14. Walls, A. W. G., Steele, J. G. 2004. The relationship between oral health and nutrition in older people. *Mech Ageing Dev.* 125: 853-857.
15. Papas, A. S., Palmer, C. A., Rounds, M. C., and Russell, R. M. 1998. The effects of denture status on nutrition. *Spec. Care Dentist.* 18:17-25.
16. Sheiham, A., and Steele, J. 2001. Does the condition of the mouth and teeth affect the ability to eat certain foods, nutrient and dietary intake and nutritional status amongst older people? *Public Health Nutr.* 4:797-803.
17. Kossioni, A., and Bellou, O. 2011. Eating habits in older people in Greece: The role of age, dental status and chewing difficulties. *Arch. Gerontol. Geriatr.* 52:197-201.

CEREALS AND THEIR PRODUCTS AS SOURCE OF FOLATE IN REPRODUCTIVE WOMEN CONSUMING VEGETARIAN AND OMNIVOROUS DIETS

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613.261 : 664.696

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ABSTRACT

Adequate folate intake plays important role in reproductive women especially in preventing and reducing the risk of neural tube and other birth defects. Cereals and their products represents major source of energy, protein, B vitamins and minerals for the world population and are assumed to contribute notably to the overall folate intake.

The aim of this study was to determinate intake of cereals and their products in reproductive women consuming vegetarian and omnivorous diets, and estimate if there is any difference in cereals and folate intake between groups. Subjects were 70 women, aged 25 to 43 years, divided in two groups according to their eating habits. Dietary method used was validated quantitative food frequency questionnaire for assessment folate intake.

This survey found no significant difference between amount of cereal consumption among groups, but found 21% higher folate intake from cereals in vegetarian group. Statistically significant difference was found in bread consumption. Wholegrain bread made more than 85% of daily bread intake in vegetarians while only 61% in omnivores - resulting in 12% higher folate intake in vegetarian population. Also found was difference in breakfast cereals intake with women following vegetarian diet have 30% higher intake resulting in more than 30% higher folate intake.

The results pointing out a necessity of the grain surface treatment, preceding the milling in wholegrain buckwheat, corn and rice flour production.

Keywords: cereals, folate, vegetarians, omnivores

INTRODUCTION

Cereals are one of the most important sources of food [1]. Together with pseudo-cereal grains are a major source of energy, good source of complex carbohydrates, phytochemicals, minerals and an important source of most B vitamins for the world population [2, 3]. High consumption of cereals has been associated with a decreased risk

of developing several chronic diseases as well as with increased intake of fiber, calcium, iron, zinc, vitamin C and folate [4].

Folate is a water soluble B vitamin that occurs naturally in wide variety of foods of plant and animal origin. As cereals and their products constitute important part of diet of the world's population it is assumed that they can contribute a lot to the overall folate intake. Besides naturally occurring folate many cereal products are fortified with folic acid which makes them very good source of folate.

Adequate folate intake plays an important role in primary prevention of several diseases [5] and is important for maintaining normal function of organism. Folate is needed for production and maintenance of new cells, which is especially important during periods of rapid cell division and growth such as infancy, adolescence, and pregnancy. Women of childbearing age should obtain adequate amount of folate from food and supplements before conception to reduce risk of NTDs and other birth defects [6]. The Dietary reference intake (DRI) for reproductive women is 400 µg/day of folic acid obtained from food, fortified foods and dietary supplements [7]. Based on all obtained findings about benefits of adequate folate intake periconceptual folate supplementation is recommendation [8].

As difference in eating habits is important cause for variations in nutrition quality it is expected that vegetarians have superior folate status compare to omnivores due to higher abundance of plant food in nutrition [9].

The aim of this research was to establish consumption of cereals and their products as source of folate in reproductive women with different eating habits (women consuming omnivorous and vegetarian diet) using validated food frequency questionnaire (FFQ) and to determine any difference in cereals and folate intake between these two groups.

SUBJECTS AND METHODS

Subjects were 70 reproductive women aged 25 to 43 years, divided in two groups according to their eating habits (vegetarians and omnivores).

Dietary method used was validated quantitative self-administered FFQ for assessment of food folate intake, containing 39 items that uses the previous month as a reference period, with frequency consumption determined on the basis of reporting: never, one/month, two-three/month, one/week, four-six/week, every day. FFQ was previously validated in Croatian populations of adult women and adult vegetarians and details were described [10, 11].

Folate intake (total and from cereals) was calculated using national food composition tables [12].

Anthropometric measurement of subjects were performed in the morning after overnight fasting. The height and weight were measured at scale with attached stadiometer (Seca, Type 710-220, Vogel & Halke GmbH & Co., Germany). During the measuring subjects were barefoot with minimal clothing and measurement were

performed according to standard instructions [13]. The height was measured with precision 0.1 cm, and body weight with precision 0.1 kg. Body mass index (BMI) was calculated from obtained measures for each subject. Waist and hip circumference were measured with flexible, inelastic measuring tape with precision 0.1 cm. Measures were obtained while subjects were standing erectly, muscles relaxed at the level of the natural waist (horizontal plane at the top of right iliac crest) for waist circumference and horizontal plane at the maximum circumference of the buttocks for the hip circumference. The waist-to-hip ratio (WHR) was calculated by dividing waist by hip circumference.

All measurement were performed by trained personnel.

Participation in the study was voluntary and informed written consent was obtained from all participants.

Program SPSS v.17 was used for the statistical analysis. Descriptive analysis of anthropometric and dietetic parameters was assessed as basic statistic method while difference in mean values of selected parameter according eating habits of woman was tested by Student's *t* test. *p* values <0.05 were considered as significant.

RESULTS AND DISCUSSION

Basic characteristic of the subjects (age and anthropometric characteristic) and their folate intake (total and from cereals) are presented in Table 1. Data are shown for total sample, and for each group of women (omnivores and vegetarians) separately. When comparing these two groups it was noticed that women following omnivore diet had higher body mass, as well as BMI, percentage of body fat and waist circumference, while vegetarians tended to have higher total folate and folate from cereals intake. According to *t* test difference between vegetarian and omnivore women were statistically significant for hip circumference, WHR and for total folate intake.

Obtained results were expected because previous study showed that vegetarian diets are associated with reduced body weight and BMI and lower rates of obesity prevalence compared to non-vegetarians [14] due to higher abundance of plant food in daily diet and resulting higher folate intake [15].

One of the good sources of folate and a food consumed by both observed groups are cereals and cereal products. In this study average consumption of cereals did not differ significant between groups - it was 134.85 g/day for omnivores and 146.21 g/day for vegetarians. According to data from the Croatian Bureau of Statistics average consumption of cereals and cereal products for year 2011 was 112.4 kg/year per capita or calculated 307.95 g/day per capita. This amount include rice, flour and other cereals, bread and other bakery products, pastry-cook products, pasta products and other cereal products [16], what differs from food that was included in this study.

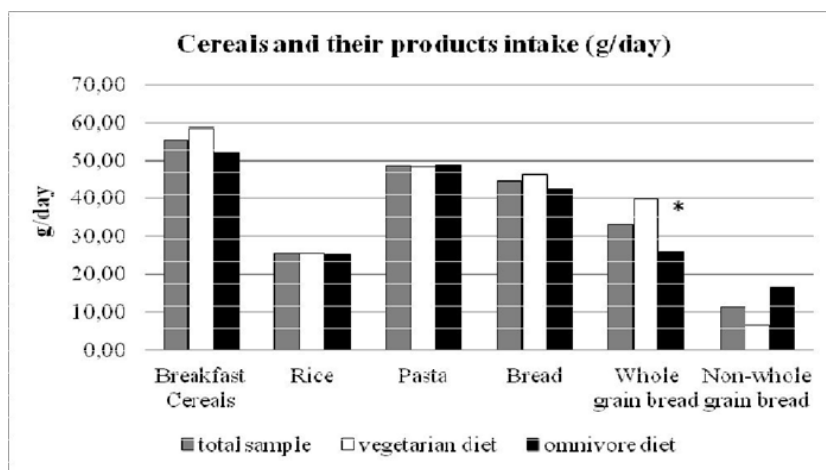
Table 1. Characteristics of subjects. Subjects defined by age, anthropometric characteristics and folate intake.

Parameters	TOTAL (n=70)	OMNIVORE women (n=35)	VEGETARIAN women (n=35)	<i>p</i>
Age (years)	34.77 ± 4.49	34.86 ± 4.43	34.69 ± 4.61	0.874
Age - range (years)	min. 25; max. 43	min. 25; n max. 43	min. 25; max. 43	/
Body mass (kg)	67.59 ± 13.88	70.56 ± 15.86	64.61 ± 11	0.072
Body height (cm)	168.42 ± 5.11	168.77 ± 4.69	168.08 ± 5.54	0.573
Body mass index (kg/m ²)	23.83 ± 4.8	24.78 ± 5.51	22.89 ± 3.82	0.100
Body fat (%)	26.3 ± 7.57	27.59 ± 8.27	25.02 ± 6.66	0.157
Waist circumference (cm)	77.59 ± 10.03	78.38 ± 11.45	76.81 ± 8.47	0.516
Hip circumference (cm)	103.60 ± 10.21	106.33 ± 11.72	100.87 ± 87	0.024*
Waist to hip ratio	0.75 ± 0.05	0.74 ± 0.05	0.76 ± 0.05	0.037*
Folate from cereals total (µg/day)	29.86 ± 19.4	26.25 ± 17.71	33.47 ± 20.58	0.121
Food Folate total (µg/day)	203.47 ± 98.96	165.05 ± 78.43	241.89 ± 103.35	0.001**

p*<0.05; *p*<0.005

Figure 1 shows average daily intake of cereals and their products (breakfast cereals, rice, paste, bread) based on obtained data while figure 2 shows corresponding amount of folate. According to the type of cereals consumed there was no big difference in intake of pasta (~48 g/day) and rice (~25 g/day) in both groups, so these foodstuff do not contribute to difference in daily folate intake. Although there was also no big difference in daily bread intake among groups, there was statistically significant difference in type

of bread consumed. In group of women following vegetarian diet wholegrain bread makes more than 85% of daily bread intake, while in women following omnivorous diet it makes 61%. Based on observed intake and a higher folate content in wholegrain breads, women consuming vegetarian diet had 12% higher folate intake compare to women consuming omnivorous diet. Also observed was difference in breakfast cereals intake with women following vegetarian diet have 30% higher intake and consequently more than 31% higher folate intake. Statistically significant difference between groups was estimated for amount of whole bread consumed and for folate intake from whole grain bread.

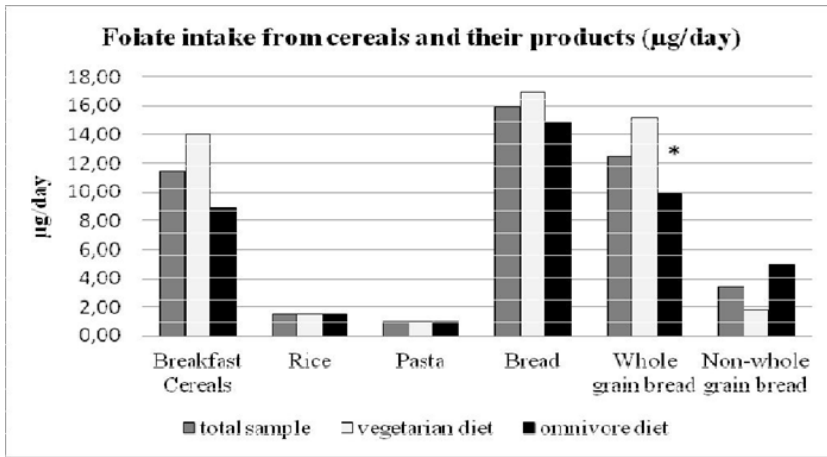


* $p < 0.05$

Figure 1. Average daily consumption of cereals and their products

This study showed that cereals contribute with 12.6% (10% for omnivores, and 15.1% for vegetarians) to total folate intake in population of Croatian women of reproductive age. This differs from previously published studies where showed that cereals contribute to total folic acid intake with 11.9-22.3% [17] and that breakfast cereals contribute with 17.7% to total folate intake [18] probably due to small sample.

Result obtained in this study were significantly lower than result obtained from study of U.S. women of reproductive age which showed that cereals contributed with 36.6% to total daily folate intake [19]. Taken into account only food that was followed up in this study stated contribution would be reduced to contribution of 20.06%, with ready to eat cereals being most strongly correlated with contribution of 19.7% to total folate intake. Our results show 6.0% contribution to total folate intake for total population, with 5.4% contribution for omnivores and 6.6% for vegetarians.



* $p < 0.05$

Figure 2. Average daily intake of folate from cereals and their products

To prevent NTD women need to achieve optimal folate status before pregnancy occurs. For that reason is important that women in their reproductive age maintain adequate folate intake.

In this survey we obtain results for daily folate intake 203.33 µg for whole population. When observed according to dietary habits of participants it was 165.05 µg for omnivore and 241.89 µg for vegetarian women. These results are similar to already published findings for folate intake in Croatian women where daily intake was 156.6 ± 72.2 µg white bread and citrus were mayor folate sources [20]. Vegetarian women in this study have folate intake more similar to mean intake of European women (247 µg/day) [21].

Table 2. Total folate intake, intake from cereals and satisfying of recommendations of subjects divided into categories according waist circumference and BMI

Parameters	Waist circumference (cm)				Body mass index (kg/m ²)				
	≥80	≥88	<80	<88	<18	18-25	25-30	>30	
Number (n)	12	8	23	27	0	22	7	6	
Number (%)	34.29	22.86	65.71	77.14	0.00	62.86	20.00	17.14	
OMNIVORE women	FOLATE INTAKE:								
	µg/day	181.51	185.85	153.89	156.80	/	171.29	109.47	200.04
	DRI (%)	45.4	46.5	38.5	39.2	/	42.8	27.4	50.0
	Croatian RDA (%)	90.8	92.9	76.9	78.4	/	85.6	54.7	100.2
	From cereals (%)	9.02	6.81	10.50	10.95	/	9.57	13.42	7.36
Number (n)	11	4	24	31	2	25	5	3	
Number (%)	31.43	11.43	68.57	88.57	5.71	71.43	14.29	8.57	
VEGETARIAN women	FOLATE INTAKE:								
	µg/day	238.66	298.04	243.37	234.65	277.64	236.13	236.49	275.04
	DRI (%)	59.7	74.5	60.8	58.7	69.4	59.0	59.1	8.8
	Croatian RDA (%)	119.3	149.0	121.7	117.3	138.8	118.1	118.3	137.5
	From cereals (%)	18.36	9.76	10.50	10.95	4.53	15.91	18.21	9.58

*DRI – Dietary reference intake = 400 µg/day [6];

National RDA– recommendation of the Republic of Croatia = 200 µg/day [22]

When folate intake is compared with DRI [6] as it is shown in Table 2, subjects meet recommendations with less than 75%. Precisely it was 50.8% for whole population, 40.9% for omnivores and 60.5% for vegetarian women respectively. When compare with national recommendations which are amount to 200 µg/day [22], vegetarians have an adequate intake, and meet recommendations with 121%. Opposite of that omnivores meet recommendations with only 81.8%. When observed whole population together it satisfy national recommendations with 101.7%. Pucarín-Cvetković et al. declared similar

results on Croatian reproductive women where women in their reproductive years consumed less than the recommendations for folate, most of them consume about half the recommending amount [20].

It is very clear that reproductive women do not intake adequate amount of food folate for life stage, same as in European population [21], therefore possible recommendation for all women of reproductive age is to consume more foodstuff rich in folate or to take supplements to obtain adequate intake.

CONCLUSIONS

This survey showed there is no significant difference in amount of cereals consumed between vegetarian and omnivore women but it showed difference in type of cereals consumed. Vegetarian women consumed more whole grain cereals and their products compare to omnivore women. According to that reproductive vegetarian women have higher total and folate from cereals intake.

REFERENCES

1. FAO (Food and Agriculture Organisation) 2002. World Agriculture: Towards 2015/2030. Summary Report. FAO, Rome.
2. Kulp, K., and Ponte JR, J.G. 2000. Handbook of Cereal Science and Technology. 2nd ed. New York, Marcel Dekker.
3. McKeivith, B. 2004. Nutritional aspects of cereals. Nutr. Bull. 29:111-142.
4. Liu, S., Stampfer, M. J., Hu, F. B., Giovannucci, E., Rimm, E., Manson, J. A. E., Hennekens, C. H., and Willett, W. C. 1999. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am. J. Clin. Nutr. 70(3):412-419.
5. Bailey, L. B., and Gregory, J. F. 2006. Folate. Pages: 278-301 in: Present knowledge in nutrition 9th ed. Bowman B. A, Rusell, R. M. (ed.) ILSI, Washington, DC.
6. Berry, R. J., Li, Z., Erickson, D., Li, S., Moore, C. A., Wang, H., Mulinare, J., Zhao, P., Wong, L-Y. C. Gindler, J., Hong, S-X, Gunter, E., and Correa, A. 1999. Prevention of neurale-tube defects with folic acid in China N. Engl. J. Med. 341:1485-1490.
7. Institute of Medicine 1998. Folate. Pages: 196-305. in: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline, Washington DC.

FEATURES AND CHARACTERISTICS OF IFS FOOD STANDARD IMPLEMENTATION IN THE MILLING INDUSTRY

UDC 664.6/7(083.74)

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ABSTRACT

International Featured Standard Food (IFS Food) aims to establish a safety management system and ensure the quality of food production. This standard is of special importance for food producers and is often a prerequisite for cooperation with retailers. The aim of this paper is to present experiences in the implementation of IFS Food/Version 6 in the milling industry, with special emphasize on the features and characteristics of the standard. IFS Food was implemented by using the methods of the food quality and safety management system. A hazard analysis and a risk assessment were conducted after which monitoring and measurement of processes that could influence food quality and safety have been made. The standard provides specific scoring of each requirement, depending on the degree of implementation and compliance. The result of implementing IFS Food in the milling industry has led to an increase in the safety and quality of raw materials and finished products and an increase in customer satisfaction. The number of non-compliant conditions or products recorded during the process of implementation and during internal audit was twofold smaller than before the implementation of the standard.

Keywords: IFS Food, food safety, milling industry

INTRODUCTION

Consumer awareness about the safety of food that they consume is increasing because foodborne diseases have become a growing health problem worldwide and their surveillance is a fundamental component of food safety systems. Foodborne zoonotic diseases are the most common causes of food poisoning and in the European Union over 320.000 human cases are reported each year, but the real number is likely to be much higher [1]. In the European Food Safety Authority's (EFSA) latest report on foodborne zoonotic disease in the European Union, the overall reporting rate was 1.12 outbreaks per 100,000 population [2] in 2011. In United States about 48 million people are affected by foodborne diseases each year [3].

