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FACULTY OF FOOD TECHNOLOGY OSIJEK**

# **PROCEEDINGS OF THE**

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**FLOUR–BREAD '19**

12<sup>th</sup> CROATIAN CONGRESS OF CEREAL TECHNOLOGISTS

**BRAŠNO–KRUH '19.**

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Osijek, 2020

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## THE EFFICIENCY OF MODERN MILL INDUSTRY CLEANING SYSTEMS ON MYCOTOXIN AND ALKALOID LEVELS IN CEREALS

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### SUMMARY

The article presents the entire process of purification of buckwheat, rye and wheat before milling with an emphasis on the removal of damaged, with mycotoxins contaminated grains, harmful seeds and their substances. The focus in modern cleaning process lies on the removal of fine and low-density impurities, removal of grain with low bulk density, optical sorting of discoloured, defective grain kernels, foreign seeds and sclerotia. This year we modernized a cleaning system for cereals in the mill and validated the process. We have tested the efficiency of our new cleaning system for cereals infected by deoxynivalenol (DON), ochratoxin A (OTA), ergot alkaloids (EA) and tropane alkaloids (TA). The infected raw materials went through the whole cleaning system. The samples were taken according to regulations after each cleaning phase and at the end of the milling process. The concentration of DON was measured with Neogen rapid tests. OTA, TA and EA in buckwheat and rye were determined by the HPLC method in an accredited external laboratory. Obtained data show that we can significantly reduce the alkaloid and mycotoxin levels and improve the health of the milling products with such a cleaning technology in our mill.

**Keywords:** mycotoxins, alkaloids, mill cleaning technology

### INTRODUCTION

The production of quality milling products begins with the acceptance of high-quality raw material and effective cleaning process. Elements of the cleaning process that affect the profitability include the costs for operating and maintaining equipment in the cleaning process and minimizing loss of good-quality raw material into the screenings. At the end of the process, it is necessary to provide a healthy milling product. Climate change and its effects on the quality of food crops, including contamination with mycotoxins, is very important issue worldwide, such as food security (Magan et al., 2011). Crop growth and its interaction with beneficiary and pathogenic and/or toxigenic microorganisms vary from year to year, mainly depending on local weather, making the agricultural sector particularly exposed to

the climate change (Frances and Lobell, 2015). Even in milling industry, we need to adapt to climate change with the development of technology and modern cleaning equipment.

In general, cleaning covers the removal of foreign material and low-quality material. Cleaning based on separation processes involves the removal of freely assorting foreign material from a raw material. Cleaning is performed prior to storage and/or shortly before processing. Typically, coarse (pre-) cleaning before storage is combined with an intensive cleaning before milling (Schaarschmidt and Fauhl-Hassek, 2018).

The aim of the current work was to validate the process of cleaning cereals (rye, buckwheat, wheat) in a mill with cleaning capacity 4 t/h regarding mycotoxin and alkaloid contamination with installed cleaning machines. The focus lies on the effectiveness of individual cleaning steps undertaken in the mill to reduce the level of contaminants from raw material. Observed mycotoxins were: DON in wheat, OTA in buckwheat and alkaloids: tropane alkaloids (TA) in buckwheat and ergot alkaloids (EA) in rye. Commission Regulation (EC) No. 1881/2006 with amending acts determine the maximum levels of mycotoxins in cereals. For ergot alkaloids maximum levels have not been established yet. Contamination with ergot alkaloids is determined (observed) with the presence of ergot sclerotia. Milling industry has a zero tolerance for presence of harmful weeds that contain tropane alkaloids (widespread weed *Datura stramonium* in buckwheat). Maximum level of TA is the limit of detection – 1.0 µg/kg.

## **MATERIALS AND METHODS**

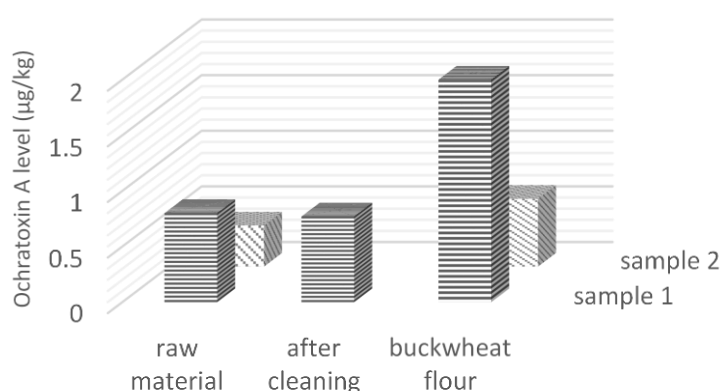
The effectiveness of cleaning steps undertaken in the mill was tested with six samples of buckwheat (two infected by OTA and four by TA); one sample of wheat infected by DON and one sample of rye infected by ergot sclerotia. The infected raw material went through the whole cleaning system of the mill. Some cleaned cereal samples were milled into flour.

The samples of input raw materials and rejected materials were taken according to Commission Regulation (EC) No. 401/2006 in different stages of cleaning process before milling – after separator, after trieur and after SORTEX. The samples of end product, flour, were also taken.

The set of analytical parameters was depended on the type of contamination. TA and EA in buckwheat and rye were determined by the LC-MS/MS method and OTA in buckwheat by the HPLC method in an accredited external laboratory. The concentration of DON was analysed with fast test Reveal Q+ for DON, with Raptor<sup>®</sup>, distributed by Neogen. Rejected material of whole wheat for determination of DON was grounded on Laboratory hammer mill 3100<sup>®</sup> (Perten, Sweden) and then homogenized. The Automatic lab mill MLU 202<sup>®</sup> (Bühler, Germany) was used to get wheat milled products (finished flour and bran).

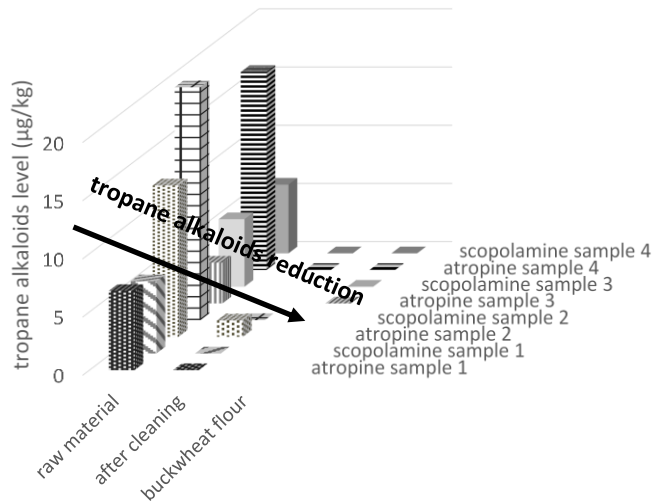
## RESULTS AND DISCUSSION

Keriere et al. (2016) determined the concentrations of mycotoxins in the non-thermal treatment (raw) and in thermal treatment (steamed) buckwheat. All the tested samples were found positive for OTA, but the levels were low. The higher content of OTA was in the husk. Between the treated or untreated buckwheat there was no difference in levels of OTA. Also in our case during the cleaning process, the concentration of mycotoxin OTA in buckwheat in these two trials did not become lower. In both tests, OTA in buckwheat flour was higher than in raw material (**Figure 1**). The reason for that could be the fact that the input value for OTA was low and did not exceed statutory limits for milling products and how the mycotoxin was distributed around the kernel. Usually higher concentrations of mycotoxins are in the outer parts of a kernel. The level of mycotoxins can increase due to the partial milling of hull into the flour.

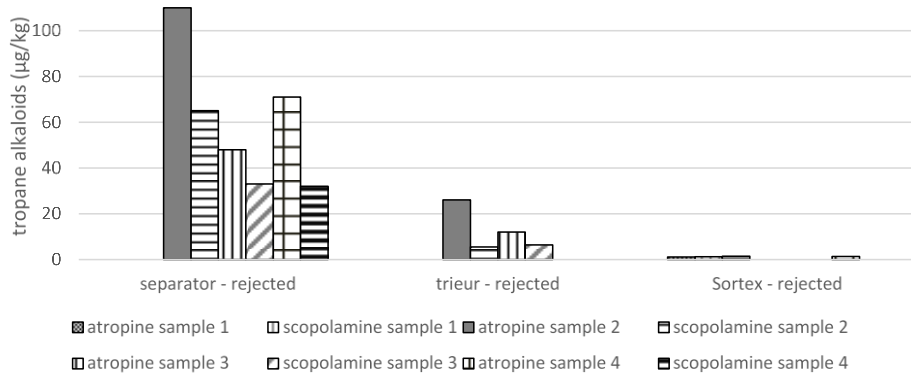


**Figure 1** Ochratoxin A concentration in the buckwheat after cleaning and in the flour, compared to the input material

The results for the reduction of tropane alkaloids show that the cleaning process reduced the quantity of tropane alkaloids below the statutory limits in the cereals (**Figure 2**). According to the analysis of rejected material, separator and trieur are very effective in lowering the TA values (**Figure 3**). The levels of TA in cleaned buckwheat and finally in flour are low or under the limit of detection.



**Figure 2** Tropane alkaloids (atropine and scopolamine) concentration in the buckwheat after cleaning and in the flour, compared to the input material

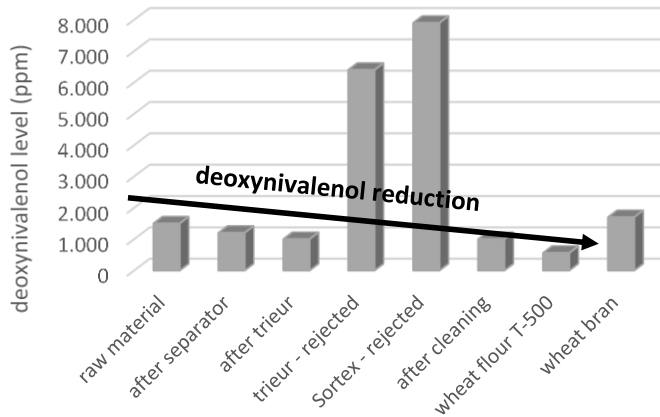


**Figure 3** Tropane alkaloids (atropine and scopolamine) concentration in the reject material

For reducing mycotoxins, our results are in agreement with other studies, which indicates that the effectiveness of the cleaning process in reducing the level of DON. In their paper, Schaarschmidt and Fauhl-Hassek (2018) collected various data on DON reduction through the cleaning process. The change in mycotoxin concentration in cleaned wheat compared to the uncleaned grain in all studies was from 35 to even 90%.

Tibola et al. (2015) monitored DON concentration in wheat. The maximum DON concentration was observed in the bran, regardless of initial contamination level. DON concentrations in bran increased by 125% compared to input material. DON in finished flour was reduced by 43% to input material.

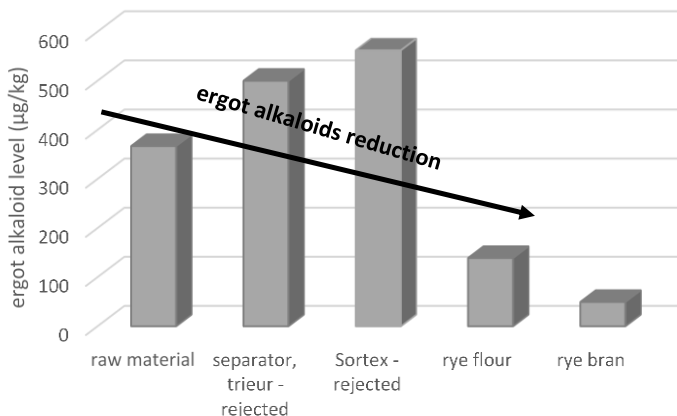
In our case the DON in wheat flour type 500 reduced by about 37% compared to used wheat. The level of DON in bran was equivalent to that of the input wheat. Results for cleaning wheat and rye (**Figure 4** and **5**) show that the optical sorting with the SORTEX reduced both DON and EA levels.



**Figure 4** The concentration of DON in wheat before cleaning, in rejected material after each cleaning phase and in end product

Values for DON and EA in rejected material from this phase are very high. The EA is not fully removed from the mill product, because ergot sclerotia breaks into small fragments (dust) which can not be removed from the material and consequently leads to higher values of EA in rye flour than in bran.

In their study Graeber et al. (2016) found out that, on average, the optical sorting with the SORTEX machine reduces EA levels, as in our case. In the end, in rye flour the EA concentration was within the safe range. That proves the effectiveness of modern cleaning process.



**Figure 5** The concentration of ergot alkaloids in rye before cleaning, in rejected material after each cleaning phase and in end product

## CONCLUSIONS

Cleaning process in the mill is effective in the reduction of EA in rye cleaning. The most important machine is optical sorting, where ergot sclerotia is mainly removed. Good results are obtained also in removing harmful weeds containing TA as *Datura stamonium* in cleaning of buckwheat. The separator and the trieur have got the main role in cleaning process regarding the removal of TA. A decrease of mycotoxin DON content when cleaning the wheat was confirmed. More samples with different contamination levels of DON must be analyzed to get relevant data. The biggest problem with the existing cleaning process is removing of mycotoxin OTA from buckwheat. Contamination with this mycotoxin can not be easily detected. Buckwheat grains are not different in shape, appearance, specific gravity and the machines involved in cleaning process do not recognize them as foreign material.

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**CULTURAL-HISTORICAL HERITAGE OF THE KARLOVAC COUNTY – HOMEMADE BREAD AS A GASTRONOMIC TOURIST PRODUCT IN RESTAURANTS IN THE KARLOVAC COUNTY**

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**SUMMARY**

Tourism can provide a strong contribution and a boost to the protection of intangible cultural-historical heritage, among which gastronomy plays an important role. In this respect, we have investigated to what extent is homemade bread recognized as an authentic indigenous gastronomic product by the owners and restaurant managers in Karlovac County. The research was conducted by the use of various scientific methods, such as field research methods, desk research methods, descriptive methods, deductions, descriptive statistics, synthesis, and semi structured interviews with target group representatives, owners, and restaurant managers in Karlovac County. The research results suggest that gastronomy, as a modern and sophisticated tool for the interpretation of intangible cultural-historical heritage, can significantly contribute to its efficient preservation, while it develops and raises the quality of the tourist product at the same time. Homemade bread, made according to the original recipe, can also make an efficient contribution to the preservation of intangible cultural-historical heritage, while raising the quality of the tourist product at the destination level at the same time. However, it has not been sufficiently exploited.

**Keywords:** gastronomic tourist product, homemade bread, intangible cultural-historical heritage, Karlovac County

**INTRODUCTION**

Nutrition essentially forms a vital part of the tourist offer of every destination, as a mixture of biological, socio-cultural and tourist aspects. Food is one of the main or autonomous incentives for tourist travels and stays and it also forms the basis of the tourist offer in the tourist destination. It is shown that food consumption in tourism can either be a top tourist experience or a stimulating consumer experience, depending on specific circumstances (Quan and Wang, 2004). The experience with food and beverages can significantly influence the perception of the whole destination and the level of guest satisfaction with the overall tourist experience.



The characterization of food and beverage as a tourist phenomenon is associated with a certain type of food and/or beverage from authentic local groceries, prepared in a special, traditional way and served to the guest, giving the particular ambience which is unique to the specific tourist destination. Several researches have documented how food contributed to tourist experiences and influenced their decisions, behaviour and satisfaction (Björk and Kauppinen-Räsänen, 2019; Getz et al., 2014). Instead of consuming food in restaurants and hotels, tourists are in constant search of local food and beverage experience, which is a part of gastronomic tourism (Sormaz, 2016). Gastronomic tourism, in general, refers to originality, indigenous food and drink, and authentic places, areas or countries of origin, and they testify to the culture and traditions of living of the local population (Groves, 2001, Green and Dogherty, 2008; Sormaz, 2016). An important point for the development of gastronomic tourism is the protection of local products in the regions they belong to. Local gastronomy can effectively help in profiling a particular region into a tourist region or enhance already existing tourism, thereby contributing to regional development and rural development respectively (Green and Dougherty, 2008; Hall et al., 2003). In this context, local dishes are becoming important tools for learning about different cultures (Kastenholz & Davis, 1999; Gyimothy et al., 2000; Joppe et al., 2001). Local, indigenous gastronomy can contribute to the sustainability of the region's resources and responsible tourism with an emerging food culture (Sormaz, 2016). Gastronomic tourism, which supports regional development by fostering the link between food and beverage and tourism, strengthens local identity and culture, as well as the economic well-being of the local community. Thus, gastronomic tourism will also contribute to the protection of historical and cultural heritage and help pass it on to future generations and ensure its sustainability (Hall et al., 2003). Changes in eating habits in societies and increasing social value of nutrition are one of the reasons for moving towards gastronomic tourism. In the professional and scientific literature on gastronomy, different types of food, wine, oils (olive, pumpkin) are studied, but there is a lack of study on bread as an important factor for branding a particular region as gastronomic. Ministry of Tourism (2011) issued the Regulations about the establishment of a special standard - Croatian Authentic Cuisine, which establishes for the first time what authentic Croatian dishes are, thus laying the foundations for the branding of Croatian cuisine and restaurants as one of the leading assets of Croatian tourism. The list includes various types of bread and pastries. According to this Regulation, the homemade bread must be prepared in the facility, and different types of flour can be used for preparation (corn, white, mixed flour, whole grain flour) with the addition of different types of cereals. In this paper, homemade bread represents the backbone of gastronomic tourism and the construction of the gastronomic brand in Karlovac County, so the role and the importance of the preparation and serving of homemade bread in restaurants in Karlovac County were examined. The purpose of this paper was to investigate the extent of recognizing homemade bread as an authentic indigenous gastronomic product, both by owners and restaurant managers in Karlovac County and the usage

of traditional recipes in this area. The paper also pointed out the role and the significance of homemade traditional bread as a tool of interpretation of the intangible cultural-historical heritage.

### **MATERIALS AND METHODS**

The main hypothesis of this research was that the profiling and branding of significant tourist regions could very quickly be achieved by encouraging the development of local (indigenous) gastronomies, in this case by reviving traditional ways of making bread. The hypothesis was tested by conducting a primary survey using a structured questionnaire sent by e-mail. The survey questionnaire consisted of 10 questions. One part of the questionnaire consisted of open-ended questions that referred to general information about the restaurant and whether the restaurant was preparing traditional homemade bread, in what quantities and based on which recipe. The other part of the questionnaire asked the respondents to evaluate individual elements of the research using Likert scale in the range of 5 marks to rate the level of agreement with the following statements: "The guests recognized bread as a high value gastronomic product of our restaurant", "Traditional local bread prepared and served at our restaurant significantly encourages the arrival of guests at our restaurant" and "The bread we prepare and serve at our restaurant is made according to the original local traditional recipe" (1 signified a low degree of agreement, while 5 indicated a high degree of agreement). The survey was conducted in Karlovac County from May to July 2019. The target group consisted of 43 restaurants in the area of Karlovac County, 12 of which won the title of "100 Best Croatian Restaurants" for several years in a row. The survey questionnaire was completed either by the owners, the restaurant managers, the kitchen managers or the chefs themselves. Besides the online survey, the field research was also conducted in order to collect as many traditional homemade bread recipes unique to Karlovac County as possible, and to compare collected recipes to those indicated to have been prepared in restaurants.

### **RESULTS AND DISCUSSION**

Karlovac County of exceptional natural beauty and cultural and historical heritage is the strongest continental tourist destination, which recorded 353 264 tourist arrivals in 2018 and 608 366 overnight stays, which represents a growth of 7.62% compared to 2017. Gastronomy is one of the offers almost all tourists are interested in. According to the results of the research conducted by the Institute of Tourism TOMAS in 2018, 29% of guests emphasized gastronomy, 26% getting acquainted with natural beauties and 20% engaging in sports and recreation as a motive for coming to Croatia. In this sense, Karlovac County has got all the necessary resources for gastronomic tourism.

The recognition of traditional homemade bread as an authentic gastronomic product by the owners and restaurant managers in Karlovac County was examined through the primary survey using a structured questionnaire. Questionnaires were sent out

to all 43 restaurants in Karlovac County, 12 of which were listed in the "100 Best Croatian Restaurants". A total of 20 restaurants responded to the survey, 7 of which were listed in the "100 Best Croatian Restaurants". Out of the total number of restaurants surveyed, 15% of restaurants do not have traditional homemade bread in their offer, including one restaurant in the "100 Best Croatian restaurants". The other 85% of the restaurants surveyed stated that they have traditional homemade bread every day, which they prepare daily in their kitchen and serve in their restaurant. Out of these, 30% of the restaurants say they prepare three or more types of traditional homemade bread daily. Out of the total number of restaurants that participated in the survey, 55% of restaurants have got a long tradition of business, over 20 years. These restaurants showed that they significantly nurture traditional gastronomy, including the way they prepare and serve bread.

The statement "The guests recognized the bread as a high value gastronomic product of our restaurant" was given a high score 5 – "I completely agree" by 77% of the respondents. The following statement, "Homemade bread prepared and served at your restaurant significantly encourages the arrival of guests at your restaurant", was responded affirmatively by 85% of respondents and 70% of respondents gave this statement a high score 4, and 30% fully agreed with this statement by giving it the highest score 5.

As previously mentioned, 85% of the restaurants that participated in the survey answered that they traditionally prepare and have homemade bread in their offer. However, the field research and the study of original recipes from different parts of Karlovac County (Kordun, Ribnik, Ozalj, Karlovac, Krnjak, Barilović, Maljevac, Mrežnica) revealed that the way of preparing traditional homemade bread in a certain restaurant is significantly different from the original traditional way of making bread. The choice of ingredients in original traditional recipes also differs significantly from those used in restaurants which participated in the survey.

A representative number of traditional homemade bread recipes from all parts of Karlovac County were collected (**Table 1**). Based on gathered recipes, it is evident that in the entire observed area corn bread was the most commonly produced homemade bread, probably due to the low cost and wide spread of corn crops in the area, together with the white wheat flour. Flour for bread making was traditionally obtained in stone wheel mills, operated by the power of water, and nowadays frequently by electricity, so the type of the homemade bread depended on the availability of the flour, time of the year and generally on the type of the crop grown in the particular area. Corn flour contains high levels of many important vitamins and minerals, including potassium, phosphorus, zinc, calcium, iron, thiamine, niacin, vitamin B6, and folate (Sabanis and Tzia, 2009). Although in each recipe for corn bread making, corn flour was pretreated with boiling water, there were significant differences in the selection of starter culture for fermentation depending on the area of Karlovac County. In most parts traditional bread making procedure was used, which involves adding sieved whole corn flour, hot water, wheat flour and yeast and/or leavened dough from the late bread making (acting as sourdough), while in

Maljevac area neither yeast nor the sourdough was added to the dough. Bread making with non-wheat flour presents considerable technological difficulty, because their proteins lack the ability to form the necessary gluten network for holding the gas produced during the fermentation (Gallagher et al. 2003; Arendt et al. 2002), so it is not surprising that in the traditional recipes usually wheat flour is the main ingredient. That is also evident in this research, while wheat flour (both white and whole grain) was used almost in every obtained recipe, whether as primary flour, or as a supplement for easier dough kneading. Wheat flour consists mainly of starch (ca. 70–75%), water (ca. 14%) and proteins (ca. 10–12%), and non-starch polysaccharides (ca. 2–3%), in particular arabinoxylans (AX), and lipids (ca. 2%), which are important minor flour constituents relevant for bread production and quality (Goesaert et al., 2005). In traditional wheat homemade bread making, flour, water, salt, yeast and/or other microorganisms (sourdough), often with the addition of non-essential ingredients, such as fat and sugar, are mixed together into a visco-elastic dough, which is fermented and baked. For Karlovac County, there was no record of using sourdough in wheat homemade bread making, only yeast usage was recorded, except for Maljevac area where both yeast and sourdough were absent and usually only baking soda was added to dough mixture. Based on the gathered recipes it was not clear whether the sourdough was really absent or the information was lost during the transfer of the recipe through generations. In wheat breads, sourdough is mainly used to improve flavour. However, the addition of sourdough has also got a major effect on the dough and the final bread structure, because sourdough fermentation results in increased mineral bioavailability and reduced phytate content, and improves the textural qualities of bread (Arendt et al., 2007). Sourdough fermentation was noticed in traditional recipes from Kordun and Ribnik area, where particular emphasis was placed on making the sourdough. Ribnik area was also specific in the choice of main grains for flour making since rye and oat grains were used. Without sourdough, whole meal rye or wheat-rye flour mixes are very difficult to process. Sourdough provides aromatic and pleasing flavour, and improves overall quality and shelf life of whole grain breads. Thus, a traditional rye sourdough process not only improves flavour and texture of the rye products but also enables consumption of whole meal rye, which is well known for its high nutritional quality and health-promoting properties and could not be used in bread making without the sourdough process (Katina et al., 2005). Also, the number of fermentations before the baking of bread varied between 0 and 2, depending on the type of bread and the used starter culture, or its absence (**Table 1**).

**Table 1** An overview of the ingredients and preparation techniques used in traditional homemade bread making in different areas of Karlovac County (NA – not applied) (part I)

Area in Karlovac County	Grains/Flour	Pretreatment	Starter culture	Supplement	Number of dough risings
Kordun	Corn flour	Boiling water treatment of corn flour	Corn flour, Whole wheat flour and baker's yeast mixed with warm water (overnight fermentation)	NA	2
Ribnik	Rye, wheat, corn and oat grains; White wheat flour	Grains milling and sieving; Boiling water treatment of flour mixture	Whole grain flour mixed with warm water (7-day fermentation)	Salt Pork fat Seeds (if available)	1
Ozalj	White wheat flour, whole wheat flour and corn flour	NA	Baker's yeast suspended in warm water	Salt Oil	1
Karlovac	White wheat flour corn flour	Boiling water treatment of corn flour	Baker's yeast suspended in warm water	Salt Oil	1
Krnjak	White wheat flour	Boiling water treatment of white wheat flour.	Baker's yeast suspended in warm water	Salt Oil	2
Barilović	Rye, wheat, corn and millet grains	Grains milling and sieving; Boiling water treatment of flour mixture	NA	salt pumpkin seed (optional)	1

**Table 1** An overview of the ingredients and preparation techniques used in traditional homemade bread making in different areas of Karlovac County (NA – not applied) (part II)

Area in Karlovac County	Grains/Flour	Pretreatment	Starter culture	Supplement	Number of dough risings
Maljevac	Corn grains	Grains milling and sieving; Boiling water treatment of corn flour	NA	NA	0
Maljevac	White wheat flour	Ice cold water treatment	NA	Baking soda	0
Maljevac	Wheat grains	Grains milling and sieving	Baker's yeast suspended in warm water	Salt	1
Mrežnica	Rye, wheat, corn and grains	Grains milling and sieving; Boiling water treatment of flour mixture	NA	Salt	0

One of the most significant findings of this research is that caterers, restaurant owners, restaurant managers, or chefs who offer or prepare homemade traditional bread in their restaurants, consider that it is sufficient to prepare and bake bread in their restaurant daily. Majority or 63% of restaurants prepare and bake white bread to which they add milk, butter or spices. Only 37% of the restaurants that participated in the survey used a variety of flours (wheat, corn, buckwheat or rye) to prepare traditional homemade bread and prepare it in the traditional way specific for their region. These restaurants are located in extremely rural areas and they point out that their guests have recognized bread as a highly valued gastronomic product and that traditional homemade bread prepared and served at their restaurant significantly encouraged the arrival of guests. However, the rest of the respondents who were found not to prepare homemade bread in the way that is considered to be traditional for their region also stated that restaurant guests recognized bread as a highly valuable gastronomic product and that traditional homemade bread prepared and served in their restaurant significantly encouraged the arrival of guests. Therefore, the experience of the guests themselves, which has not been examined by this research, is an important factor.