Current European legislation requires all food businesses to introduce food management systems to ensure food safety based on the internationally recognized World Health Organization's (WHO) Codex Alimentarius and the principles of Hazard Analysis and Critical Control Point [4, 5]. In Croatia, the legislative framework for ensuring food safety and quality consists of the Food Act (2013) [6] and Law on Food Hygiene and Microbiological Criteria for Foodstuffs (2013) [7] which are fully compliant with European legislation. Food safety is a concept that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use [8]. To assure the safety of food, establishing a system based on continuous management including total quality management, hygiene and good manufacturing practices, is essential.

In the last ten to twenty years private standards have been launched on the global market, all with the same objective - to protect consumer health through integrated food safety management systems. At the same time, the trend that major retailers develop their own-brand range of food products was evident on the global market [9]. In view of the above, due to the increasing demands of customers, stricter legal requirements and responsibility of retailers, it was necessary to create a uniform quality assurance and Food Safety Standard, as a pre-requisite for manufacturers and suppliers to become brand manufacturers for certain retailer(s). Consequently, in 2000 several big leading international retailers and suppliers launched the Global Food Safety Initiative (GFSI) with the aim to ensure convergence amongst food safety standards, to benchmark existing certification frameworks for food safety and to maintain a benchmarking process for food safety management systems. This is a non-profit foundation managed by The Food Business Forum (CIES). CIES stands for Comité International d'Entreprises à Succursales, which is the French name for International Committee of Food Retail Chains. Retailers could accept any of the recognized GFSI certification schemes as a proof of "due diligence" in food safety procedures [10].

Consequently, the International Featured Standard (IFS) was developed, an internationally accepted audit standard for all parties involved in the supply chain and its aim was to ensure safety and quality of food and non-food products and related services. IFS Food is a standard for auditing quality and food safety of food products. It can be implemented only in companies where the product is processed, or if there is a danger of product contamination during the primary packaging. IFS Food defines unique and complex requirements for content, procedures and evaluation systems as well as for audit criteria. It was developed by German and French retailers in order to provide international security standards for companies which produce private label brands for large retailers. In developing the final IFS Food version 6, retailers, stakeholders, and representatives of industry food services and certification bodies were also involved, in addition to German, French and Italian working groups. During its development, IFS Food gained input from a recently formed IFS North America working group and retailers from Spain, Asia and South America. The new IFS Food version 6 has been in force since July 2012 [11].

The main objectives of IFS Food, and other IFS standards [11], are:

- to establish a common standard with a uniform evaluation system,
- to work with accredited certification bodies and qualified IFS approved auditors,
- to ensure comparability and transparency throughout the entire supply chain,
- to reduce costs and time for both suppliers and retailers.

The aim of this paper is to present experiences in the implementation of IFS Food/Version 6 in the milling industry, with special emphasize on the features and characteristics of the standard. As a case study we have deliberately chosen one small enterprise (SE), because even though small and small and medium-sized enterprises (SMEs) are said to contribute significantly to the economies of most countries, including Croatia, they are the least likely to comply with regulatory requirements because of resource constraints. Consequently, there is a paucity of published work in this area.

MATERIALS AND METHODS

Enterprise description

IFS Food ver. 6 was implemented in a small enterprise located in the northern part of Croatia, owned by the German-Hungarian mill group. The production facility was renovated in 2007 and equipped with new devices. This is important to point out because the infrastructure condition of a building is often a crucial factor for the implementation of the IFS Food standard. Installed production capacity of the mill is 150 tons per day. Wheat used as a raw material is purchased from local contract manufacturers and also on the EU market, and it is used for manufacturing different types of flour.

The mill was organized into two production units – wheat grinding, and packaging and storage of flour. Business monitoring was completely computerized with program MS Navision. Production was carried out according to ten specifications of products sold as its own brand as well as a brand of international retailers in the domestic market. The total number of employees was 29, working in three shifts with no temporary workers. Raw materials were stored in a floor warehouse, while finished products were stored in silos with a capacity of 60 tons and a floor warehouse.

The mill possessed its own laboratory for the analysis of raw materials and finished products. Analyses of raw materials and finished products were carried out in accordance with the Regulations on quality of cereals, mill and bakery products, pasta, dough and dough products [12].

Implementation of IFS Food Standard

The enterprise implemented and certified a quality management system according to ISO 9001:2008 and HACCP system in 2010. Additionally, two standards, Kosher and Halal were implemented in 2012 and 2013, respectively.

The IFS Food Standard was implemented over a period of 8 months during 2012 in collaboration with a consulting company. Certification was conducted by an accredited company in January 2013. The procedure of IFS Food implementation was carried out according to the time frame which is summarized in Table 1.

Generally speaking, risk assessment comprises four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. The process provides a framework for the systemic and objective evaluation of all available information pertaining to foodborne hazard. Although HACCP was implemented in 2010, due to the some changes in the manufacturing procedure and also due to the requirements of IFS Food standard, a new manufacturing flow diagram needed to be designed upon which risk assessment was made. The risk assessment was conducted considering the probability of hazard occurrence (values 1 to 3) and the risk degree (values 1 to 3). Hazard occurrence is estimated as: 1–may occur; 2–a rare occurrence, and 3–a common occurrence. Risk degree is estimated as 1–small (causes repulsion but does not require medical intervention), 2–medium (characteristic symptoms, requiring medical intervention) and 3–large (can cause death and permanent illness).

Implementation of the IFS Food standard is conducted by scoring the fulfilment of a particular requirement [11] (Table 2).

Evaluation of IFS Food Standard effectiveness

With the aim of verifying the effectiveness of the Standard implementation, the parameters of safety and product quality, customer satisfaction and the number of non-conforming conditions/products were monitored during implementation and eight months after certification.

Raw materials and the finished product must comply with current legislation (in terms of security) and internal standards (in terms of quality).

Customer satisfaction was measured by the number of complaints received concerning products and by conducting a customer survey using a questionnaire. Topics covered by the questionnaire were: product quality, delivery time, resolving complaints and courtesy of employees.

All products / conditions that do not meet the requirements relating to the quality management system, the IFS system, HACCP system, legislation, standards / requirements of customers and internal standards were considered inconsistent.

Table 1. Time frame for IFS Food implementation

AIM OF THE PROJECT IMPLEMENTATION	Implementation of IFS Food with the final goal of certification by an accredited certification company. Covered area: Products of grain - wheat flour
PROJECT PHASES	<p>PHASE I</p> <ul style="list-style-type: none"> a) assessment of the current conditions of: building, organization and documentation b) defining: activities for preparation and correction of system documentation; responsibilities in the implementation of activities (infrastructural, organizational and documentation) and deadlines <p>Period of implementation: May 2012.</p>
	<p>PHASE II</p> <ul style="list-style-type: none"> a) preparation and correction of system documentation b) educational activities – team education for implementation and system maintenance <p>Period of implementation: June-July 2012.</p>
	<p>PHASE III – system application and auditing</p> <ul style="list-style-type: none"> a) practical consulting (checking the degree of requirements implementation, working on improvements) b) educational activities - employees training in the workplace c) correction of prepared documentation (optional) <p>Period of implementation: August-September 2012.</p>
	<p>PHASE IV - preparing for system certification</p> <ul style="list-style-type: none"> a) internal audit b) rejection of non-compliance / work on improvements <p>Period of implementation: October-December 2012.</p>
	<p>PHASE V – system certification</p> <ul style="list-style-type: none"> a) attendance at the certification audit b) rejection of non-compliance / work on improvements upon the certification audit <p>Period of implementation: January 2013.</p>
DURATION OF THE PROJECT	<p>Eight months.</p> <p>Dates of the activities are defined by client service provider. Responsibility for the implementation of certain phases and activities will be specified during Phase I.</p>

Table 2. Evaluation of requirements

Result	Explanation	Points
A	<i>Full compliance</i>	20
B (deviation)	Almost full compliance	15
KO requirement scored with B	Almost full compliance	15
C (deviation)	Small part of the criteria is implemented	5
D (deviation)	Requirement has not been implemented	- 20
Major	When there is a substantial failure to meet the requirements of the Standard, which includes food safety and/or the legal requirements of the production and destination countries. A major can also be given when the identified non-conformity can lead to a serious health hazard. A major can be given to any requirement which is not defined as KO.	15% of the possible total amount of points is subtracted
KO requirement scored with D	<i>Knock-Out</i> <i>The KO requirement has not been implemented</i>	50% of the possible total amount of points is subtracted
NA	Not applicable Requirement not applicable for a company	N/A requirements will be excluded from the final scoring

The total score is calculated as follows [11]:

Total number of points = (total number of IFS requirements – requirements scored with N/A)x20.

Final score (in %) = number of points awarded / total number of points.

RESULTS AND DISCUSSION

The first phase of the project implementation was the assessment of the current state of the organization, processes and documentation. According to the obtained results, the required activities, responsibilities and timeline for improvement were defined. The infrastructural state of a facility is an important and often crucial factor for implementing IFS Food. In this case, due to the previously mentioned data on the status of the building, its condition was estimated as satisfactory and only minor investments were required to achieve full effectiveness.

The complexity of IFS Food requires the existence of prerequisite programs (PRPs), including good manufacturing (GMP) and good hygienic practice (GHP), at the highest level of efficiency. The prerequisite programs represent the conditions and/or necessary basic activities to maintain a hygienic environment for the production, handling and the provision of safe finished products all along the food product process [13].

Documents preparation and educational activities to the required skill levels were performed according to the time frame and training plan. Educational activities for the food safety team involved initial training on the IFS Food requirements as well as continuous training through the stages of system implementation by the consultant. Training for workers involved the application of the IFS Food requirements in individual workplaces. Educational activities included all workers, and were conducted by a consultant.

During the IFS Food implementation process, a new flow diagram concerning flour manufacturing was established and after verification it served as a base for risk assessment. Risk assessment was conducted stage by stage from the reception of the raw material up to the despatch of the finished products. The HACCP offers a scientific, rational and systematic approach for identification, assessment and reduction of hazards during production, processing, manufacturing, preparation and use of food and is one of the most successful quality management concepts worldwide [14]. However, implementation of IFS Food had some additional requests: allergenic substances needed to be define, the function of magnet was more precisely defined because mill did not have a metal detector (and this request was KO), and the possibility of contamination due to the usage of compressed air was also monitored.

By applying the methodology of risk assessment considering the probability of hazard occurrence and the risk degree (potential health impacts), prerequisite programs (PRP) were determined in the case that the multiplication score was 1 to 3, and control points (CT), in the case that the multiplication score was 4. Critical control points (CCPs) in the case that the multiplication score was 6 were not established.

The outcome of risk assessment is an estimation of the magnitude of human health risk in terms of likelihood of exposure to hazards in a food and the likelihood and impact of any adverse health effects after exposure [15].

As a result of the hazard analysis and risk assessment, three control points were determined: (1) receipt of wheat, auxiliary materials and packaging, (2) white cleaning,

and (3) dosage of auxiliary materials. The necessities to control the appearance of biological and physical hazards in the raw materials, chemical hazards at packaging's reception, physical hazards in process of white cleaning and chemical hazards in dosage of auxiliary materials were established (Table 3.).

Table 3. Hazard analysis conducted during manufacturing

Stages	Hazards	Description of CP	Critical limits	Process	Monitoring	Frequency
CP1 1a. Wheat's reception	Biological Physical	Humidity control Visual inspection	Humidity <13.5% Absence of all particles	Wheat's visual inspection and humidity analysis	Wheat's visual inspection and humidity analysis	Every reception
CP1 1b. Packaging's reception	Biological Chemical	Requirements of requests for packaging's reception	Within legal limits	Analytical reports	Analytical reports	Every reception
CP1 1c. Auxiliary materials' reception	Biological	Requirements of requests reception	Within legal limits	Expiration date inspection Analytical reports	Expiration date inspection Analytical reports	Every reception
CP2 10. White cleaning	Physical	Control of magnet	Separation of all metal particles	Control of magnet	Control of magnet	Once per week
		Control of control sieve	Particles > 250 µm	Control of control sieve within Program of preventive maintenance	Control of control sieve within Program of preventive maintenance	Four times per year
CP3 17. Dosage	Chemical	Control of concentration of additives	Within legal limits	Weighting of additives	Weighting of added additives	Every weighing

Physical hazards mainly included foreign bodies (metal, plastic, wood, glass, stones and rocks etc.), which could have originated from the raw material but also from machines or transport. The term biological hazard was mainly related to cereal insects and mycotoxines (aflatoxins, ochratoxin etc.). Chemical hazards were related to the traces of heavy metals (in the stage of packaging's reception) and additives which could be added in higher concentrations than recommended (in the stage of dosage). Corrective measures were defined in every stage and they included: withdraw of raw material, packaging or auxiliary materials (CP1a, CP1b, CP1c), stopping the manufacturing process unless the hazard is removed (CP2) and storage of a single batch in a separate warehouse (CP3).

Having only four established control points is in accordance with expectations since the flour milling industry is considered a low risk area within the food handling enterprises, because flour is mainly used for baking purposes. However, there are pathogens, which might produce toxins heat stable and can cause illness even after the product has been baked, such as *Bacillus* and *Staphylococcus* species. Flour is generally regarded as a microbiologically safe product because it is a low water-activity commodity. Although the growth of pathogenic bacteria may not be supported under such conditions, pathogens that contaminate flour may survive for extended periods [16].

The specific features of the milling industry – such as the common practice to provide the required amount of raw material from a large number of suppliers, storage of raw materials in bulk, the need for mixing raw materials of different parameters in order to ensure customer demands (producer specifications, often for different applications in the bakery and confectionery industry) and storage of finished products in silos – significantly complicate the fulfilment of requirements. Consequently, meeting the requirements of the system includes a high level of documentation and training for workers. Ensuring traceability represents a component of the procedures for revocation and withdrawal. Traceability (the ability to trace products) means that the flow of material and information within a company and/or supply chain can be followed [17]. Traceability can be useful not just to optimize production planning and scheduling, e.g. minimize waste and ensure optimal use of raw materials [18], but also as a part of a competitive strategy [19].

Requirements that a company must meet in order to receive an IFS Food certification are arranged into six chapters: (1) Senior Management Responsibility, (2) Quality and Food Safety Management, (3) Resource management, (4) Planning and Production Process, (5) Measurements Analysis and Improvements and (6) Food defence (IFS, 2012). A summary overview of scoring requirements is presented in Table 4.

Figure 1 shows the final score for each of the six chapters. Senior Management Responsibility (1) received a final score of 96.59% and fulfilled the greatest number of requirements. Planning and Production Process (4) was the group with the larger number of requirements and requirements scored as not applicable for the company. The final score for this group of requirements was 76.33%, which was the lowest result.

Table 4. Summary overview of scoring requirements

	Senior management responsibility	Quality and food safety management	Resource management	Planning and production process	Measurements analyses, improvements	Food defence
KO	0	0	0	0	0	0
Majors	0	0	0	0	0	0
A	21	26	18	86	37	6
B	0	1	3	7	6	0
C	1	2	5	12	1	1
D	0	0	0	8	0	0
N/A	0	4	2	32	1	1

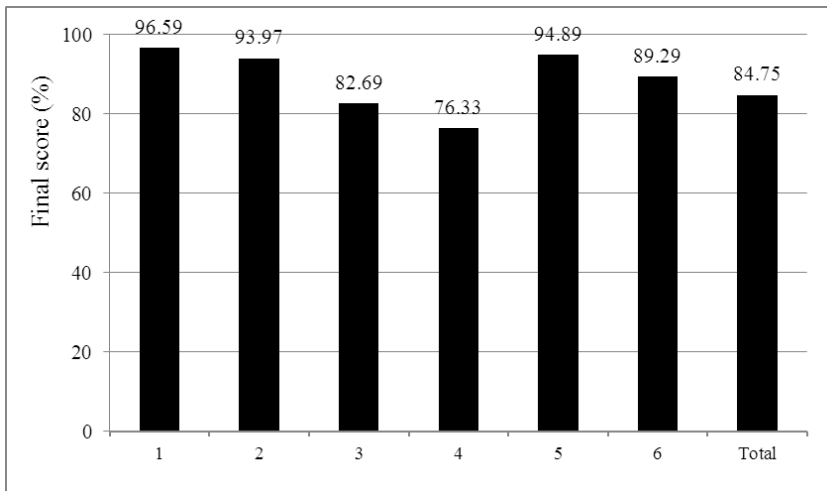


Figure 1. Final scores for all chapters

As a result of an audit, a certificate was awarded at the foundation level with a total score of 84.75%. For all requests that were not assessed with a maximum number of points, a corrective plan was formed with clear actions aiming to improve the fulfilment of requirements and define responsible persons and deadlines. To obtain a certificate, the

final score of all requirements should be a minimum of 75%. With a total score $\geq 75\%$ and $< 95\%$, the achieved certification is at foundation level, while with $\geq 95\%$, certification is at higher level [11].

Efficacy results of IFS Food implementation based on customer satisfaction and reported non-conforming products / conditions are shown in Table 5 and Figure 2.

Table 5. Comparing the results of the safety and quality of products based on customer satisfaction and registered non-conforming condition during and after the implementation of IFS Food Standard

	May 2012 – January 2013	February 2013 – September 2013
<i>Customer satisfaction</i>		
Number of received complaints	8	2
Questionnaire	3.8	4.4
<i>Registered non-conforming products /conditions</i>	7	3

From the obtained results it is evident that the implementation of IFS Food has led to an increase in customer satisfaction expressed by a drop in the number of product complaints by 75% and an increase in the overall score from the questionnaire by 12%. The number of non-conforming products / conditions decreased by 57% during the implementation process and during internal auditing.

Figure 2 shows the results of safety and quality evaluation of raw materials and products during and after the IFS Food implementation. The security and quality of raw materials increased by 5 percentage points and after implementation of IFS Food was at 95%, while security and quality of products increased by 4 percentage points and was at 99%.

Further improvements, as indicated by the results of the certification and internal audits, can be achieved by developing a methodology for monitoring process parameters/key performance indicators (KPI) and by further raising the awareness and competence of workers. Planned changes in terms of increased production and storage capacity of the mill and the development of new products will require further efforts in reviewing and developing system performance.