## CONCLUSIONS

Gastronomy in tourism is an excellent tourist experience or a stimulating consumer experience, depending on specific circumstances, and food is one of the main or autonomous incentives for tourist travels and stays. It also forms the basis of the tourist offer in a tourist destination. Considering that tourists are in constant search for local food experience, we can conclude that the caterers from Karlovac County area have not sufficiently recognized this and that they do not use traditional gastronomy, that is, traditional ingredients and the traditional way of preparing and serving bread. Only 37% of the total number of respondents said that they prepared traditional homemade bread daily, in a traditional way, and that restaurant guests recognized bread as a highly valuable gastronomic product. Moreover, this bread, prepared and served at their restaurant, significantly encouraged the arrival of their guests. However, the rest of the respondents who do not prepare bread in the traditional way or with traditional ingredients also endorsed the same attitude, indicating the need to examine guests' views on bread in some future research. The results of this study confirmed that preparing and serving traditional homemade bread can serve as a powerful tool for creating and profiling of particular region into a gastronomically recognizable region. In this way, it is possible to contribute to the regional and rural development of Karlovac County as far as gastronomy and tourism is concerned. The process of developing successful gastronomic tourism is the protection of local products in the regions they belong to, in this case traditional ingredients and a traditional way of making bread, which should also be considered in the future. The results of the research also indicate that gastronomy, as a modern and sophisticated tool for the interpretation of the intangible cultural and historical heritage, can significantly contribute to its effective preservation and the development and improvement of the quality of the tourist product. Traditional homemade bread made according to the original recipe can significantly contribute to effectively preserving the intangible cultural and historical heritage while raising the quality of the tourist product at destination level, but it is not used to a sufficient extent. Finally, we also confirm the main hypothesis of this research, stating that by encouraging the development of local, indigenous gastronomy, in this case the revival of old, traditional ways of making bread, the profiling and branding of a particular tourist region, can effectively be achieved.

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## CEREAL BASED FOOD INDUSTRY IN EU COUNTRIES - ECONOMIC POINT OF VIEW

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### SUMMARY

Present globalization has brought many new directions to the eating habits of the population. By eliminating cultural, informational, and other boundaries, there are many opportunities to improve life. A prerequisite for improvement is an economy that enables the development of the food industry on the basis of market preferences. The manufacturing industry is a significant factor in every economy, with a focus on the food industry, which takes a significant position, both in terms of the number of employees and the share of the gross domestic product. In Europe, bakeries have a very important position in the national economy. Data on the annual income of bakeries in 2012 supports this, which amounts to 98.4 billion euros. In the same year, in Europe, the bakery sub-sector was the first sub-sector in the food industry by the number of companies (53.7% of all food companies) and by job positions (1.36 million jobs, 32% of all jobs in the food industry in Europe). In recent years, during the crisis and in the period emerging from the crisis, bakeries have been affected by a demand for low-cost products, which has led to a reorientation of the business, with the focus on reducing the cost component in order to achieve positive business results. In addition to technological changes, there is a change in the supply of materials, primarily raw materials. Life habits of the population are changing, with a growing emphasis on health-oriented lifestyles. Very often there is a strong need for gluten-free food. All this significantly impacts the decision on engaging in an entrepreneurial venture in the bakery sector. Starting from the general economic situation, influenced by many changes in the business environment, the aim of this article is to further expand the theoretical knowledge on entrepreneurship in the bakery sector, in terms of the fundamental components of entrepreneurship and bakery.

**Keywords:** food industry, bakery, bread, entrepreneurship

### INTRODUCTION

The bakery sector is an important component of the food industry, which has undergone significant changes under the influence of technological advancements. In recent years, during the crisis and after the crisis ended, the bakery sector has

been influenced by the demand for low-priced products, which has led to a reorientation of business to focus on reducing component costs, in order to achieve positive business results. In addition to technological changes, there were also changes in the procurement of raw materials. The lifestyle of the population has changed, with the focus on an increasingly nutrition-oriented lifestyle, which resulted in the increase of the product range. All of that has also significantly influenced the decisions regarding entrepreneurial ventures.

### ***Historical review of bread production***

According to the available data, first bread was made by the Egyptians (1500 BC). For ancient Greeks, bread was a significant food item and even back then it was recognized as an important diet ingredient, as well as a valuable nutritional component. Ancient Romans treated it the same, and were the first to start adding supplements such as laurel, poppy, almonds, cheese, and olives to the bread. In the early Middle Ages, bread was distinguished by "colour". Specifically, white bread was a privilege of the rich. At that time, communion bread (unleavened bread) started to be prepared in the Church. Starting in the nineteenth century, bakeries began to appear in cities. Their work was strictly supervised and at the time they were operating within guilds. It was not until the 12th century that machines started to be used in the preparation of bread, which turned bread making into an industrial activity regulated by law. At the same time, bread was also a means of payment for work done (Benson, 2013). In 1602, bread arrived in America from England and mass wheat production started (Benson, 2013). The production of bread has become more sophisticated over time, especially with the introduction of yeast into the production process in 1868. When it comes to bread that usually implies mixing flour with water (milk, whey, or another liquid), salt (sometimes sugar), fat, eggs, and yeast or sourdough. It is possible to use various types of flour, so nowadays wheat bread (white, semi-white, black), as well as bread made from beans, rye, potatoes, corn, buckwheat, etc. is available. In Croatian history, bread was also made by adding carob, oak acorns, or some other plants.

The perception of bread has changed throughout history. In the past, black bread was the bread of the poor, while today it is consumed by individuals who are oriented towards healthy nutrition, due to its higher nutritional value, yet it is also more expensive. The perception of white bread has also changed, which is now a symbol of poorer eating habits. With the development of society and the advancement of the economy, as well as the ability to use and distribute information, consumer demands and interests become a key issue for businesses. A satisfied consumer contributes to the continued growth and development of the business. Technological development has made it possible to meet different consumer demands, but the quality of food products, including bread, should not be neglected. Production has become far simpler than it has been in history, production is massive, products are attractively packaged, and their durability is extended. Nutrition cognition is constantly being disseminated, scientific research is being conducted on

nutrition, healthy foods, and their usefulness for human health. All this influences the acquisition of additional knowledge, which also affects the consumer's choice for final consumption. Food safety issues are becoming more and more pronounced. Commonly mentioned nutritional safety issues include: the presence of preservatives, antibiotics, artificial colours, pesticides, or growth hormones, bacteriological contamination, the impact of food on obesity, baby foods, food irradiation, and the use of biotechnology. (Nefat, Pamić, 2008, p. 117). The described historical sequence of events, as well as the changes in consumer perceptions and changes in the technological and technological predispositions of production, were present all over the world.

### ***Cereals and the food industry in numbers***

Flour, as a basic component of bread, combines three different industry sectors: agriculture, flour production (milling), and bakery (Benson, 2013). Until the industrialization of bread, farmers had the greatest impact on bread production. They dictated the production of cereals, depending on the yields and the profit they could make, taking into account any adverse situations that could cause a negative result (such as various bugs and destroyers).

After industrialization, the situation has changed. It was the farmers who listened to the market, the millers, and the bakeries. This three-way relationship has been, and will always be, intertwined, at points that have different levels of power, but all work towards one goal, profit (Benson, 2013). Consuming bread was not just about the food industry anymore, the importance of bread was also manifested as the use of bread in advertising various products (Benson, 2013). From the point of view of the economy of Europe, baking industry takes a very significant position in national economies. In 2012, the annual revenue of bakeries was 98.4 billion euros. The same source points out 154 803 enterprises in the bakery sector. In the same year, in Europe, the bakery sub-sector is the first sub-sector of the food industry by the number of companies (53.7% of all food companies) and by jobs (1.36 million jobs, 32% of all jobs in the food industry in Europe). Regarding the topic, bread is one of the key final products of this industry. The most important ingredient in bread is cereals (flour). The European flour milling industry is a leading food processing industry in the cereal industry. It uses approximately 45 million tonnes of common wheat and rye, plus 2 million tonnes of oats per year, producing approximately 35 million tonnes of flour annually (EU-27). According to the same source, more than 3800 businesses (small and medium-sized) are engaged in flour milling. This industry employs approximately 45 000 people and represents a turnover of 15 billion euros, with an average capacity utilization of about 65%. The produced flour is used as follows: small bakeries 30%, industrial bakeries 30%, biscuit makers 14%, bakeries in shopping malls 12%, flour in households 12%, other users 5%.

From the aspect of the Republic of Croatia, the food industry is one of the leading industries. It accounts for about 24% of the entire manufacturing sector and employs

about 20% of the employees (Butorac, Vizek, 2015). Consequently, the great importance of the food industry for the development of agriculture is evident.

Ubiquitous globalization has brought many new directions in the nutrition habits of the population. By deleting cultural, informational, and other boundaries, there are more opportunities to improve lives. The prerequisite for this improvement is an economy that enables the development of the food industry on the basis of market preferences.

From the point of view of the EU economy, the bakery sector has a very significant position in the national economies. In 2012, the annual revenue of bakeries was 98.4 billion euros (Bakery and Bake-off, Market Study, [http://leo-fp7.eu/files/Bakery\\_and\\_Bakeoff\\_market\\_study.pdf](http://leo-fp7.eu/files/Bakery_and_Bakeoff_market_study.pdf), 30/04/2019). The same source points out information on 154 803 businesses in the bakery sector. In the same year, in Europe, the bakery sub-sector is the first sub-sector of the food industry by the number of companies (53.7% of all food companies) and by jobs (1.36 million jobs, 32% of all jobs in the food industry in Europe) (Bakery and Bake-off, Market Study, [http://leo-fp7.eu/files/Bakery\\_and\\_Bakeoff\\_market\\_study.pdf](http://leo-fp7.eu/files/Bakery_and_Bakeoff_market_study.pdf), page 19, 30/04/2019). In the Republic of Croatia, the bakery business can be performed in many ways, such as: Production of bakery products, wholesale of finished products to other legal and natural persons, and retail sale to end consumers (from shops, kiosks, or through mobile sellers) (Vranar, page 43, 2016).

Regarding the topic, bread is one of the key final products of this industry. The most important ingredient in bread is cereals, flour. The European flour milling industry is a leading food processing industry in the cereal industry. It uses about 45 million tonnes of common wheat and rye, plus 2 million tonnes of oats per year, producing approximately 35 million tonnes of flour annually (EU-27) (European Flour Millers, Facts & Figures, <http://www.flourmillers.eu/page/facts/>, 09/06/2019). At the EU level, more than 3800 enterprises (SMEs) are engaged in flour milling as a core business. The industry employs approximately 45,000 people and represents a turnover of 15 billion euros, with an average capacity utilization of about 65% (European Flour Millers, Facts & Figures, <http://www.flourmillers.eu/page/facts/>, 09/06/2019). The flour produced is used as follows: small bakeries 30%, industrial bakeries 30%, biscuit makers 14%, bakeries in shopping malls 12%, flour in households 12%, other users 5% (European Flour Millers, Facts & Figures, <http://www.flourmillers.eu/page/facts/>, 09/06/2019).

This paper analyses agricultural production, especially the part that constitutes the components of bread production.

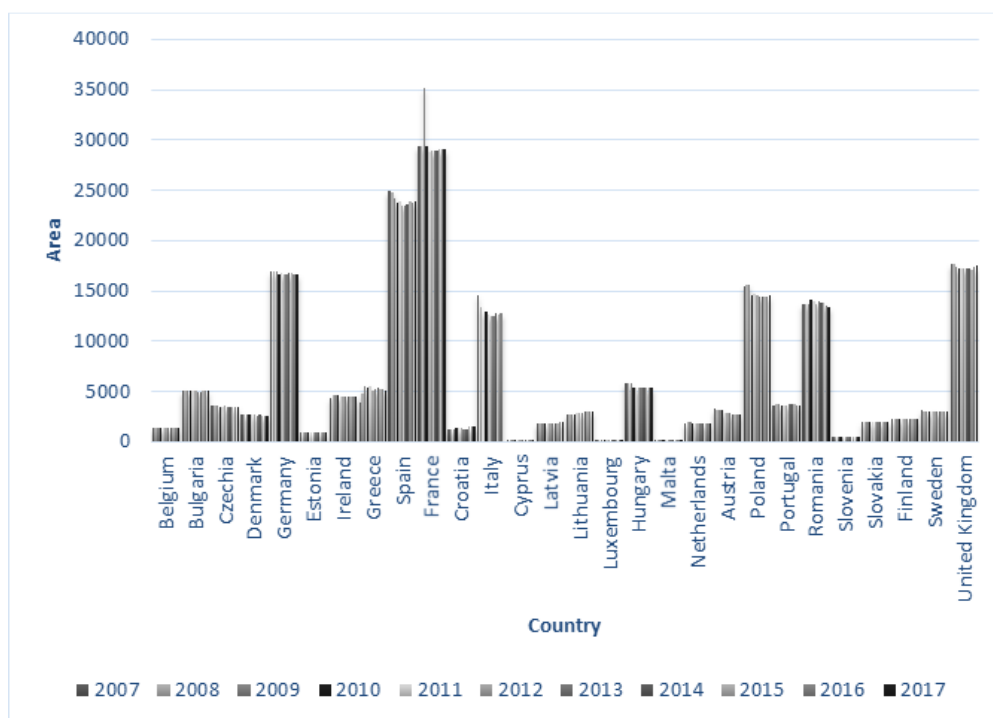
## **MATERIALS AND METHODS**

We used the method of analysis and synthesis in elaborating the current knowledge in the field of entrepreneurship and bakery, as well as descriptive statistics in the numerical representation of numerically measurable quantities from the bakery industry as a component of the food industry. Secondary data available on the Eurostat website (Eurostat, <https://ec.europa.eu/eurostat/data/database>, 2019-05-

15) and the Central Bureau of Statistics of the Republic of Croatia (CBS, www.dzs.hr, 2019-05-12) was used as a data sample. Considering that cereals used in bread production are one of the most common foodstuffs in everyday life, data on the selling prices of wheat, oats, corn, potatoes, and barley for the period 2007-2017 was used. Data was also collected on the land area used for production, the yield of the agricultural industry, and the yield of crops as the key component of the entire agricultural industry. Overcoming the increasing orientation of the population towards healthy nutrition, data from the field of organic farming was also observed. Data was observed for 28 EU countries.

## RESULTS AND DISCUSSION

According to available Eurostat data, there were no significant changes in the utilized agricultural area by categories in the EU in the last ten years. Most of the utilized agricultural area in 2017 refers to France, 29101.33 thousand ha, which represents a 1.1% decrease compared to 2007. After France, the most significant agricultural area is in Spain, followed by Germany and England. The least amount of used agricultural area was recorded for Cyprus, Malta, and Luxembourg (Figure 1).

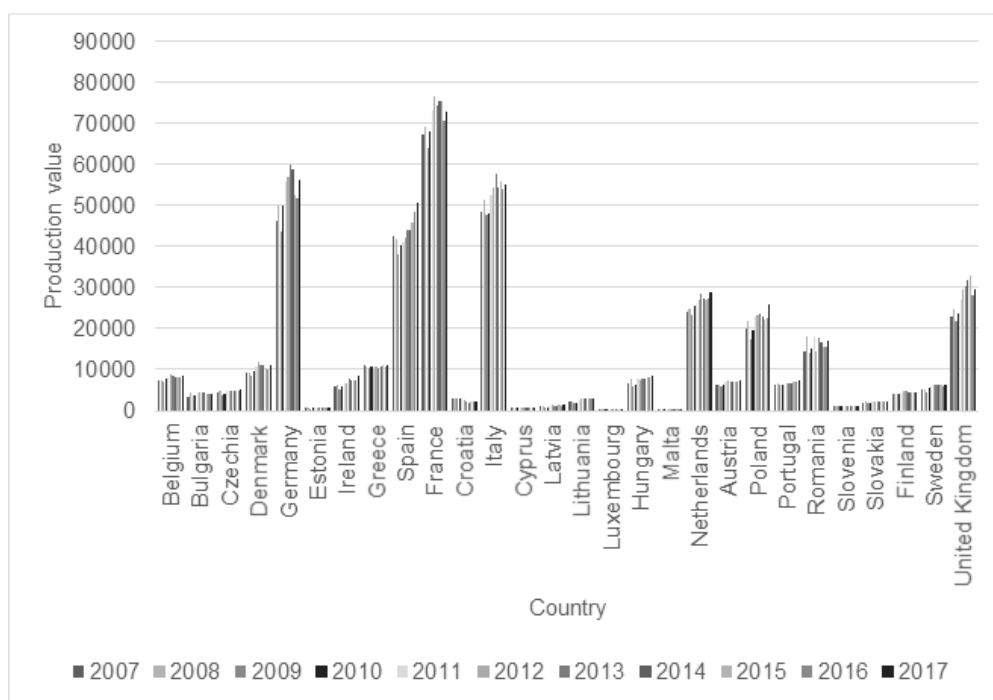


**Figure 1** Utilized agricultural area (thousands ha) in EU countries

The initial observation years were 2007, the year of the evident crisis, and 2017, as the year in which national economies achieved stability and the crisis period ended. As can be seen from **Figure 1**, there were no significant fluctuations in the utilization

of agricultural land in the observed period. The most significant change was recorded for Greece, specifically in 2017, when the utilized agricultural area was 29.8% higher than in 2007. This is followed by Croatia with an increase of 24.5% in 2017 (1496.66 thousand ha of utilized agricultural area).

The next significant position for observing the importance of agricultural production is the production of the agricultural industry - basic and producer prices. Production is valued at basic prices. Basic price is defined as the price received by the manufacturer after deduction of all product taxes, but including all subsidies on products. The production of the agricultural industry consists of the sum of the production of agricultural products, the agricultural services, and the goods and services produced in inseparable non-agricultural secondary activities (Eurostat). **Figure 2** shows the production of the agricultural industry - basic and producer prices, expressed in millions of EUR.

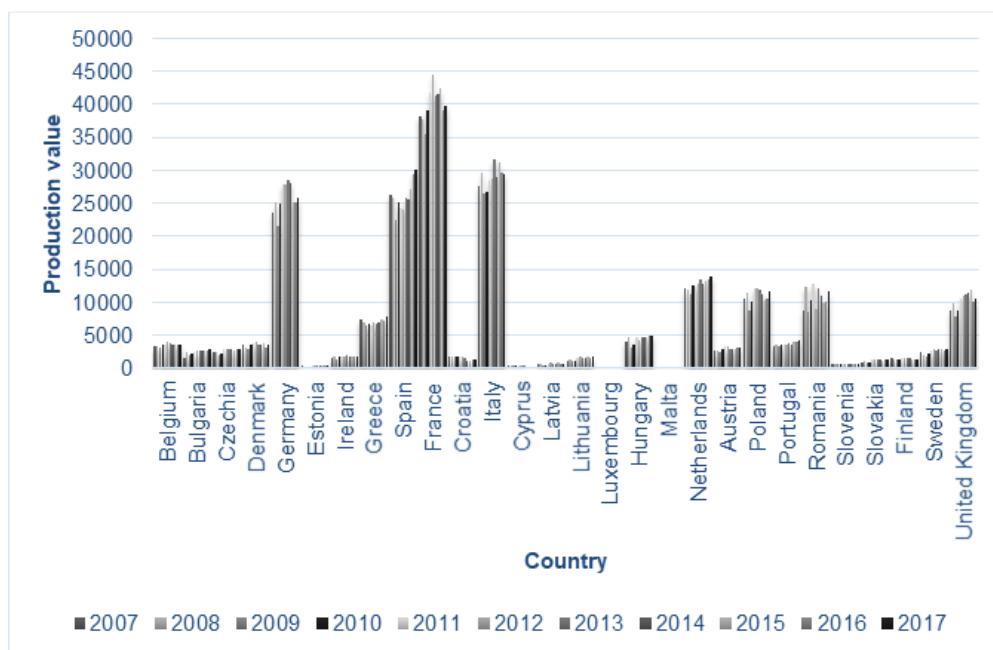


**Figure 2** Production of the agricultural industry (basic and producer prices) (million EUR) in EU countries

The production of the agricultural industry - basic and producer prices was the most significant for France in 2017, with EUR 72642.31 million. Compared to 2007, this is an increase of 8.2%. In the observed period, Ireland recorded the most significant increase of 41.3%, i.e. in absolute terms, from EUR 5975.44 million in 2007 to EUR 8443.72 million in 2017. The production of the agricultural industry for Croatia in 2007 amounted to EUR 2873.14 million, while for 2017 it amounted to EUR 2203.85

million (a decrease of 23.3%), which is the most significant decrease for the observed countries.

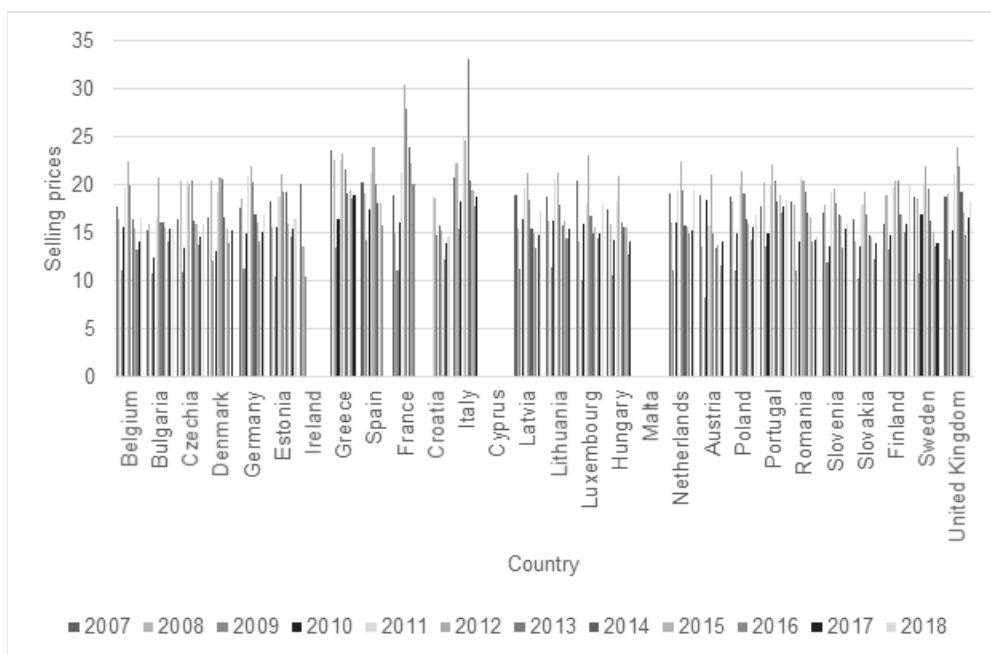
Crop yields and selling prices for individual cereals as raw materials for bread production are shown in figures 3 to 7. Crop production was estimated at basic prices, which were defined as the prices received by the producer after the deduction of all taxes on products, but including all subsidies on products. The concept of production included sales, as well as changes in stocks and crops used as feed, for processing, and for own use by the manufacturer (Eurostat).



**Figure 3** Crop production - basic and producer prices (million EUR) in EU countries

According to the available data, the most significant increase in crop production for 2017, compared to 2007, was noticed for Bulgaria (81.8%), followed by Lithuania (52.7%), Latvia (40.40%), and Romania (35.2%). Crop production declined in Croatia, Cyprus, Finland, and Slovenia. It should be noted that the figure is given in relation to basic and producer prices, which should be analysed in relation to the pricing policy of other participating industries and the fiscal policy of each country. Below is an overview of the selling prices of wheat and producer prices without fiscal levies (for Croatia excluding VAT).



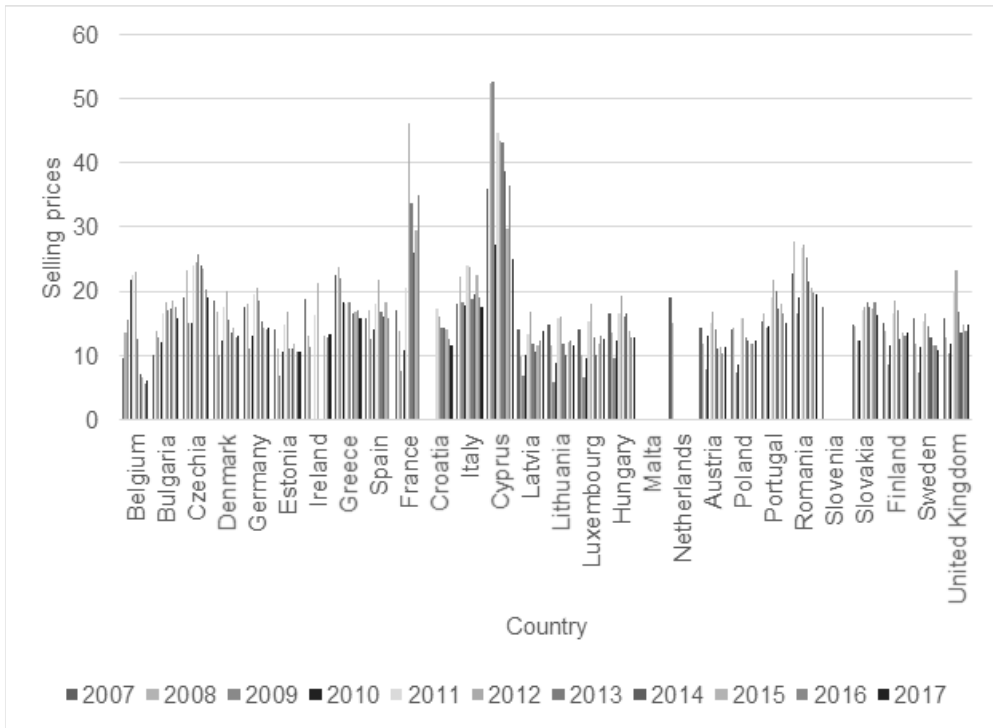


**Figure 4** Selling prices of wheat (EUR per 100 kg) in EU countries

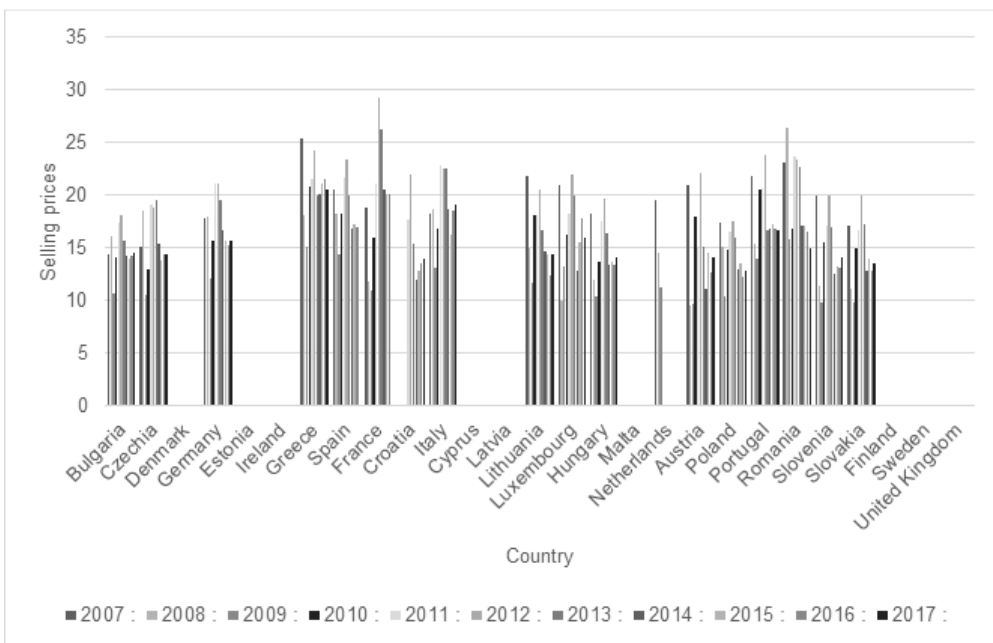
Netherlands, Portugal, and Finland registered a wheat price increase in 2017 compared to 2007, while the other countries have been stagnating or slightly declining. For Croatia, data was available from and including 2011. Given the available data, it is notable that the price of wheat in 2017 (EUR 14.62 per 100 kg) was 22.3% lower than in 2011, when the price of wheat was 18.8 EUR per 100 kg. The highest wheat price per 100 kg in 2017 was recorded for Finland, Portugal, and England. The average price of wheat was EUR 16.9 per 100 kg.

An increase was noticed in 2017 for the price of oats in Bulgaria (58.9%) and Slovakia (9.2%), compared to 2007, while all other countries saw a decrease in the price of oats, which was approximately the same in each country.

For Croatia, data was available from and including 2011. Given the available data, it can be concluded that the price of oats in 2017 (EUR 11.63 per 100 kg) was 32.8% lower than in 2011, when the price was 17.32 EUR per 100 kg. The highest price of oats per 100 kg in 2017 was recorded for Cyprus (EUR 25 per 100 kg), Romania (EUR 19.4 per 100 kg), and the Czech Republic (EUR 19.11 per 100 kg). The average price of oats was EUR 14.2 per 100 kg.

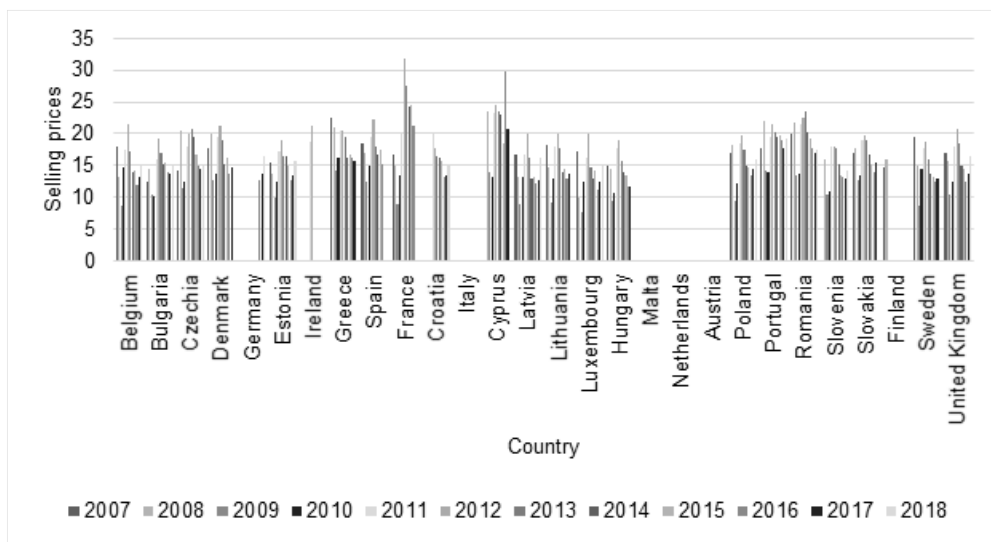


**Figure 5** Selling prices of oats (EUR per 100 kg) in EU countries



**Figure 6** Selling price of corn (EUR per 100 kg) in EU countries

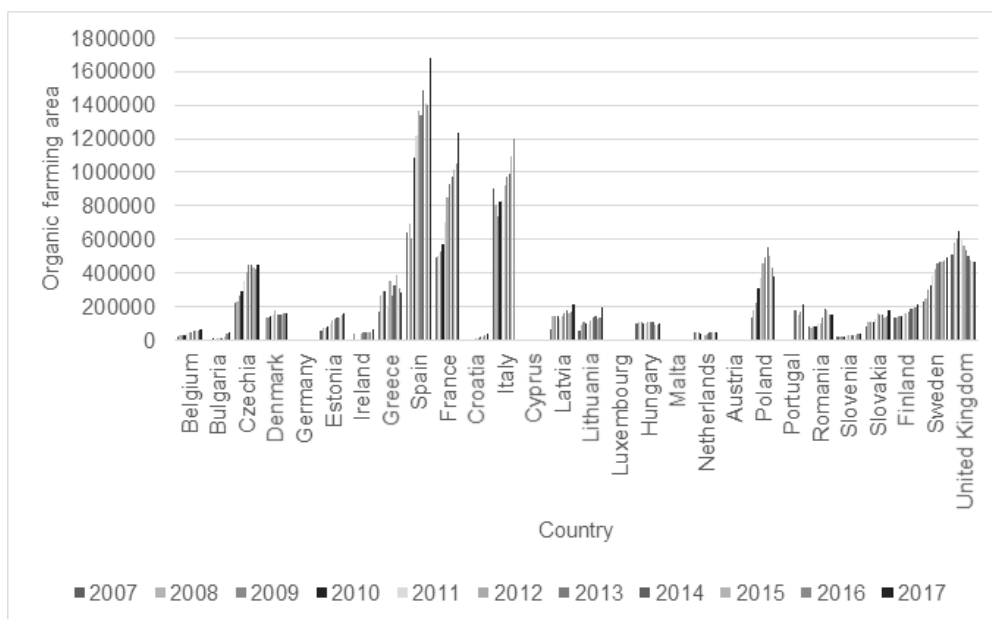
For the observed countries, the prices of maize have reduced or remained approximately at the same level in 2017, compared to 2007. For Croatia, given the available data, it can be said that the price of corn in 2017 (EUR 13.94 per 100 kg) was 20.9% lower than in 2011, when it was 17.62 EUR per 100 kg. The maximum corn price per 100 kg was recorded in 2017 for Greece (EUR 20.55 per 100 kg). The average price of corn in the same year was EUR 15.24 per 100 kg.



**Figure 7** Selling prices of barley (EUR per 100 kg) in EU countries

For the observed countries, the price of barley has largely reduced in 2017, or it remained at approximately the same level, compared to 2007. Significant price increases were recorded for Bulgaria (21.3%). In Croatia, given the available data, it can be concluded that the price of corn in 2017 (14.91 EUR per 100 kg) was 25.6% lower than in 2011 (20.04 EUR per 100 kg). The highest barley price per 100 kg in 2017 was recorded for Portugal (EUR 19.21 per 100 kg), while the average price of barley in the same year was EUR 15.94 per 100 kg.

The historical aspect of the origin and consumption of bread is outlined in the introduction, with an emphasis on changes that are occurring in consumer perceptions, especially in the part that refers to healthy eating. Hence, the land area for organic farming (completely converted area) in ha is presented below. An area which fulfils all the conditions of production laid down in Council Regulation (EC) No. 1782/2003 834/2007 can be considered environmentally friendly. Detailed rules for the implementation of this Regulation are set out in the Commission Regulation (EC) No. 889/2008 (Eurostat) (**Figure 8**).



**Figure 8** Land area for organic farming (fully converted area) in ha, in EU countries

All the observed countries, except England, significantly increased the land area for organic farming in 2017, compared to 2007. The most significant increase was recorded by Croatia and Bulgaria (available data for Croatia for the comparative year 2009). In 2017, France and Spain recorded the most significant land area for organic farming, while the land area for organic farming in Croatia was 42348 ha.

## CONCLUSIONS

In conclusion, notwithstanding the significant economic changes that followed the beginning of the crisis, no significant changes have occurred in the area of agricultural land use. The most significant changes were recorded in Greece and Croatia, which indicates that significant changes in the utilization of agricultural land were occurring in countries that were significantly affected by the crisis. At the same time, in terms of crop production (at producer prices), Croatia has recorded a decrease in production, which is influenced by the pricing policies of other participating industries. From the point of view of each individual cereal, it can be noted that the prices of the cereals decreased in the observed period. For 2017, the average price of wheat was higher than the price of other observed cereals. From the Croatian perspective, it should be mentioned that numerous regulatory changes have occurred in the observed period, which have legally framed the procedures in agricultural production, particularly bread and other bakery products. International food production standards are implemented in business processes. The quality of food and bakery products is becoming one of the most important issues in this industry. Amendments are made to laws that indirectly affect the intensity of agricultural production, and incentive mechanisms are installed in relation to arable

land. Changes in consumer awareness about the consumption of healthy food are leading to changes in organic farming, and that is significant in all European countries.

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## THE EFFECT OF FREEZING CONDITIONS AND FROZEN STORAGE ON THE PHYSICAL AND SENSORY PROPERTIES OF PUFF PASTRY

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### SUMMARY

The aim of this paper was to investigate the impact of freezing conditions on the quality of puff pastry. The impact of blast freezing temperatures (-25, -30, -35 °C) and the temperatures in the center of the dough piece (-10, -15, -20 °C) on the quality of frozen puff pastry after storage at -18 °C, during 1, 7, and 30 days, was investigated by means of response surface methodology. The desirability function was employed to optimize the effects of freezing conditions. The responses were physical properties (pastry volume) and sensory score (flakiness, flakiness uniformity, pore size, layer thickness, and color uniformity). The highest quality score was attributed to the samples frozen at -35 °C that reached -10 °C in the center. These samples exhibited 4.5 times higher pastry lift during baking and accordingly, the highest volume and the best sensory properties. After 7 days of storage of the frozen dough, the pastry lift and volume deteriorated. However, the sensory quality of puff pastry remained unchanged, retaining the same level as that after 1 day of frozen storage (the total score was 17.1 points out of the maximum 20 points). Thermogravimetric analysis (TGA) was performed to analyze the release of water from the pastries. The deconvolution of TGA curves revealed that there was an alteration in water populations associated with gluten among the samples. After 7 and 28 days of storage, in the best scored samples, the peak associated with water bound to gluten exhibited a shift to a significantly higher evaporation temperature. In contrast, other samples showed lower evaporation peaks related to water population bound to gluten, which implies that freezing conditions affect the water redistribution and the water-binding ability of the gluten network.

**Keywords:** blast freezing, quality, pastry lift, volume, sensory score, TGA, water population

## **INTRODUCTION**

Puff pastry is a special category of bakery products characterized by a layered structure and delicate, flaky texture. The layered structure is achieved by repeated lamination of dough layers separated by fat layers, without the use of yeast or leavening agents. Fat is important for the development of the layered structure, lift, appearance, and volume, so puff pastry usually contains high amounts of fat. With the increased consumers' requests for healthier products, attempts have been made to modify the formulation of puff pastry in terms of reducing fat content, changing the composition of the fat fraction by reducing the saturated fat content, reducing the content of sodium, using whole meal flour instead of refined wheat flour, addition of soy flour, etc.

Puff pastry is commonly distributed in the form of "bake-off" frozen dough. Dough freezing technology has been widely accepted in the industry due to reduced economic losses resulting from the increased shelf-life of bakery commodities and due to the convenience of offering fresh products to the consumers. However, longer periods of frozen storage deteriorate the quality of the products. The delicate nature of the structure of puff pastry makes it particularly vulnerable to frozen storage. The optimization of freezing conditions is usually necessary in order to preserve the sensory and textural properties as close as possible to those of conventional pastry.

The loss in quality of frozen products has been related to damage in the gluten network, water redistribution during freezing, and water sublimation from surface layers (Cauvain, 1998; Meziani et al. 2012; Loveday et al., 2012; Wang et al., 2015). In this study, puff pastry was made from refined wheat flour partially substituted with whole meal flour in proportion 70:30 w/w. The aim was to study how different conditions during blast-freezing and subsequent frozen storage effect the quality of puff pastry, in order to define the optimum conditions for puff pastry. Also, it was hypothesized that different freezing conditions and frozen storage periods would affect water-binding properties of gluten and that gluten water-binding abilities would be more preserved in an optimally treated sample.

## **MATERIALS AND METHODS**

### ***Materials***

The puff pastry dough formula used in this study was: refined and whole meal wheat flour (700 g + 300 g, respectively, total 1000 g), water (59.9), salt (20 g), sugar (10 g), vital gluten firm Fidelinka-Skrob, Subotica, Serbia (10 g), improver-S500 Puff Pastry from Puratos, Belgrade, Serbia (5 g), laminating fat-margarine Argenta Pastry HF from Puratos, Belgrade, Serbia (600 g). Puff pastry was produced according to procedure described in Soronja Simović et al. (2009).

### ***Experimental procedure***

Puff pastry dough pieces (29 g) were frozen in a pilot-scale freezer (Bongard Model BSP46.15, France). The freezer was monitored to produce 3 different air-blast temperatures: -25, -30 and -35 °C. The puff pastry dough pieces were removed from the pilot-freezer after reaching -10, -15, and -20 °C core temperature. Freezing times to reach the core temperatures are given in Table 1. The samples were transferred to a storage freezer and kept at -18°C. After 7 and 28 days of frozen storage, the samples were thawed at +4 °C during 16 h and baked for 17 min at 220 °C in a deck oven (Mac-Pan MD/CO/S/B18, Italy). After cooling to ambient temperature, the baked puff pastry was subjected to analysis: volume measurement, sensory scoring, and TGA.

### ***Volume measurement***

Volume measurement was performed on a laser scanner VolScan (Stable Micro Systems, UK).

### ***Sensory evaluation***

Sensory analysis was performed according to a score-based method described in Soronja Simović et al. (2009). The quality attributes assessed during evaluation included: appearance (shape, crust color, and properties), crumb structure (flakiness, uniformity, cell size, cell wall thickness, and color uniformity), odor, and taste. The maximum score number was 20 and the quality categories were: excellent (17.6-20 points), very good (15.2-17.5 points), good (13.2-15.1 points), acceptable (11.2-13.1 points), and unacceptable (< 11.2 points).

### ***Thermogravimetric analysis (TGA)***

About 95 mg of puff pastry obtained from the center of the pastry piece was placed in a previously tared ceramic crucible inside a thermogravimetric analyzer model TGA701 LECO (Leco Corporation, Michigan, US). The samples were heated from room temperature ( $\approx 25^{\circ}\text{C}$ ) to up  $180^{\circ}\text{C}$ , at the rate  $1^{\circ}\text{C}/\text{min}$  and held isothermally for 5 min. Thermograms of the sample weight as the function of temperature and its first derivative (DTG) were analyzed. In order to determine different water populations, the DTG curve was deconvoluted to a sum of two Gaussian peak functions. Fitting procedures were accomplished using Origin 8 Lab software (OriginLab Corporation).

### ***Statistical analysis***

All experiments were done in triplicate. Statistica 13 (TIBCO Software Inc., CA) was used to carry out two-way ANOVA procedures, with Tukey's post-hoc test to evaluate significant differences ( $p < 0.05$ ).

Two factors, three levels full factorial experimental design was used to study the effects of freezing conditions on the quality of puff pastry. Statistical analysis of the



experimental data and the optimization procedures were performed using the Design Expert 8 statistical software (Stat-Ease Inc. MN).

## RESULTS AND DISCUSSION

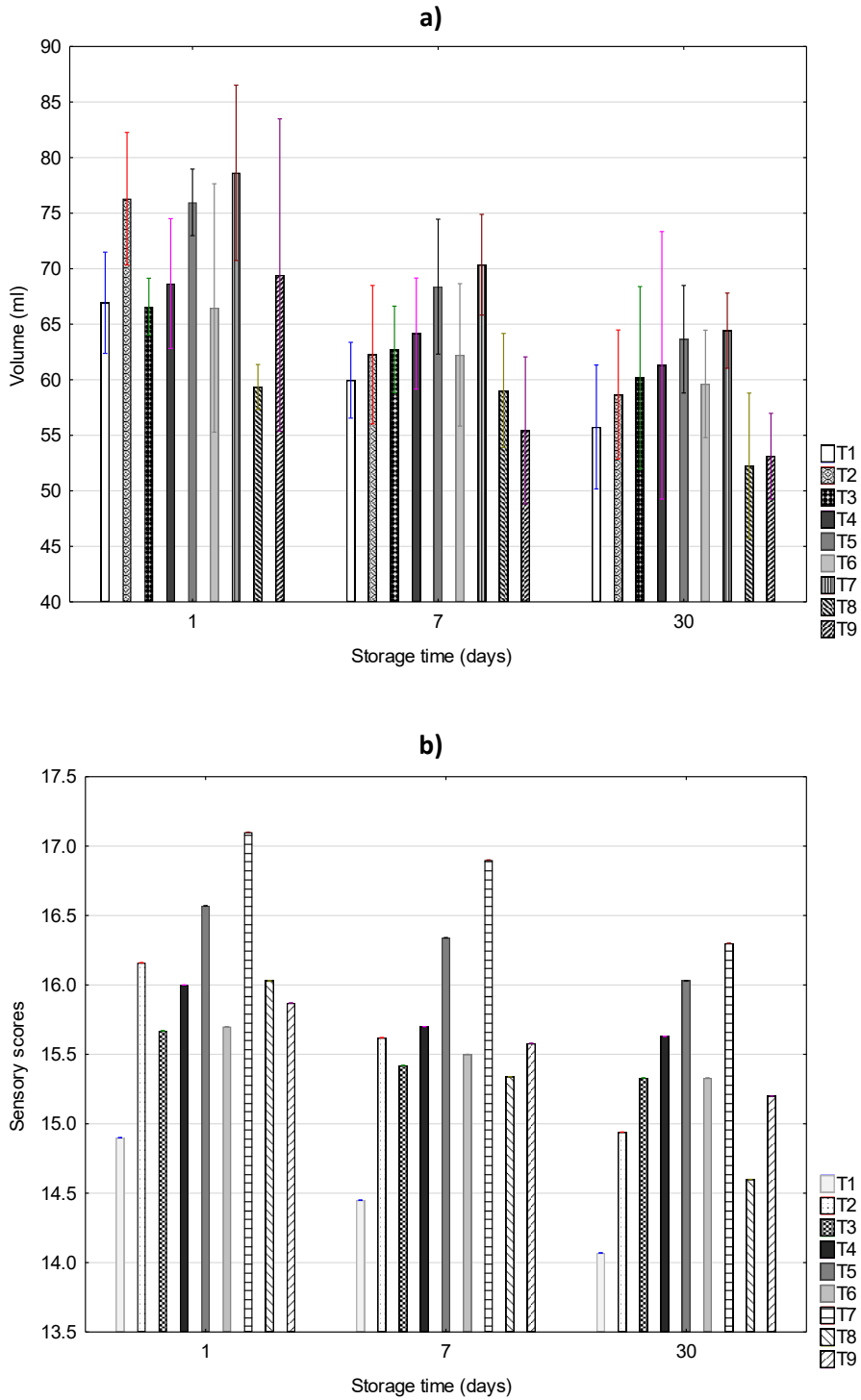
### ***Effect of freezing conditions on the quality of puff pastry and optimization***

The effect of freezing conditions on the quality of puff pastry was investigated according to the full-factorial 3<sup>2</sup> experimental design. Individual parameters were the final temperature of blast freezing and the temperature reached in the core of the pastry dough piece. The actual experimental settings are presented in **Table 1**. In this experiment, maximum volume and the maximum sensory score were prioritized. Optimization of the freezing regime was performed using the desirability function approach (data not presented here). Within the storage period of 28 days, treatment T7 reached the highest desirability value, followed by treatments T5 and T9. Thus, the optimum freezing conditions included the following temperature combinations -35 °C/-10 °C.

**Table 1** Experimental design (full-factorial 3<sup>2</sup>)

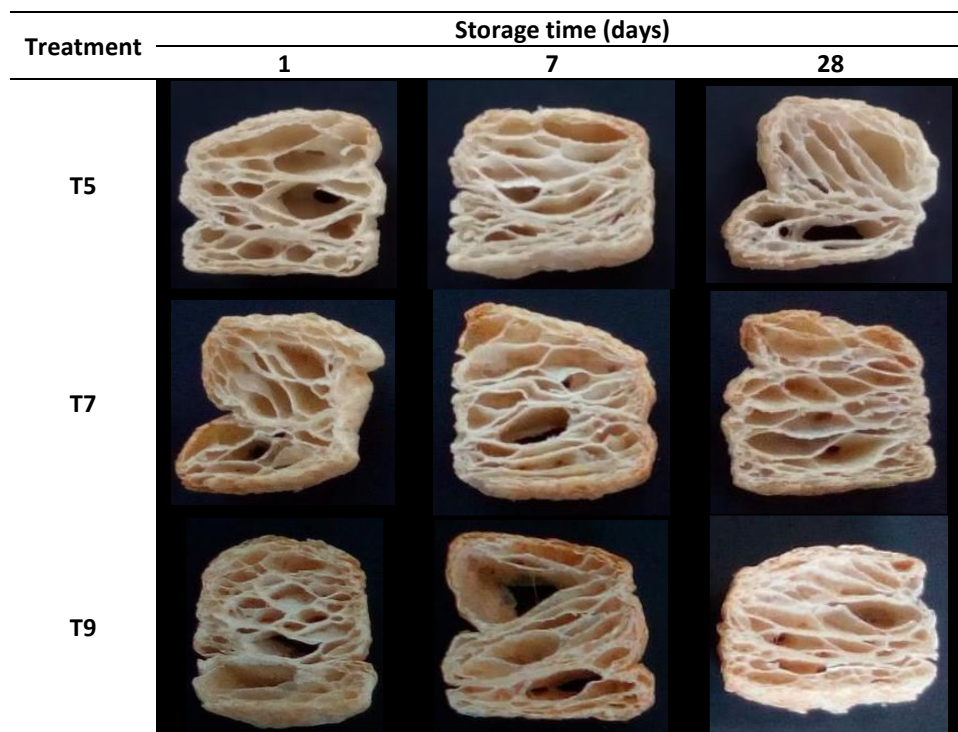
<b>Treatment</b>	<b>Temperature of blast freezing (°C)</b>	<b>Temperature in the center of a pastry piece (°C)</b>	<b>Freezing time (min)</b>
<b>T1</b>	-25	-10	29
<b>T2</b>	-25	-15	34
<b>T3</b>	-25	-20	41
<b>T4</b>	-30	-10	23
<b>T5</b>	-30	-15	34
<b>T6</b>	-30	-20	40
<b>T7</b>	-35	-10	18
<b>T8</b>	-35	-15	27
<b>T9</b>	-35	-20	29

The changes in selected quality attributes of the puff pastry during frozen storage are displayed in **Figure 1 (a, b)**. As it can be seen from the diagrams, the volume of puff pastry gradually decreased over storage time. A similar trend was noticed for the sensory score, but the decline was less expressed in the T7 and T5 samples. These samples retained a relatively high sensory score (> 16 points, out of the maximum 20 points). Gerrard et al. (2000) reported an almost 20% loss of puff pastry lift over 3 months of frozen storage.



**Figure 1** Effect of different freezing regimes on the quality attributes of puff pastry:  
 a) volume; b) sensory attributes

To get a better insight into the structure of puff pastry, cross sections were made (**Figure 2**). A good layered structure is a desirable attribute which reflects good lift and thin layers. Thick layers and fewer large holes are undesirable and reflect undeveloped layers and thick cell walls. The T7 sample showed uniform, well developed layers, good lift and well-expressed cells over storage time. The sample T5 was also characterized by fine layering but less lift (after 7 days), while after 28 days it exhibited strong lift during baking, which led to a slightly deformed structure due to crushing. By the end of storage time, T9 was characterized by lower lift and somewhat less developed layers (smaller cell size), but without undeveloped, watery layers.



**Figure 2** Cross sections of pastries

The quality of puff pastry depends on many factors, among which the quality of gluten holds primary importance (Wickramarachchi et al., 2015). During freezing and frozen storage, gluten undergoes changes that detrimentally effect the quality of puff pastry. Freezing and frozen storage promotes weakening of the gluten network and the modification of its rheological and viscoelastic properties. This internal structure damage is due to the formation and growth of ice crystals. During frozen storage, gluten polymer proteins with high molecular weight undergo depolymerization and increased solubility (Silvas-García et al., 2014). Freezing conditions cause water to leach out from the gluten matrix and affect the water distribution in glutenin/gliadin systems (Wang et al., 2015).

### **TGA analysis of baked pastry**

TGA is a useful tool that allows studying the interactions among dough components in the sense of bonding ability. Previous studies based on the work of Fessas and Schiraldi (2001), undertaken in flour-water mixtures, dough, and baked products, revealed that TGA can be used to discriminate different water fractions, which is useful in understanding how water is retained by different components of the macromolecular matrix. Different water fractions have different binding strengths and, depending on the ease of their removal and their amount, conclusions can be made on their distribution between the macromolecules in a matrix.

The deconvolution of the DTG data (first derivative of TGA data) revealed that the overall DTG trace can be reproduced as the sum of 2 main populations: One population that evaporates at lower temperature (Water 1) and the second that evaporates at higher temperature (Water 2). **Table 2** displays the main attributes of these water populations after 7 and 28 days of pastry frozen storage. Average area indicates the amount of water contributing to each of the water populations. Peak temperature indicates the evaporation temperature of the corresponding water population. Low peak temperature population (Water 1) can be linked to the evaporation of water bound to carbohydrates (starch). Water evaporation at temperatures around and above 100° C can be associated with water retained by the gluten network (Water 2). Most of the water belonged to "easily removable" water (76-86%), which evaporated in the temperature range 70-75 °C. In T5 and T7, 23% of water was trapped by gluten, whereas in T9, gluten retained less water (13%). The sample with the optimal freezing treatment (T7) had the highest evaporation temperature (117.4 °C), showing that water was strongly bound by gluten in comparison to other samples, particularly T5.