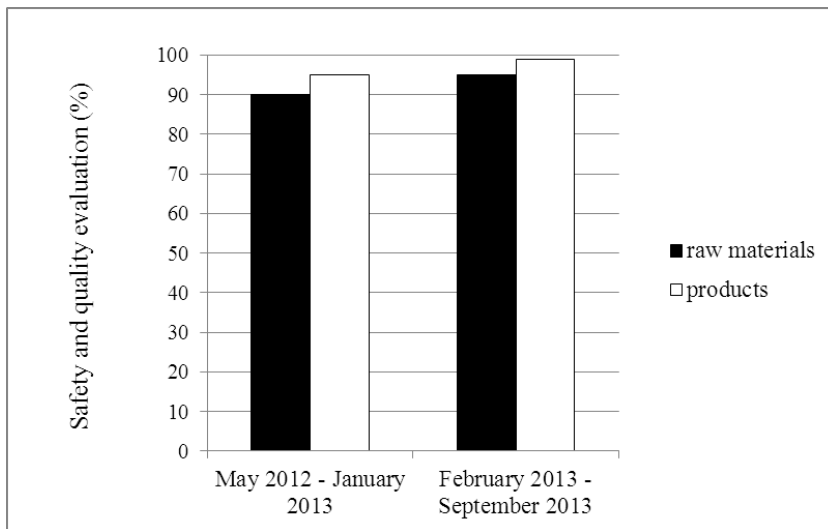


Figure 2. Safety and quality of raw materials and products during and after implementation of IFS Food

CONCLUSIONS

Implementation of IFS Food was conducted by using the elements of a previously implemented quality management system and HACCP system, along with the necessary documentation and organizational improvement according to IFS Food, ver. 6. As a result of hazard analysis and selected risk assessment methodology, three control points were established: receipt of wheat, auxiliary materials and packaging; white cleaning; and dosing auxiliary materials. Based on the achieved final score of 84.75%, a certification was awarded at the foundation level. During the implementation process, focus was placed on the application of specific requirements in the milling industry in terms of quality variability of raw materials, the implementation of pest control and ensuring the traceability system. Implementation of IFS Food has led to an increase in the safety and quality of raw materials and finished products and an increase in customer satisfaction expressed by a decrease in the number of product complaints and higher overall score from the questionnaire. The number of non-compliant conditions or products recorded during the process and internal audit was twice as small as before the implementation of the system.

REFERENCES

1. EFSA – European Food Safety Authority. 2013a. Foodborne zoonotic diseases. Available at: <http://www.efsa.europa.eu/en/topics/topic/foodbornezoonoticdiseases.htm>.
2. EFSA – European Food Safety Authority. 2013b. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2011. *EFSA Journal* 11(4):3129, 250 pp. doi:10.2903/j.efsa.2013.3129. Available at: <http://www.efsa.europa.eu/en/efsajournal/doc/3129.pdf>.
3. CDC – Centre for Disease Control and Prevention. 2012. Trends in Foodborne Illness in the United States. Available at: <http://www.cdc.gov/features/dsfoodnet2012/>.
4. Codex Committee on Food Hygiene. 1997. HACCP System and Guidelines for its Application. In: *Food hygiene Basic Texts*. Rome: Food and Agriculture Organization of the United Nations, World Health Organization, pp. 33-45.
5. Commission of the European Communities. 2000. White Paper on Food Safety. Available at: http://ec.europa.eu/dgs/health_consumer/library/pub/pub06_en.pdf.
6. Food Act. 2013. Official Gazette of the Republic of Croatia, NN 81/13.
7. Law on food hygiene and microbiological criteria for foodstuffs. 2013. Official Gazette of the Republic of Croatia, NN 81/13.
8. ISO 22000:2005. Food safety management systems – Requirements for any organization in the food chain.
9. Lawrence, G., Simmonds, G., and Vass, P. 2002. The regulatory framework. Institutional responsibilities and relationship. Vol 13., Bath, UK: University of Bath.
10. CIES- The Food Business Forum. 2009. The global challenge: Food safety report. Available at: <http://www.globalfoodsafetyresource.com/GFSI%20Report%202009.pdf>.
11. IFS Food/Version 6. 2012. Available at: http://www.ifs-certification.com/images/ifs_standards/ifs6/IFS_Food_V6_en.pdf.
12. Regulations on quality of cereals, mill and bakery products, pasta, dough and dough products. 2005. Official Gazette of the Republic of Croatia, NN 78/05.
13. Gaaloul, I., Riabi, S., and Ghorbel, R. E. 2011 Implementation of ISO 22000 in cereal food industry „SMID“ in Tunisia, *Food Control*. 22:59-66.
14. WHO, World Health Organization. 1997. HACCP – Introducing the Hazard Analysis and Critical Control Point System. Geneva: WHO; 1997. Available from: http://www.who.int/foodsafety/fs_management/haccp_intro/en/index.html.
15. Lammerding, A. M. 1997. An overview of microbial food safety risk assessment. *J. Food Prot.* 60:1420-1425.
16. Berghofer, L. K., Hocking, A. D., Miskelly, D., and Jansson, E. 2003. Microbiology of wheat and flour milling in Australia. *Int. J. Food Microbiol.* 85:137-149.
17. Karlsen K. M., Dreyer, B., Olsen, P., Elvevoll, E. O. 2013. Literature review: Does a common theoretical framework to implement food traceability exist?. *Food Control*. 32:409-417.
18. Moe, T. 1998. Perspectives on traceability in food manufacture. *Trends Food Sci. Technol.* 9(5):211-214.
19. Canavari, M., Centonze, R., Hingley, M., and Spadoni, R. 2010. Traceability as part of competitive strategy in the fruit supply chain. *Br. J. Nutr.* 112(2):171-184.

PRODUCTION OF MAGLICA

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ABSTRACT

Pasta is a permanent product of flour or semolina and water, with the possible addition of some ingredients to improve the nutritional and organoleptic properties of the finished product. Today is produced a large number of different types of pasta, which vary according to the raw material composition, method of manufacture, primarily design and purpose. In the group of noodles and similar products part of them is also crust or phyllo pastry for pies and rolls and grinders and maglica.

Maglica is the product which is obtained by kneading the dough from flour, water and salt as for pie, rolling pastry around the rolling pin, winding the rolling pin and in the end puling it off. That kind of prepared dough is cut into pieces up to 2 centimeters and baked in the oven.

The aim of this thesis is to describe and implement production process of the maglica, along with monitoring all parameters and changes during the kneading, shaping, baking and storage of the finished product.

Keywords: pasta, maglice

INTRODUCTION

Pasta is a permanent product made from wheat flour which obtained by mixing, forming specific shapes and drying. Can be made only from flour and water or with additions that enrich the nutritional value of the product. On the market the pasta can put a fresh, dried or roasted. The basic raw material in the production of pasta: semolina, water and salt.

A large number of bakery products are made of very thin flat layer or bark, and there are pies, strudels, baklava, ružica, etc. The bark is made with a thickness of 0.2 to 0.5 mm, thinner for confectionery and thicker for bakery products. Basic raw materials are flour, water and salt, and rarely use other raw materials. Flour used in this product has a high content of gluten, great extensibility and flexibility. Improving the properties of flour inappropriate quality is achieved by the addition of wheat gluten or L-cysteine.

The manufacturing process of production crust from dough is composed of the following phases:

- preparation of flour and kneading in the standard high-speed bakery kneading tools, rarely mixers with cold water;
- sharing dough into pieces;
- rest 20-30 minutes to gluten completely swollen - pieces sprinkled a small amount of oil and cover in order to prevent the formation of crust on the surface;
- bed bark by hand or machine;
- drying, molding and packaging as an intermediate product that is sold or used in the production of pasty, pie and similar products.

Fully mechanized and automated production line for bark of dough includes the following operations: mixing, thinning and stretching, cutting, drying and molding.

Organoleptic evaluation of maglica

Organoleptic evaluation and sensoric analysis is a mandatory part of the activities in identifying and defining the quality of food products. Experts in the field of food production generally agree that, at list in the near future again time, the human senses can not be adequately replaced by appropriate instrumental techniques and analytical methods, and this can be achieved only partially.

MATERIALS AND METHODS

Materials

Materials which used for maglica production are: flour T-500 (64%), eggs (20%), water (13%), salt (1%) and vegetable oil (1%).

On the Table 1. shows raw material composition dough for maglica production.

Table 1. Raw material composition dough for maglica production

Flour (kg)	25
Eggs (pieces)	120
Water (L)	5
Salt (kg)	0,5
Oil (L)	6,5

Methods

Determination of the chemical composition of flour:

- determination of water;
- determination of ash;
- determination of degree of acidity;
- production of maglica.

Preparation of raw materials includes tempering, separation of impurities and weighing flour. At slow speed mixing flour temperature should be higher, and at high speed mixer with the mixer should be lower. Eggs "S" class (65–79 grams) are stored in a cool, dry and ventilated place. Salt is usually added dissolved in water for kneading. Oil is tempered to room temperature. The water temperature must be adjusted according to the temperature of the dough to be achieved according to the number rpm and kneading used.

Mixing and forming the dough is different from a few minutes to an hour, depending on the type of product and type of machines which is done kneading. Temperature mass of dough ranges from 18 to 22 °C. Use single phase or direct kneading in which to mix all the raw materials together with temperature control, and kneading the dough takes to obtain the desired quality. On the end of mixing mass of dough rests 20 to 30 minutes on temperature 20–22 °C.

Mass of dough after completion mixing rest 20–30 minutes at 20 to 22 °C.



Figure 1. Dough for maglica

Forming of maglica

Upon completion of resting the dough, it slipped into one before the drafting and design maglica. For shaping dough to get the shape of maglica must be already obtained mass of diugh cut into smaller pieces to make it easier could attenuate the dough. Smaller pieces of dough is stretched rolling pin, then the obtained crust that is coated with oil and overlaps a portion of which is coated, then re-coated folded part and wrapped in a roll. Obtained roll of bark to be cut to a length of 1.5 to 2 cm (Fig. 3 and 4). Formed maglica remains are placed in a baking tray, which is also coated with grease.



Figure 3. and 4. Cutting, forming of maglica and preparation for baking



Figure 5. Packaging of maglica

Baking and packaging

The oven temperature is 220 °C, and the time for which maglica at that temperature to be ready 30 minutes, if the temperature is 250 °C, then it takes 20 minutes. Baked maglica are cooled and packaged in PVC or PE packaging (Fig. 5).

Finished packaged product has a shelf life of three months, testing and analysis of the sample maglica that were made a year ago, showed that the product was microbiologically and nutritionally valuable and suitable for consumption.

RESULTS AND DISCUSSION

Table 2. Results of flour analysis

	Wheat flour T-500
Water (%)	12.95
Ash (%)	0.472
Degree of acidity (%)	2.16

Table 3. Organoleptic evaluation of maglica

Factor of quality	Grade	Factor of importance	Point
Shape and color	4.7	0.8	3.76
Consistency, structure and texture	4.7	0.8	3.76
Chewing	4.9	0.8	3.92
Odor	4.8	0.6	2.88
Taste	4.9	1.0	4.9
The total sum of points			19.22

The organoleptic evaluation of maglica participated in a group of 16 students who were evaluated: shape, color, consistency, structure, chew, odor and taste grades of 1–5, which multiplied by factor of importance given finally points.

From the Table 4 it can be seen that maglica received highest grade for the taste and chewing (4.9 of 5). Grade of odor was 4.8 out of a maximum 5. The shape and color as well as consistency, structure and texture were given a slightly lower grade 4.7.

Grades multiplied by a factor of importance given the final score. Thus, the most points they had to taste maglica 4.9 out of 5 maximum, and the odor 2.88 of 3 maximum. Other properties are evaluated had a slightly smaller number of points and chewing 3.92, shape and color and consistency, structure and texture 3.76 from 4 maximum.

The final score and the total sum for evaluating maglica was 19.22.

CONCLUSIONS

- Maglica are food products that belong to the group of pasta.
- The flour used to produce mist in their composition with literature data.
- Organoleptic evaluation of maglica is shown that the product is accepted by the examiner.
- To chew and taste nebulae have received the highest grade (4.9) and the highest number of points for taste (4.9 of 5) and odor (2.88 of 3).
- The lowest grade and the lowest number of points were in shape and color, and for consistency, structure, texture (grade 4.7 and 3.76 point).
- The final score and the total sum for evaluating maglica was 19.22 which puts maglica high-quality product.

Finally, we can conclude that maglica is very promising and high quality product that is made from flour and it can be used in different ways in the diet.

REFERENCES

1. Bešlagić, S. 1999. Tehnologija prerade žita, skroba i šećera, IP Svjetlost, Sarajevo.
2. Đaković, Lj. 1997. Pšenično brašno, Novi Sad.
3. Hosney R. C. 1994 Principles of Cereal Science and Technology, Published by the AACC, St.Paul, Minnesota, USA, 1-411.
4. Kill, R. C. 2001 Turnbull, K.: Pasta and semolina technology, Blackwel Science, USA.
5. Miličević, D. 2011. Tehnologija pekarskih i pekarsko – konditorskih proizvoda, Tuzla.
6. Žeželj, M. 2005 . Tehnologija žita i brašna, prerada brašna, Knjiga I i II, Beograd.
7. <http://www.food-info.net/hr/products/pasta/production.htm>
8. <http://en.wikipedia.org/wiki/Pasta>
9. <http://www.marodi.com/>

HMW-GS AS PROTEIN MARKERS IN DIFFERENTIATION OF WHEAT CULTIVARS

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ABSTRACT

12 wheat (*Triticum aestivum* L.) cultivars differing in high molecular weight-glutenin subunits (HMW-GS) composition were collected from experimental field of the Agricultural Institute Osijek in 2009 and 2010. HMW-GS was analyzed by SDS-PAGE method and according to the catalogue of alleles the Glu1-score was calculated. The most frequently occurring HMW-GS were 2* 7+8/9 5+10. The proportion of HMW-GS in total extractable proteins of grains was analyzed by RP-HPLC and their average values in 2009 and 2010 were 8.91 and 9.55%, respectively. Comparing the cultivars, a several groups of related HMW-GS attributes were separated. Cultivar Divana as the Croatian bread improver with favorable HMW-GS composition (2* 7+9 5+10) and the highest HMW-GS proportion (11.98%) had a distinct position. In contrast, the Croatian yielding cultivar Sana was positioned on the opposite side due to unfavorable HMW-GS composition (2* 6+8 2+12) and the lowest HMW-GS proportion (6.24%). Cultivars Soissons, Felix, Aida and Ilirija with the highest Glu-1 score (10) belonged to the same cluster and their HMW-GS proportion varied between 8.90 and 10.01%. Cultivars Srpanjka and Golubica were due to higher proportion of HMW-GS (9.80% and 10.01%, respectively) and similar Glu-1 score (6 and 5, respectively) positioned very close.

Keywords: wheat, HMW-GS, Glu-1 score, RP-HPLC

INTRODUCTION

For many years cereal scientists have made considerable efforts in better understanding of the biochemical basis of wheat viscoelastic properties. Glutenins are, based on electrophoretic mobility recognized as high molecular weight glutenins (HMW-GS) with molecular weight from 80 to 130 kDa and low molecular weight glutenins (LMW-GS) with molecular weight from 30 to 50 kDa. Of particular importance are HMW-GS which are coded by polymorphic genes at Glu-1 loci (Glu-A1, Glu-B1 and Glu-D1) present on the long arms of the group 1 chromosomes [1]. Although HMW-GS account only 10% of the wheat storage proteins, they are one of the most important genetic factors in determining the dough forming properties. It is well established that that HMW-GS 1 and 2* at Glu-A1, 7+9 and 17+18 at Glu-B1 and 5+10 at Glu-D1 loci are related to higher dough strength and loaf volume, whereas HMW-GS null (N) at Glu-A1, 6+8 at Glu-B1

and 2+12 at Glu-D1 loci have negative effects. In dough formation, the glutenins become covalently-linked into large elastic networks promoting dough elasticity [2-5]. The objective of this study was to examine the HMW-GS attributes in the function of differentiation of common wheat cultivars.

MATERIALS AND METHODS

Materials

12 common wheat cultivars (Srpanjka, Žitarka, Divana, Aida, Felix, Zlata, Ilirija, Sana, Seka, Golubica, Soissons and Olimpija) differing in high molecular weight-glutenin subunits (HMW-GS) composition and baking quality characteristics were collected from the experimental field of the Agricultural Institute Osijek in 2008/209 and 2009/2010.

Methods

Composition of HMW-GS was analyzed by SDS-PAGE (Hoefer SE 600). HMW-GS bands were identified according to the Payne and Lawrence alleles catalogue, 1983. The HMW-GS were extracted following the procedure of Wieser et al., 1998 [6]. The analytical separation of gluten proteins was carried out on a Supelco Discovery Bio Wide Pore C18 column (5 µm, 150 mm x 4.6 mm) using Perkin Elmer LC 200 chromatograph equipped with autosampler, column thermostat (50 °C) and DAD detector (210 nm). The gradient was performed by 0.1 (v/v) % TFA in ACN/WATER. The peak area under albumins and globulins, gliadins and glutenins chromatogram was used as a direct measure for amount of total extractable grain proteins and their subgroups and consequently the proportion (%) of HMW-GS was calculated. Values were the average of two replicates for each sample. Total-Chrom software package (Perkin Elmer Instruments, USA) and Statistica for Windows 6.0 (StatSoft, USA) were used for statistical evaluation and principal components analysis (PCA).

RESULTS AND DISCUSSION

According to the catalogue of alleles [1], HMW-GS 1, 2* and N (null) were detected at the Glu-A1 locus (Table 1).

At the Glu-B1 locus, HMW-GS 7+8, 7+9, 17+18 and 6+8 were identified, while at the Glu-D1 locus HMW-GS 2+12 and 5+10 were found. These findings are in accordance with our previous work [7, 8].

The most frequent HMW-GS at the Glu-A1 locus was 2*, at the Glu-B1 locus 7+9 and at the Glu-D1 locus 5+10. The domination of HMW glutenins combinations 2* 7+9 5+10 in European winter wheat cultivars have been also reported by others [9, 10].

Glutenin fractions analyzed by RP-HPLC were eluted according to different surface hydrophobicity in the series HMW-GS and LMW-GS (Fig. 1). The proportion of HMW-

GS in total extracted proteins of wholemeal grains in 2009 and 2010 were 8.91% and 9.55%, respectively, and there is no significant difference between years (Table 1).