During storage, peak temperatures for both water populations decreased, showing that the main components (starch and gluten) had a lower ability to retain water. The optimally treated sample (T7) showed the highest peak temperature for Water 2 associated with gluten (113.3 °C), showing that gluten continued to retain water more strongly in T7. The amount of water released by starch declined with longer storage time. On the other hand, the amount of water released by gluten increased after a 28-day storage period, in comparison to that after a 7-day storage period. This may be attributed to water redistribution between starch and gluten; the more mobile water population (Water 1) moved towards sites where it is more tightly bound, thus increasing the amount of the other fraction (Water 2) (Schiraldi and Fessas, 2003). Wang et al. (2014) showed that the weight loss of hydrated gluten increased with prolonged frozen storage, due to the formation of a more open, weak structure affected by recrystallization. Fessas et al. (2008) explained that the temperature gap between the peaks of 2 water populations can be related to the looseness of the gluten network; the narrower the gap, the higher the looseness. Looser gluten network suggests higher accessibility of SH groups (Fessas et al., 2008) and indicates the depolymerization of the gluten polymer. Zhao et al. (2012) reported an increase of SH and a decrease of SS bonds in gluten during frozen storage

and suggested that water redistribution and ice recrystallization lead to the breakage of SS bonds. In our study, the temperature gaps between two peaks decreased over the storage period, except in the case of T7, where it remained similar to the condition after 7 days of frozen storage, which may indicate less destabilization.

**Table 2** Characteristics of water populations (water peak distribution and peak temperature of the deconvoluted DTG curves) obtained for the optimal samples

Sample	Average area (%)		Average peak temperature (°C)	
	Water 1	Water 2	Water 1	Water 2
<b>Storage day 7</b>				
<b>T5</b>	76.5±5.6 <sup>c</sup>	23.5±5.6 <sup>b</sup>	69.7±2.8 <sup>b</sup>	111.1±1.4 <sup>b</sup>
<b>T7</b>	76.2±1.2 <sup>c</sup>	23.8±1.2 <sup>b</sup>	74.2±0.1 <sup>c</sup>	117.4±1.0 <sup>c</sup>
<b>T9</b>	86.7±0.1 <sup>b</sup>	13.3±0.1 <sup>a</sup>	74.6±0.2 <sup>c</sup>	116.5±0.6 <sup>c</sup>
<b>Storage day 28</b>				
<b>T5</b>	56.7±6.2 <sup>a</sup>	43.3±6.2 <sup>d</sup>	64.7±0.2 <sup>a</sup>	98.8±0.6 <sup>a</sup>
<b>T7</b>	63.4±3.7 <sup>b</sup>	36.6±3.7 <sup>c</sup>	70.1±0.3 <sup>b</sup>	111.3±0.8 <sup>b</sup>
<b>T9</b>	78.0±1.3 <sup>c</sup>	22.0±1.3 <sup>b</sup>	64.1±0.3 <sup>a</sup>	97.6±0.1 <sup>a</sup>

<sup>a,b,c,d</sup> Means with different subscript letters in a column indicate significant difference ( $p < 0.05$ ).

## CONCLUSIONS

The effects of freezing conditions and storage time on the properties of frozen puffed pastry were investigated in this study. During frozen storage, the quality attributes of puff pastry declined. Full factorial 3<sup>2</sup> design and the desirability function approach was used to optimize the conditions of blast freezing. The optimal blast freezing conditions to obtain puff pastry with maximum volume and highest sensory score were: freezing temperature -35 °C and the temperature at the core of the pastry -10 °C. By the end of the storage period, puff pastry treated with the optimum freezing treatment showed well developed layers, good lift, and high sensory score (16.4 points), close to the maximum sum of scores (20 points).

Thermogravimetric analysis (TGA) was shown to be a potentially useful tool in differentiating between the variously treated puff pastry. Freezing and frozen storage detrimentally affect the gluten network, which reflects in the differences in water availability and bonding strength. TGA data showed that in the optimally treated puff pastry, water population associated to gluten evaporated at a higher temperature in comparison to other samples, showing stronger bonding, over a 28-day frozen storage period. During storage, the gap between the peak evaporation temperatures increased, except in the case of the optimal sample, implying that the

gluten network retained its tightness in the optimal sample, which may be associated with a less destabilized network.

#### ACKNOWLEDGMENT

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## COMPLEX COMPARISON OF THE EFFECT OF QUINOA AND CANAHUA WHOLEMEAL FLOURS ON THE QUALITY OF COMMERCIAL WHEAT FLOUR

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### SUMMARY

Quinoa and canahua (kaniwa) wholemeals, substituting 10 or 20 wt.% of wheat, were prepared at laboratory conditions. Both non-traditional plant materials weakened protein technological quality and fermentation ability, as determined by Zeleny and Falling Number Test, respectively (about 10% decrease in both cases). Amylograph test results were affected by very low amylases activity in wheat flour, as well as by high absorption capacity of canahua and quinoa – samples differed in gelatinization temperature at the beginning. Rapid Visco Analyser profiles of pure flours differed clearly – both alternative materials did not demonstrate viscosity peak and differed in starch retrogradation rate during cooling phase of the test. Farinograph water absorption was partially increased by quinoa addition, and reversely by canahua ones. Effect of both plant materials on stability of dough consistency and on dough cohesiveness was similar – therefore prolongation and weakening were observed. Extensigraph test confirmed positive effect of alternative crops on dough elasticity for 10% addition of both, but composite dough with twofold dosage had worsened machinability (dough was too tough). Optimal times of dough leavening and maturation, as well as dough volumes were somewhat longer and higher for dough variants containing quinoa wholemeal as shown by the fermentograph and maturograph records. In the first phase of baking, reversely, wheat-canahua flour composites reached statistically better results. Baking test results correlated with fermentograph and the maturograph ones, i.e., better specific bread volumes were determined for wheat-quinoa bread variants. Colour of bread crumb was influenced by yellow and black-red colour of quinoa and canahua seeds. Both alternative materials introduced their typical aroma and taste, less unpleasant for the 10% addition of both, as well as for quinoa canahua wholemeal. All data, along with the sensory scores, pointed to maximal tolerable quinoa or canahua dosage up to 15 wt.%.

**Keywords:** wheat composite flour, quinoa, canahua, rheology, bread, PCA



## INTRODUCTION

In ancient times, humans were hunter-gatherers and perhaps along with the herbivore animals, people systematically explored which plant seeds and fruits were eatable. In the area of the Central America, such raw materials were found in tiny seeds of plants *Salvia hispanica*, *Chenopodium quinoa* and *Chenopodium palidicaule*, all over the world now known as chia, quinoa and canahua (kaniwa), respectively. For pre-Columbian Inca and Aztec civilisations, the crops sorted among pseudocereals represented staple food with religious connotations – quinoa was called "mother of grain" and was considered a sacred gift from the gods.

Compared to wheat in terms of nutrition composition, both *Chenopodium* genus members are richer in dietary fibre (6.1% and 4.0% vs. 2.5%, respectively), as well as in lipid and mineral contents (Rosell et al. 2009). Quinoa is a good source of lysine and histidine (Bavec and Bavec 2007). From other phytochemicals, saponins, phenolics, and flavonoids could be pointed out (Vega-Gálvez et al. 2010; Pérez et al. 2016).

The present paper compared quinoa and canahua wholemeal effect on the technological quality of commercial wheat flour, including basic analytics, pasting properties, rheological behaviour of non-fermented as well as fermented dough, and finally consumer's quality of leavened wheat bread. Any of naturally gluten-free plant materials has a potential to lower baking value of wheat flour – one of the paper's goal was the evaluation of recommended alternative material dosage.

## MATERIALS AND METHODS

Standard wheat flour samples, coded WF1 and WF2, were produced by the Jaroslav Chochole – Delta company, Czech industrial mill located in Prague in the years 2015 and 2017, respectively. Samples WF1 and WF2 were used as controls, as well as bases for the preparation of flour composites with quinoa and canahua wholemeals. Basic technological quality of both controls WF1 and WF2 corresponds to a long-term average of the Central European region; for example, protein content, Zeleny value and Falling number 13.1%, 40 ml and 421 s, respectively, were determined for the item WF1 and for the item WF2. Quinoa and canahua were bought in a specialised retail shop in Prague in the form of whole (hulled) seeds. For three quinoa varieties and four canahua ecotypes and using factor 6.25, Steffolani et al. (2013) determined protein content in ranges 13.64-14.51% and 12.02-17.55%, respectively. By using a two-step chemical digestion (by sulphuric acid and sodium hydroxide), recorded ranges of crude fibre were 1.95-2.00% and 3.42-5.71%, respectively. To produce quinoa and canahua wholemeal flour type of fine granulation, comparable to WF, a laboratory grinder Concept KM 5001 (Elko Valenta, Czech Republic) was used (abbreviations *Qui* and *Can*, respectively). Grains were disintegrated in a batch regime with raw material dosage around 25 g during 3 min.

In tested flour composites, non-traditional materials *Qui* and *Can* replaced 10 or 20 wt.% of wheat flour (sample codes WF1+10*Qui*, WF1+20*Qui*, WF2+10*Can*, WF2+20*Can*).

Within the cereal laboratory of the UCT Prague, the complex assessment of technological quality "from grain to bread" was developed; employing proper parts of the procedure, analytical and rheological parameters of control and composite flours, as well as bread quality features were evaluated. The Zeleny test and the Falling number methods describe technological potential of proteins and starch-amylose complex, and were carried out according to the norms ISO 5529 and ISO 3093, respectively (in two replications). Rapid Visco Analyser (RVA), a pair Farinograph – Extensograph were used to explore viscosity of flour suspension and visco-elastic properties of non-fermented dough during mixing-overmixing and during unidirectional deformation. By using a triplet Fermentograph – Maturograph – Oven spring recorder (OTG), rheological behaviour of leavened dough during dough fermentation, proofing and the first stage of baking was monitored; within bakery praxis, the first two mentioned phases of yeasted bread production are also called leavening and maturation (or proofing), respectively.

Within a laboratory baking trial, full-recipe leavened dough was prepared using the farinograph; internal method of the baking test was published earlier (Švec and Hrušková 2010). In the tempered incubator, dough fermentation takes 50 min. Dough splitting and bun moulding is carried out manually, followed by dough proofing phase which takes 45 min. Baking is carried out in a laboratory oven preheated to 240 °C for 14 min, with steaming after full baking plate insertion. After 2 h of cooling at room conditions, bread shape as height-to-diameter ratio is described, specific volume is determined and bread crumb penetration as well as sensory scoring is appointed.

Observed data statistical analysis was performed in the Statistica 13.0 software, using two-factor analysis of variance (Tukey's HSD test at  $P = 95\%$ ) and principal component analysis. Observed factors were non-traditional material type and its addition.

## RESULTS AND DISCUSSION

### *Analytical properties of flour and flour composites*

In terms of protein technological quality, used wheat controls WF1 and WF2 differed in the Zeleny test values significantly (about 20%; **Table 1**), what reflects qualitatively different grain input into mill, as well as diverse agroclimatic conditions in the proper harvest years. The same as quinoa, canahua lowered sediment volumes gradually too, disrupting gluten structures up to statistically similar values. Also, further non-traditional plant materials as naturally gluten-free, especially acorn and nopal flour, demonstrated a similar negative impact (Švec and Hrušková, 2017).

Estimated amylases activity in quinoa and canahua wholemeals were clearly lower than in wheat flour; with respect to the accuracy of the Falling number  $\pm 10\%$ , only quinoa increased the value of WF1 provably (**Table 1**). It corresponds also with higher dietary fibre content.

**Table 1** Effect of quinoa and canahua wholemeals on analytical-technological quality of wheat flour (WF)

Composite flour	Addition (%)	Zeleny test (ml)			Falling Number (s)		
WF1 / WF2	0	55 e	-	40 cd	317 a	-	421 ab
Qui / Can	100	43 d	-	43 d	900 d	-	900 d
WF1+Qui	10 / 20	34 ab	-	30 a	474 bc	-	569 c
WF2+Can	10 / 20	36 bc	-	31 ab	414 ab	-	478 bc

**Rheological properties of flour and non-fermented dough**

Pasting profiles of WF1 and WF2 at the RVA test were considered as typical for such type of wheat flour (semi-bright smooth flour, or soft white flour). Partial replacement of wheat flour brought differences in peak viscosities only, while pasting temperatures and final viscosities (at the end of the test, i.e. in 16<sup>th</sup> min) did not depend on the composite flour tested (**Table 2**). Diverse maximal viscosities reflected a difference in a starch particle size/shape, as well as in ratios of amylose/amylopectin (Pérez et al., 2016).

**Table 2** Effect of quinoa and canahua wholemeals on the selected pasting features of wheat flour (WF)

Composite flour	Addition (%)	Peak temp. (°C)	Peak viscosity (mPa.s)	Final viscosity (mPa.s)
WF1 / WF2	0	87.3 a - 84.7 a	2171 c - 2106 bc	2952 a - 2863 a
WF1+Qui	10 / 20	87.2 a - 86.4 a	2010 ab - 1920 a	2790 a - 2801 a
WF2+Can	10 / 20	86.5 a - 86.5 a	2034 abc - 1947 a	2856 a - 2777 a

WF1, WF2 – wheat flour controls for blends with quinoa and canahua, respectively.

Qui, Can – quinoa and canahua wholemeal, respectively.

a-c: values signed by the same letter are not statistically different ( $P = 95\%$ ).

Farinograph testing (**Table 3A**) confirmed two generally known facts – non-traditional gluten-free plant materials demonstrate usually larger ability to retain water and thus their addition to wheat flour increases water absorption during non-fermented dough preparation. Along with this, a partial prolongation in dough development time, as well as in dough stability occurs. Furthermore, a higher extent of dough consistency weakening (higher Mixing Tolerance Index) could be expected. Although samples WF1+10Qui and WF1+20Qui did not differ in water absorption, higher amount of quinoa was reflected in a somewhat higher decrease of consistency in the latter case only. In case of incorporation of 20% canahua, water absorption unexpectedly decreased about 3 percent, resulting in two-time prolongation of dough stability (the less water, the harder the dough and likely longer stability; data not shown). Within tested flour composites, dough softening as the Mixing Tolerance Index had partially risen to a comparable extent (e.g. from 50 to 80 BU for WF-Q composites; **Table 3A**). From the empirical point of view, values lower than 100 BU are still considered as acceptable – leavened bread volumes likewise maintain comparable.

**Table 3** Effect of quinoa and canahua wholemeals the selected rheological features of wheat flour (WF)

*A. Farinograph test*

Composite flour	Addition (%)	Water absorption (% flour base)				Mixing tolerance index (BU)					
WF1 / WF2	0	62.5	ab	-	65.0	c	50	b	-	24	a
WF1+Qui	10 / 20	63.4	b	-	63.4	b	60	bc	-	80	c
WF2+Can	10 / 20	65.0	c	-	62.0	a	20	a	-	80	c

*B. Extensograph test*

Composite flour	Addition (%)	Ratio ela - ext (-)				Energy (cm <sup>2</sup> )					
WF1 / WF2	0	1.90	a	-	1.78	a	130	b	-	105	ab
WF1+Qui	10 / 20	2.70	b	-	2.90	b	117	ab	-	84	a
WF2+Can	10 / 20	1.74	a	-	4.21	c	81	a	-	102	ab

WF1, WF2 – wheat flour controls for blends with quinoa and canahua, respectively.

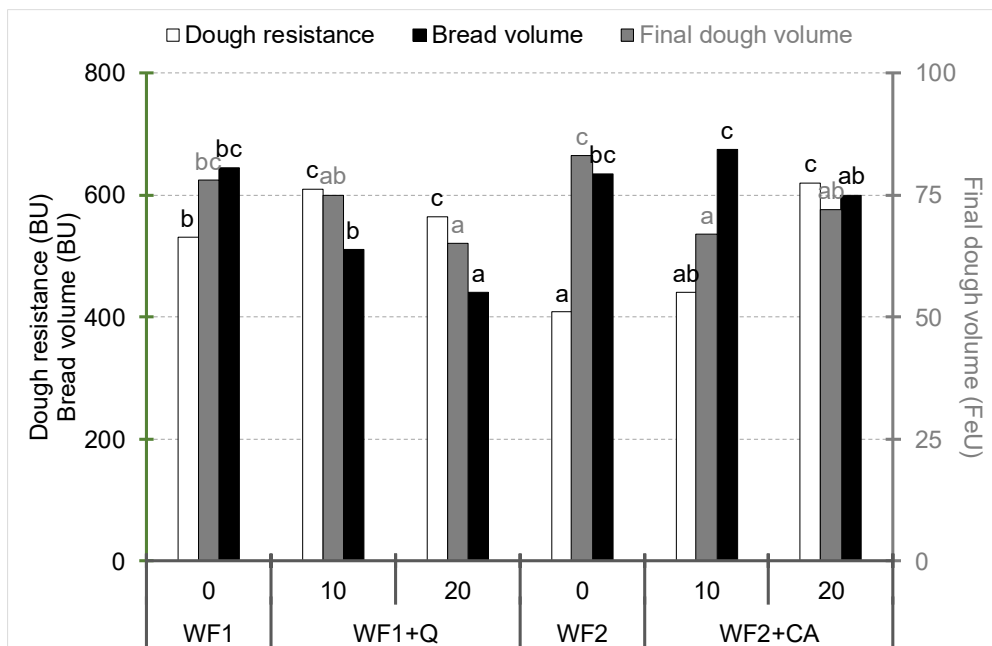
Qui, Can – quinoa and canahua wholemeal, respectively.

a-c: values signed by the same letter are not statistically different ( $P = 95\%$ ).

For wheat flour, produced for leavened bakery goods, optimal values of extensograph elasticity-to-extensibility ratio is between 2.0 and 2.2, and extensigraph energy higher than 100 cm<sup>2</sup>. Considering that practical experience, control WF1 demonstrated higher technological quality than WF2. After 60 min of dough resting, its extensigraph ratio 1.90 was closer to optimum and the extensigraph energy was higher than for WF2 sample, too (130.0 and 104.9 cm<sup>2</sup>, respectively; **Table 3B**). Addition of quinoa affected extensibility of wheat dough mainly, as indicated by the ratio rise (Table 3B). Ten percent dosage of quinoa improved wheat dough machinability close to optimum. Compared to that, 10% of canahua wholemeal in a dough recipe had rather insignificant effect on both elasticity and extensibility of WF dough (energy values 1.74 vs. 1.78). At the same time, areas under curves (energy levels) dropped about 25% (similar values between WF2+10Can and WF1+20Qui). Twofold portion of canahua wholemeal changed dough machinability to an unacceptable state, dough elasticity increased almost threetimes and thus the ratio was multiplied to 4.21 (**Table 3B**).

***Rheological behaviour of fermented dough***

Differences among tested samples during three technological stages of the fermentation process (i.e. leavening, proofing and the first stage of baking) were noticed especially in dough volumes (**Figure 1**). It could be said, that dough prepared from bakery stronger WF1 was partially weakened by quinoa addition, and reversely the WF2 control by canahua addition.



**Figure 1** Effect of quinoa and canahua wholemeals on dough volumes during three consecutive technological phases of fermentation process. Fermentation (leavening): Final dough volume (Fermentograph test), maturation (proofing): Dough resistance (Maturograph test), the first stage of baking: Bread volume (Oven rise apparatus test).

a-c: columns signed by the same letter are not statistically different ( $P = 95\%$ )

### **Baking trial results**

During bread dough preparation, water addition corresponded with the farinograph water absorption in a reversal mode, and in average, they were higher for WF2 and its blends with canahua wholemeal (56.0% vs. 57.2%, respectively; repeatability  $\pm 0.2\%$ ; **Table 4**). Lower dosage of quinoa had an improving effect on the bread volume, although bread shape was partially worsened. At a higher level of addition, it demonstrated a disrupting of gluten skeleton and bread size decrease about 25%. Mentioned results are in agreement with the ones published by Iglesias-Puig et al. (2015). Replacement of wheat flour by 25% whole quinoa flour resulted in generally denser bread – specific volume fell from 448 to 346 cm<sup>3</sup>/100 g and crumb firmness increased twice (0.77 vs. 1.55 N). For all three parameters of bakery quality, the canahua dosages meant stepwise lowering of bread quality (**Table 4**, **Figure 2**). In terms of a sensory profile, wheat-quinoa bread reached a somewhat better score, as more homogenous porosity was identified, weaker stickiness during crumb mastication as well as intensity of strange flavour. On the other hand, Iglesias-Puig et al. (2015) determined worsening of the composite bread acceptability (from 7.94 to 7.58 points). Benefits from quinoa nutrition were confirmed, as well as the increase in fibre ratio and in portion of calcium, iron and zinc.

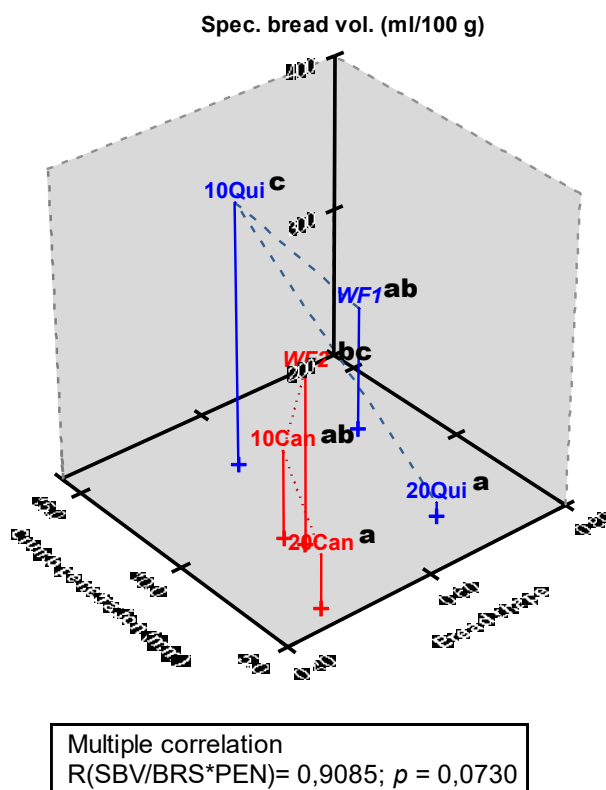
**Table 4** Effect of quinoa and canahua wholemeals on the bread baking test results (WF)

Composite flour	Addition (%)	Recipe water addition (%)	Specific bread volume (cm <sup>3</sup> /100 g)	Bread shape (-)
WF1 / WF2	0	58.0 d - 56.5 bc	282 ab - 313 bc	0.72 c - 0.53 abc
WF1+Qui	10 / 20	55.5 ab - 54.5 a	371 c - 208 a	0.57 abc - 0.68 bc
WF2+Can	10 / 20	57.6 cd - 57.5 cd	258 ab - 235 a	0.52 ab - 0.47 a

WF1, WF2 – wheat flour controls for blends with quinoa and canahua, respectively.

Qui, Can – quinoa and canahua wholemeal, respectively.

a-d: values signed by the same letter are not statistically different ( $P = 95\%$ ).

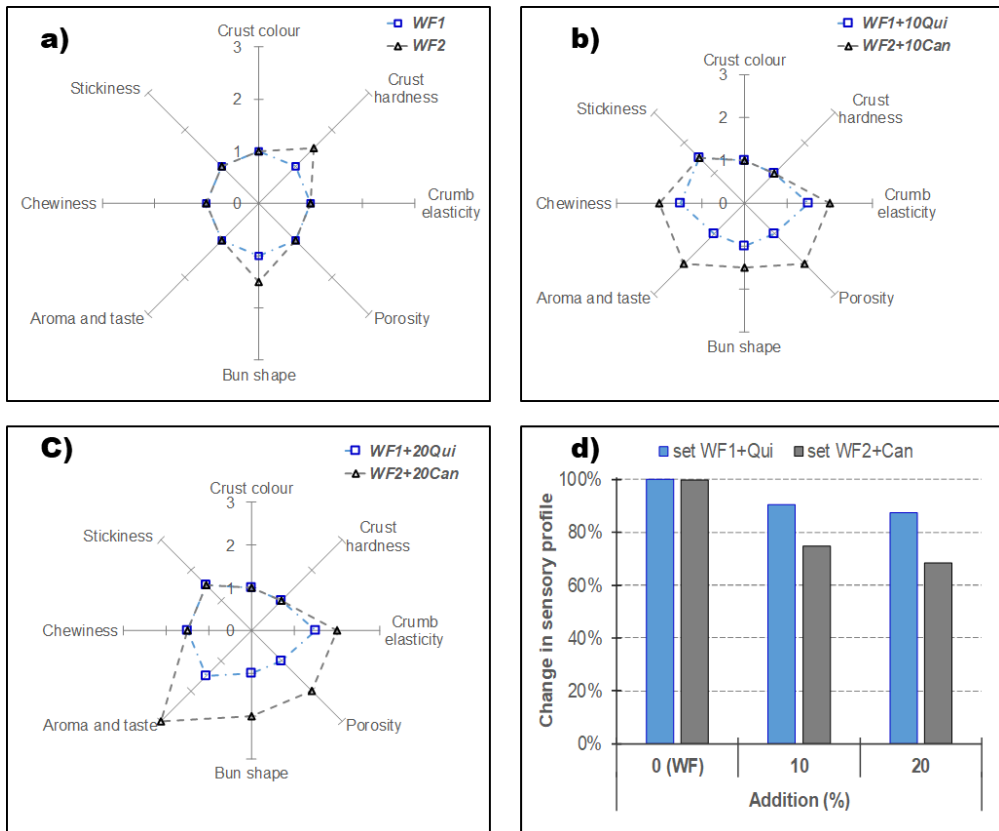


**Figure 2** Enhancement effect on wheat bread properties. Variance of the specific bread volumes.

SBV – specific bread volume, BRS – bread shape (height-to-diameter ratio), PEN – crumb penetration; a-c: samples signed by the same letter are not statistically different ( $P = 95\%$ )

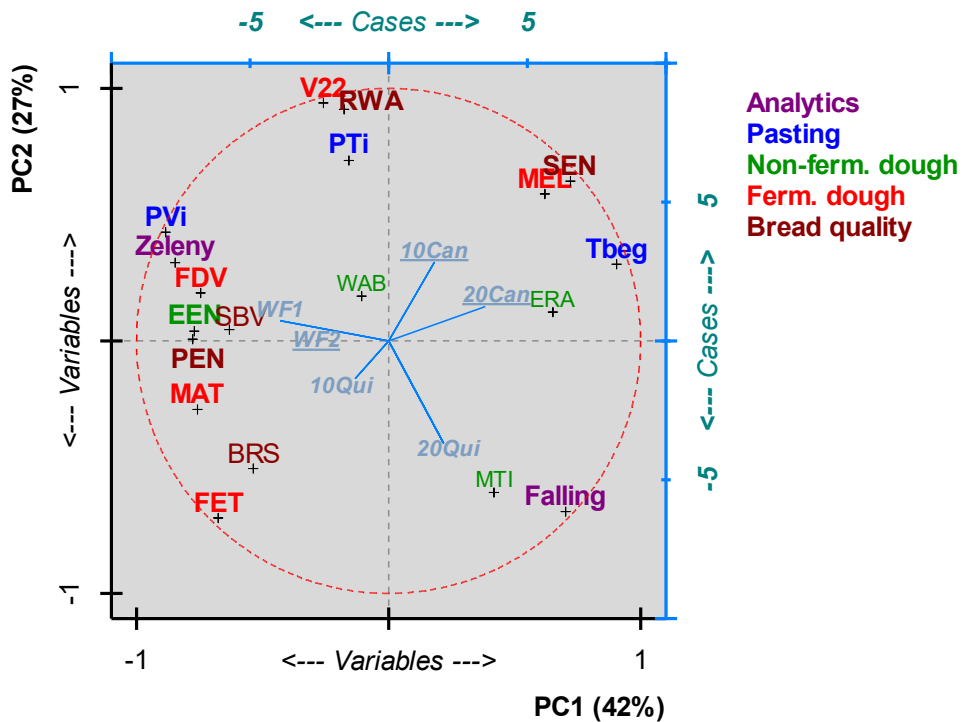
**Statistical analysis**

The first two principal components explained 69% of data variability, grouping determined quality parameters into three or four clusters. From a technological point of view, the largest cluster confirmed dependence of the specific bread volume and crumb penetration rate on characteristics through the whole complex quality assessment (namely Zeleny test, RVA Peak viscosity, extensograph energy, fermentograph final volume of dough and on maturograph optimal leaving time).



**Figure 3** Sensory profiles of wheat bread with added quinoa and canahua wholemeals (in points).

a) non-fortified wheat controls, b) addition 10%, c) addition 20%; d) relative worsening of sensory score of wheat bread by additions of quinoa and canahua wholemeals. Notice: 1 point means typical, normal, or usual; 3 points mean negative, almost unacceptable change



**Figure 4** Principal components biplot of loadings (variables, indicated by points) and score plot (cases – samples, indicated by vectors). Analytical parameters: Zeleny – Zeleny test, Falling – Falling number; Farinograph parameters: WAB – water absorption, MTI – mixing tolerance index (degree of dough softening); Extensigraph parameters: ERA –elasticity-to-extensibility ratio, EEN – energy; Amylograph parameter: Tbeg – temperature of flour gelatinization; RVA parameters: PTi – (viscosity) peak time, PVi – peak viscosity; Fermentograph parameters: FET – optimal fermentation time, FDV – final dough volume; Maturograph traits: MAT – optimal maturation time, MEL – maturograph dough elasticity; OTG parameter: V22 – Final bread volume. Baking trial parameters: RWA – recipe water addition, SBV- specific bread volume, BRS – bread shape (height-to-diameter ratio), PEN – crumb penetration, SEN – bread sensory profile. Notice: variables less important for tested samples distinguishing are indicated by smaller non-bold letters.

## CONCLUSIONS

Replacement of wheat flour by wholemeals from quinoa and canahua seeds at levels 10 or 20 wt.% provably modified technological quality of both proteins and polysaccharides, quantified by analytical and rheological proofs and directly by baking trial. The results showed worsening of dough machinability by the addition of 20%, confirmed clearly by depreciation in specific bread volumes and other quality features. In this regard, 10% of both quinoa and canahua wholemeals was



considered as an acceptable enrichment rate. Properties of such doughs and breads were almost comparable to wheat control, but nutrition score was supported in terms of dietary fibre and some minerals.

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## ULTRASOUND SONICATION EFFECTS ON THE PASTING PROPERTIES OF BUCKWHEAT FLOUR

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### SUMMARY

Buckwheat (*Fagopyrum esculentum* Moench) is a non-cereal plant classified as a pseudocereal due to grain containing a significant amount of starch. As a naturally gluten-free plant, it is perfect raw material for the formulation of gluten-free products, although its specific taste and aroma can hinder its widespread usage. Although the specific aroma, which is especially pronounced after a thermal treatment, may restrict its application in food products, buckwheat flour can be used as a thickener in many semi-liquid products. However, high protein content, as well as the presence of other polysaccharides results in high viscosity of buckwheat flour gels.

The main objective of this study was the assessment of the pasting properties of buckwheat flour made from hulled buckwheat grains subjected to ultrasound processing at 40 kHz in different solid:liquid ratios (1: 10, 1:5, 1:2.5).

The ultrasound treatment was observed to have a different impact depending on the used solid:liquid ratio. The 1:10 ratio seemed to have no strong impact on flour pasting properties, while the 1:5 ratio increased, and the 1:2.5 ratio decreased peak viscosity. The final viscosity was the lowest in the sample with the 1:2.5 solid:liquid ratio. The breakdown and setback viscosity were similar for 1:10 and 1:2.5 samples, while the 1:5 ratio samples were highest and lowest, respectively. The pasting curves seemed to be flattened, indicating more stable vs. temperature changes behaviour.

**Keywords:** buckwheat flour, ultrasound treatment, pasting properties

### INTRODUCTION

In order to meet the needs of the growing population, new foods are continuously being studied. The other objective of food engineering is to improve nutritional and healthy characteristics of food. An excellent solution for the formulation of new gluten-free products is the use of alternative crops or underused species. Pseudocereals largely meet these requirements due to their excellent nutritional profile, being rich in phenolic and phytochemical compounds and the absence of

gluten proteins in their composition. In addition, the amino acid profile and nutritional properties, such as the essential amino acid index, biological value, protein efficiency ratio and nutritional index of pseudocereals are higher than in conventional cereals, such as rice and maize. Therefore, the use of pseudocereals in the diet would help to fight various health issues, and would also represent a progress towards an adequate supply of nutrients in the diet of people with celiac disease (Mir, 2018).

As known, gluten network is essential to obtain soft baking goods with special texture and good sensory characteristics. Consumers that must eat gluten-free bakery products (e.g. bread) demand products having quality characteristics as similar as possible to those obtained with wheat. For this purpose, different types of ingredients/additives are normally used in the formulations of gluten-free foods in order to imitate the viscoelastic properties of gluten and thus to improve the overall characteristics of the finished products. However, the presence of additives and usually the higher fat content in gluten-free products often do not satisfy consumer demand, as consumers are now more dedicated to simple and minimally treated foods, made with few and usual ingredients. As an alternative to the use of ingredients and additives, ultrasound (US) technology is also successfully used during the processing of gluten-free types of dough to improve their physical characteristics. The main objective of this study was the assessment of the pasting properties of buckwheat flours obtained from the dehulled buckwheat grains subjected to ultrasound processing at 40 kHz in different solid:liquid ratios (1: 10, 1:5, 1:2.5).

## **MATERIALS AND METHODS**

The Polish buckwheat (*Fagopyrum esculentum* Moench) of *Kora* variety was purchased locally from the farmers' association named Dolina Gryki Sp. z o.o. Grupa Producentów Ekologicznych, Międzylesie, Poland. All the reagents were of an analytical grade and distilled water was used for dilutions.

Buckwheat grain samples were treated in ultrasound bath of 5 l capacity (WAH LUEN ELECTRONICS, China) of working frequency 45Hz and maximum power 100 W for 15 min in different solid:liquid ratio – 1:10, 1:5 and 1:2.5. For the evenly exposure to ultrasound, the grain was agitated gently with two RW20 stirrers (Janke&Kunkel, Germany) at a speed of 90 rpm. Precisely, 250 g, 500 g and 1000 g of buckwheat were weighed respectively and immersed in the treatment tank containing 2.5 L of distilled water. Two control samples were also prepared; the control sample of the untreated grain and the control sample of grain were washed in distilled water for 15 min in ratio 1:10 in with same agitation as treated samples. The second control was made with the purpose to distinguish the impact of ultrasound on grains from the impact of grain soaking and washing. Finally 5 different samples were prepared as follows:

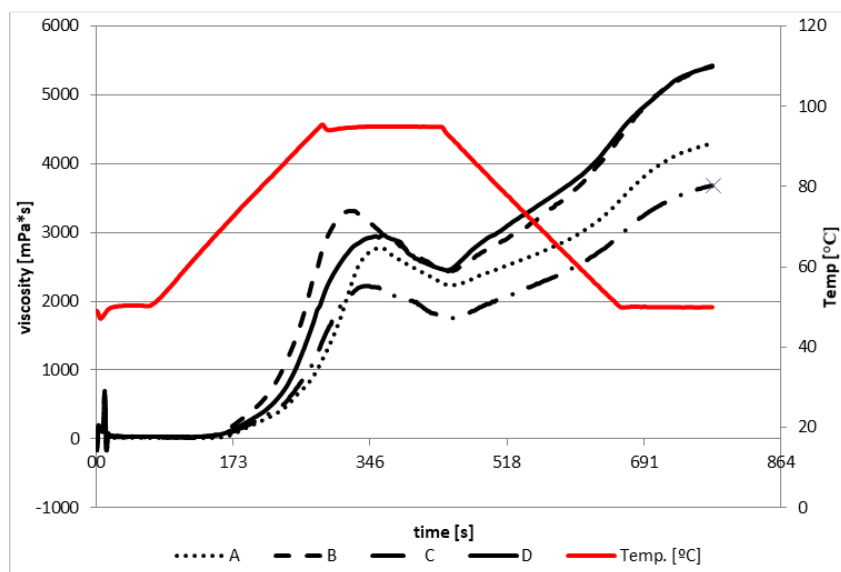
- A. Polish buckwheat ultrasound treated in a water tank in a ratio of 1:10;
- B. Polish buckwheat ultrasound treated in a water tank in a ratio of 1:5;

- C. Polish buckwheat ultrasound treated in a water tank in a proportion of 1:2.5;
- D. Untreated Polish buckwheat – control sample;
- E. Polish buckwheat mixed in the water tank in a ratio of 1:10.

After all treatments, the grain samples (A, B, C, E) were dried for 24 hours at 55° C in the laboratory oven (SML, Zalmed, Poland). After drying, all samples were milled on a stone mill Fidibus21 (KoMo, Germany) and sieved for fraction unification on the 500 mm mesh sieve (CISA, Spain) on the sieve shaker LAB-11-200/UP (EKO-LAB, Poland).

## RESULTS AND DISCUSSION

The pasting curves of buckwheat flours after ultrasonication in different water:solid ratios are presented in **Figure 1**. It is clearly seen that the buckwheat flour pasting profiles were modified vs. the native one regardless the sample. The ratio 1:5 of solid:liquids was most effective for peak viscosity increase, while both ratios of 1:10 and 1:2.5 resulted in lowering of peak viscosity. The sample from ultrasound treatment of 1:2.5 ratio showed the lowest values of both peak and final viscosity.



**Figure 1** Pasting curve of buckwheat flours after ultrasonication in different solid:liquid ratios.

A - buckwheat treated in a ratio of 1:10; B - buckwheat in a ratio of 1:5; C - buckwheat treated in a ratio of 1:2.5; D - buckwheat – control sample

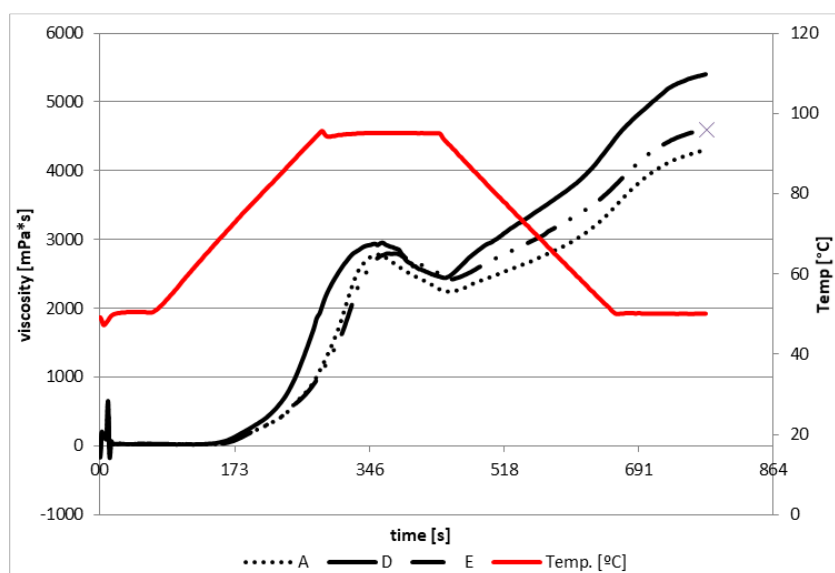
The pasting parameters of buckwheat flour are shown in **Table 1**. The peak viscosity was lowered for 1:10 and 1:2.5 solid:liquid ratio samples about 6% and 3% respectively, as well as final viscosity which was lowered for 18% and 16%. The unique sample with the both increased values was sample of 1:5 solid:liquid ratio.

**Table 1** Pasting parameters of buckwheat flours after ultrasonication in different solid:liquid ratios

	I	A	B	C	D
PV [mPa*s]		2645.00±29.70 a	3063.50±0.71 d	2789.50±19.09 b	2860.50±13.44 c
TV [mPa*s]		2224.00±104.65 a	2375.00±15.56 b	2218.50±55.86 a	2428.00±22.63 b
FV [mPa*s]		4434.00±108.89 a	5014.50±13.44 b	4535.00±42.43 a	5267.00±42.43 c
BD [mPa*s]		421.00±74.95 a	688.50±114.85 c	571.00±74.95 bc	432.50±36.06 a
SB [mPa*s]		2210.00±4.24 a	2639.50±2.12 c	3216.50±13.44 b	2839.00±65.05 d
PT [°C]		84.40±0.14 d	79.90±0.01 a	83.05±0.07 c	81.95±0.07 b

A - buckwheat treated in a ratio of 1:10; B - buckwheat in a ratio of 1:5; C - buckwheat treated in a ratio of 1:2.5; D - buckwheat – control sample; PV – peak viscosity, TV – through viscosity, FV – final viscosity, BD – breakdown viscosity, SB – setback viscosity, PT – peak temperature, mean values with different lowercase letters imply significant differences between means in rowat  $p < 0.05$ .

Every processing of the sample can impact the characteristics of pasting properties of flour. The supporting process of soaking and agitation was also able to lower the pasting characteristic parameters. The pasting curves of flour samples treated in 1:10 solid:water ratio are shown in **Figure 2**.



**Figure 2** Pasting curve of buckwheat flour after ultrasonication in 1:10 solid liquid ratio vs. wet and dry control sample. A - buckwheat treated in a ratio of 1:10; D - buckwheat – control sample; E – buckwheat – wet control sample.

The modification of flour pasting profile only after mixing sample in water was more pronounced by the ultrasound treatment. However, the basic change was obtained just after water treatment. Such behaviour is related to washing out the broken particles of starch granules which release amylose into the solution when left in the sample.

Both peak and final viscosities were significantly lowered compared to the dry untreated sample, while through viscosity was similar in all samples. Also the breakdown viscosity was not changed, but the significant difference was visible for setback viscosity parameter. This parameter reflects retrogradation or re-ordering of starch components like amylose and amylopectin. The value of setback is highly correlated with texture of different products because high setback value means more pronounced syneresis. The highest setback was observed for native untreated buckwheat flour, while lowest and more stable sample was obtained from an ultrasound treated sample.

**Table 2** Pasting parameters of buckwheat flour after ultrasonication in 1:10 solid liquid ratio vs. wet and dry control sample

I	A	D	E
PV [mPa·s]	2645.00±29.70 a	2860.50±13.44 c	2802.00±29.70 b
TV [mPa·s]	2224.00±104.65 a	2428.00±22.63 b	2321.00±36.77 ab
FV [mPa·s]	4434.00±108.89 a	5267.00±42.43 c	4832.50±23.33 b
BD [mPa·s]	421.00±74.95 a	432.50±36.06 a	481.00±7.07 ab
SB [mPa·s]	2210.00±4.24 a	2839.00±65.05 c	2511.50±60.10 b
PT [°C]	84.40±0.14 b	81.95±0.07 a	85.35±0.49 c

A - buckwheat treated in a ratio of 1:10; D - buckwheat – control sample; PV – peak viscosity, TV – through viscosity, FV – final viscosity, BD – breakdown viscosity, SB – setback viscosity, PT – peak temperature, mean values with different lowercase letters imply significant differences between means in rowat  $p < 0.05$ .

## CONCLUSIONS

The pasting properties were significantly changed after the ultrasound treatment. The impact of the treatment strongly depended on solid:liquid ratio during ultrasonication. The peak viscosity was lowered for 1:10 and 1:2.5 samples and also final viscosity was lowered for 18% and 16%, respectively. The sample with opposite behaviour was sample of 1:5 solid:liquid ratio where both peak and final viscosity were increased.

The pasting properties play a very important role in the choice of the different types of flours depending on the production needs, for the use in industry as a thickener, or any other use. Ultrasound treatment smoothens the pasting curves of buckwheat

flour and offers opportunity to obtain stable behaviour of this valuable ingredient widening its potential application in food industry.

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## POSITIVE EFFECTS OF BARLEY ON HUMAN HEALTH

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### SUMMARY

Significant increase in cardiovascular (coronary heart disease, hypertension and stroke) and metabolic diseases (type 2 diabetes, metabolic syndrome and insulin resistance) as well as the growth of global obesity represent major problems of today. A proper and nutritiously balanced diet is one of the main prerequisites for the healthy way of life. Due to the increased quantity of scientific evidence that the wholegrain and wholegrain-based products not only provide energy and nutrition, but also have a positive effect on human health, there are more and more recommendations to include these ingredients in our everyday diet. Barley is the fourth most often produced cereal in the world with the annual production of around 140 million tons. It is rich in nutrients and functional ingredients and is an important source of beta-glucan.

Based on scientific opinions, the European Food Safety Authority (EFSA) has approved three health claims pursuant to the Article 13(1) and two health claims pursuant to the Article 14(1)(a) of Regulation (EC) No 1924/2006 which are connected with barley and beta glucan from barley. Although barley has been used since ancient times, new scientific findings give additional stimulation to users to consume barley, but also to the food industry to develop barley-based products.

The paper gives an overview of the most important barley producers in the world and the state of barley production in the Republic of Croatia as well as the most important health benefits of barley and barley products with approved health claims by the European Food Safety Authority (EFSA).

**Keywords:** barley,  $\beta$ -glucan, cardiovascular and metabolic diseases, health statement, functional food

### INTRODUCTION

Progress in the science of nutrition, technology and food production has greatly reduced illnesses that are caused by nutrient deficiencies, but at the same time it faces the growing challenges of obesity, non-contagious diseases and aging (Shao et



al., 2017). Epidemiological studies have shown that regular consumption of whole grains protects against cancer, cardiovascular diseases, diabetes and obesity (Slavin, 2004). The consumption of whole grain and a healthy lifestyle is greatly associated with a lower incidence of chronic diseases or the metabolic syndrome (Hrg, 2017). Cereals and grain products are an important source of energy and a significant part of a balanced diet. In spite of the recommendations that it is necessary to consume three servings of whole grains daily, in western countries the consumption is often much lower, around one portion per day (Slavin, 2004). However, these recommendations apply to people who do not avoid cereals in their diet for medical reasons.

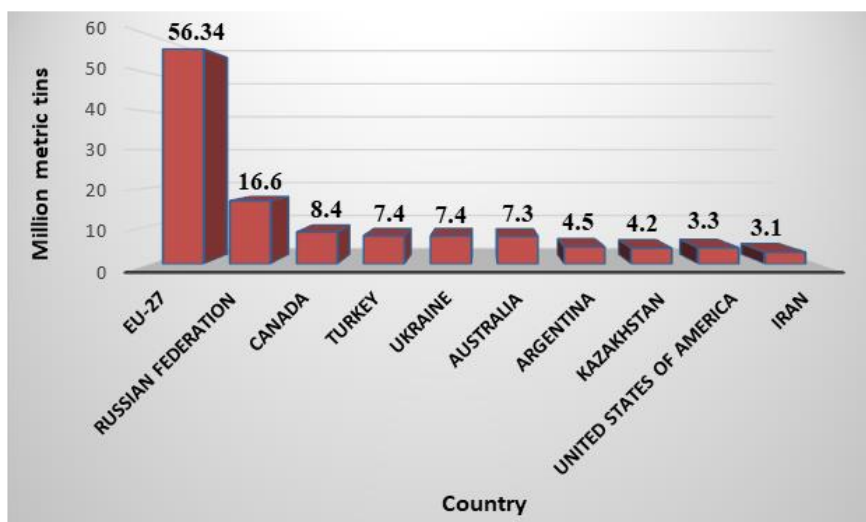
Barley is a grain that has been used in human nutrition for generations. Except for human nutrition, it is used for animal feed, barley malting, in pharmaceutical industry, etc. Recently, the European Food Safety Authority (EFSA) has approved health claims related to barley and barley beta-glucan which can be considered „functional foods“. According to Regulation (EC) 1924/2006 on nutrition and health claims made on foods, health products must be labeled in an appropriate manner. Claims on such products are emphasized on the label and suggest that the product can be nutritious and healthy and can assist in alleviating, improving, regulating, increasing, and / or decreasing certain organism states (Pollak, 2008).

The paper gives an overview of the most important barley producers in the world and the state of barley production in the Republic of Croatia as well as the most important health benefits of barley and barley products with approved health claims by the European Food Safety Authority (EFSA).

#### **WORLD PRODUCTION OF BARLEY**

Barley (*Hordeum vulgare* L.) is a one-year grass plant (*Poaceae*), and it is considered to be one of the oldest grains in Europe. With corn, wheat, rice and millet, this grain is a dominant culture in more than two thirds of the world's cultivated area, and it occupies about 9% of farmland surfaces (Leff et al., 2004). There are around 55 million ha in the world that produce a yield of about 140 million tons of barley, making it fourth most produced cereal, after corn, rice and wheat (Zhou, 2009). The largest producers in the world are the European Union, Russia, Canada, Ukraine, Turkey and Australia (**Figure 1**). The EU, Australia, Ukraine and Russia are exporters, while Saudi Arabia, China and Iran are the largest importers globally (Global Barley Market and Current Situation, 2019). Among grains, barley has the largest range of distribution, 10 to 70° north latitude, which is explained by high polymorphism and resistance to unfavourable conditions of cultivation. It also grows at high altitudes, over 4000 meters (Gagro, 1997). In 2017, in Croatia, the harvesting area of barley was 53 950 ha with the production of 260 426 t/ha, which is a natural yield of 4.8 t/ha (CBS, 2018). Unlike wheat and corn, which showed a significant decline in production, barley production was approximately the same as in 2016 (**Table 1**). Although barley has a long tradition of growing in Croatia, its production is less than 0.5% of the total world production (Agroklub, 2017). After analyzing the long-term

meteorological data, it was concluded that the yield of barley is more influenced by weather conditions than by varieties/hybrids or agrotechnical interventions (Klink et al., 2011).



**Figure 1** The largest producers of barley in the world (Web 1)

The introduction of more productive varieties and modern agrotechnics certainly contributed to the increase of yield. Choosing barley variety primarily requires considering its purpose. Approximately 65% of cultivated barley is used as animal feed, 33% for malt production, while only 2% is used directly for human consumption (Sullivan et al., 2013). However, lately, we are witnessing the results of numerous studies that indicate a positive impact of barley on human health. Therefore, some new barley varieties with increased beta-glucose content, desirable starch composition and improved grinding/processing properties have been created especially for use in human nutrition (Ames and Rhymer, 2008), in order to maximize the nutritional and health benefits of barley.

**Table 1** The production of the most important cereals in Croatia, 2013-2017 (Web 2, Web 3, Web 4)

	2013	2014	2015	2016	2017
<b>Wheat</b>	998 940	648 917	758 638	960 081	682 322
<b>Maize</b>	1 874 372	2 046 966	1 709 152	2 154 470	1 559 638
<b>Barley</b>	201 339	175 592	193 451	263 165	260 426
<b>TOTAL</b>	<b>3 074 651</b>	<b>2 871 475</b>	<b>2 661 241</b>	<b>3 377 716</b>	<b>2 502 386</b>

## **HEALTH BENEFITS OF BARLEY AND PRODUCTS OF BARLEY**

It is an indisputable fact that the lifestyle of the majority of the world's population represents a great danger for the development of modern age diseases. Poor nutrition and the lack of physical activity, obesity and smoking are risk factors aiding the development of cardiovascular diseases. Coronary Heart Disease (CHD) is the primary cause of mortality on a global scale and is considered a direct or indirect cause of a quarter of all deaths in developed countries (Lloyd-Jones et al., 2009). In Croatia, arterial hypertension is the leading risk factor for overall mortality, and cerebrovascular diseases, which represent the most important complication of hypertension, have been the second on the list of individual causes of deaths (HZJZ, 2017) for years. According to the World Health Organization, cardiovascular diseases are the cause of death of 17.3 million people worldwide or cause 30% of total mortality (Kralj, 2012). In Croatia, there were 23,504 deaths caused by cardiovascular disease (CHD) in 2017 which accounted for 44% of total mortality (HZJZ, 2017). As much as 80% of premature deaths from CHD could be avoided by modifying and controlling risk factors – refraining from smoking, consuming healthy food and controlling arterial hypertension, diabetes, and elevated lipid levels (Benjamin et al., 2019).

Physical inactivity, poor diet and sedentary lifestyle also significantly contribute to the development of obesity, metabolic insulin resistance and type 2 diabetes (Metelko and Crkvenčić, 2004). By changing lifestyle, the frequency of metabolic disorders that make up the metabolic syndrome could be drastically reduced (Kuzmanić et al., 2008).

The increasing concern about the growth of obesity and non-chronic diseases at both global and national levels has prompted scientists to focus their work and research on prevention, in cooperation with the industry and relevant institutions. Throughout the world, food guidelines recommend the inclusion of whole grain and grain based products in people's daily diet due to their positive health effects (Herforth et al., 2019). The latest scientific knowledge and consumer information on positive health effects of those products has contributed to a greater interest in consumption and investment in the development of new products in the area of functional food and functional food ingredients. In this respect, barley has been recognized as a suitable food with a numerous identified positive effects on health. Barley is a rich source of nutrients due to its high concentration of carbohydrates, a moderate protein concentration, high dietary fiber content, especially  $\beta$ -glucan, and is also a good source of phosphorus and potassium (Kumari and Kotecha, 2015). Barley grain, based on dry matter, contains 59-68% of nitrogen-free extract, 1.39-3.9% of fat, 9-17% of protein, 12.6-22.6% of fibers and 2.3-3% ash (Pospišil, 2010). Barley is a particularly important source of fiber and beta-glucan. Beta-glucan is a linear D-glucose polymer bound to  $\beta$ - (1-4) and  $\beta$ - (1-3) glucosidic bonds (Wang and Ellis, 2014). Beta-glucan is found in all grains, but its concentration is the highest in oats (4.6-4.9%) and barley (1.8-6%) (Schönlechner and Berghofer, 2006). The variety of barley, especially rich in beta-glucan fibers, is digested more slowly than

standard barley varieties and can be very useful for obese and diabetic patients (Lifschitz et al., 2002).

Research on laboratory animals showed that male rats, fed with barley bran over 8 weeks, even in two low doses, showed a decrease in hyperlipidemia caused by cholesterol intake; total cholesterol, triglycerides, LDL cholesterol and VLDL particles in the blood decreased while HDL cholesterol levels were elevated in the treated groups. The addition of barley bran has beneficial effects on the liver, the heart, and the kidneys, and some authors have noted the increase of dose-dependent effects (Abulnaja and El Rabey, 2015).