Table 1. Composition and proportion of HMW-GS in wheat samples

Genotype	HMW-GS at the Glu-1 loci			Glu-1 score	HMW-GS (%) ^a
	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>		
Srpanjka	N	7+8	2+12	6	9.80 ^b
Žitarka	N	7+8	2+12	6	8.25
Divana	2*	7+9	5+10	9	11.98
Aida	2*	17+18	5+10	10	9.46
Felix	2*	7+8	5+10	10	9.27
Zlata	2*	7+9	5+10	9	9.24
Ilirija	2*	7+8	5+10	10	9.52
Sana	2*	6+8	2+12	6	6.24
Seka	1	7+9	5+10	9	9.34
Golubica	N	7+9	2+12	5	10.01
Soissons	2*	7+8	5+10	10	8.90
Olimpija	2*	7+9	5+10	9	8.79
Year					
2009					8.91ns
2010					9.55
Means^c					9.23

^a Proportion of HMW-GS in total extracted proteins; ^b Means (2009-2010; n=4) at P<0.05;

^cMeans (2009-2010; n = 48) at P < 0.05

Comparing cultivars, a several groups of related HMW-GS attributes were separated. Cultivar Divana as Croatian standard for the highest quality have a distinct position (Table 1, Fig. 2) due to favourable HMW-GS composition (2* 7+9 5+10) and the highest HMW-GS proportion (11.98%).

In contrast, Croatian high yielding cultivar Sana is positioned on the opposite side (Fig. 2) due to less favourable HMW-GS composition (2* 6+8 2+12) and the lowest HMW-GS proportion (6.24%) (Table 1). In our previous studies this cultivar was also characterized by a lower proportion of HMW-GS and weaker dough rheological properties [7, 8].

Cultivar Žitarka, as Croatian standard for baking quality, with lower Glu-1 score (6) and HMW-GS proportion (8.25%) is also isolated from the others. Cultivars Srpanjka and Golubica in spite of lower Glu-1 score (6 and 5, respectively) are more distant from

cultivars Žitarka due to higher proportion of HMW-GS (9.80% and 10.01%, respectively) (Table 1, Fig. 2). According to our previous findings [7,8], cultivars Srpanjka and Golubica consistently performed better baking quality compared to cultivar Žitarka.

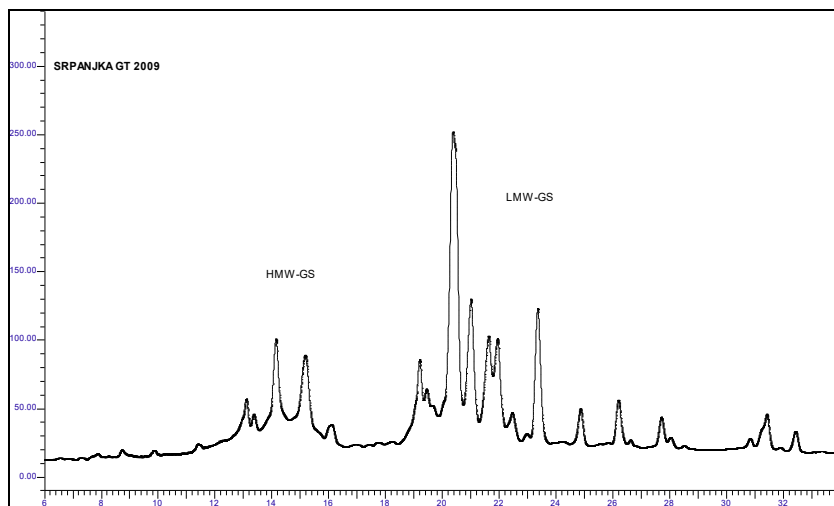


Figure 1. RP-HPL chromatogram of glutenin fractions (cultivar Srpanjka)

Cultivars Soissons, Felix, Aida and Ilirija with the highest Glu-1 score (10) belong to the same cluster and their HMW-GS proportion varied between 8.90% and 10.01% (Table 1, Fig. 2). According to previous gluten strength classification, these cultivars belong to the strong group with good and very good baking characteristics [8].

With medium to strong gluten, cultivars Zlata and Seka belong the same cluster due to the same Glu-1 score (9) and similar HMW-GS proportion (9.24% and 9.34%, respectively) (Table 1, Fig. 2). In relation to these two cultivars, cultivar Olimpija with the same Glu-1 score was slightly distant because of the lower HMW-GS proportion (8.79%). Generally, cultivar Olimpija is characterized by a high protein content and excellent viscoelastic dough properties.

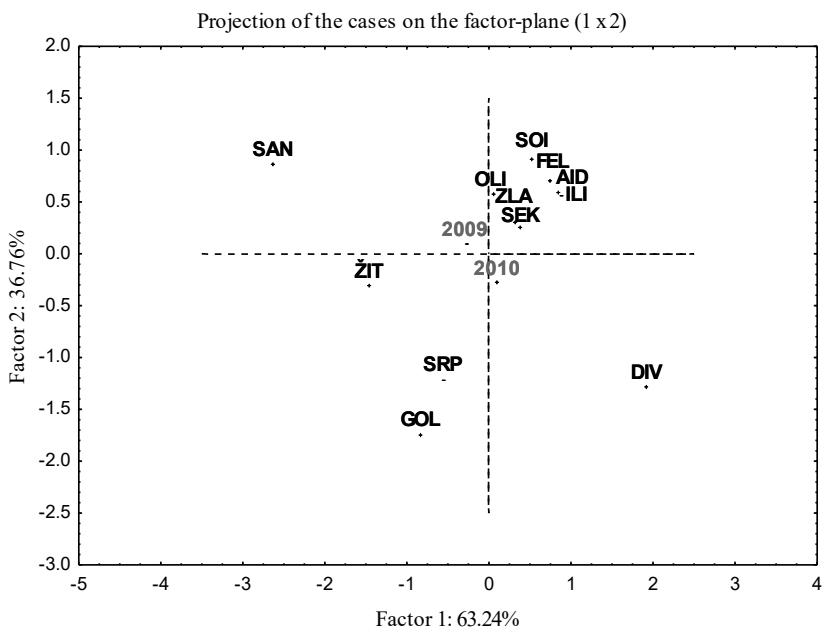


Figure 2. Cultivar clusters on the plane of the two first principal components. HMW-GS attributes determine the clusters. Cultivars are marked with letters: SRP=Srpanjka; ŽIT=Žitarka; DIV=Divana; AID=Aida; FEL=Felix; ZLA=Zlata; ILI=Ilirija; SAN=Sana; SEK=Seka; GOL=Golubica; SOI=Soissons; OLI=Olimpija

CONCLUSIONS

Analysed cultivars were clearly separated in relation to the composition of HMW-GS and their proportion in the total extracted protein.

Considering the significant impact of HMW-GS on the baking quality, cultivar classification by HMW-GS attributes significantly contributes to the improvement of breeding work towards the creation of high quality wheat cultivars already in the early stages of breeding (F4 - F6) when most of the standard analysis for assessing baking quality cannot be made due to the limited amount of wheat sample.

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REFERENCES

1. Payne, P. I., and Lawrence, G. J. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high-molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun.* 11:29–35.
2. Shewry, P. R., Tatham, A. S., and Halford, N. G. 2001. Nutritional control of storage protein synthesis in developing grain of wheat and barley. *Plant Growth Regulat.* 34: 105-111.
3. Pena, E., Bernardo, A., Soler, C., and Jouve, N. 2005. Relationship between common wheat (*Triticum aestivum* L.) gluten proteins and dough rheological properties - Gluten proteins and rheological properties in wheat. *Euphytica* 143:169-177.
4. Dupont, F. M., and Altenbach, S. B. 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *J. Cereal Sci.* 38:133-146.
5. Anderson, O. D., Bekes, F., and D'Ovidio, R. 2011. Effects of specific domains of high-molecular-weight glutenin subunits on dough properties by an in vitro assay. *J. Cereal Sci.* 54:280-287.
6. Wieser, H., Antes, S., and Selmeier, W. 1998. Quantitative determination of gluten protein types in wheat flour by reverse-phase high-performance liquid chromatography. *Cereal Chem.* 75:644-650.
7. Horvat, D., Kurtanjek, Z., Drezner, G., Simic, G., and Magdic, D. 2009. Effect of HMM glutenin subunits on wheat quality attributes. *Food Tech. Biotechnol.* 47:253-259.
8. Horvat, D., Drezner, G., Sudar, R., Magdić, D., and Španić, V. Baking Quality Parameters of Wheat in Relation to Endosperm Storage Proteins. 2012. *Croat. J. Food Sci. Technol.* 4:19-25.
9. Tsenov, N., Atanasova, D., Todorov, I., Ivanova, I., and Stoeva, I. 2009. Allelic diversity in Bulgarian winter wheat varieties based on polymorphism of glutenin subunit composition. *Cereal Res. Comm.* 37:551-558.
10. Dencic, S., Obreht, D., Kobiljski, B., Štatkic, S., and Bede, B. 2008. Genetic determination of breadmaking quality in wheat. Pages 278-281 in: *Proceedings of 43rd Croatian and 3rd International Symposium on Agriculture, Opatija, Croatia.*

DEVELOPMENT OF ENZYMATIC ACTIVITY IN WHEAT GRAIN DURING GERMINATION PROCESS

UDC 633.11 : 577.15

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ABSTRACT

The aim was to determine the optimal germination time in order to achieve the best results for investigated indicators in relation to the recommended values. Wheat samples underwent germination process in controlled conditions, regarding temperature and air humidity, over a period of 7 days. Obtained results for total diastatic power were in range from 240 °WK in starting sample to 420 °WK at the end of the germination process. α -amylase activity was in range from 0 DU/dm in starting sample to 60 DU/dm at the end of germination process. It was determined that germination time ranging from 158 to 168 h is optimal to obtain recommended values for wheat malt (diastatic power 250–420 °WK, α -amylase activity 40–60 DU/dm). Satisfying results were achieved on the half of the 4th day of germination. In conclusion, obtained results indicate that optimal germination time for synthesis of enzymes that affect the total diastatic power of malt (except β -amylase which is activated only during the germination process) and α -amylase synthesis, is between 156–168 h. In case of compromising these indicator values with the rest of important malt quality indicators, the optimal germination time ranges from 120–140 h.

Keywords: wheat, germination, α -amylase activity, diastatic power, malting optimization

INTRODUCTION

The development of enzymatic activity during the germination of wheat grain was determined. Malting consists of three major phases: steeping, germination and kilning. Germination is a crucial process where most of the targeted changes, such as malt modification and the synthesis of enzymes, take place. When the germination of kernels has reached a desired stage, germination is terminated by the controlled drying of the seeds, or in other words, kilning the green malt by blowing hot air through the grain bed. Malt wort fermentability is dependent on an adequate supply of the essential nutrients required by yeast. Levels of the nutrients will ultimately depend on other factors, particularly levels of enzymes such as starch-degrading enzymes, proteases, and β -glucanases. Starch-degrading enzymes play a key role in saccharification of mash and

supply yeast with fermentable sugars. It is generally considered that the rapid and efficient conversion of starch to fermentable carbohydrates during brewing is dependent on a number of parameters including the level of the already mentioned starch degrading enzymes in the malt, the gelatinisation temperature of the starch and the actual temperature program of the mash. Starch hydrolysis is carried out by a number of malt enzymes working together (including α -amylase, β -amylase, limit dextrinase and α -glucosidase). Although α -amylase is able to hydrolyse intact starch granules, the rate of hydrolysis is very slow compared to that of solubilised starch [1, 2]. Effective hydrolysis by α -amylase, therefore, occurs only after the starch has been solubilised (or gelatinised). Effects of starch-degrading enzymes on fermentability became more significant in better modified malts with α -amylase showing stronger effects than total diastatic power [3]. The poorer fermentability of malt was predominately due to low levels of α -amylase, although, free amino nitrogen (FAN) also appeared to be an important factor. The reason why it is important to provide sufficient enzymatic activity in malt (neither too high nor too low, rather optimal) is because the relationships between fermentability and levels of starch-degrading enzymes. They have also been controversial but several fermentability studies have shown a need for adequate modification [4, 5]. Recently there has been some consensus on the importance of these enzymes but only when allowances were made for individual enzymes, such as α -amylase, β -amylase and limit dextrinase versus diastatic power [6], and when allowances were made for differences in β -amylase thermal stability [5-7]. Research has shown that positive effects of increased enzyme levels can be negated by other factors such as starch gelatinisation temperature, [8] (starch gelatinized at lower temperatures is more completely hydrolysed to fermentable sugars as starch degrading enzymes are still active), although, others have found this effect insignificant [5]. Gjertsen and Hartlev [9] also found enzyme levels had little effect on fermentability when enzymes were present at high levels, in which case modification was more important. Bathgate et al., [10] though, found fermentability could be limited by over modification because of increased levels of soluble protein and reduced levels of fermentable sugars due to elevated malting losses. MacGregor [11] indicated starch granules readily gelatinize during mashing in malts with good β -glucan breakdown but with only adequate, not complete protein breakdown because good modification also ensures adequate levels of amino acids for the yeast, although, in low gravity, or all malt worts, free amino nitrogen (FAN) is seldom limiting [12]. Achieving optimal enzyme activity in the grain can be considered very important for the proper conduct of the malting process. Suppression of excessive production of starch-degrading enzymes in the grain is particularly important in wheat because they are established varieties that have a diastatic power of up to 160 °L (about 600 °WK). Therefore the objective of this work was to determine minimum required time of germination, which is necessary for the development of optimal enzyme activity in grain.

MATERIALS AND METHODS

Ten kg of wheat Golubica from trial fields of Agricultural Institute Osijek was obtained during season 2010. Grain samples were collected as untreated and conditioned grain, scaled and packed into in double-walled paper bags (1 kg). Until micromalting, the material was stored for two months in a dry and cool place (20 °C) to overcome post-harvest grain dormancy. To avoid the influence of microbiological contamination on malt quality, raw material control concerning *Fusarium graminearum* and *Fusarium culmorum* contamination was conducted, according to MEBAK procedure. Micromalting was conducted in steeping vessel and at drier Seeger micromaltery according to MEBAK [13], and germination was conducted in Climatic test chamber (Climacell 222, Medcenter Einrichtungen GmbH). Degermination was performed manually. Malt was stored for one month in order to stabilize. α -amylase activity and total diastatic power were determined in the samples. Other amylolytic activity indicators in grain such as extract, extract difference, viscosity of laboratory mash, filterability of laboratory mash were also measured. All indicators were determined according to EBC (methods 4.5.1.; 4.5.2.; 4.8.; 4.12.; 4.13.) [14].

Table 1. Micromalting scheme of wheat samples

1 st day	Immersion steeping for 5 hr, t = 14,0 °C; Dry steeping for 19 hr, t = 14,5 °C
2 nd day	Immersion steeping for 4 h, t = 14,0 °C; Dry steeping for 20 hr, t = 14,5 °C
3 rd day (*)	Immersion steeping for 2 hr, t = 14,0 °C;
3 th day to 7 th day	germination was carried out at 14,5 °C during 3-7 day on relative humidity of air in each procedure: r.H. = 85%; sampling during germination was performed daily
8 th day	duration of kilning was 19 hr, performed according to standard procedures for pale malt (MEBAK) after last hour of germination, draying finished, malt degermination, measuring and packing in paper bags and stored

(*) control of the degree of soaking at the beginning of the third day and every hour of soaking under the water, when it was found that the grain does not tolerate any further soaking under water, moisture content of 44,5% was adjustment with sparging (spray steeping) in germination box (1st day of germination)

The determination of the combined activity of $-\alpha$ and $-\beta$ amylase of germinated grain was performed under standardized reaction conditions was carried out as follows: malt

enzymes were extracted with water at 40 °C. Standard starch solution was hydrolysed by the malt enzyme extract, the amount of reducing sugars formed by amylolytic action was estimated iodometrically. The result was expressed as grams of maltose produced under the specified conditions by 100g of malt (EBC 4.12.). The determination of α -amylase activity of germinated grain was performed as dextrinization time of standardized starch solution in the presence of excess β -amylase. Malt α -amylase is extracted with 5 g/L Na-chloride solutions at 20 °C. Buffered limit dextrin is hydrolysed by the grain enzyme extract. The amount of α -amylase is estimated visually using a colour standard which corresponds to the concentration of starch remaining in solution at the specified end point of the dextrinization process. The result is calculated as the quantity of α -amylase which will dextrinize soluble starch, in the presence of an excess of β -amilase, at the rate of 1g per hour at 20 °C (EBC 4.13.). Micromalting of the wheat samples was carried out according to the procedure shown in Table 1.

RESULTS AND DISCUSSION

Wheat varieties appropriate for malting have not yet been commercialized. For that reason, local bread varieties are being used for malting. In accordance to analytical quality parameters, optimizing of process conditions for malting has been performed only for Northeuropean varieties (mostly German) that genotypically differ from domestic varieties. This is not appropriate since agro climatic conditions during cultivation are significantly different from country to country. Some researches indicate that most domestic varieties (especially bread varieties) can be sorted into B quality group which is characterized with higher N share in grain and relatively low mash viscosity (caused by lower share of soluble pentosanes) [15, 16]. Enzymes synthesis is extremely energetically demanding set of anabolic reactions. For that reason it is necessary to determine duration of that process so it would not harm the malt quality, and cause huge economic losses during malting. Total diastatic power should include α -amylase activity and activity of other amylolytic enzymes in the grain, especially β -amylase. α -amylase is synthesized during germination process and it is genotypically determined. Expression of its potential depends on process parameters during germination [17, 18]. Total diastatic power implies activation of other amylolytic enzymes that are present in wheat grain, and are hugely dependent on agro climatic conditions to which the crop was exposed. Stated facts indicate that duration times of these processes are of most importance to gain favorable α -amylase activity and total diastatic power, without damaging the rest quality indicators. Connection of activation time duration and enzyme synthesis (total diastatic power) to other quality indicators is shown in fig. 1. α -amylase synthesis, and its correlation to other malt quality indicators is shown in fig. 2.

In amylolytic complex activation, also known as diastase, a significant activity has been observed from the very beginning (240 °WK) and also even activity increase till sixth day. After that the curve shows that saturation has been achieved. From technological point of view, total diastatic power should not exceed 250 – 420 °WK, so it is obvious

that optimal values have been achieved between third and fifth day. The malting process cannot be stopped at that time because α -amylase activity is not fully developed (fig. 2). Stopping the process would inevitably lead to fermentability extract decrease because α -amylase activity is an important precursor for formation of limit dextrinases who are important for β -amylase activity. Seventh day of germination, saturation of diastase activity occurs, and that is what Sacher noticed during his experiment [17, 18] and he enrolled this to β -amylase activity decrease (fig. 1). In case of α -amylase synthesis during germination (*de novo*), it is extremely important to achieve optimal values for enzyme activity since, due to the nature of starch in raw materials intended for brewing, it is not possible to achieve enough fermentability extract and that can be detrimental to economic aspects of malt and beer production.