Researchers from the University of California fed 11 healthy men with two test meals, both with beta-glucan. One meal was high-fiber barley pasta (15.7 g), while the second meal was wheat pasta with low fiber content (5.0 g). The obtained results showed that the barley pasta removed the insulin response for up to four hours after the meal and that the tested men had a significantly lower cholesterol concentration than after consuming wheat pasta (Bourdon et al., 1999). Also, the increased consumption of barley based food products can be considered a dietary approach to reducing LDL-cholesterol concentrations (AbuMweis et al., 2010). The European Food Safety Authority (EFSA) has approved three health claims in accordance with Article 13 (1) and two health claims in accordance with Article 14 (1) (a) of Regulation (EC) No. 1924/2006 related to barley and barley beta-glucan (**Table 2**).

Considering the increasing awareness of the growing importance of preventing all diseases of modern civilization, quality and nutritionally balanced diet will be one of the prerequisites of quality and healthy living. However, there is a need for additional efforts and the cooperation of health and nutritional experts in order to contribute to a healthy lifestyle or to healthy eating habits through joint actions and synergy.

**Table 2** Approved health claims (part I) (Web 5)

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<b>Art.13(1)</b>	Barley grain fibre	Barley grain fibre contributes to an increase in faecal bulk	The claim may be used only for food which is high in that fibre as referred to in the claim HIGH FIBRE as listed in the Annex to Regulation (EC) No 1924/2006.	No increase in faecal bulk
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**Table 2** Approved health claims (part II) (Web 5)

<b>Art.13(1)</b>	Beta-glucans	Beta-glucans contribute to the maintenance of normal blood cholesterol levels	The claim may be used only for food which contains at least 1 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources per quantified portion. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these beta-glucans.	maintenance of normal blood cholesterol concentrations
<b>Art.13(1)</b>	barley	Consumption of beta-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal	The claim may be used only for food which contains at least 4 g of beta-glucans from oats or barley for each 30 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained by consuming the beta-glucans from oats or barley as part of the meal.	reduction of post-prandial glycaemic responses

**Table 2** Approved health claims (part III) (Web 5)

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<b>Art.14(1)(a)</b>	Barley beta-glucans	Barley beta-glucans has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease	"Information shall be given to the consumer that the beneficial effect is obtained with daily intake of 3 g of barley beta-glucan. The claim can be used for foods which provide at least 1 g of barley beta-glucan per quantified portion."
<b>Art.14(1)(a)</b>	Barley beta-glucans	Barley beta-glucans has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.	"Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of barley beta-glucan. The claim can be used for foods which provide at least 1 g of barley beta-glucan per quantified portion."

## CONCLUSIONS

Improving nutritional habits, moderate physical activity, and healthy lifestyle have shown positive effects on human health, especially in reducing chronic non-contagious diseases. Approved health claims related to barley are a scientific proof of its important role in nutrition. The food industry's investment in product development and consumer education has an important role in promoting healthy eating habits and is an inevitable partner for health institutions in improving and preserving health. Only through the joint efforts and actions of all relevant factors can we achieve satisfactory results for the improvement of consumer habits and healthy lifestyle which will ultimately result in a better health status of the population and better quality of life.

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## POSSIBLE APPLICATIONS OF BREWER'S SPENT GRAIN IN THE PRODUCTION OF BREAD AND PASTRY

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### SUMMARY

Brewer's spent grain (BSG) represents up to 85% of the total residue from the brewing process (approximately 20 kg/hl of beer), and if including wastewater, it is the second largest by-product in brewing industry. It is usually landfilled or used as animal feed, but as a lot of breweries are situated in urban areas, they often have problems in getting rid of this by-product. Having in mind that approximately 3.4 million tons of BSG is produced in the European Union annually, it is important to consider other applications of this interesting raw material. Some of research has been done on investigating different possibilities of incorporating cereal by-products as functional ingredients of various foods, including breads, cereal bars, extrudates and also pasta. Brewer's spent grain has very good nutritional properties, not only for livestock, but also for humans. It is lignocellulose material rich in hemicellulose, proteins, cellulose, lipids and lignin, but can also be characterized as a good source of phenolic compounds and some minerals. Considering that, it has a great potential to be used as a good source of dietary fibre in human nutrition. When dried and milled into flour it can easily be used in bakery products as a rich source of functional ingredients. Finding other possible applications for BSG, especially in bread and pastry production, can lead to the minimization of environmental impact, can be beneficial for businesses as a cheap or no-cost material and can have improved value for consumers.

**Keywords:** brewer's spent grain, raw material, bread, functional food

### INTRODUCTION

Brewer's spent grain (BSG) is produced in a brewhouse of the brewery during wort production, and represents used malt grains which were previously milled depending on subsequent lautering method. Majority of the starch, including some proteins, lipids, polyphenols and other compounds are washed from malt grains during the mashing and sparging processes. What is left is a seed coat, i.e., a husk including more or less starchy endosperm and walls of empty aleurone layer,

depending on the efficiency of the mashing process. Because of several factors which affect its chemical composition, BSG can be quite a heterogeneous substance. The reasons can be: various amounts and contents of different types of malt which sometimes include unmalted cereals; time of barley harvesting; type of hops added; the malting and mashing regime and possible addition of adjuncts or other brewery by-products such as spent yeast and auxiliary filtration agents (Steiner et al., 2015). Considering the variations of different brewing recipes that use various amounts and contents of different types of malt which sometimes include unmalted cereals, time of barley harvesting, type of hops added, the malting and mashing regime and possible addition of adjuncts or other brewery by-products such as spent yeast and auxiliary filtration agents, BSG can be quite a heterogeneous substance (Steiner et al., 2015). However, Santos et al. (2003) have investigated different samples of BSG from one brewery and concluded that different lots of spent grain from the same brewery have only small variations in composition. BSG is composed of protein (31% dry weight), pentosans (19%), lignin (16%), starch and  $\beta$ -glucans (12%), cellulose (9%), lipids (9%) and ash (4%) (Prentice and Refsguard, 1978). **Table 1** shows the diverse chemical composition of BSG which is the result of the aforementioned factors.

**Table 1** Chemical composition of brewer’s spent grains (Kieran et al., 2016)

Component	1	2	3	4	5	6	7	8	9	10	11
Hemicellulose (arabinoxylan)	21.8	n.d.	29.6	41.9	28.4	22.5	40	n.d.	22-29	22.2	19.2
Cellulose	25.4	n.d.	21.9	25.3	16.8	0.3	12	31-33		26.0	21.7
Starch	n.d.	n.d.			n.d.	1	2.7	10-12	2-8		
Protein	24	31	24.6	n.d.	15.2	26.7	14.2	15-17	20-24	22.1	24.7
Lignin	11.9	16	21.7	16.9	27.8	n.d.	11.5	20-22	13-17	n.d.	19.4
Lipids	10.6	3-6	n.d.		n.d.	n.d.	13	6-8	n.d.		
Ash	2.4	4.0	1.2	4.6	4.6	3.3	3.3	n.d.	n.d.	1.1	4.2
Phenolics	n.d.	1.7-2.0	n.d.		n.d.	n.d.	2.0	1.0-1.5	0.7-0.9		

All values are expressed in g per 100 g dry matter (%w/w); n.d. – not determined  
 1-11 – research papers from which the values were taken

With up to 40% of dry weight, the main constituent of BSG is hemicellulose which is primarily made up from arabinoxylan, the main non-cellulose polysaccharide in cereals and grasses. Cellulose is another abundant polysaccharide in BSG, and low levels of  $\beta$ -glucan and starch can also be present. Another significant constituent of BSG is lignin, representing about 10-28% of total dry weight. It is poly-phenolic macromolecule with complex structure, important in maintaining the structural rigidity and integrity of plant cell walls (Lynch et al., 2016). Depending on barley variety, possible addition of adjuncts and other factors, protein content in BSG can vary considerably, but is typically present at levels of around 20% on dry weight basis. The most abundant are hordeins, gluteins, globulins and albumins (Celus et al., 2006). Approximately 30% of the total protein content is represented by the essential amino-acids, with lysine being the most abundant (14.3%) (Waters et al., 2012) what can be significant as this amino acid is usually deficient in cereal foods. BSG is possibly the most underutilized by-product of the brewing industry, as it is generally used as low-value animal feed which has very low market value of around €35 per tonne. Due to its good chemical composition and potential good nutritional value (high fibre and protein content), it is more and more considered as a rich food supplement in human diet, but can also be used in non-food applications. This is especially interesting because of the abundance of this brewery by-product or raw material. It is estimated that annual global production of BSG is around 39 million tons, with approximately 3.4 million tons produced in the European Union (Mussatto, 2014). Therefore, due to its low cost, abundance and nutritional value, it can be used in products with improved value for consumers, bringing benefits for businesses and minimizing environmental impact and risk to human health.

## **VERSATILITY OF BREWER'S SPENT GRAINS**

### ***Non-food and animal feed applications***

More and more studies are carried out on finding the possible treatment techniques for utilizing BSG in energy production. Pinheiro et al. (2019) have concluded that combined intensification approaches qualify BSG waste as a valuable renewable resource for cost-effective ethanol production. Because of its high carbon content BSG can be used for the production of hydrochar using hydrothermal carbonization (HTC), a thermochemical wet process for the production of a variety of products, higher energy density fuels among others, at moderated temperatures. Arauzo et al. (2019) extracted protein fraction from the BSG biomass before HTC and evaluated the effect of the protein extraction on the hydrochar properties. They found out that hydrochar yield from HTC processing at 220 °C was decreased after protein extraction, but carbon/nitrogen ratio increased, making it better for energy production.

Except for the energy production, hydrochar can also be used as an efficient adsorbent, but producing it from BSG and using it for this purpose would not be very cost-effective. Fortunately, this is not necessary as "raw" BSG has good adsorption

properties itself, which were proven by several research groups (Low et al., 2000; Silva et al., 2004).

The low ash content and high amount of fibrous material make BSG suitable for the production of building materials (Russ et al., 2005). The same attributes made it an interesting material for paper production (Ishiwaki et al., 2000).

High polysaccharide, protein and mineral content, make BSG quite valuable substrate for various biotechnological processes. It can be used as a substrate for the cultivation of microorganisms. This is especially true for mushrooms, not only because of its high protein and polysaccharide content, but also its high water content, with conjunction with some of its physical properties such as particle size, volume weight, specific density, porosity and water-holding capacity (Wang et al., 2001). BSG is also suitable nitrogen and energy source which makes it a good substrate for enzyme production, for example, xylanase by *Streptomyces* isolate from Brazilian cerrado soil (Nascimento et al., 2002) and alpha-amylase by *Aspegillus oryzae* NRRL 6270 in solid state fermentation (Francis et al., 2003). Cellulosic and non-cellulosic polysaccharides from barley cell walls can be degraded into their corresponding constituents by hydrolytic procedures – hydrothermal, enzymatic or acidic. Some of the products obtained by these procedures are of industrial significance as precursors of food grade chemicals or as energy sources in microbial fermentations. Currently, a number of added-value bioproducts such as organic acids, amino acids, vitamins, ethanol and butanediol are produced by fermentation, using glucose or xylose as substrates (Mussatto et al., 2006).

As stated before, the majority of BSG is today used as animal feed, mainly for cattle, because of its high fibre and protein content. Other important reason is the fact that it is the easiest and cheapest method of disposing this brewery by-product. Belibasakis and Tsirgogianni (1996) reported that when BSG was incorporated into the diet of cows, milk yield, milk total solids and milk fat yield were increased. Study performed by Jayant et al. (2018) suggest that BSG can serve as an alternate protein source with 50% level substitution of soybean meal without any adverse effect on the growth, nutrient utilization and feed conversion, when used as feedstuff for striped catfish *Pangasianodon hypophthalmus* fingerlings.

### **Applications in human nutrition**

According to the Codex Alimentarius "dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by endogenous enzymes in small intestine of human beings and belong to following categories: Edible carbohydrate polymers naturally occurring in food as consumed; Carbohydrate polymers, which have been obtained from raw material in food by physical, enzymatic or chemical means and which have been shown to have physiological effect of benefit to health by generally accepted scientific evidence to competent authorities; Synthetic carbohydrate polymers, which have been shown to have physiological effect of benefit to health by generally accepted scientific evidence to competent authorities" (Cummings et al., 2009).

The compounds in BSG that can be associated with potential health benefits include dietary fibre (e.g. arabinoxylans,  $\beta$ -glucans) and phenolic compounds (e.g. hydroxycinnamic acid). In comparison with other cereals, protein fraction of BSG has relatively high content of the essential amino acid, lysine (Lynch et al., 2016). As mentioned earlier, arabinoxylans are the primary constituents of the hemicellulose fraction of BSG which can account for up to 25% on dry weight basis. These compounds are considered to be dietary fibre and can act as prebiotic in the large intestine. They are selectively degraded in the colon by intestinal bacteria expressing xylanases and arabinofuranosidases and represent a new class of prebiotics. A healthy population of gut bacteria (e.g. bifidobacteria and lactobacilli) is important for the maintenance of gut health, because bifidobacteria produce short chain fatty acids through the fermentation of dietary fibres. The production of those fatty acids is generally considered beneficial, because fatty acids protect the host against pathogens, induce immune responses, reduce cholesterol synthesis, stimulate colonic blood flow, enhance muscular contraction and may protect the colon against cancer development (Grootaert et al., 2009).

**Table 2** Recommendation for daily dietary fibre intake (Delzenne et al., 2019)

Reference	Region	Recommendation	Issuing body
EFSA (2010)	EU	25 g/day for adults	EFSA
		2 g/MJ for children from the age of one year	
Scientific advisory committee on nutrition of United Kingdom (2015)	UK	30 g/day for adults	UK's SACN
		15 g/day (age 2-5)	
		20 g/day (age 5-11)	
		25 g/day (age 11-16)	
		30 g/day (age 16-18)	
Nordic Council of Ministers (2012)	Nordic countries	25-25 g for adults	The Nordic Council of Ministers
USDA (2015)	US	33.6 for men	USDA
		28 g for women (14 g/ 1000 kcal)	
NHMRC (2017)	Australia and New Zealand	30 g for men	NHMRC
		25 g for women	

EFSA -European Food Safety Authority; EU, European Union;

FSAI - Food Safety Authority of Ireland;

NHMRC - National Health and Medical Research Council;

SACN - Scientific Advisory Committee on Nutrition; UK, United Kingdom;

USDA - United States Department of Agriculture.

Numerous studies have been made about the effects of dietary fibres on human health, but before making definitive conclusions, several factors have to be taken into account. Some of them are: quantitative and qualitative analysis of dietary fibre are missing in most food composition tables; the complexity of human gut microbiota; most intervention studies were done with purified or isolated dietary fibres, at relatively high doses; reported problems of discomfort linked to the consumption of DF etc. (Delzenne et al., 2019). The recommended daily doses of dietary fibre, taken from several sources, are shown in **Table 2** Soluble dietary fibre, which includes (1/3, 1/4)- $\beta$ -glucan, appears to be important in lowering plasma cholesterol and postprandial serum glucose levels (Brennan and Cleary, 2005).

### ***Brewer's spent grain in bread, pastry and pasta production***

Before using it in bakery products, BSG has to be properly prepared. First of all, it has to be dried in order to prevent spoilage and ensure the longevity and prolonged shelf life. After removing excess water and drying procedure (drying at high temperatures; freeze-drying), BSG has to be finely milled because of its coarse structure that comes from the barley/malt husk. Other ways of preserving BSG from spoilage are freezing and the use of a mixture of benzoate, propionate and sorbate at a concentration of 0.2-0.3% (w/w). The first is not very energy efficient and requires a lot of storage space in brewery, and the latter will preserve BSG for only 4 to 5 days, and can only be used for animal feed (Bayerische Landesanstalt für Landwirtschaft, 2008).

Some of the interesting and useful properties of BSG flour in foods are: the ease of blending; calorie content is about half that of most cereal flours; high water absorption capacity; provides valuable minerals such as Ca, P, Fe, Cu, Zn and Mg; low-fat absorption (beneficial for batters and coating); uniform tan colour, bland flavour, and mildly roasted aroma; high fibre content and high protein content (Huige, 1994).

When used in human nutrition, BSG flour is generally used as fibre rich substitution in bread and pastry production. In general, when wheat flour is substituted with flours with higher fibre content, the result is the weakening of the gluten network, which becomes less elastic. The possible reasons are interactions between hemicellulose constituents with gluten forming proteins (gliadin and glutenin) and also the reduction of the total gluten content. The addition of hemicellulases can reduce both issues by solubilizing arabinoxylan chains and allowing the formation of viscous solutions, thus producing softer bread (Wang et al., 2004). Brazilian group compared the enzymatic and extrusion treatments on BSG and their effect on breadmaking. They concluded that the elasticity of bread varied and generally decreased with storage time that bread colour parameters were little affected by the different formulations and that, after 72 hour storage, the specific volume was not affected by the difference in formulations. "The addition of spent grain flour to bread resulted in larger water absorption. The enzymatic treatment of spent grains in an extruder did not affect the characteristics of bread when compared to the untreated spent grain flour. However, bread made from untreated spent grain flour with

enzymes directly added to the dough had better texture and volume when compared to bread made without the addition of enzyme or untreated BSG. The direct addition of enzyme to the dough resulted in some dough alterations, which, nevertheless, were difficult for untrained testers to detect" (Steinmacher et al., 2012).

Ktenioudaki et al. (2015) carried out a comprehensive investigation into the potential of bioprocessing techniques (such as sourdough fermentation and technological aids) on the technological and sensory properties of BSG breads. They evaluated the shelf-life and staling kinetics of BSG breads, including the thermophysical properties, examined the effect of sourdough fermentation on the aromatic properties of BSG breads and determined the in vitro antioxidant capacity and phenolic composition of BSG flour and breads. "Xylanase and dough conditioner improved the specific volume, texture, sensory properties of breads, affecting also the staling kinetics. Sourdough fermentation influenced the final aroma BSG breads through liberation of amino acids necessary for the formation of volatile compounds. The complete phenolic profile (free and bound) and the mineral composition of BSG flour and breads were determined. Sourdough and enzymes did not alter the phenolic profile but increased the in vitro antioxidant capacity of breads. Sourdough fermentation reduced the phytic acid content of BSG breads, hence potentially increasing the bioavailability of the minerals found in BSG breads." The overall conclusion was that the applied bioprocessing techniques improved the technological qualities of BSG breads. It showed that there is the potential for percentages of BSG higher than 15% that were used in this study, to be included in baking formulations to further enhance the nutritional properties of breads.

In similar research, wheat bread formulations with up to 10% of added BSG or sourdough made from BSG resulted in dough with improved handling properties compared to the wholemeal control and based on rheological analyses. These breads also had similar technological properties to wholemeal bread and had improved softness and staling. From the sensory perspective, the biggest difference between test and BSG breads was in sour taste and flavour perspective, with both increasing with the increasing levels of added BSG or sourdough made with BSG. Consequently, sweetness decreased as substitution levels increased and was lower for breads substituted with fermented BSG. Regardless of these changes in taste, the sensory panel concluded that breads made with BSG and BSG sourdough had completely acceptable aroma (Waters et. al., 2012).

Stojceska and Ainsworth (2008) found out that the addition of BSG to bread dough formulations at the level of 0-30% significantly ( $P < 0.001$ ) increased the total dietary fibre level from 2.3% to 11.5% and fat level from 3.4% to 4.4%. Protein content varied between 10.7% and 11% and was not related to the addition of BSG. Similar results were obtained by Kteundaki et al. (2012) who incorporated BSG at the level of 0-35% into breadsticks and found out that the protein level increased from 14.3% to 18.4%, fat level from 0.3% to 2.1%, and dietary fibre level from 7% to 27%. Taking into account the composition of BSG, the results obtained in two aforementioned papers are not surprising.



The addition of BSG to bread dough affects the loaf volume, texture and shelf life, probably due to an increased level of arabinoxylans, the main polymers in BSG. It can be generally concluded that the greatest loaf volume reduction happens at the addition of 30% BSG. Fibre addition significantly ( $P < 0.001$ ) increases crumb firmness in samples containing 20 and 30% of BSG, but the addition of 10% BSG does not make any significant difference (Stojecka, 2019).

Romanian research group investigated nutritional properties and volatile profile of the brewer's spent grain supplemented bread and found out that the substitution of wheat flour with 5-20% BSG resulted in bread formulations with enhanced nutritional value (increased fibre, protein, fat and minerals content) and with pleasant flavour attributes imparted by the characteristic volatile compounds. The results from sensory analysis revealed good organoleptic attributes for the samples with up to 10% BSG flour (Fărcaș et. al., 2014).

Except for the bread production, BSG is also used as valuable and nutritious supplement for pasta production. As in breads produced with the addition of BSG, pasta enriched with the same brewery by-product has similar properties, i.e., total dietary fibre content increases significantly (up to 135%) with the moderate to high increase of  $\beta$ -glucan (up to 85%), resistant starch (up to 57%) and total antioxidant capacity levels (up to 19%). The sensory and instrumental analyses on the quality of pasta show that spaghetti samples with the addition of 5 g/100 g BSG demonstrated sensorial properties fairly similar to durum semolina pasta, especially for the overall quality and firmness. Nevertheless, pasta with the addition of 10% BSG provided the best compromise in terms of technological performances along with nutritional and sensorial aspects (Nocente et al., 2019). The valorisation of BSG in the production of fibre-enriched fresh egg pasta was investigated by Cappa and Alamprese (2017). For the improvement of pasta structure, they used egg white powder. They found out that the addition of BSG significantly lowered the average brake strain of pasta with respect to a standard formulation produced without fibre (26±10% vs. 54±4%, for raw sheets; 25±8% vs. 54±1%, for cooked sheets). On the other hand, by adding egg white powder the mechanical properties of cooked pasta improved due to a tighter protein network developed by ovalbumin. With the highest amount of egg white powder added to pasta formulation, break load (6.5±0.4 N) and strain (33±4%) of cooked pasta were significantly higher than in samples without this additive (1.4±0.1 N and 18±1%, respectively). After experiments with different formulations, they concluded that the one with BSG amount of 6.2 g/100 g and egg white powder content of 10.2 g/100 g had the best properties.

## CONCLUSIONS

Bread and other various bakery products are one of the most consumed foods in the world, so their fortification with other nutritionally rich adjuncts makes much sense. This is especially true if these adjuncts are abundant and readily available, have very low price and excellent nutritive and good sensory properties. All of these properties/qualities can be attributed to brewer's spent grain. Of course, BSG also has

some drawbacks. The first one is its very high water content. The moisture has to be removed before any milling or preparation procedures, which can be costly and energy consuming. Because of the high water and carbohydrate/protein content, it is very susceptible to microbiological spoilage, which leads very fast to the deterioration of sensory properties and its inability to be used as foodstuff. High moisture content corresponds to high volume, which makes the transport of the BSG more expensive. From the sensory point of view, to be acceptable in any type of products, including bread and pastry, it has to be finely milled to break down the coarse structure that comes from the malt husk. Research has shown that, because of rather/quite coarse structure of the BSG flour and its high fibre content, the best rheological, mechanical and sensory properties have bakery products in which wheat flour was replaced by maximum of 10-15% of BSG flour. This does not mean that new approaches and processing techniques will not make it possible to be used in higher percentages in future. Nevertheless, because of its advantages, BSG remains a very potent and nutritionally rich by-product which will certainly have a bigger role in food production in future. All of the shortages of the BSG could be overcome by clever planning and better techniques used in its processing. The thing that should not be ignored is the positive ecological impact that the usage of BSG, as a valuable raw material, has.

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**CEREAL PRODUCTS CONSUMPTION FREQUENCY IN RELATION TO  
ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS IN HOSPITALIZED  
SCHIZOPHRENIC PATIENTS**

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**SUMMARY**

Cereal products are important components of human nutrition. Whole grains and refined cereals have different structure and physiological effects. Inadequate dietary habits, as a characteristic of schizophrenic patients, may affect life expectancy and disease complications. This study is aimed at assessing the cereal products consumption frequency and at determining its association with anthropometric and biochemical parameters of hospitalized schizophrenic patients. The intake of selected cereal products (wheat/mixed-wheat bread, rye/whole-wheat bread, pastries, breakfast cereals, pasta, rice) was assessed in 259 subjects (aged  $\geq 18$  years) using the nutrition section of Dlugosch & Krieger's General Health Behaviour Questionnaire. Body weight and height, waist and hip circumferences, and % body fat were measured. Lipid profile and blood glucose were determined from blood samples. Daily consumption of wheat/mixed-wheat bread was reported by 208 participants (80.3%), whereas only six participants (2.3%) consumed rye/whole-wheat bread equally often. Pastries were eaten few times a week by 87 participants (33.6%). Rye/whole-wheat bread was consumed less frequently among the participants with triglycerides ( $p=0.014$ ), total cholesterol ( $p=0.006$ ), and low-density lipoprotein cholesterol ( $p=0.003$ ) in the normal range, while rice was consumed more frequently among those with glucose ( $p=0.041$ ) and total cholesterol ( $p=0.021$ ) in the normal range. No association was found between other selected cereal products and observed anthropometric and biochemical parameters. The results indicated that there is no major impact of cereal products, as an isolated food group, on the observed anthropometric and biochemical parameters.

**Keywords:** cereal products, lipid profile, blood glucose, schizophrenia

## INTRODUCTION

Cereals are, according to the definition, grains or edible seeds that belong to the grass family, *Gramineae* (Bender and Bender, 1999). The group "cereals" does not only cover the grass members, such as wheat, rice, maize, millet, barley, oat, and rye but also includes food like bread, pasta, and flour (Sarwar et al., 2013). As the main source of energy and numerous essential nutrients, cereals and cereal products represent an important component of human nutrition (McKevith, 2004). However, whole grains and refined cereals have different structure and physiological effects (Tayyem et al., 2016). While whole grains contain all three key components of the grain (endosperm, germ, and bran), refined cereals have two key components (germ and bran) removed mechanically during the process of milling (Slavin, 2003; Aune et al., 2016). This is important from the nutritional point of view since refined cereals have less dietary fibre and less essential micronutrients, when compared to whole grains (Kelly et al., 2017).

Various studies have focused on the role of cereals in diet and their impact on human health (McKevith, 2004). Adequate consumption of cereals, primarily whole grain cereals, has been associated with a positive impact on the prevention and treatment of chronic diseases, such as diabetes, hypertension, cardiovascular diseases (CVDs), and cancer (McKevith, 2004). Although cereals and cereal products in general have low-fat content, the pastries subgroup may contain considerable amounts of salt and fat, especially saturated (SFA) and trans fatty acids (TFA) (Albuquerque et al., 2017; Olatona et al., 2018), and thus are connected with an increased risk for non-communicable diseases (Olatona et al., 2018).

Current findings on dietary habits of individuals with schizophrenia are limited and insufficient. Nevertheless, when compared to the population of healthy people, schizophrenic patients tend to have poorer dietary habits (Amani, 2007). In comparison to the general population, schizophrenic patients are more prone to the consumption of supper snacks, instant meals, and unhealthy foods (Roick et al., 2007). Furthermore, the diet of schizophrenic patients is generally characterized by the excessive consumption of foods rich in fat and sugar, but insufficient in dietary fibre (Brown et al., 1999; Ratliff et al., 2012; Ito et al., 2015).

It is also well-known that schizophrenic patients have reduced life expectancy when compared to people without mental disorders (Laursen et al., 2012). One of the reasons for reduced life expectancy in this specific population group lies in their inadequate dietary habits (Jahrami et al., 2017).

Despite the aforementioned, and to the best of our knowledge, there are no published studies focused on the assessment of cereal products consumption frequency and its relationship with cardiovascular risk observed through lipid profile and blood glucose in hospitalized schizophrenic patients.

We hypothesized that inadequate cereal dietary pattern will be determined among the study participants and that it would be associated with increased cardiovascular risk.

## **SUBJECTS AND METHODS**

The present cross-sectional study was conducted during the period from May to November 2017 in Psychiatric Hospital Ugljan, Croatia.

### ***Participants***

All patients, both men and women, hospitalized in Psychiatric Hospital Ugljan at the time of the research recruitment were considered eligible to enter the study if they were aged  $\geq 18$  years and had the diagnosis of schizophrenia (F20.0 - F20.9) according to the 10th Revision of the International Classification of Diseases (ICD-10). Hospitalized patients without the diagnosis of schizophrenia were not included in the study. Additionally, participants were excluded if they refused to provide all the requested data, if they or their legal guardians refused to give written informed consent, or if they had any physical or cognitive impairment that could negatively influence collection of the relevant data and the overall participation in the study. During the recruitment process and before providing written informed consent, the researcher explained all the aims, study procedures and protocols to the potential participants. All of the study procedures were approved by the Ethics Committee of the Psychiatric Hospital Ugljan and the Central Ethics Committee of the Medical School, University of Zagreb.

A total of 294 patients met the inclusion criteria and were therefore considered eligible to enter the study. Out of those, 35 patients (11.9%) were excluded because they or their legal guardians refused to give written informed consent or because of significant physical or cognitive impairments. Therefore, the final sample consisted of 259 hospitalized schizophrenic patients, out of which the vast majority were men (209 participants; 80.7%). The mean age of the final sample was  $52.4 \pm 9.2$  years, with four participants (1.6%) aged between 18 and 30 years, 27 participants (10.4%) aged between 31 and 40 years, 64 participants (24.7%) aged between 41 and 50 years, 112 participants (43.2%) aged between 51 and 60 years, and 52 participants (20.1%) older than 60 years.

### ***Methods***

The consumption frequency of selected cereal products was assessed using the nutrition section of Dlugosch & Krieger's General Health Behaviour Questionnaire (Dlugosch and Krieger, 1995). Translations of the questionnaire from German to Croatian language were independently conducted by one professional in the field of nutrition with the knowledge of German language and one professional German – Croatian translator. Afterwards, the obtained translations were checked for dissimilarities. The original questionnaire was, with the written permission of the authors, partially modified in order to give better insight into eating patterns of study participants. To make necessary modifications, the original questionnaire was pilot tested on a group of randomly chosen schizophrenic patients. Based on the validation results, several food items, for which it was shown to be consumed often by the target population, were added to the questionnaire, and vice versa. Out of



the overall 32 food items listed in the questionnaire (questionnaire available upon request), six belong to a group of cereal products: wheat/mixed-wheat bread, rye/whole-wheat bread, pastries, breakfast cereals, pasta, and rice. The participants were asked to indicate the consumption frequency of each item in the previous three months. They were able to choose between the following consumption frequencies presented on a four-point Likert scale: every day (1), few times a week (2), rarely (3), and never (4). The questionnaire was administered by the researcher in the form of face-to-face interview.

Moreover, we have performed basic anthropometric measurements, including the assessment of body weight (BW), body height (BH), waist circumference (WC), hip circumference (HC), and % body fat (% BF). BW and BH were measured according to standard instructions (Lee and Nieman, 2010) using a digital medical scale with stadiometer Seca 799 (Vogel & Halke GmbH & Co., Hamburg, Germany). From the measured values, body mass index (BMI) was calculated by the formula:  $BW \text{ (kg)}/BH^2 \text{ (m}^2\text{)}$ . WC and HC were also measured according to standard instructions (Lee and Nieman, 2010) using a non-stretchable measuring tape. From the measured values, the waist-to-hip ratio (WHR) was calculated by the formula:  $WC \text{ (cm)}/HC \text{ (cm)}$ . Body composition analysis was performed using bioelectric impedance on the OMRON BF500 analyzer (Omron Healthcare, Vernon Hills, Illinois, USA). All anthropometric measurements were performed by the researcher.

The concentrations of serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and glucose (GLC) were determined from blood samples. The analyses were performed on the Cobas c 111 analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland), following the manufacturers' instructions. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated from the obtained values for TC, HDL-C, and TG, using the Friedewald formula (Friedewald et al., 1972). Blood samples were collected in the morning, after a 12-hour fast. Blood sampling and blood sample analysis were carried out by the trained medical staff in the Department of Medical Biochemistry, Psychiatric Hospital Ugljan.

### **Statistical analysis**

Data analysis was performed using statistical software Statistica v. 6.1. (StatSoft Inc., Tulsa, OK, USA). Participants were grouped depending on whether the measured values of each of the studied anthropometric and biochemical parameters were below or above the cut-off values. There were two groups for each of the studied health parameters: BMI [(1) < 25 kg/m<sup>2</sup> and (2) ≥ 25 kg/m<sup>2</sup>] (WHO, 1995), WC [cut-off values for Europids: (1) < 80 cm (women); < 94 cm (men) and (2) ≥ 80 cm (women); ≥ 94 cm (men)] (IDF, 2005), WHR [(1) < 0.85 (women); < 0.90 (men) and (2) ≥ 0.85 (women); ≥ 0.90 (men)] (WHO, 2011), % BF [(1) < 32% (women); < 25% (men) and (2) ≥ 32% (women); ≥ 25% (men)] (ACE, 2009), TG [(1) < 1.7 mmol/L and (2) ≥ 1.7 mmol/L] (IDF, 2005), HDL-C [(1) < 1.3 mmol/L (women); < 1.0 mmol/L (men) and (2) ≥ 1.3 mmol/L (women); ≥ 1.0 mmol/L (men)] (IDF, 2005), LDL-C [(1) < 3.0 mmol/L and (2) ≥ 3.0 mmol/L] (Hockley and Gemmill, 2007), TC [(1)

< 5.0 mmol/L and (2) ≥ 5.0 mmol/L] (Hockley and Gemmill, 2007), and GLC [(1) < 5.6 mmol/L and (2) ≥ 5.6 mmol/L] (IDF, 2005). In order to get a more detailed insight into the association between pastries’ consumption frequency and studied health parameters, participants were additionally grouped depending on the established pastries’ consumption frequency. The participants who have reported to consume pastries every day or few times a week were classified as frequent consumers. Less frequent consumers were those participants who reported rare or no intake of pastries.

The assumption of normality was investigated using the Kolmogorov-Smirnov test for normality. All study data were presented using descriptive statistics (mean ± standard deviation and percentage). Statistical tests used to analyze data were *t*-test for independent samples and Difference test: difference between two means. The *P* values < 0.05 were considered significant.

## RESULTS AND DISCUSSION

Certain inadequacies in cereal products consumption have been determined. Vast majority of the participants (208 participants; 80.3%) reported daily consumption of wheat/mixed-wheat bread. Contrariwise, the equally frequent consumption of rye/whole-wheat bread was documented in only six participants (2.3%). Intake of pastries as frequent as few times a week was reported in one-third of the study participants (87 participants; 33.6%), while 20 participants (7.7%) consumed them on a daily basis. Under the term “pastries” in the questionnaire, the following products were listed: burek, puff-pastries, donuts, and strudels. The results of the consumption frequencies of all studied cereal products are listed in **Table 1**.

**Table 1** Cereal products consumption frequency

Cereal product; <i>n</i> (%)	Consumption frequency			
	every day	few times a week	rarely	never
wheat/mixed-wheat bread	208 (80.3)	40 (15.4)	9 (3.5)	2 (0.8)
rye/whole-wheat bread	6 (2.3)	24 (9.3)	30 (11.6)	199 (76.8)
pastries	20 (7.7)	87 (33.6)	94 (36.3)	58 (22.4)
breakfast cereals	3 (1.2)	36 (13.9)	156 (60.2)	64 (24.7)
pasta	3 (1.2)	229 (88.4)	22 (8.5)	5 (1.9)
rice	0 (0)	220 (84.9)	34 (13.1)	5 (1.9)

Abbreviations: *n* – number of participants

Most importantly, the participants were prone to everyday consumption of wheat/mixed-wheat bread, classified also as white bread, for which it is already well-known to be linked with an unhealthy lifestyle (Prättälä et al., 2001). On the other hand, the consumption of whole-grain bread was associated with a healthier lifestyle and numerous positive outcomes related to blood lipid profile (Jacobs et al., 2001). Additionally, the diet of whole-grain bread consumers was characterized by lower

total daily fat intake, along with the lower daily intake of SFA (Jacobs et al., 2001). Therefore, despite the fact that the present study was focused just on the intake of one of the major food groups, the obtained results speak in favour of poor dietary habits characteristic for schizophrenic patients.

The distribution of participants by groups is presented in **Table 2**. In the case of 27 participants (10.4%), it was not possible to determine % BF due to the tremor that is a recognizable side effect of antipsychotic treatment. It was confirmed by a statistical analysis that the absence of this data would not influence the research results and therefore the participants were not excluded from the study. Furthermore, in the case of four participants (1.5%) the LDL-C concentration could not be calculated using Fiedewald formula due to the TG level being too high (> 4.6 mmol/L). For the same reason as aforementioned, the participants were not excluded from the study.

**Table 2** Cereal products consumption frequency

Studied parameters		n (%)
BMI (kg/m <sup>2</sup> )	< 25	106 (40.9)
	≥ 25	153 (59.1)
WC (cm)	< 80 cm (women); < 94 cm (men)	63 (24.3)
	≥ 80 cm (women); ≥ 94 cm (men)	196 (75.7)
WHR	< 0.85 (women); < 0.90 (men)	34 (13.1)
	≥ 0.85 (women); ≥ 0.90 (men)	225 (86.9)
% BF	< 32% (women); < 25% (men)	127 (49.0)
	≥ 32% (women); ≥ 25% (men)	105 (40.5)
	data missing	27 (10.4)
TG (mmol/L)	< 1.7	170 (65.6)
	≥ 1.7	89 (34.4)
HDL-C (mmol/L)	< 1.3 (women); < 1.0 (men)	115 (44.4)
	≥ 1.3 (women); ≥ 1.0 (men)	144 (55.6)
LDL-C (mmol/L)	< 3.0	131 (50.6)
	≥ 3.0	124 (47.9)
	data missing	4 (1.5)
TC (mmol/L)	< 5.0	157 (60.6)
	≥ 5.0	102 (39.4)
GLC (mmol/L)	< 5.6	174 (67.2)
	≥ 5.6	85 (32.8)

Abbreviations: n – number of participants; BMI – body mass index; WC – waist circumference; WHR – waist-to-hip ratio; % BF – % body fat; TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TC – total cholesterol; GLC – glucose

According to the results listed in **Table 2**, more than half of the study participants (153 participants; 59.1%) were overweight or obese (BMI  $\geq$  25 kg/m<sup>2</sup>) (WHO, 1995). Additionally, the vast majority of the study participants had WHR (225 participants; 86.9%) and WC (196 participants; 75.7%) above the cut-off values (IDF, 2005; WHO, 2011). The obtained results should be taken quite seriously as BMI, WHR, and WC are indicators of abdominal obesity and important predictors of leading chronic non-communicable diseases (Vazquez et al., 2007; Paniagua et al., 2008; Ahmad et al., 2016). Moreover, numerous published studies have confirmed that people with mental disorders, including schizophrenia, have higher all-cause mortality rates and thus reduced life expectancy when compared to the general population (Ösby et al., 2016).

It is already well established that inadequate dietary habits represent one of the leading causes of excess mortality and reduced life expectancy (Jahrami et al., 2017) and are also considered as one of the major contributors to the deterioration of metabolic risk factors (Olatona et al., 2018). Therefore, we examined the association between the consumption frequencies of selected cereal products established in the present study and anthropometric and biochemical parameters of hospitalized schizophrenic patients (**Table 3**).

**Table 3** The association between selected cereal products consumption frequencies and studied health parameters (part I)

Studied parameters; M $\pm$ SD		Selected cereal products					
		wheat/mixed- wheat bread	rye/whole- wheat bread	pastries	breakfast cereals	pasta	rice
BMI (kg/m <sup>2</sup> )	< 25	1.3 $\pm$ 0.6	3.7 $\pm$ 0.7	2.8 $\pm$ 0.9	3.1 $\pm$ 0.7	2.1 $\pm$ 0.4	2.1 $\pm$ 0.4
	$\geq$ 25	1.2 $\pm$ 0.5	3.6 $\pm$ 0.8	2.7 $\pm$ 0.9	3.1 $\pm$ 0.6	2.1 $\pm$ 0.4	2.2 $\pm$ 0.4
	p value <sup>†</sup>	0.679	0.162	0.550	0.848	0.967	0.136
WC (cm)	< 80 cm (women); < 94 cm (men)	1.2 $\pm$ 0.5	3.6 $\pm$ 0.8	2.8 $\pm$ 0.9	3.0 $\pm$ 0.6	2.1 $\pm$ 0.5	2.2 $\pm$ 0.5
	$\geq$ 80 cm (women); $\geq$ 94 cm (men)	1.3 $\pm$ 0.6	3.6 $\pm$ 0.8	2.7 $\pm$ 0.9	3.1 $\pm$ 0.7	2.1 $\pm$ 0.4	2.2 $\pm$ 0.4
	p value <sup>†</sup>	0.681	0.946	0.541	0.603	0.734	0.919
WHR	< 0.85 (women); < 0.90 (men)	1.3 $\pm$ 0.5	3.5 $\pm$ 0.8	2.9 $\pm$ 0.9	3.1 $\pm$ 0.6	2.1 $\pm$ 0.3	2.2 $\pm$ 0.6
	$\geq$ 0.85 (women); $\geq$ 0.90 (men)	1.2 $\pm$ 0.6	3.6 $\pm$ 0.7	2.7 $\pm$ 0.9	3.1 $\pm$ 0.7	2.1 $\pm$ 0.4	2.2 $\pm$ 0.4
	p value <sup>†</sup>	0.386	0.280	0.405	0.755	0.713	0.336

**Table 3** The association between selected cereal products consumption frequencies and studied health parameters (part II)

Studied parameters; M ± SD		Selected cereal products					
		wheat/mixed-wheat bread	rye/whole-wheat bread	pastries	breakfast cereals	pasta	rice
% BF	< 32% (women); < 25% (men)	1.3 ± 0.6	3.6 ± 0.8	2.7 ± 0.9	3.1 ± 0.7	2.1 ± 0.5	2.2 ± 0.4
	≥ 32% (women); ≥ 25% (men)	1.2 ± 0.5	3.6 ± 0.8	2.7 ± 0.8	3.0 ± 0.6	2.1 ± 0.4	2.2 ± 0.4
	p value <sup>†</sup>	0.274	0.708	0.974	0.526	0.600	0.291
TG (mmol/L)	< 1.7	1.2 ± 0.5	3.7 ± 0.7	2.8 ± 0.9	3.1 ± 0.7	2.1 ± 0.4	2.1 ± 0.4
	≥ 1.7	1.3 ± 0.6	3.5 ± 0.9	2.7 ± 0.8	3.1 ± 0.6	2.1 ± 0.4	2.2 ± 0.5
	p value <sup>†</sup>	0.476	0.014*	0.359	0.774	0.737	0.233
HDL-C (mmol/L)	< 1.3 (women); < 1.0 (men)	1.2 ± 0.5	3.6 ± 0.8	2.7 ± 0.8	3.0 ± 0.7	2.1 ± 0.4	2.2 ± 0.4
	≥ 1.3 (women); ≥ 1.0 (men)	1.3 ± 0.6	3.7 ± 0.7	2.7 ± 1.0	3.2 ± 0.7	2.1 ± 0.4	2.2 ± 0.4
	p value <sup>†</sup>	0.584	0.288	0.929	0.061	0.561	0.892
LDL-C (mmol/L)	< 3.0	1.2 ± 0.5	3.8 ± 0.6	2.8 ± 0.9	3.1 ± 0.6	2.1 ± 0.3	2.1 ± 0.4
	≥ 3.0	1.3 ± 0.6	3.5 ± 0.9	2.7 ± 0.9	3.1 ± 0.7	2.1 ± 0.5	2.2 ± 0.5
	p value <sup>†</sup>	0.381	0.003*	0.682	0.749	0.371	0.101
TC (mmol/L)	< 5.0	1.2 ± 0.5	3.7 ± 0.6	2.7 ± 0.9	3.1 ± 0.6	2.1 ± 0.3	2.1 ± 0.4
	≥ 5.0	1.3 ± 0.6	3.5 ± 0.9	2.8 ± 0.9	3.0 ± 0.7	2.2 ± 0.5	2.2 ± 0.5
	p value <sup>†</sup>	0.072	0.006*	0.758	0.477	0.078	0.021*
GLC (mmol/L)	< 5.6	1.2 ± 0.5	3.7 ± 0.7	2.8 ± 0.9	3.1 ± 0.7	2.1 ± 0.4	2.1 ± 0.4
	≥ 5.6	1.3 ± 0.6	3.6 ± 0.9	2.6 ± 0.9	3.1 ± 0.7	2.2 ± 0.5	2.2 ± 0.5
	p value <sup>†</sup>	0.811	0.332	0.278	0.445	0.253	0.041*

Abbreviations: M ± SD – mean ± standard deviation; BMI – body mass index; WC – waist circumference; WHR – waist-to-hip ratio; % BF - % body fat; TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TC – total cholesterol; GLC – glucose

\* statistically significant

<sup>†</sup> t - test for independent samples

Selected cereal products consumption frequency data were gathered using a four-point Likert scale: 1- every day; 2 – few times a week; 3 – rarely; 4 – never.

As the numbers on the Likert scale were inversely proportional to the consumption frequencies they represented, the higher mean values listed in **Table 3** indicate the lower consumption frequencies, and vice versa. Rye/whole-wheat bread was consumed less frequently among the participants with TG ( $p=0.014$ ), TC ( $p=0.006$ ), and LDL-C ( $p=0.003$ ) in the normal range, while rice was consumed more frequently among those with GLC ( $p=0.041$ ) and TC ( $p=0.021$ ) in the normal range. For all the

other studied anthropometric and biochemical parameters there were no statistically significant differences in the consumption frequencies of selected cereal products depending on whether the measured values of specific parameters were above or below the prescribed cut-off values. Our findings do not support the initial hypothesis we had. Contrary to our findings, in the research conducted by Mertens et al. (2017), the dietary pattern that included, among other characteristics, the higher intake of white bread, along with the lower intake of whole-grain bread, positively correlated with CVD incidence. In the same research, the dietary pattern with the higher intake of whole-grain breakfast cereals was associated with lower CVD incidence, while there was no significant association between the dietary pattern with higher intake of pasta and rice, and CVD incidence (Mertens et al., 2017). Żbikowska et al. (2015) concluded in their research that the excessive consumption of pastries could have negative health implications due to the high intake of trans fatty acids and toxic products of lipid oxidation. Even though pastries are usually rich in SFA and TFA, surprisingly, our results did not show any association between higher consumption frequency and studied health parameters.

Differences between studied anthropometric and biochemical parameters of frequent and less frequent consumers of pastries are summarized in **Table 4**. As we had assumed, the group of less frequent consumers of pastries had significantly lower BMI, WC, and GLC, and significantly higher HDL-C (all  $p < 0.001$ ) when compared to the group of frequent consumers. Contrary to our expectations, less frequent consumers of pastries also had significantly higher WHR, % BF, TC, and LDL-C (all  $p < 0.001$ ) than the frequent consumers of pastries. There was no significant difference in the TG level ( $p = 1.000$ ) between the two groups.

To the best of our knowledge, this is the first study to investigate the relationship between cereal products consumption frequency and anthropometric and biochemical parameters in hospitalized schizophrenic patients. Presented findings are part of the clinical trial primarily focused on the impact of nutrition intervention program on metabolic syndrome parameters in this specific population group, which will hopefully provide important insights in the role of nutrition on comorbidities in schizophrenic patients.

With the aim to reduce the possibility of a recall bias, the questionnaires were administered by the researcher in the form of face-to-face interviews. At the same time, the questionnaire used for the assessment of selected cereal products consumption frequencies did not include portion sizes which could be considered as a limitation of this study. It is noteworthy that, based on the obtained results, the intake of other food groups that were not in the focus of the present research could potentially have a more important role on the observed anthropometric and biochemical parameters.

## **CONCLUSIONS**

The results of the present study indicated that there is no major impact of cereal products, as an isolated food group, on the observed anthropometric and biochemical parameters.

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## INFLUENCE OF HIGH-INTENSITY ULTRASOUND ON BIOACTIVE COMPOUNDS OF BUCKWHEAT HULLS

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### SUMMARY

Buckwheat hulls are by-products of the milling industry with a potential to be used as a functional food or in pharmaceutical preparations. The main advantage of hulls is their high dietary fibre and bioactive compound content, which have numerous positive health effects. This study aimed to investigate the effect of high-intensity ultrasound (400 W, 24 kHz) on the extractability of free bioactive compounds, antioxidant activity, water swelling and activity of enzyme polyphenol oxidase of buckwheat hulls milled to a particle size of  $\leq 56 \mu\text{m}$ . A 10% aqueous suspension of buckwheat hulls with different initial temperatures (9.7; 20.0; 45.0; 70.0 or 80.4 °C) was treated (2.9; 5.0; 10.0; 15.0 or 17.0 min) by direct immersion of the ultrasonic probe ( $d = 22 \text{ mm}$ ) at 100% amplitude following a central composite design. In comparison to the control sample, it was found that high-intensity ultrasound treatment increased the content of total phenolic compounds, antioxidant activity, water swelling and polyphenol oxidase activity. Optimum treatment conditions should be defined depending on the intended application of the treated hulls in cereal foods production.

**Keywords:** bioactive compounds, buckwheat hulls, high-intensity ultrasound, polyphenol oxidase

### INTRODUCTION

Although buckwheat cultivation has been neglected for years, due to recognition of its multiple health benefits and potential as a functional food, its production is steadily increasing (Li and Zhang, 2001; Christa and Soral-Smietana, 2008). However, so far only dehulled grains have been used in food products, while the hulls still represent a waste by-product (Huang et al., 2017). Buckwheat contains compounds that show potential in reducing the development of certain diseases (Mazza and Oomah, 2005) due to its antioxidant and prebiotic properties. *In vitro* and animal studies (Liu et al., 2001) have shown that proteins and bioactive compounds of



buckwheat (primarily rutin and quercetin) effectively lower blood pressure, cholesterol and blood sugar levels, maintaining the nervous system, anti-inflammatory and anti-cancer effects. Besides, buckwheat does not contain gluten and is suitable for people with celiac disease (Saturni et al., 2010). As said, buckwheat hulls are not as used in food industry, but they do have a great potential as a dietary source of rutin or can be incorporated into the food products as a dietary fibre rich in flavonoids (Oomah and Mazza, 1996).

Polyphenol oxidase (PPO) plays a role in the physiology of plant photosynthesis and in the protection of plants from the invasion by pathogens and insects. It catalyses processes that lead to hydroxylation of monophenols to *o*-diphenols and oxidation of *o*-diphenols to highly reactive *o*-quinones (Okot-Kotber et al., 2002). The generated *o*-quinones can further react with amino, sulfhydryl, thioether, phenolic, indole and imidazole groups of the proteins, which can lead to a protein cross-linking, dough-strengthening and structural improvement of bakery products (Matheis and Whitaker, 1984). In some other food systems, *o*-quinones may participate in the formation of melanoid coloured products (melanins) which can be undesirable (Okot-Kotber et al., 2002). In cereals, PPO exists in a latent form which may be activated with different physicochemical treatments such as exposure to acids, detergents, fatty acids, and alcohols (Matheis and Whitaker, 1984). Recently, Čukelj Mustač et al. (2019) showed that PPO of proso millet bran can be activated by high-intensity ultrasonic (HIU) treatment.

A large amount of waste material is generated in industrial processes and ways of extracting bioactive substances from biological sources are being explored for their future use in food and pharmaceutical industries (Drmić and Režek Jambrak, 2010) with an aim to conserve global resources and improve energy efficiency. High-intensity ultrasound treatment can potentially increase the extractability of specific bioactive compounds, such as polyphenols. Huang et al. (2017) successfully developed a method of isolating rutin from buckwheat hulls with a combination of HIU and green solvents. It also aids in reducing or completely avoiding the use of solvents and shortens the treatment time (Drmić and Režek Jambrak, 2010). Considering that high-intensity ultrasonic waves cause physical and chemical changes in cellular constituents (Drmić and Režek Jambrak, 2010), HIU treatment is a promising technology that could release bioactive compounds, improve the technological properties of buckwheat hulls, and enable their application in human consumption. Therefore, this study aimed to investigate the effect of HIU treatment on the release of free phenolic compounds, antioxidant activity, water swelling, and PPO activity of buckwheat hulls. The study was conducted on micronized buckwheat hulls. Micronization causes a redistribution of the building units of dietary fibres, increases total phenolics and yields in higher antioxidant activity (Zhu et al., 2014). Water was used as a dispersing agent in this study, since we wanted to obtain buckwheat hulls that could be used for the enrichment of bakery products.

## MATERIALS AND METHODS

### ***Buckwheat hulls***

Buckwheat hulls (*F. esculentum*, Slovenian cultivar Ljiljana, harvest year 2017) were obtained during the milling process at the Pukanic mill (Velika Gorica, Croatia).

### ***Sample preparation***

Buckwheat hulls were first coarsely ground on an ultra-centrifugal mill at a speed of 6000 rpm with a sieve diameter of 500 µm. Then, they were ground on a ball mill "CryoMill" (Retsch, Haan, Germany) using liquid nitrogen to keep the temperature at -196 °C. The sample (8.0 g) was placed in a 50 mL container with a 25 mm diameter ball. Sample pre-cooling was performed on an automatic programme followed by one grinding cycle for 4 min at a frequency of 30 Hz. The sample (50.0 g) was continuously sieved for 10 min on a vibrating machine with a 56 µm sieve.

### ***High-intensity ultrasound treatment***

The separated fraction of ≤ 56 µm was treated with HIU with a total processor power of 400 W and a frequency of 24 kHz using a 22 mm diameter probe. Sample treatments were performed according to a central composite design obtained with the help of the Design Expert 10 (StatEase, USA). The experimental plan included two independent variables: initial sample temperature (°C) and HIU treatment time (min). The central point (45 °C, 10 min) had 5 repetitions, which ultimately resulted in 13 experiments (**Table 1**). The HIU treatment amplitude was fixed at 100%.