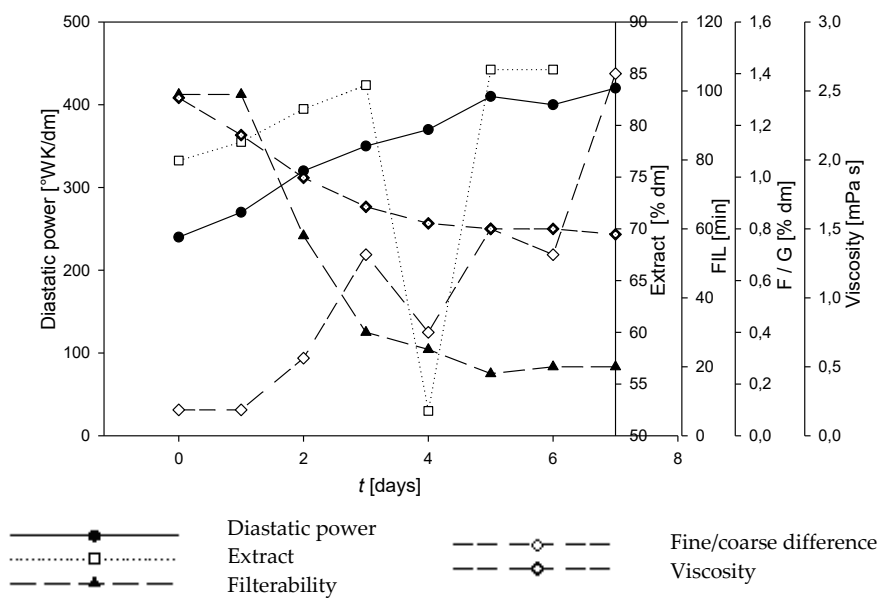


Figure 1. Development of malt grain total diastatic power during germination and its relation to other indicators of quality

In fig. 2 it is noticeable that α -amylase activity shows decreased growth velocity during first three days and sudden increase during fourth, fifth and sixth day. After the sixth day a saturation and decrease of activity occurs and this can be attributed to decreased β -amylase activity (and total diastatic power). Also a surplus of limit dextrines in wort appears (product inhibition). Optimal α -amylase activity has been

achieved between fifth and sixth day (fig. 2). Considering the rest observed indicators (extract, extract difference, viscosity of laboratory mash, filterability of laboratory mash) and recommended values for listed parameters, it can be concluded that during malting wheat by standard procedure, optimal values for total diastatic power and α -amylase activity ranges from 120 - 140 h.

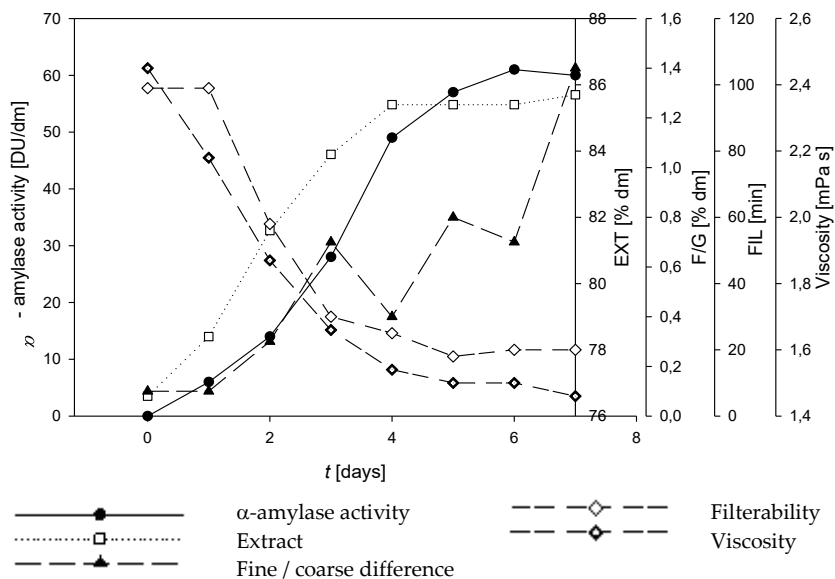


Figure 2. Development of malt grain α -amylase activity during germination and its relation to other indicators of quality

CONCLUSIONS

In conclusion, obtained results indicate that optimal germination time for synthesis of enzymes that affect the total diastatic power of malt and α -amylase synthesis is between 156-168 h. In case of compromising these indicator values with the rest of important malt quality indicators, the optimal germination time ranges from 120–140 h.

REFERENCES

1. Sandstedt, R. M., and Gates, R. L. 1954. Raw starch digestion: a comparison of the raw starch digesting capabilities of the amylase systems from flour alpha-amylase sources. *Food Res.*19:190.
2. Slack, P. T., and Wainwright, T. 1980. Amylolysis of large starch granules from barleys in relation to their gelatinisation temperatures. *J. Inst. Brew.* 86:74-77.
3. Edney, M. J., Eglinton, J. K., Collins, H. M., Barr, A. R., Legge, W. G., and Rossnagel, B. G. 2007. Importance of Endosperm Modification for Malt Wort Fermentability. *J. Inst. Brew.* 113:228-238.
4. Cole, N. W., Nischwitz, R., Powell, G. E., and Lee T. S. P. 1992. Predicting the fermentation potential of individual malts Page 89-95 in: Proceedings of the 22nd Convention, The Institute of Brewing, Australia and New Zealand Section, Melbourne, Australia.
5. Gunkel, J., Voetz, M., and Rath, F. 2002. Effect of the malting barley variety (*Hordeum vulgare* L.) on fermentability. *J. Inst. Brew.* 108:355-361.
6. Evans, D. E., Collins, H., Eglinton, J., and Wilhelmson, A. 2005. Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability. *J. Am. Soc. Brew. Chem.* 63:185-198.
7. Eglinton, J. K., Langridge, P., and Evans, D. E. 1998. Thermostability variation in alleles of barley beta-amylase. *J. Cereal Sci.* 28:301-309.
8. Stenholm, K, Wilhelmson, A., and Home, S. 1998. Starch gelatinization temperature as a malt quality characteristic Page 242-245 in: Proceedings of the 5th Aviemore Conference on malting, brewing and distilling, Institute of Brewing, London, United Kingdom.
9. Gjertsen, P., and Hartlev, P. 1980. Influence of malt amylase activities on extract yield and fermentability Page 186-192 in: Monograph-VI, EBC Symposium on the relationship between malt and beer, Helsinki, Finska.
10. Bathgate, G. N., Martinez-Frias, J., and Stark, J. R. 1978. Factors controlling the fermentable extract in distiller's malt. *J Inst Brew.* 84:22-29.
11. MacGregor, A. W. 1991. The effect of barley structure and composition on malt quality Page 37-50 in; Proceedings of the European Brewery Convention Congress, Lisbon, Portugal.
12. O'Connor-Cox, W. S. C., and Ingledew, W. M. 1989. Wort nitrogenous sources – Their use by brewing yeasts: A review. *J. Am. Soc. Brew. Chem.* 47:102-108.
13. MEBAK: Middle European Brewing Analysis Commission, Brautechnische Analysenmethoden Band I. 1997. 3rd ed. Methods 2.6.1., Selbstverlag der MEBAK, Freising-Weihenstephan.
14. European Brewery Convention, *Analytica.* 1998. 5th ed. Fachverlag Hans Carl, D-Nürnberg.
15. Krstanović, V., Grujić, O., Ugarčić-Hardi, Ž., Marić, V. 2003. Research on possibilities of wheat malt production from domestic bread-wheat varieties Page 80-87 in: Proceedings of ICC 4th Croatian Congress of Cereal Technologists, Opatija, Croatia.

16. Unbehend, Lj., Unbehend, G., Lindhauer, M. G. 2003. Comparison of the quality of some Croatian and German wheat varieties according to the German standard protocol. *Nahrung*. 47:140-144.
17. Sacher B. 1998. Trials for the optimisation of use of soft-wheat varieties in malting and brewing. Page 43-48; 173-179 in: Dissertation, TU München-Weihenstephan, Germany.
18. Narziss L. 1999. Die Technologie Der Malzbereitung. Page 25-65; 422-429 in: *Die Bierbrauerei*, F. Enke Verlag, Stuttgart, Germany.

FUNCTIONALITY OF ENZYMATICALLY TREATED CORN STARCHES ABOVE GELATINIZATION TEMPERATURE

UDC 664.25 : 577.15

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ABSTRACT

Starch is the major component in the human diet and has also unique functionality that has permitted its wide use in food products and industrial applications. In its native form, does not always fulfill the requirements for certain types of processing. However, enzymatic modification can be an alternative method to meet the specific needs of industrial processes. The aim of this study was to evaluate the effect of two different enzymes, fungal α -amylase (AM) and amyloglucosidase (AMG), on corn starch above gelatinization temperature, with special emphasis on biochemical features and structural analyses of treated starches. Amylase decreased significantly the peak viscosity of the starch, whereas amyloglucosidase reduced the final viscosity and setback. Functional properties of starch are directly influenced by hydrothermal (heat and moisture) treatment or processing conditions. Differences were observed among hydration properties. Specifically, AMG activity has more influence breaking the degree of association between intermolecular bonds, and more soluble compounds are leached out. It was also found through scanning electron microscopy that the gel structure displayed as a network matrix was highly perforated with big cell pores and thick walls when treated with AM, and some elongated structures from gelatinized starch were observed in the AMG treated starch.

Keywords: corn starch; enzymatic modification; hydrothermal properties; SEM

INTRODUCTION

Starch is the most abundant storage carbohydrate in staple foods such as cereals, roots and tubers [1]. It is an essential energy source in the human diet and constitutes an excellent raw material for modifying the texture of many processed food-stuffs [2]. Native starches have their own unique properties, however, sometimes their features are not the required for industrial applications and they lack the versatility necessary for the range of food products available in the marketplace. Enzymatic modification can achieve functional properties not found in native starches, in order to meet the specific needs of industrial processes. Normally, enzymatic hydrolysis of starch is aiming at obtaining

sugars and maltodextrins from the complete hydrolysis and rarely is focused on the resulting modified starch. Enzymatic hydrolysis of starch typically requires the coordinated action of different enzymes activities. Enzyme modification of starch may be carried out using one or more enzymes under appropriate conditions of temperature and pH. Native starches are resistant to hydrolysis because enzymes cannot access linkages [3]. The addition of water and heat allows the granule to become disorganized. In the process known as gelatinization, the granule swells, and the amylose seeps from the granule. During gelatinization, foods containing starch become softer in texture, making them more palatable. After gelatinization, digestive enzymes can easily access the glycosidic bonds. The enzymatic and thermic pretreatment would be expected to change the distribution of substitution groups and consequently influence the paste stability and hydrothermal properties of corn starch samples. Differences among corn starches in granule swelling (onset of viscosity), peak temperature, peak viscosity, shear thinning during pasting, and gel firmness during storage, have been mostly attributed to differences in amylopectin structure, whereas differences in setback and final viscosity during pasting have been attributed to amylose structure [4]. Pasting occurs after gelatinization, when starch swells and becomes thick. The diversity of the modern food industry and the enormous variety of food products require that starch be able to tolerate a wide range of processing techniques. Because of these demands it is of particular interest to modify native starches by enzymatic modification and to determine biochemical features of starch and how they affect its functional properties

MATERIALS AND METHODS

Materials

Corn starch samples were generously supply by Huici Leidan (Huarte (Navarra), Spain). The enzymes used in our study for specific roles are α -amylase (AM) from fungi (Fungamyl 2500 SG), obtained from Novozymes (Bagsværd, Denmark) (0.50 g/ml) and amyloglucosidase (AMG). Amyloglucosidase 1100 (3300 U/mL) from Novozymes (Bagsværd, Denmark).

Sample preparation

Preliminary assays were carried out for optimizing enzymatic reactions (starch quantity, pH, and enzyme activity). The quantities of enzyme chosen were based on the level of enzyme necessary for hydrolyzing 50% of the suspended starch. The following conditions were selected: AMG-1100 (4 U/g starch), Fungamyl 2500 (5 U/g starch).

Corn starch (5.0 g) was suspended in 25 mL of 20mM NaH₂PO₄ buffer at pH 6.0 or in sodium acetate buffer at pH 4.0, depending on the AM or AMG reaction, respectively. For obtaining the enzymatic treated starches, enzyme levels previously detailed were added to the starch suspension. Starch suspensions without adding enzymes, as controls, were carried out in parallel. Enzymatic treatment was carried out in a rapid viscoanalyzer (RVA) (Newport Scientific model 4-SA, Warriewood, Australia) by

following the ICC Approved Standard 162 (ICC-Standard No.162 Approved, 1996). Pasting properties were recorded using the ThermoLine software supplied with the device. Then, samples were washed twice to remove the enzyme and the released hydrolysis products. Briefly, 50 mL of water were added and homogenized with a Polytron ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, USA) during 1min at speed 3. Samples were centrifuged for 15 min at 7,000×g, and a temperature of 4 °C. Supernatants were pooled together and boiled in a water bath for 10 min to inactivate the enzymes before any further analyses. Sediments containing starch were freeze-dried and kept into the freezer at -25 °C for further analyses. Four batches were prepared for each treatment.

Starch hydration properties and iodine binding values

Swelling parameters and water soluble compounds of modified corn starch samples were determined following the method reported by [5], with slight modification as reported by Rosell, Yokoyama, & Shoemaker, 2011 [6]. Briefly, the supernatant was decanted into an evaporating dish and the weight of dry solids was recovered by evaporating the supernatant at 80 °C till constant weight. Four replicates were made for each sample. Residues (W_r) and dried supernatants (W_s) were weighed and swelling power (SP), solubility index (SI) and swelling capacity (SC) were calculated as follows:

$$\text{Swelling Capacity (g/g)} = W_r / W_i \quad \text{Eq. (1)}$$

$$\text{Solubility Index (\%)} = W_s / W_i \times 100 \quad \text{Eq. (2)}$$

$$\text{Swelling Power (g/g)} = W_r / W_i (100 - SI) \quad \text{Eq. (3)}$$

where W_i was the sample weight (g, db).

Iodine binding values are indicative of amylose complex formation. The iodine binding value was determined in the soluble supernatant after heat treatment and centrifuging the samples as described above. The soluble supernatant (40 μ L) was mixed with 2 mL of an aqueous solution of 0.2% KI and 0.65% I₂. The absorbance at 690 nm was measured using a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan). Paste clarity was directly measured from the supernatant at 650 nm using a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan).

Values were the average from four replicates.

Scanning electron microscopy (SEM)

The structural properties of the samples were studied using a JSM 5200 scanning electron microscope (JEOL, Tokyo, Japan). The corn starch powders were stick on a specimen holder using cuprum tape, and then coated with gold in a vacuum evaporator

(JEE 400, JEOL, Tokyo, Japan). The obtained specimens were examined at an accelerating voltage of 10 kV.

Statistical analysis

Multiple analyses of variance for the identification of all single effects were performed by using Statgraphics Plus v. 7.1 (Statistical Graphics Corporation, UK). Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

RESULTS AND DISCUSSION

Pasting properties

The pasting properties from corn starch samples at 95 °C are presented in Table 1 and the viscosity curves can be displayed in Figure 1. Pasting properties were affected by enzyme activity. The data showed that starches pretreated with enzymes exhibited lower pasting temperature, lower viscosity, through and setback values compare with control starch samples. The breakdown viscosity (Figure 1) recorded as the difference between the peak viscosity and the minimum viscosity point at 95 °C increased significantly with the AMG treatment but the opposite trend was observed with the AM.

Table 1. Pasting properties of corn starch samples measured by RVA

	Control-4	AMG	Control-6	AM
Peak viscosity(cP)	12614±536	9586±742	12800±503	5123±205
Trough(cP)	6780±373	819±174	7381±636	1699±71
Breakdown(cP)	5834±188	8767±907	5419±188	3424±249
Final Viscosity(cP)	10758±425	1369±281	12479±776	2636±92
Setback(cP)	3979±84	550±110	5098±275	937±41
Pasting Temp(°C)	71±7	55±24	62±13	54±25
Hydrolysis 95°C(%)	0±0	21±6	0±0	59±2
Hydrolysis 50°C(%)	0±0	87±3	4±5	79±1

Considering that high breakdown viscosity indicates granule disruption or the less tendency of starch to resist shear force during heating [7], these results suggest that sample treated with AMG is less stable during gelatinization process and showed higher resistant to shear force than AM. Similar results for the setback values were reported by [8] where, corn starch granules were allowed to react with alfa-amylase. The setback

value, an index of retrogradation tendency in the starch paste, also decreased after pretreatment with enzymes. Analyses were performed above gelatinization temperatures. Several events take place during the gelatinization of starch: the molecular order and thus birefringence disappears, the starch granule loses crystallinity (melting of crystallites), water is absorbed, swelling of the granule is followed by leaching of mainly amylose, and when further heated, starch granules are disrupted and partial solubilization is achieved [9, 10]. Therefore results showed different characteristics influenced by thermal treatment at 95 °C, as mentioned above.

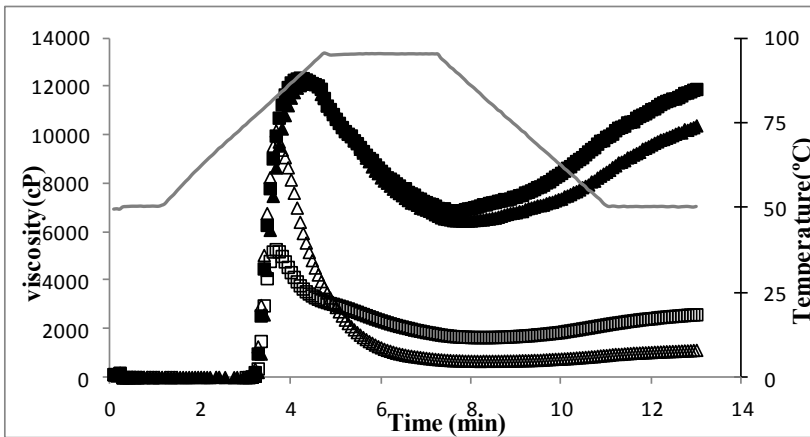


Figure 1. Pasting curves of corn starch samples measured in RVA. Legend: ▲ Control pH 4; △ AMG; ■ Control pH 6; □ AM

Experimental conditions were optimized for obtaining partial hydrolysis of the starch, mainly referring to the maximum viscosity at 95 °C, which is reflected in the hydrolysis percentage (Table 1). Hydrolysis at 95 °C showed lower values for AMG treated samples whereas at 50 °C greater hydrolysis is obtained with AMG. It is generally accepted that granules contain amorphous and crystalline domains that create a semicrystalline environment within the granule. It is also understood that the crystalline domains are mainly composed of amylopectin while bulk amorphous domains are made up of amylose traversed by non-crystalline regions of amylopectin. AMG is an exoamylase, which action a sequential manner starting from the non-reducing end of the starch molecule to produce well defined end products. Due to its saccharific activity, AMG cleaves every glycosidic bond from the non-reducing end successively to produce glucose [11]. This can explain the higher percentage of hydrolysis obtained at 50 °C. Corn starch samples were more rapidly hydrolyzed by AMG. On the other hand, AM is

an endoamylase which acts randomly at the interior of starch molecule to release linear and branched oligosaccharides of varying chain lengths that breaks every alternate glycosidic linkage due to its amylolytic activity [11]. Heat treatment of the samples at 95 °C promotes changes in the amorphous areas of granules, making them more accessible to enzymatic activity by AM.

Amylose content- Hydration properties

The results from hydration properties and amylose content are presented in Table 2. Enzymatic treatment clearly influence results from paste clarity, solubility index and amylose content. No significant differences were found from the results of swelling capacity and swelling power. When starch granules are heated in the presence of water, the starch granules absorb water and swell but enzymatically modified starch samples did not differed from the control samples and did not cause difference in the water absorption from starch granule. However, the results showed significant differences due to the enzyme activity for others properties.

Table 2. Effect of different enzymes on the solubility index (SI), swelling power (SP), swelling capacity, and iodine binding absorbance of corn starch. Paste clarity is expressed as absorbance at 650 nm. Iodine binding is expressed as absorbance at 690 nm.

	Control-4	AMG	Control-6	AM
Paste clarity (Abs 650nm)	0.15±0.12	0.54±0.20	0.05±0.02	0.50±0.47
SI (g/100g)	2.92±1.67	26.74±3.25	3.72±0.41	22.08±1.36
Swelling power (g/100g)	9.93±1.78	10.85±0.27	9.56±1.93	9.22±0.75
Swelling capacity (g/g)	8.74±0.79	7.95±0.23	8.33±1.00	7.19±0.61
Amylose content (Abs 690nm)	0.47±0.38	2.66±0.11	0.16±0.14	2.68±0.10

Enzymatically modified starches showed increased paste clarity values compared to their controls. The released water soluble compounds from the enzymatic treatments increased significantly the paste clarity. This behavior is related to the dispersion state of the sample which is clearly influenced by the enzyme activity. Starch granule swelling is known to begin in the bulk relatively mobile amorphous fraction and in the more restrained amorphous regions immediately adjacent to the crystalline region [12]. Enzymatic treatment influenced results from swelling index, which determines the

quantity of soluble solids obtained from treated starch that are formed with diminutive structure. More soluble solids are formed in enzymatically modified samples at 95°C. When aqueous suspension of starch granule is heated, the starch molecules hydrate and swell with a consequent leaching of some soluble starch into the liquid. AMG and AM activities have more influence breaking the degree of association between intermolecular bonds, and more soluble compounds were leached. The amount of amylose leached to supernatant was verified. Results showed higher values of amylose were detected in the supernatant of enzymatically modified corn starches. Enzymatic modification of corn starch did cause change in apparent amylose content in either of the starches. These results indicated that the amount of leached material in the supernatant after enzymatic and thermal treatment at 95 °C was significant, and that gelatinization of the starch granules did occur during the process. It was also observed an increase of free sugar content released in enzymatic modified corn starch samples, being more notable in samples treated with AMG. This enzyme is characterized for the capacity to hydrolyze the linkage α -1,4 of non-reduced extremes from polysaccharides for the glucose formation; generates free sugars released by its saccharifying activity; even though samples were washed, more residual quantity still remained in starch.

Microstructure SEM of the starch

The scanning electron micrographs (SEM) of corn starches samples are shown in Figure 2 and 3. SEM investigation showed that enzymatic modification of the samples caused several changes in the structure of starch granules compared with control starch samples. Fig 2a shows the morphology of control samples at pH 4. The morphology from AMG enzymatically modified corn starch samples are shown in Fig 2b, which are magnified 1000, 2000 and 3500 times, respectively. Control sample at pH 4 showed a uniform gel structure with cell pores with 5 microns diameter. It can be observed numerous threads or strands and thin structures scattered over the walls. Enzymatic treatment of starch with AMG resulted in a gel structure more distorted with bigger pores and some elongated structures from gelatinized starch. Some small rounded particles can be observed likely from released hydrolysis products. On the other hand, Fig 3 shows the morphology of control samples at pH 6 (Fig 3a), and of AM enzymatically modified corn starch (Fig 3b). At the different magnification (1000, 2000 and 3500 times), it can be observed in the control sample a homogeneous distribution of the molecules and a continuous uniform gel with smaller cell pores than the ones observed at pH 4.0. Enzymatic treatment with AM yielded a highly perforated network with big cell pores and thick walls. From SEM images a few dextrans could be observed, which confirms the hydrolysis activity of AM. As was mentioned above, AM is an endo-enzyme that randomly hydrolyzes the α -1,4 glycosidic linkages in polysaccharides, resulting in short chains [13], as released products such as dextrans.

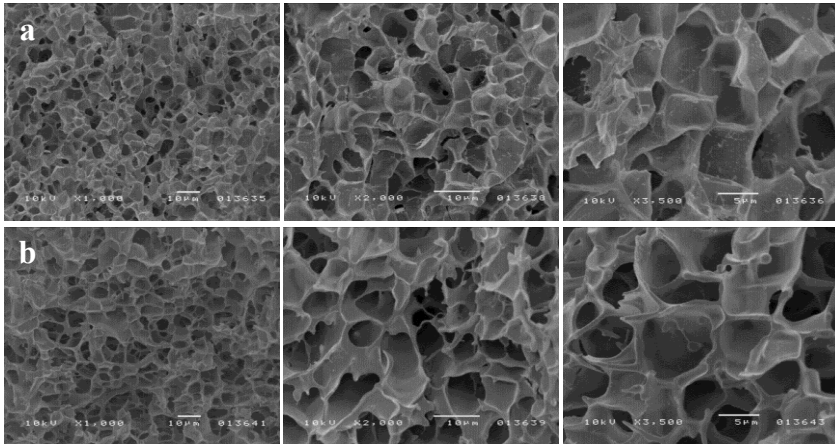


Figure 2. Scanning electron micrographs of corn starch samples. 1000x, 2000x and 3500x. Control at pH 4 (a) Amyloglucosidase (b)

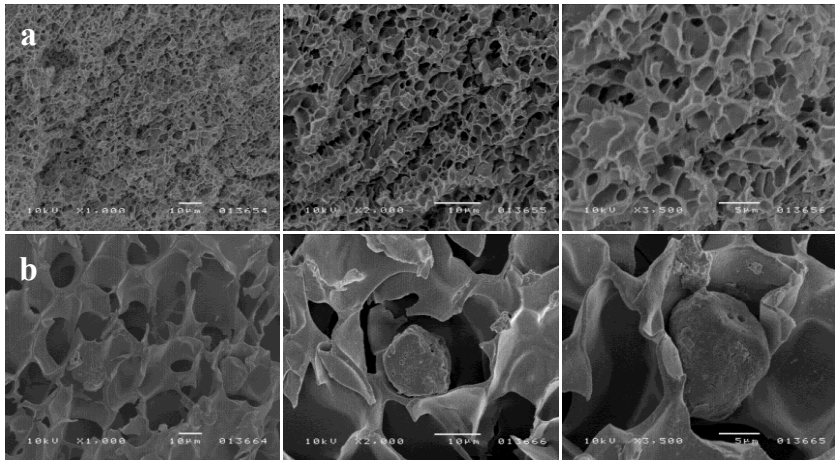


Figure 3. Scanning electron micrographs of corn starch samples. 1000x, 2000x and 3500x. Control at pH 6 (a) α -Amylase (b).

CONCLUSIONS

Enzymatic modification of corn starch significantly affects functional properties and starch features. Generally, hydration properties are significantly affected by enzymatic modification being further influenced by AMG activity. Results confirmed that the loss of granular structural order and changes in both amorphous and crystalline domains during gelatinization can be more influenced by the enzymes and that samples enzymatically treated showed higher values of final product obtained from the hydrolyzed process. Microscopic techniques showed that the main factor in the changes of starch granule microstructure were the enzymes, where network from enzymatically modified corn starch samples showed structures more perforated and a great alteration and disruption of the gel matrix.

ACKNOWLEDGEMENTS

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REFERENCES

1. Kasprzak, M., et al. 2012. Effect of enzymatic treatment of different starch sources on the in vitro rate and extent of starch digestion. *Int. J. Mol. Sci.* 13(1):929-942.
2. Biliaderis, C. G. 2009. Structural transitions and physical properties of starch.pdf. *Starch: Chemistry and Technology*, Third Edition.
3. Haralampu, S. G. 2000. Resistant starch - a review of the physical properties and biological impact of RS3. *Carbohydr. Polym.* 41(3):285-292.
4. Tziotis, A., et al., Functional properties of starch from normal and mutant corn genotypes. *Carbohydr. Polym.* 2005. 61(2):238-247.
5. Toyokawa, H., et al. 1989. Japanese noodle qualities. 1. Flour components. *Cereal Chem.* 66(5):382-386.
6. Rosell, C. M., Yokoyama, W., and Shoemaker, C. 2011. Rheology of different hydrocolloids–rice starch blends. Effect of successive heating–cooling cycles. *Carbohydr. Polym.* 84(1):373-382.
7. Huang, Q., et al. 2010. The effect of enzymatic pretreatments on subsequent octenyl succinic anhydride modifications of cornstarch. *Food Hydrocoll.* 24(1):60-65.
8. Huber, K.C. and BeMiller, J.N. 2000. Channels of maize and sorghum starch granules. *Carbohydr. Polym.* 41(3):269-276.

9. Eerlingen, R.C. and Delcour, J.A. 1995. Formation, analysis, structure and properties of type-III enzyme resistant starch. *J. Cereal Sci.* 22(2):129-138.
10. Kim, S.-K. and Kwak, J.-E.. 2009. Formation of Resistant Starch in Corn Starch and Estimation of its Content from Physicochemical Properties. *Starch – Stärke.* 61(9):514-519.
11. Kumar, P. and Satyanarayana, T. 2009. Microbial glucoamylases: characteristics and applications. *Crit. Rev. Biotechnol.* 29(3):225-55.
12. Donovan, J. W. 1979. Phase-transitions of starch-water system. *Biopolymers.* 18(2):263-275.
13. Rosell, C. M., et al. 2001. Experimental Approach To Optimize the Use of α -Amylases in Breadmaking. *J. Agric. Food Chem.* 49(6):2973-2977.

STARCH CHARACTERISATION USING SPECTROPHOTOMETRY AND DIRECT POTENTIOMETRY

UDC 664.23 : 543.4

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ABSTRACT

Starch consists of two polymer types; amylose and amylopectine. Their ratio is starch origin-dependent. Triiodide ions bind characteristically to the amylose and amylopectin molecules of the starch. This can be monitored using spectrophotometry, but recently also direct potentiometry with platinum redox sensor. The absorbance and electrical potential change of the starch-triiodide complex were measured for wheat, potato, corn, rye, barley, rice, tapioca and commercial starch. The results showed characteristic curves for each starch type, corresponding to the specific amylose/amylopectine ratio. The curves were used to determinate starch type-specific parameter values; for spectrophotometry: starch-triiodide peak wavelength maximum (λ_{\max}/nm), maximum absorbance change for λ_{\max} (ΔA) and for the direct potentiometry: slope (S) for the linear response region, maximum potential change (ΔE) and relative sensitivity (mV/mg) for potential change in the corresponding starch concentration. Data comparison using these two methods revealed that methods serve to distinguish starch types based on specific triiodide bounding to starch components, but when absolute data changes between starches were compared, no correlation between them has been found.

Keywords: spectrophotometry, direct potentiometry, starch triiodide complex

INTRODUCTION

Starch is a semicrystalline biopolymer and it is stored in various plant locations, such as in cereal grains, roots, tubers, stempiths, leaves, seed, fruit and pollen.

The general properties of a starch, such as gelatinization, solubilization, swelling, granule size, chemical constitution, crystal type, and enzymatic degradation, differ because of the origine of starches. The starch granules from different botanical sources vary in size, shape, and content of amylose and amylopectin, influencing their chemical and physical properties [1].

The interaction of starch and iodine results in the formation of complexes [2] with characteristic colors. The color of the starch–triiodide complex has been shown to vary with starch chain length [3]. Because starch assumes a helical structure, iodine molecules occupy the central cavity of the helical molecule in the complex [4]. Many physicochemical properties of starch, such as its iodine binding capacity and degree of polymerization (DP), depend on the starch's botanical origin [5, 6].

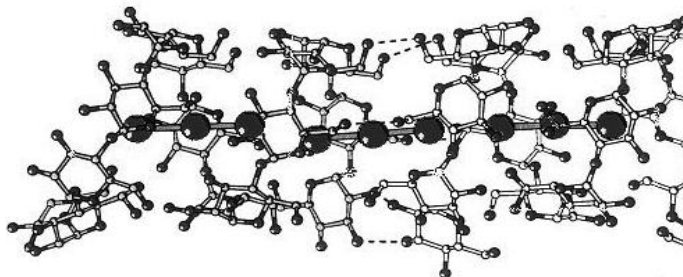


Figure 1. Starch-triiodide complex

In our previous paper [7], we described a method for the determination of starch based on direct potentiometric measurements, where the response of the triiodide ion in starch-triiodide complex is measured.

The aim of this work was to determine and compare the differences in various starches, concerning amylose/amylopectine ratio and starch-triiodide complex formation by using direct potentiometry and UV-VIS spectrophotometry.

EXPERIMENTAL

Reagents and solution preparation

Starch samples were isolated from wheat (Srpanjka), potato, maize, rye (Barun), barley (Conduct), rice, and tapioca, which were obtained at the local market store in Croatia, and the commercial model (control) starch was obtained from Kemika (Croatia).

The samples' seed coats were peeled off, and an alkali steeping method [8] was used to prepare the purified starch samples. The starch solution was prepared in a concentration range of 1-5 g/L. Starch solutions were prepared fresh each day to avoid microbial degradation. Solutions with starch concentrations greater than 5 g/L form a starch gel. As a consequence, such solutions are difficult to manipulate and could cause quantitative errors.

Apparatus

Direct potentiometric measurements were performed on 780 Metrohm pH Meter (Metrohm, Switzerland) combined with IJ64 platinum redox electrode (Ionode, Australia) as a detector and a silver/silver (I) chloride reference electrode. Spectrophotometric measurements were performed using AvaSpec 2580 UV-Vis spectrophotometer (Avantes, Netherland).

Procedure

Spectrophotometric

For spectrophotometric measurements, five independent series of starch triiodide solutions were prepared. The previously prepared potassium triiodide solution was then added to the starch-filled (wt 5%) 50 mL volumetric flasks. The volumetric flasks were filled to the mark with deionized water, stirred in a sonic bath for 5 minutes and were ready for further investigation.

Potentiometric

Triiodide solution was transferred to the titration vessel. The responses of the platinum redox electrode were measured by accurate, incremental additions of the prepared starch solutions. The solutions were continuously stirred during the addition of starch and during the measurements. Five independent series of starch triiodide solutions were measured.

RESULTS AND DISCUSSION

Spectrophotometric

Starch samples were characterized by measuring starch triiodide complex absorption spectra. The commercial starch was used as a reference model. The amylose-amylopectin ratio in starch depends directly on botanical starch origin, there is a considerable difference in the starch-triiodide spectra for different starch types. The difference is noticeable in the wavelength area of the starch-triiodide complex where peaks vary in their heights and maximum wavelength values. These parameters from starch-triiodide complex spectra have been used for raw pre-statistical starch type differentiation (Table 1). A model starch was used as a reference for λ_{\max} comparison to other starch types (shown as $\Delta\lambda$). Rye (Conduct) showed the highest positive shift (+71 nm), Waxy corn starch the highest negative (-44) and Potato (Sigma) (+7) the lowest shift towards reference starch. The absorbance increase (ΔA) at each λ_{\max} was calculated. Potato (Sigma) and rice starch showed the highest absorbance increase, 0.474 and 0.422, respectively. This indicates that the highest amount of triiodide is complexed with starch.

Table 1. Measured parameters from the absorbance spectra for all starch types

Starch origin	λ_{\max}/nm	$\Delta\bar{A}(\lambda_{\max})$ for 5% starch solution	$\Delta\lambda/\text{nm}$ (shift λ_{\max})
Model starch	564	0.337	0
Potato (Sigma)	571	0.474	+7
Corn	610	0.321	+46
Corn waxy	520	0.155	-44
Tapioca	600	0.398	+36
Rye (Conduct)	630	0.310	+66
Wheat (Srpanjka)	630	0.197	+66
Rice	590	0.422	+26

Potentiometric

The platinum redox electrode showed a decrease in the response potential for each starch sample, and the curve shapes and slopes differed between starch samples. This is shown in Table 1; slope (S), linear response region per added starch mass, maximum potential change (ΔE) in linear response area and relative sensitivity (mV/mg) for potential change in the corresponding starch sample. Rye starch showed the highest slope -6.716 , maximum potential change 47 mV and the highest relative sensitivity which indicated it bounded the more triiodide than other starches. Barley starch showed the longest linear response area. Model starch showed the lowest relative sensitivity 2.97 mV/mg. The response data reflect the profile characteristics in the differences in the origins of the starches and amylose amylopectine ratio.

Table 2. Measured parameters from the redox electrode potential response change

Starch origin	slope (S)	linear area / mg starch	ΔE mv / linear	relative sensitivity mV / mg
Rice	-4,964	9	43,2	4,80
Rye (Conduct)	-6,716	7,5	47	6,27
Tapioca	-6,381	7,5	46	6,13
Corn	-5,022	7,5	37	4,93
Wheat (Srpanjka)	-3,542	12	39,8	3,32
Barley (Vanessa)	-3,961	10,5	39,6	3,77
Potato (Sigma)	-5,169	7,5	37,3	4,97
Model starch	-3,035	9	26,73	2,97

CONCLUSIONS

The results showed characteristic data for each starch type, corresponding to the specific amylose/amylopectine ratio, for both techniques.

Data comparison using these two methods revealed that methods serve to distinguish starch types based on specific triiodide bounding to starch components, but when absolute data changes between starches were compared, no correlation between them has been found.

REFERENCES

1. Smith, A. M. 2001. The Biosynthesis of Starch Granules. *Biomacromolecules*. 2:335–341
2. Teitelbaum, R. C., Ruby, S. L. and Marks, T. J. 1978. On the structure of starch-iodine. *J. Am. Chem. Soc.* 100:3215–3217
1. Bailey, J. M. and Whelan, W. J. 1961. Physical properties of starch. *J. Biol. Chem.* 236:969–973
2. Hinrichs, W., Büttner, G., Steifa, M., Betzel, C.H., Zabel, V., Pfannemüller, B. and Saenger, W. 1987. An Amylose Antiparallel Double Helix at Atomic Resolution. *Science* 238:205–208
3. Chatel, S., Voirin, A., Luciani, A. and Artaud, J. 1996. Starch identification and determination in sweetened fruit preparations, *J. Agric. Food Chem.* 44:502–506
4. Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N. and Hisamatsu, M. 2005. Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. *Carbohydr. Polym.* 62:25–34
5. Sakač, N., Sak-Bosnar, M. and Horvat, M. 2013. Direct potentiometric determination of starch using a platinum redox sensor. *Food Chem.* 138:9–12
6. Baik, M.Y., Kim, K.J., Cheon, K.C., Ha, Y.C. and Kim, W.S. 1997. Recrystallization Kinetics and Glass Transition of Rice Starch Gel System. *J. Agric. Food Chem.* 45:4242–4248

EFFECT OF HIGH INTENSITY ULTRASOUND ON PROPERTIES OF STARCH PASTES

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ABSTRACT

Starch has an abundant use in the food industry because it is a thickening, stiffening and gluing agent. Also, nowadays there is an emphasis on the need to develop mild non thermal methods for food processing and in this view ultrasound has great potential. Therefore it is interesting to determine the effect of high intensity ultrasound on functional properties of the most common carbohydrate in the human diet. In this study we reviewed the effect of one and two minute treatment of rice and corn starch pastes with 24 kHz probe, 400 W of nominal output power, at 20%, 60% and 100% amplitude. The results showed that 60% and 100% ultrasound treatments increase the swelling power of starch pastes but increase the resistance to refrigeration of only corn starch pastes while leaving the rice starch paste unchanged. Apparent viscosity was measured at three different temperatures, as well as the hardness of starch pastes differ depending on the conditions of the ultrasound treatment. By reviewing the influence of ultrasound on starch pastes, it was determined that no deteriorating effect occurred and this treatment could be helpful for freezing of food stuffs.

Keywords: US; starch paste; swelling; texture; refrigeration; viscosity

INTRODUCTION

Except being an essential energy source in the human diet, starch is also a very good raw material for modifying the texture of many processed food products [1]. Differences in granule composition and microstructure among starches of different botanical sources (corn -sphere/polyhedral 2-30 μm and rice polyhedron 2-8 μm), have been known to result in differential starch granule reactivates, reaction patterns and various functionalities among starches [1, 2]. Swelling, gelatinization, pasting and retrogradation of granules are important aspects of starch functionality. Gelatinization is the process in which heated starch becomes soluble in the presence of sufficient moisture, binds water and forms a gel. Starch granules swell as a result of absorbing water, and the crystalline organization of starch becomes irreversibly disrupted, and at this point breakage of hydrogen bonds occurs [1, 3]. Molecular interactions (hydrogen bonding between starch chains) after cooling of the gelatinized starch paste are called retrogradation [1].

New and innovative methods in food science present a challenge in order to improve the technological processes as well as to improve the characteristics of food products [2]. Ultrasound treatment (UST) can be safely used in different phases of food production in order to acquire microbiological safe food products with enhanced physical-chemical properties [4]. Ultrasound (US) assisted freezing can improve the quality of frozen food because cavitations can promote nucleation and because subjecting ice crystals to alternating acoustic stress leads to products of smaller crystal size distribution and small ice crystal formation [5].

Modification of starch is carried out to enhance the positive attributes and eliminate the shortcomings of native starches. Aim of this research is to determine the effect of US on the rheological and refrigeration properties of corn and rice starch pastes. We wanted to examine, if the beneficial effect of short time treatment of starch pastes with US in the US assisted freezing, is exclusively due to the creation of small ice crystals, or does it affect the properties of starch granules.

MATERIALS AND METHODS

Sample preparation

Corn starch (C*GEL 03401, Cargill) contained 86.5-89.5% of starch and rice starch (S7260 – 500G, Sigma Aldrich) contained 87.0-92.5%. Starch pastes were prepared by suspending 2/3 of 25 g of starch in 500 ml of distilled water and mixing them on a magnetic mixer with constant heating. Once the temperature of the suspension reached the gelatinization temperature for the given starch (65 °C for corn and 70 °C for rice starch), it was cooled down to 50 °C at which time the remaining of the sample was mixed in and reheated to the gelatinization temperature where it was held for the next 30 minutes. The suspension of starch was done gradually in order to avoid incomplete suspension or gelatinization. Starch pastes prepared in this way were regarded as reference samples to which the samples treated with US were compared.

Ultrasound treatment

Starch pastes were submitted to UST at different time intervals (1 and 2 minutes) and at different amplitudes (20%, 60% and 100%) with an US probe (Hielscher GmbH, Ultraschallprozessor UP 400s, Germany) with 400 W of nominal output power and 24 kHz frequency, always with a full cycle. The samples were always treated in a 1000 ml laboratory flash and the probe was inserted 1 cm into the suspension so that the effect of US would be the same during each treatment.

Table 1. Abbreviations for the tested 5% corn and rice starch pastes depending on the applied UST (amplitude and time of treatment)

Starch	Native suspension (5%)	UST (% - amplitude; min - time)					
		20%		60%		100%	
		1 min	2 min	1 min	2 min	1 min	2 min
Corn	CN	C1/10	C2/20	C1/60	C2/60	C1/100	C2/100
Rice	RN	R1/10	R2/20	R1/60	R2/60	R1/100	R2/100

Determination of rheological properties

Starch paste viscosity was determined on a rotary viscosimeter Rheometric Scientific RM-180 (Mettler Toledo, US). Viscosity was measured at the temperature of gelatinization for the given starch, at 90 °C and finally, the backward viscosity was measured after cooling it to 50 °C.

Texture profile analysis was performed on the texture analyzer TA.HDplus (Stable Microsystems, UK) according to [6] using SMS P/O-5R polyethylene probe and 5 kg load cell test, speed of 1 mm/s, strain 40%. For the measurement of hardness, starch pastes were transferred to Petri dishes after they have been heated to 90 °C. Samples were left to cool down at room temperature for 15 minutes and later put to refrigeration for 24 hours before testing. Filling the Petri dishes to the top and allowing them to harden in this shape ensured that the dimensions of all the samples were equal.

Determination of the resistance to refrigeration and thawing and swelling power

Resistance to thawing and refrigeration was determined by modification of method by Rondán-Sanabria (2009) [7]. When the starch pastes were cooled down to 50 °C (during measurement of viscosity), 5 samples of 10 mL were taken for measurement of resistance to repeated thawing and refrigeration, and 2 samples of 40 mL for the measurement of resistance to one cycle of refrigeration and thawing. Due to limitations of our centrifuge (Rotina 35, Hettich Zentrifugen, Germany), only the 40 mL samples could be analyzed according to the method. 10 ml samples were centrifuged at 4000 rpm for 20 minutes, while the 40 ml samples were centrifuged at 10 000 rpm for 15 minutes. Samples were frozen to -18 °C. Syneresis of water was measured by weighing the samples prior and after centrifuge and decantation of water.

In order to determine the damage done to starch granules by UST, we measured their swelling power. After preparation of the sample (for native samples) and UST treatment, 8 mL of starch pastes were transferred into glass tubes and put in a water bath at gelatinization temperature for the given starch (65 °C for corn, 70 °C for rice starch). After 30 minutes, samples were centrifuged at 4000 rpm for 15 minutes and the decanted

water was weight. Finally, samples were left in a drying oven at 105 °C until they reached a constant mass. 4 parallel measurements were made for each sample. From the differences in the mass of sample, according to Douzals (1998) [8], the amount of absorbed water was calculated.

Statistical analysis of regression and correlations was done in Microsoft Excel 1998.

RESULTS AND DISCUSSION

Table 2. Resistance to refrigeration measured during five consecutive days on the 10 mL samples of corn and starch native and US treated starch pastes

	SINERSIS %				
	1. day	2. day	3. day	4. day	5. day
CN	63.6	55.0	61.4	61.1	65.6
C1/20	21.4	7.3	15.2	15.1	9.4
C1/60	31.1	30.9	17.8	34.1	31.4
C1/100	32.7	0.2	19.8	29.9	0.4
C2/20	29.7	24.7	5.2	0.1	0.9
C2/60	26.2	21.8	15.7	13.3	21.1
C2/100	22.9	3.7	6.4	8.9	0.0
RN	41.6	52.8	57.8	48.0	59.2
R1/20	44.5	54.2	57.8	62.9	64.6
R1/60	-	49.9	57.9	59.5	61.3
R1/100	33.6	53.2	60.4	59.5	-
R2/20	38.8	51.7	55.3	61.0	60.2
R2/60	-	53.3	62.1	62.7	62.5
R2/100	45.3	44.9	60.6	64.3	65.6

Syneresis represents the separation of a liquid from gel, but can also signify the separation of solvent in polymer suspensions and it is an indicator of a samples resistance to freezing [9].

UST of corn starch pastes before freezing them resulted in a decrease of syneresis during thawing and therefore an increased resistance to refrigeration. When examining the resistance to refrigeration during the 5 consequent days (Table 2) of thawing, the best results were gathered with 20% amplitude and 2 minutes of treatment. Longer treatment time was always more successful.

Second measurement with the 40 mL samples (Figure 1) showed that the 60% amplitude is the most successful treatment for corn starch pastes, causing a 21.9% decrease in water syneresis after first refrigeration and thawing. Improvement in the resistance to refrigeration is especially strong with the increasing numbers of thawing and repeated refrigeration. Corn starch has a large share of amylose (22.3-32.5) and this causes larger retrogradation during storage and consequently syneresis [10]. UST of rice starch pastes showed no effect on their resistance to refrigeration and thawing (Table 2, Figure 1). Swelling of starch granules relates to the magnitude of the interaction between starch chains within the amorphous and crystalline domains and we determined a strong correlation between the swelling power and resistance to refrigeration (corn 0.99, $p=0.09$; rice 0.94, 0.05).

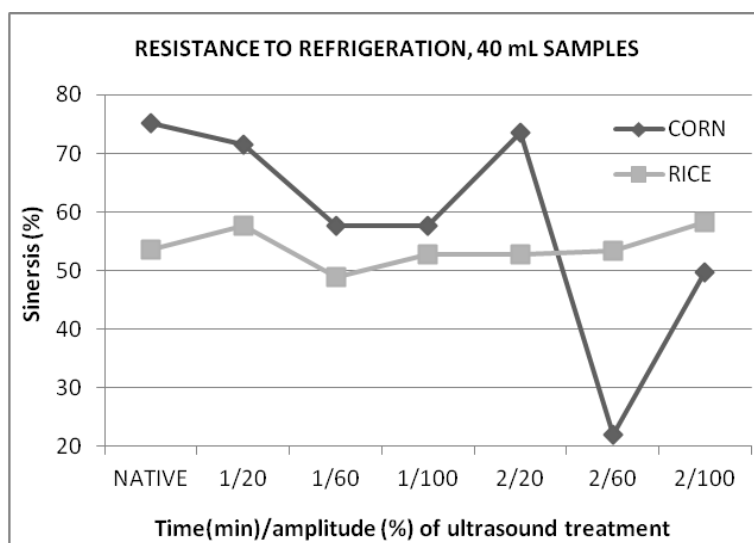


Figure 1. Resistance to refrigeration measured during the first thawing on the 40 mL samples of corn and rice native and US treated starch pastes

Swelling power of both corn and rice starch granules linearly grow with the increase of amplitude of US during treatment (Figure 2), resulting in the largest power to swell in

the samples that were treated with 100% amplitude. Increase of the number of damaged starch granules is in a linear correlation with the increase of amplitude of US applied and therefore results in better water absorption, solubility and swelling power than the one found in native, untreated samples [11,12]. Therefore, even a short treatment with US, like the one that is applied during US assisted freezing, is likely to change the swelling ability of starch, and a time difference of 1 and 2 minutes treatment does not make a significant difference.

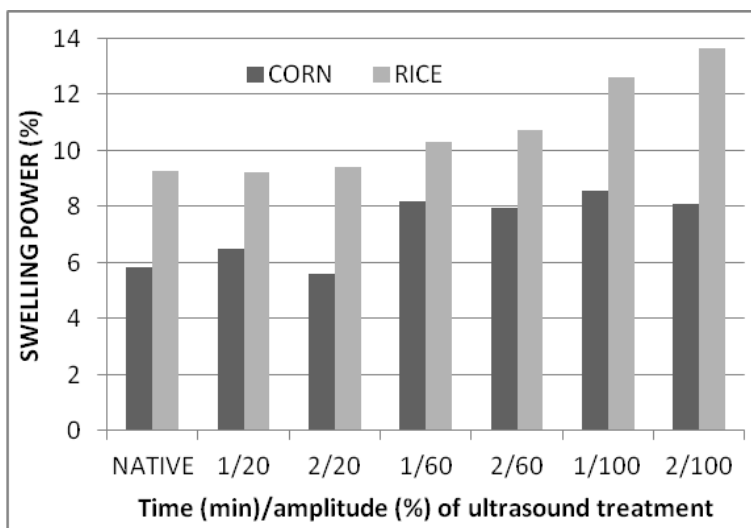


Figure 2. Swelling power of native and US treated corn and rice starch pastes

Starch gel hardness is generally a consequence of retrogradation which is linked to amylopectin crystallization and water syneresis [13].

Treatment of corn starch paste with 60% amplitude, 2 minutes, same as rice starch paste 100%, 2 minutes, caused the largest increase in starch gel hardness (Table 3).

Treatment of both starch pastes with 20% amplitude, 1 minute, caused a slight decrease of starch gel hardness. Decrease in starch gel hardness could possibly be a result of water retention caused by UST, which causes a delay in formation of links between amylose units which form firmer gels [12] or a result of breakage of starch polymers and their reorganization which cause a decrease in their ability to form a firm gel [14]. This indicates that the hardness of gels is dependent on the degree of starch damage caused with UST and a strong correlation was found for all treatments of rice starch (0.76, $p=0.01$) while only for 1 minute treatments of corn starch (0.73, $p=0.27$)

Table 3. Results of rheology measurement of native US treated corn and starch gels and pastes

	VISCOSITY (mPa)			HARDNESS (N)
	65 °C	90°C	50°C	
CN	6.7	37.3	50.0	22.6
C1/20	7.7	21.9	28.2	7.8
C2/20	5.9	30.5	38.8	27.5
C1/60	8.4	30.7	38.4	36.4
C2/60	7.3	26.6	35.9	55.6
C1/100	9.5	22.3	27.8	32.1
C2/100	8.9	16.0	19.7	29.3
RN	10.4	24.4	39.0	10.9
R1/20	10.9	30.7	32.8	8.7
R2/20	11.6	37.1	41.2	8.3
R1/60	11.9	37.2	47.9	7.4
R2/60	12.3	29.9	39.1	15.9
R1/100	11.9	29.1	35.3	13.9
R2/100	12.9	20.5	25.0	53.0

Depending of the starch source and its gelatinization temperature [1], we determined viscosity of starch pastes at three different temperatures -65 °C for corn starch, 70 °C for rice starch, and 90 °C and 50 °C for both (Table 3). Since viscosity of starch is highly dependent on temperature, results should be examined for each temperature separately.

At gelatinization temperature for a given starch, there is a moderate growth in viscosity with the increase of US amplitude, while the time of treatment does not make a significant difference.

At 90 °C there is an increase of amplitude applied causes a decrease in viscosity which is even more emphasized at 50 °C. With rice starch paste, this treatment is especially stressed with 2 minutes of treatment.

Considering that gelatinization will cause an increase of viscosity, it could be expected that at gelatinization temperature, US causes an increase in the number of damaged starch granules but cannot cause the same effect at 90 °C where starch is pregelatinized. Therefore, we examined if there exists a correlation between the swelling power and

viscosity of starch pastes determined at their gelatinization temperature. For corn starch Persons coefficient was 0.95 for the 1 minute treatments and 0.85 for the 2 minutes treatment, while for rice starch coefficient amounted to 0.78 for the one minute treatment and 0.83 for the two minute treatment. Similar results as the ones we found for rice starch pastes that show that the viscosity is not linearly dependent on the intensity of US, were found by [14]. They explained this by damage that UST causes to starch granules because it causes an increase in the swelling power which results in breakage of the starch granules and deterioration of the crystalline structure of the starch granule. Amylose and amylopectin exit the granule as a result of breakage and enter the suspension in which they establish new intermolecular links during cooling [3, 15].

CONCLUSIONS

UST has a varying effect on starch pastes depending on their botanical origin (corn and rice). Treatment of starch pastes with US that could be useful during US assisted freezing, does not have a deteriorating effect on starch granules. Never the less treatment with 60% and 100% amplitude increases their ability to swell and changes their viscosity. Tested UST has a beneficial effect on syneresis and therefore resistance to freezing of corn starch pastes while further research is required to define the conditions of UST that would cause the same effect in rice starch pastes.

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REFERENCES

1. Singh, N., Singh, J., Kaur, L., Singh Sodhi N., and Singh Gill, B. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chem.* 81:219-231
2. Kim, H. S., Kim, B. Y., and Baik, M. Y. 2011. Application of ultra high pressure (UHP) in starch chemistry. *Crit. Rev. Food Sci. Nutr.* 52(2):123-141
3. Lida, Y., Tuziuti, T., Yasui, K., Towata, A., and Kozuka, T. 2008. Control of viscosity in starch and polysaccharide solutions with US after gelatinization. *Innov. Food Sci. Emerg.* 9:140-146
4. FDA. 2000. Kinetics of microbial inactivation for alternative food processing technologies. <http://www.cfsan.fda.gov/~comm/ift-toc.html>
5. Zheng, L. Y., and Sun, D. W. 2006. Innovative applications of power ultrasound during food freezing process. *Food Sci. Technol.* 17:16-23

6. Jang, Q., Xu, X., and Jin, Z. 2011. Physico-chemical properties of rice starch gels: effect of different heat treatments. *J. Food Eng* 107:353-357
7. Rondán-Sanabria, G. G., and Finardi-Filho, F. 2009. Physical-chemical and functional properties of maca root starch (*Lepidium meyenii* Walpers). *Food Chem.* 11: 492-498.
8. Douzals, J. P., Perrier Cornet, J. M., Gervais, P., and Coquille, J. C. 1998. High-Pressure Gelatinization of Wheat Starch and Properties of Pressure-Induced Gels. *J. Agric. Food Chem.* 46:4824-4829
9. Sandhu S. K., and Singh, N. 2007. Some properties of corn starches II: Physicochemical, retrogradation, pasting and gel textural properties. *Food Chem.* 101:1499-1507
10. Gunaratne, A., and Hoover, R. 2002. Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydr. Polym.* 49:425-437
11. Režek-Jambrak, A. 2010. US effect on physical properties of corn starch. *Carbohydr. Polym.* 79:91-100
12. Izidoro, D. R., Sierakowski, M. R., Isidoro Haminiuk, C. W., de Souza, C. F., and Scheer, A. P. 2011. Physical and chemical properties of ultrasonically, spray-dried green banana (*Musa cavendish*) starch. *J. Food Eng.* 104:639-648
13. Miles, J., Morris, V. J., and Ring, S. G. 1985. Gelation of amylose. *Carbohydr. Res.* 135:257-269.
14. Ljubić Herceg, I., Režek Jambrak, A., Šubarić, D., Brnčić, M., Rimac Brnčić, S., Badanjak, M., Tripalo, B., Ježek, D., Novotni, D., and Herceg, Z. 2010. Texture and pasting properties of ultrasonically treated corn starch. *Czech J. Food Sci.* 28(2):83-93
15. Jackson, D. S., Waniska, R. D., and Rooney, L. W. 1989. Differential water solubility of corn and sorghum starches as characterized by high-performance size-exclusion chromatography. *Cereal Chem.* 66:228-232

A KNOWLEDGE MANAGEMENT MODEL AIMED AT CONTINUOUS QUALITY ASSURANCE IN BAKERIES

UDC 664.61 : 005.94

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ABSTRACT

Product quality is one of the dominant factors of survival in the complex economic conditions marked by overabundance of products and keen market competition. Still, it is quite rare to see a systematic approach to the system of quality attainment and maintenance, which is also the case in the baking industry. The issue is usually left to individual technologists in charge of the production process and their capabilities. Consistent quality and efficient production imply a systematic approach to gathering, recording and distributing of knowledge in an organisation, which is the domain of knowledge management. Today, knowledge is not only a characteristic of human beings; it is also a constituent of intelligent computer systems that are responsible for managing production processes. Knowledge management has become more efficient with the development of computer programming systems for knowledge capturing and recording. These systems are not only devices to record and further distribute knowledge through modern computer networks; rather, their function is to help in making important decisions in the production process, based on the information from the environment. The basis of such systems is an ontological model that systematically structures the knowledge on a particular element of the structure or a particular business process. One of the tasks of modern scholarship is to provide systematic knowledge structuring so that it can be efficiently distributed through modern communication channels, and then used in real-life conditions. Accordingly, this research was carried out to create a framework model of knowledge management aimed at achieving continuous quality in the baking industry.

Keywords: knowledge management, bakery production, intelligent web, ontology, knowledge model

INTRODUCTION

Generally speaking, the purpose of science is to explore and identify lawful relationships in the world in which we live. From a scientific point of view, systematic research results, i.e. systemised lawful relationships detected represent knowledge. Recording, systematization and distribution of knowledge was made possible by script, whose

invention is connected to the first technological revolution, i.e. the rise of the earliest civilizations about 3.500 years before Christ, at a time when people began to systematically grow and use grains in their diet, and the technology of metals was developed for the purpose of land cultivation. This was the age of the first technological revolution.

The first technological revolution has radically changed the way the society functioned at the time, and the principles of social organization established during that period still exist today. During World War II, mainly due to the advent of missile technology followed by space technology, as well as computer technology, the second major technological revolution began, striving to move the mankind from the age of metals that began with the first major technological revolution, to the age of space travel, and the age of information and knowledge. It is to be expected that the effects of these technologies on human civilization will be as significant as the effects of the first technological revolution.

The effects of the second technological revolution reflect in the exponential multiplication of knowledge and continuous improvement in the quality of life of every individual. Knowledge management has become a social imperative that has an impact on all spheres of human activity. Baking industry, being under pressure to keep up with the technological knowledge and meet the growing knowledge-based needs of the population (e.g. nutritional), requires a systematic and organized approach to knowledge management in order to ensure a continued quality of baked goods production tailored to customers' needs.

MATERIALS AND METHODS

Modern baking industry, as all other branches of industry, can gain its competitive advantage primarily through knowledge. The modern age is the age of exponential growth of knowledge, which makes knowledge management vital to the success of any industry, including the baking industry. In order to approach this matter systematically, adequate knowledge management models need to be developed. Taking into consideration this research problem, the following hypothesis was constructed:

H1: It is possible to create a knowledge management model in the baking industry that will ensure a continued quality of baked goods production tailored to customers' needs

Based on the proposed hypothesis, the following research objectives were defined:

1. Explore the concept of knowledge management and consider its application in real-life production conditions.
2. Consider developments expected in the baking industry as a result of evolving social processes
3. Describe and evaluate a knowledge management model in the baking industry that will ensure a continued quality of baked goods production tailored to customers' needs

Overall, a deductive method of investigation was used as the initial hypothesis was explored in a mental experiment. The main method utilized to accomplish the research goal is the modelling method. In order to correctly define the analogies in the process of modelling between the original and the model we will apply the systematic approach. Other scientific research methods have also been used in addition to the above-mentioned.

RESULTS AND DISCUSSION

Knowledge is one of the concepts for which modern science has no single definition or a unified view. In the course of human history, various philosophers offered various interpretations of the term knowledge, balancing between subjective and objective views of this concept. Just how essential the concept of knowledge is can be observed in the fact that in the history of philosophy a separate branch emerged called epistemology, which was concerned with knowledge. The terms episteme (knowledge) and logos (explanation) are often confused in scientific definitions, even today.

Epistemology, as a theory of knowledge (episteme + logos), was introduced by the Scottish philosopher, James Frederick Ferrier. Epistemology juxtaposes the objective against the subjective.

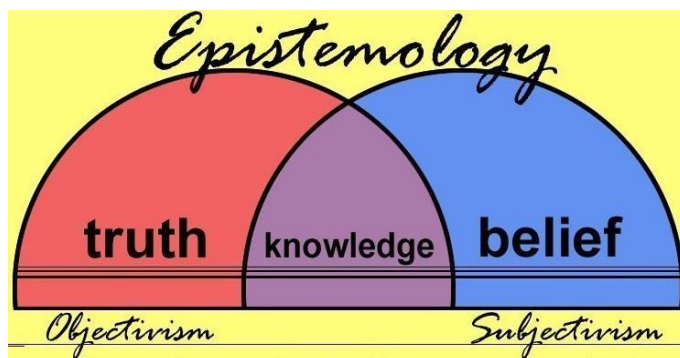


Figure 1. The concept of knowledge according to epistemologists (Source: <http://siamtownhouse.com/?p=8569>; 1.10.2013)

According to epistemologists, knowledge occurs at the intersection of the subjective and the objective, as shown in Figure 1.

Without aspiring to give a deeper analysis of the origin of the concept of knowledge, these are the most common definitions of the term:

- facts, information and skills that a person has gained through experience or education;
- the totality of all the facts and information in a particular field;
- a reliable understanding of an item, with the potential ability to use it for a specific purpose.

Instead of looking at the definition of knowledge, it is much more important to understand how knowledge is derived, i.e. how one acquires knowledge. Knowledge comes from data or information, and from knowledge comes wisdom. Figure 2 shows the relationship between knowledge, data, information and wisdom.

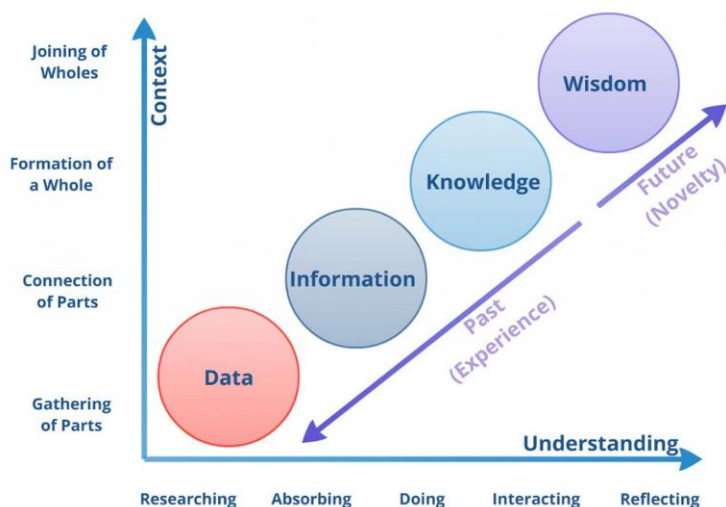


Figure 2. Relationship between data, information, knowledge and wisdom (Source: <http://www.easterbrook.ca/steve/2012/09/what-is-climate-informatics/>, 1.10.2013.)

An important aspect of the relationship, as shown in Figure 2, is the relationship of data, information, knowledge and wisdom to future events. People have been seeking to understand their environment in order to be able to better accept and manage future

events. It is not possible to know the future with certainty; however, based on available information linked through knowledge, one can consider possible future scenarios, which makes people more able to interact with future events. This means that the data are a reflection of the past. When context, i.e. the metadata is added to the data; in other words, when the data is linked into a meaningful whole, information is generated. Information represents a description of an item, its definition or a view of that item. Usually, the information can be recognized by the fact that it gives an answer to a question beginning with what, who, where, or when. Information is transformed into knowledge when it has a particular meaning to someone, because it describes the relative position of an item in its environment. When the information answers the question beginning with how, such information is considered to be knowledge. Knowledge is essentially part of methods used to analyze the world that surrounds us, and thus knowledge is manifested in the way we approach a particular problem. It is the purpose of science to recognize isomorphic systems and then systematically record how they operate. Knowledge, like data and information, is focused on the past. However, by recognizing lawful relationships and generalizing them, thereby shifting from the concrete case to all identical and similar cases, wisdom is created, which, unlike previous factors - data, information and knowledge, focuses on the future. In general, wisdom defines the principles, fundamentals, moral principles and prototypes. However, without data, i.e. systematised information explaining the principles by which isomorphic systems function, there is no wisdom.

The spiral of knowledge opened up in the twentieth century, thereby enabling exponential multiplication of knowledge that is now available for people to use and acquire. Moreover, knowledge has become an important factor in the individual and social survival. While capital, raw materials and technology used to be important factors in building competitive advantage, information and knowledge play a pivotal role today. However, individual management of a vast amount of knowledge is almost impossible. Gone are the days when knowledge was concentrated in the minds of individuals on whom the success of the community depended. Today, there is global pressure on the distribution of structured knowledge so that knowledge can be available to all at any time. The Internet has practically become a universal base of unstructured knowledge that seeks to become structured as soon as possible. General knowledge-related issues affect all spheres of human activity, including industrial production, which means the baking industry, too. As in any system, knowledge management in the baking industry is an issue that needs addressing.

In order to adequately look into the issue of knowledge management in general, and to define the descriptive model of knowledge management in the baking industry that will ensure a continued quality of baked goods production tailored to customers' needs, it is necessary to analyse the manifestations of knowledge, knowledge-related processes and methods for efficient recording of knowledge.

There are two types of knowledge, in terms of its holder: knowledge inherent to people, called explicit or tacit knowledge, and knowledge inherent to machines, i.e. implicit knowledge. Knowledge inherent to people comes mainly from experience and relates to

thinking and behavioural patterns, as well as competencies acquired mostly through formal and self-education. On the other hand, knowledge inherent to machines is stored on various media, from, until recently popular, print or analogue media, to currently ubiquitous digital media. There are various estimates on the tacit to explicit knowledge ratio ranging from 5%:95% to 55%:65%. However, there is a general consensus that the level of knowledge in people is still higher than it is explicitly recorded. Figure 3 shows the ratio of implicit (tacit) to explicit knowledge, using the iceberg analogy.

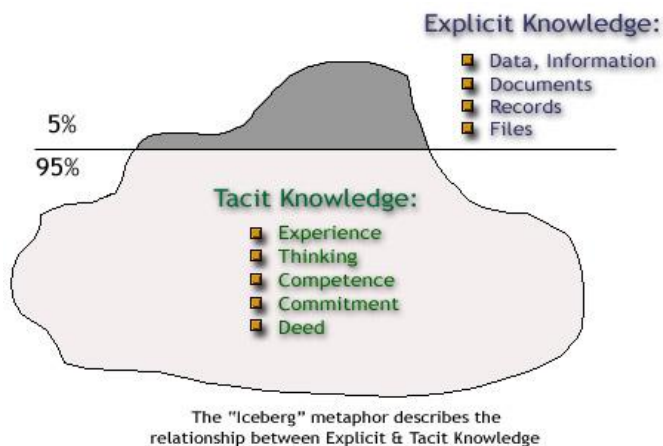


Figure 3. Estimated implicit (tacit) to explicit knowledge ratio (Source: <http://www.cognitivedesignsolutions.com/KM/ExplicitTacit.htm>, 5.10.2013)

As a result of the need for systematic transformation of implicit knowledge into explicit knowledge, an independent scientific discipline emerged, called knowledge management. Symbolically speaking, knowledge management tends to create self-learning organizations in which tacit-to-implicit knowledge transformation occurs in a spiral process for the benefit of an organization. Figure 4 shows the knowledge spiral in an organization.

The reasons for considering the possibility of knowledge management is the growing volume of knowledge; the need for knowledge externalization, so that an organization is not dependent on individuals; the need for combining the knowledge of a number of people; the need for faster access to knowledge; the need for knowledge to be understandable to all; the need for knowledge to be used economically, the need for knowledge to provide a competitive advantage over others; and the need for knowledge

to be a collective concept. Considering the mentioned general requirements, the fact that the baking industry processes will have to evolve as a result of market-driven need to meet customers' needs, it is to be expected that this industry will start with the systematic implementation of knowledge management software systems in the near future.

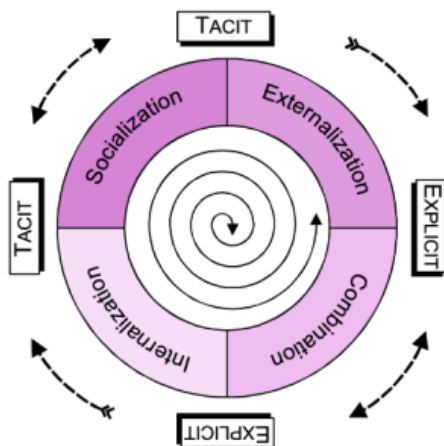


Figure 4. Knowledge spiral in an organization (Source: <http://kmbkteam.wordpress.com/>, 3.10.2013)

It is also to be expected that as a result of the evolution of nutritional science and development of general knowledge and people's needs for a better and longer life, the technological sciences will be required to develop the baked goods production. In such conditions, when new knowledge appears, enriched with practical experience, the technological process of bread making will have to be approached in a systematic manner, taking into account the need for structuring knowledge using ontological tools and the Internet, as a social medium for the distribution of knowledge.

Based on the above considerations, the basic principles were developed for creating a descriptive structural model of knowledge management in the baking industry that will ensure a continued quality of baked goods production tailored to customers' needs. The model combines the following elements:

$$KM_{pi} = eT2E_{pi} (\sum TehK_{pi} + MR_{pi} + \sum BKK_{pi} + \min(T + TRZ)_{pi})$$

where:

- KM_{pi} – stands for knowledge management in the baking industry
- $eT2E_{pi}$ – stands for externalisation, i.e. transformation of tacit knowledge into explicit knowledge
- $\sum TehK_{pi}$ – stands for the totality of technological knowledge
- MR_{pi} - stands for marketing research of customers' needs
- $\sum BKK_{pi}$ - stands for biochemical (medical) sciences in the function of customers' needs
- $\min(T + TRK)_{pi}$ – stands for minimizing the costs and time required to access knowledge

CONCLUSIONS

The most important resource of the modern era requires a systematic approach by all, including the baking industry. It involves the analysis of the structure and events that make knowledge management processes, as well as finding the model that is optimal for a particular area of human activity. As can be seen from the attached model, developed based on research, the baking industry's model of knowledge management should, besides standard elements, i.e. externalisation (transformation of tacit into external knowledge (outside business organizations), minimisation of knowledge management costs and access time, also include adequate marketing research to respond to the needs of the modern consumer. Biochemical sciences combined with modern medicine and nutritional sciences should offer solutions in this regard. Appropriate technological solutions are required so that a product like that, based on the synergy of knowledge, could get its physical equivalent at the end of the production process. The structural model of knowledge management in the baking industry does not show the method of structuring knowledge. For this purpose, one should use appropriate ontological tools because they ensure low cost of knowledge management and an adequate speed of access to knowledge as a precondition for economical use of knowledge in the baking industry. The presented model is the basis on which knowledge structuring processes in the baking industry should be developed.

REFERENCES

1. Daniel, B., Schwier, R. A., McCalla, G. 2003. Social Capital in Virtual Learning Communities and Distributed Communities of Practice. *Canadian Journal of Learning and Technology*. 29(3). <http://cjl.csj.ualberta.ca/index.php/cjlt/article/view/85/79>, 2.10.2013.
2. Kmbkteam. 2011. The Difference Between KM (Knowledge Management) & KMB (Knowledge Mobilization). <http://kmbkteam.wordpress.com/>, 3.10.2013.

3. Siam Town House. <http://siamtownhouse.com/?p=8569>; 1.10.2013.
4. Cognitive Design Solutions. 2003. Explicit & Tacit Knowledge.
<http://www.cognitivedesignsolutions.com/KM/ExplicitTacit.htm>, 5.10.2013.
5. Easterbrook. 2012. What is Climate Informatics?
<http://www.easterbrook.ca/steve/2012/09/what-is-climate-informatics/>, 1.10.2013.
6. Frost, A. 2013. Knowledge Management. <http://www.knowledge-management-tools.net/>;
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