**Table 1** Central composite design experimental plan of high-intensity treatment of separated fraction

<b>Sample temperature (°C)</b>	<b>Duration of treatment (min)</b>
45.0*	10.0
45.0*	10.0
70.0	15.0
20.0	15.0
45.0*	10.0
80.4	10.0
70.0	5.0
20.0	5.0
45.0	2.9
45.0*	10.0
45.0	17.0
9.7	10.0
45.0*	10.0

\*central point

High-intensity ultrasound treatment was carried out by immersing the probe of 1 cm into a 10% aqueous suspension (10.0 g of the sample in 100 mL of distilled water) in a 600 mL beaker. The temperature was recorded during the treatment. After treatment, the samples were lyophilized and stored at -20 °C until analysis. The HIU untreated sample represented the control.

#### ***Determination of water content and water swelling capacity***

The water content of buckwheat hulls was determined according to AACC International Method 44-19.01.

Water swelling capacity was determined according to the method previously described by Robertson et al. (2000).

#### ***Determination of the content of total phenolic compounds, antioxidant activity and polyphenol oxidase activity***

The extraction of free compounds, as well as the determination of total phenolic compounds (TPC) antioxidant activity, Ferric Reducing Antioxidant Power (FRAP) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was carried out as previously described by Čukelj Mustač et al. (2019). The polyphenol oxidase activity was determined according to AACC International Method 22-85.01 (2000) modified as described in Čukelj Mustač et al. (2019).

#### ***Determination of rutin content***

The concentration of rutin in the same extract was determined as TPC according to Li et al. (2009) with the modification of chromatographic conditions. High-performance liquid chromatograph (HPLC) was equipped with a quaternary pump with a UV/VIS PDA (Photo Diode Array) detector. Measurements were made at room temperature with a flow rate of 0.9 mL/min, a pressure of 250 bar and gradient elution. Two different mobile phases were used: A - 0.1% formic acid solution in water and B - 0.1% formic acid solution in methanol. Extract was injected into the chromatographic column in quantity of 100 µL. The rutin was quantified with an external standard using computer programme LC/MSD ChemStation.

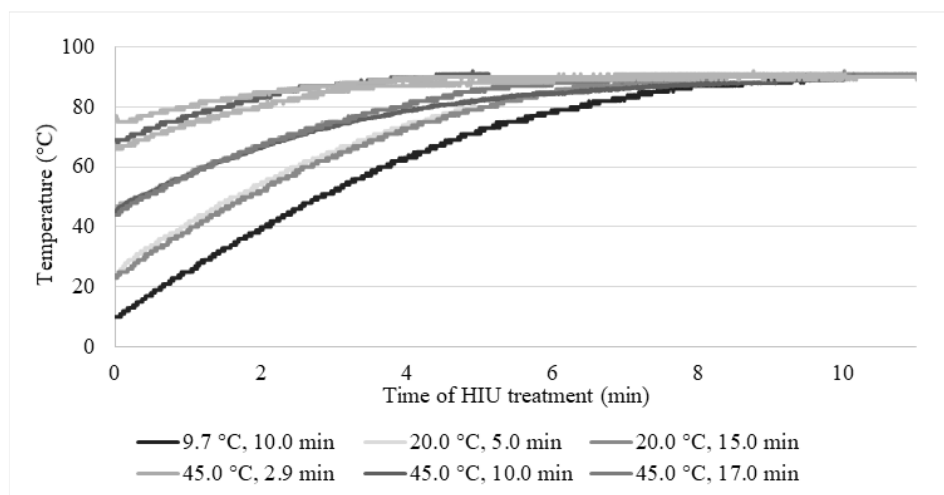
#### ***Statistical data processing***

The results obtained in this study are shown as mean ± standard deviation, expressed on the dry matter of the sample. Microsoft Office Excel 2010 and Design Expert 10 (StatEase, USA) were used for the experimental design, to statistically process the experimental results, and to produce graphs. One-way analysis of variance with Tukey post-hoc test and correlation analysis were performed in Statistica 8 (StatSoft Inc., Tulsa, Oklahoma, USA) at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### **Temperature kinetics during the ultrasound treatment**

The overall temperature difference from the beginning until the end of HIU treatment was dependent on the initial temperature and the square of the treatment time ( $p = 0.009$ ). However, regardless of the initial temperature, the temperature of the samples at the end of the treatment was approximately equalized (from 87.0 to 90.0 °C; **Figure 1**).



**Figure 1** Kinetic of temperature change during the HIU treatment

### **Influence of HIU treatment on total phenolic compounds and antioxidant activity of buckwheat hulls**

**Table 2** shows the TPC and antioxidant activity of buckwheat hulls determined by the DPPH and FRAP methods. No statistically significant differences in TPC or DPPH were found between the treated samples, while the antioxidant activity measured by the FRAP method was dependent on the treatment duration ( $p = 0.016$ ).

The TPC in control sample (180 mg GAE per 100 g sample dry matter) was substantially lower than previously reported by Quettier-Deleu et al. (2000) (333 mg GAE per 100 g of sample dry matter). The obtained difference can be attributed to the different cultivation area and environmental conditions as demonstrated by Oomah and Mazza (1996).

The HIU treatment with an initial temperature of 80.4 °C and treatment duration of 10.0 min caused a significant ( $p < 0.05$ ) increase in the DPPH antioxidant activity, which is 47% higher compared to the control sample. Similar to DPPH, the highest increase in TPC (36%) and antioxidant activity determined by the FRAP method (34%) compared to the control was also achieved with the ultrasound treatment at an initial temperature of 80.4 °C and treatment duration of 10.0 min, but this difference was not statistically significant ( $p > 0.05$ ). The initial temperature of the sample before

HIU treatment did not significantly affect TPC or antioxidant activity, probably because temperatures were approximately equalized by the end of the treatment. Since the tests for determining antioxidant activity should complement each other, Pearson's correlation coefficients ( $r$ ) with the corresponding  $p$ -values were identified (\*  $p < 0.05$ ; \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ ). A linear correlation between the TPC content and the antioxidant activity was established with a correlation coefficient between the TPC and DPPH ( $r = 0.83^{***}$ ) and between the TPC and the FRAP method ( $r = 0.69^{**}$ ). Also, a strong correlation between the antioxidant activity measurement assays (DPPH and FRAP method) ( $r = 0.83^{***}$ ) was found, despite the fact that the mechanisms of these reactions are different (Apak et al., 2013).

**Table 2** TPC and antioxidant activity of buckwheat hulls determined by DPPH and FRAP methods

Sample	TPC (mg GAE/100 g)	DPPH (mg TE/100 g)	FRAP (mg TE/100 g)
Control sample	(180.05 ± 1.31) <sup>ab</sup>	(155.13 ± 18.15) <sup>a</sup>	(133.78 ± 1.65) <sup>abc</sup>
9.7 °C; 10.0 min	(206.37 ± 4.02) <sup>ab</sup>	(187.18 ± 2.87) <sup>ab</sup>	(135.63 ± 10.47) <sup>ab</sup>
20.0 °C; 5.0 min	(186.54 ± 0.89) <sup>ab</sup>	(184.33 ± 10.01) <sup>ab</sup>	(126.52 ± 3.12) <sup>a</sup>
20.0 °C; 15.0 min	(238.68 ± 20.13) <sup>ab</sup>	(214.92 ± 10.69) <sup>ab</sup>	(168.08 ± 6.24) <sup>abc</sup>
45.0 °C; 2.9 min	(183.52 ± 11.33) <sup>a</sup>	(150.20 ± 5.60) <sup>a</sup>	(115.18 ± 7.51) <sup>a</sup>
45.0 °C; 10.0 min	(217.04 ± 23.73) <sup>ab</sup>	(183.84 ± 24.50) <sup>ab</sup>	(142.87 ± 9.25) <sup>abc</sup>
45.0 °C; 17.0 min	(219.54 ± 6.63) <sup>ab</sup>	(193.93 ± 3.67) <sup>ab</sup>	(166.25 ± 11.57) <sup>abc</sup>
70.0 °C; 5.0 min	(205.21 ± 24.39) <sup>ab</sup>	(207.62 ± 11.27) <sup>ab</sup>	(145.90 ± 12.89) <sup>abc</sup>
70.0 °C; 15.0 min	(202.93 ± 1.18) <sup>ab</sup>	(188.87 ± 5.87) <sup>ab</sup>	(158.44 ± 4.25) <sup>abc</sup>
80.4 °C; 10.0 min	(245.63 ± 4.93) <sup>b</sup>	(228.34 ± 4.92) <sup>b</sup>	(179.39 ± 3.33) <sup>abc</sup>

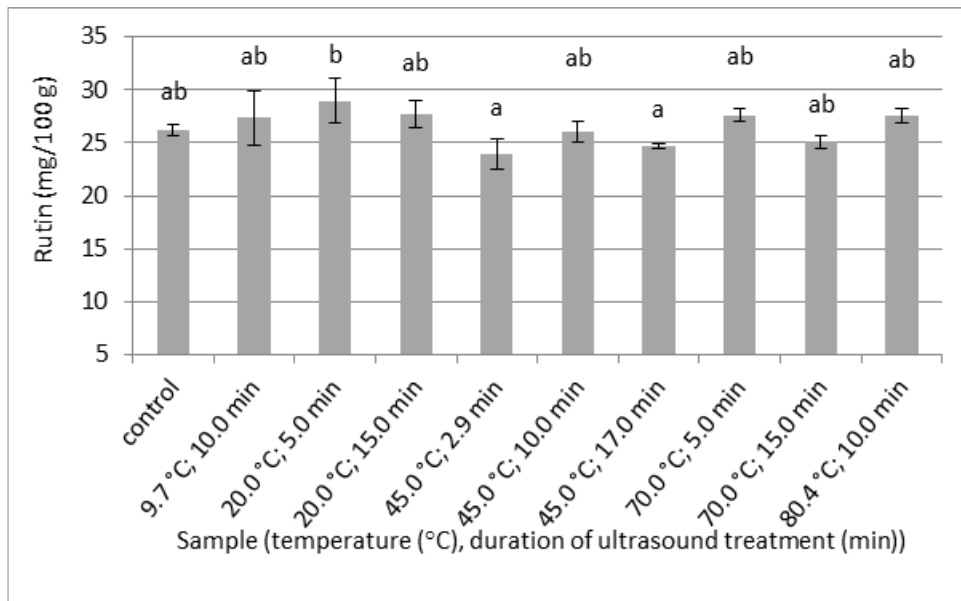
GAE, gallic acid equivalents; TE, Trolox equivalents

<sup>abc</sup> Different letters indicate significant differences between means in the same column ( $p > 0.05$ )

### **Rutin content**

Compared to the control sample, HIU treatment did not cause a statistically significant increase in the content of rutin in buckwheat hulls (**Figure 2**). Nevertheless, a small increase of the rutin content was observed in the sample with an initial temperature of 20 °C and a treatment duration of 5 minutes which was 6% higher than in the control sample. The use of a different solvent in HIU treatment could potentially be more effective in releasing rutin, since Peng et al. (2013) found a correlation between methanol content as a solvent and extraction efficiency. Thus, when using 50-70% methanol, the proportion of rutin was higher compared to the

usage of 10-50% methanol, and they explain the rapid degradation of rutin at lower methanol concentrations as a possible cause of such results. Nevertheless, considering high content of rutin, buckwheat hulls have a great potential as a dietary source of rutin.

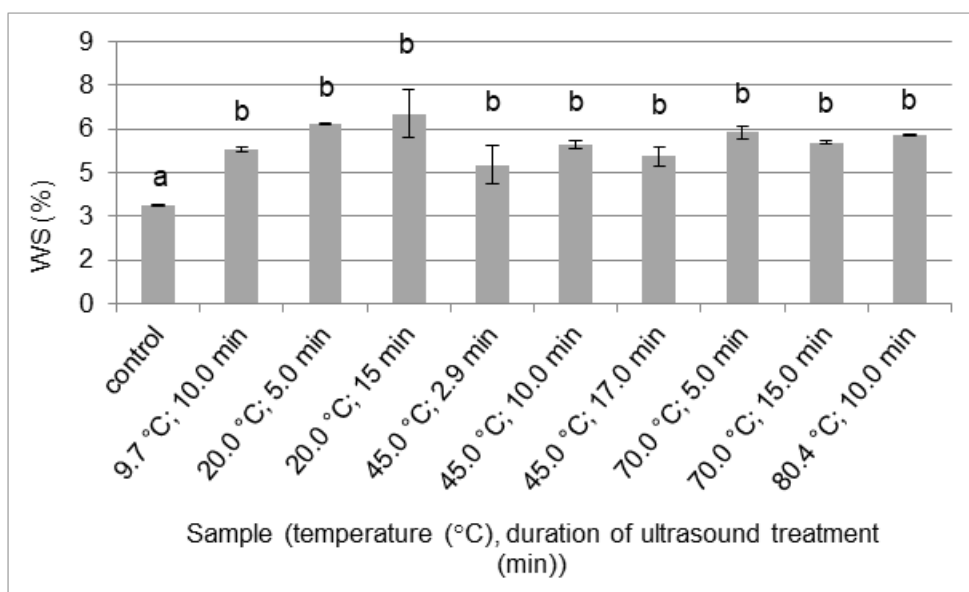


**Figure 2** The proportion of rutin depending on HIU treatment compared to control. Results are expressed as mean  $\pm$  standard deviation ( $n = 3$ ) per 100 g of sample dry matter. Different letters indicate a statistically significant difference between the obtained values ( $p < 0.05$ )

### ***Influence of HIU on water swelling ability of buckwheat hulls***

Dietary fibres have been extensively studied for their ability to regulate transit time due to the increase of stool bulk and other benefits, such as hydration properties as swelling, water-holding and water retention capacities (Robertson et al., 2000). The water swelling capacity of our control sample (3.38 mL/g) was lower compared with the results (5.09 mL/g) from Zhu et al. (2014) for the swelling capacity of water-insoluble dietary fibre of buckwheat hulls after ultrasonic bath treatment followed by the addition of enzymes ( $\alpha$ -amylase and protease) and ethanol anhydride (acetic acid anhydride). The difference could be attributed to the difference in the treatment of buckwheat hulls, since the treatment in ultrasonic bath combined with enzymes may greatly enhance the water swelling ability compared to the HIU treatment used in this study. Our results show that all HIU treated samples have significantly ( $p < 0.05$ ) higher water swelling ability compared to the control sample. The highest water swelling abilities compared to the control sample were obtained in treatments with initial temperature 20 °C and treatment duration of 5 (83%) and 15 min (93%). Considering the fact that dietary fibres make up 90% of the chemical

composition of buckwheat hulls (Skrabanja et al., 2004), it can be assumed that the action of HIU resulted in the cracking and/or redistribution of chemical bonds between building molecules of dietary fibres, which led to better water absorption. Higher swelling ability indicates higher content of soluble dietary fibre, which is characterised by the capacity to increase viscosity and to reduce the glycaemic response and plasma cholesterol (Elleuch et al., 2011). Increased water swelling indicates improved capacity for the reduction of blood cholesterol.

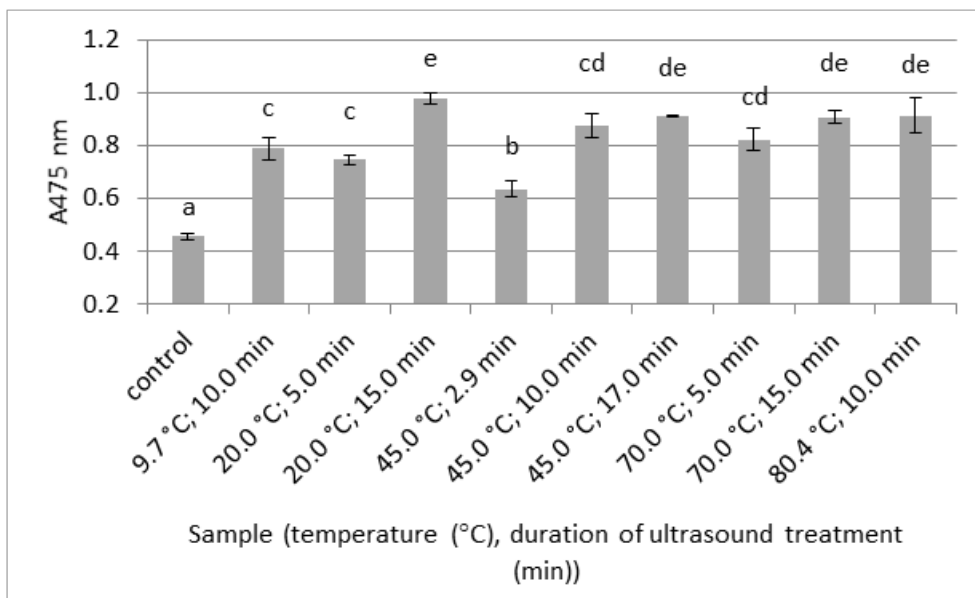


**Figure 3** Water swelling capacity (WS) depending on the HIU treatment compared to the control sample. Results are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). Different letters indicate a statistically significant difference between the obtained values ( $p < 0.05$ )

### ***Polyphenol oxidase activity***

The results show that HIU treatment caused a significant ( $p < 0.05$ ) increase in PPO activity in all samples compared to the control sample (**Figure 4**). The lowest increase was observed in the sample with the shortest duration of HIU treatment (2.9 min) and an initial temperature of 45.0 °C (39%), while the highest increase (114%) was observed in the sample of initial temperature 20.0 °C with a HIU treatment duration of 15 min. This reaffirms the importance of the duration of HIU treatment relative to the initial temperature of the sample and also on PPO activity ( $p = 0.0014$ ). The obtained results are in accordance with the results on proso millet bran by Čukelj Mustač et al. (2019), and by Bi et al. (2015) who showed that HIU treatment causes an increase in PPO activity in degreased avocado puree, which is attributed to its release from the cell upon cell damage caused by HIU. Okot-Kotber et al. (2002) also obtained similar results by using different organic solvent and ionic detergents as PPO activators in wheat bran extracts, which confirms the suitability of HIU as a

water extraction pretreatment. Combination of water extraction and HIU treatment results in a significant increase in PPO activity without the usage of organic solvents. Matheis and Whitaker (1984) confirmed that PPO has the same oxidizing effect as the chemical oxidizing agent potassium iodate whose addition during mixing procedure reforms the disulphide bonds and increases dough strength, resulting in a better quality of bakery products. The same authors pointed out the possibility of using PPO-rich natural extracts in the production of gluten-free bakery products as a substitute for commercially available chemical additives.



**Figure 4** Activity of polyphenol oxidase in treated samples compared with the control expressed as absorbance at 475 nm

## CONCLUSIONS

In this study, high-intensity ultrasound (HIU) was used as a pretreatment of an aqueous suspension of buckwheat hulls, whose further application would be intended for the production of functional cereal products. This research has shown that micronized buckwheat hulls are naturally rich in bioactive compounds and as such can be used to enrich food products, bakery products in particular. The HIU has a particularly positive effect on the content of total phenolic compounds and on the antioxidant activity of buckwheat hulls. The highest content of total phenolic compounds and the highest antioxidant activity were observed in the sample with an initial temperature of 80.4 °C and HIU treatment of 10 minutes. HIU treatment also slightly increases the extractability of the most important flavonoid of buckwheat hulls - rutin and the ability to absorb water, and the highest increase was observed in the sample with an initial temperature of 20.0 °C and HIU treatment duration of 5 minutes. High-intensity ultrasound increases the activity of the polyphenol oxidase enzyme, to a greater or lesser extent, depending on the duration



of the treatment rather than the initial temperature, with the highest increase observed at 20.0 °C and HIU treatment duration of 15 minutes (114%). Treatment optimization should be carried out concerning the type of product that is intended to be enriched with buckwheat hulls. In the production of bakery products, increased activity of polyphenol oxidase is desirable because it can strengthen the dough; yet, in some other products, it is desirable to have a lower polyphenol oxidase activity to maintain the stability of phenolic compounds. Future studies should address the applicability of treated buckwheat hulls in cereal products.

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## EFFECTS OF CEREAL CONSUMPTION ON GUT MICROBIOTA COMPOSITION

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### SUMMARY

The intake of whole grain cereals has long been linked to decreased risks of metabolic syndrome, cardiovascular diseases, and diabetes type II. It is believed that a complex range of components in whole grain cereals creates a synergistic action resulting in protective effects. Some of those components, such as resistant starches and arabinoxylans, which are not digested in the upper gastrointestinal tract, serve as a food source for gut microbiota. Generally, it is believed a diet rich in whole grain cereals (fibres) is good for the microbiota, as it increases diversity and specifically increases the bacterial genera believed to be beneficial, such as *Bifidobacterium* and *Lactobacillus*. In this study, the intake of cereals was assessed using the 3-day food dietary method among the *MicroEquilibrium* study cohort, consisting of 40 subjects, both male and female, average age  $27.75 \pm 0.66$ , distributed according to their degree of body mass index (underweight, healthy, and overweight/obese). The composition of microbiota was determined by sequencing the V3-V4 region of the 16S rRNA gene, and the taxonomic composition and alpha diversity of the subjects were correlated with the individual cereal intake. The results showed an increase in gut microbiota diversity in subjects with higher whole grain cereal intake.

**Keywords:** diet, cereal intake, fibre, gut microbiota

### INTRODUCTION

Whole grain cereals have been introduced into the human diet over 10 000 years ago, after the development of agriculture (Spiller, 2002) and today they make up the main proportion of almost all diets. The health benefits of cereal intake have long been known. In the modern age, in the early 1800s and mid-1900s, whole grain cereals were recommended for constipation prevention, especially after the 'fibre hypothesis' was published and health benefits of fibre foods were established (Trowell, 1972; Slavin, 2004). Whole grain cereals are rich in nutrients and phytochemicals with known health benefits, e.g. dietary fibre, resistant starch, and oligosaccharides, accompanied by an abundance of antioxidants, including trace minerals and phenolic compounds, all known to be beneficial for disease prevention.

Whole grain food has been associated with a lower incidence of cardiovascular disease, which is also confirmed by meta-analyses showing a consistent, inverse association between whole grains from the diet and the incidence of cardiovascular disease in epidemiological cohort studies (Mellena, et al., 2008). Epidemiological studies also show that the intake of whole grains also offers protection against various cancers, diabetes type II, and obesity (Mann, et al., 2017). Despite recommendations that say that we should consume three servings of whole grains daily, the usual intake in Western countries is not satisfactory (Slavin, 2004).

In the last decade, one new factor influencing health and well-being is becoming more recognized - microbiota. Gut microbiota represents a complex network of microorganisms playing an important role in maintaining human health. Its determined function has been ranging from helping with the digestion of certain foods and the synthesis of vitamins, to training the immune system and being a factor influencing the onset of some diseases. For microbiota to be beneficial, it is important for the host to maintain its diversity. Of the environmental factors affecting microbiota composition, food/nutrition is the one having the most impact. Some of the food components indigestible to humans, such as resistant starches, arabinoxylans, and bran, serve as a food source of gut microbiota. Generally, it is believed that a diet rich in whole grain cereals (fibres) is good for the microbiota, as it increases diversity and specifically increases the bacterial genera believed to be beneficial, such as *Bifidobacterium* and *Lactobacillus* (Koecher, et al., 2019).

The objective of this research is to determine if there is a correlation between the gut microbiota composition and whole grain cereals intake.

## **MATERIALS AND METHODS**

### ***Subjects***

The study was conducted as part of the larger study MicroEquilibrium - Research of the Balance of the Gut Microbiome, funded by the Croatian Science Foundation, in the period from January to April 2018 in Zagreb.

Subjects were recruited for the study through public flyers, advertisements on the official website of the Project, and through personal acquaintances of researchers involved in the project.

The inclusion criteria were; both genders; age from 18 to 35; body mass index (BMI) lower than 18.5, between 20 and 22 and above 27 kg/m<sup>2</sup>; healthy subjects (without known chronic diseases). The only exclusion criterion was antibiotics use in the period of three months prior to the start of the study.

Participation in the research was on a voluntary basis. Before enrolment in the research, every subject was fully informed about the research goals and the methodology used, and has given their consent to participate in the research.

The total number of applied volunteers was 136, out of which 56 were eligible to participate according to the given criteria, and out of those, 40 subjects responded to the survey, fulfilled all the necessary questionnaires, and did all the

measurements. The study involved 13 underweight subjects, 10 adequately nourished subjects, and 17 subjects with excessive body mass or obesity, both genders included.

### **Methods**

In this study, dietary, anthropometric, genomic, and statistical methods were used. Also, each subject had to collect a stool sample prior to the anthropometric measurements and had to evaluate the consistency of the stool using the Bristol stool scale, which consists of seven types of stools based on their consistency and indicating the state of our digestion (O'Donnell et al., 1990).

The dietetic method used was a three – day food diary record. This method was used to gather and analyse the intake of energy and macronutrients of the subjects. The subjects received detailed instructions for filling out the three – day food diary with an example. It had to be recorded for three consecutive days prior to giving the stool sample and conducting anthropometric measurements. The subjects estimated the quantity of consumed food and drinks by using kitchen utensils (e.g. tablespoons, teaspoons, glasses, cups, bowls, and plates) and/or weight (g,) as well as the data stated on the declaration for packaged food. They also had to record the type and the amount of the dietary supplements taken, if any. Subjects were instructed to provide the recipes for complex dishes and if those were missing, standard recipes were used (Coolinarika, 2018). All collected food records were analysed using the Croatian National Nutrition Table (Kaić-Rak, 1990), which, due to insufficient data, was supplemented with data from the declarations of consumed food products.

The size and the composition of the body was established using anthropometric methods. Body mass, body height, and body composition (shown as the amount and mass of body fat and lean tissue) were measured. Body height was measured with a stadiometer Seca 217 (Seca Ltd.) with a precision of 0.1 cm. During the measurement, subjects were barefoot, standing upright, relaxed, and touching the scale in four points (scalp, shoulder blades, backside, and heels). Also, the position of the head was in the Frankfort horizontal plane. The measurements of body mass, the percentage and mass of fat tissue, muscle mass, the percentage of lean body mass, bone mass, total body water mass, the level of visceral tissue, basal metabolism (BM), and BMI were determined using a body composition analyser (TANITA DC-430MA), which works on a principle of bioelectric impedance (BIA). During the measurement, the subjects wore minimal clothes and were barefoot.

Deoxyribonucleic acid (DNA) was isolated from the frozen stool samples using the QIAamp® PowerFecal® DNA kit according to the manufacturer's instructions. The quantity of isolated DNA was determined using the BioSpec Nano-drop spectrophotometer (Shimadzu Biotech). The isolated DNA was sent to sequencing in the Molecular Research Lab (MRDNA LAB), Texas, USA. The sequencing method used was Illumina MiSeq, using the paired-end protocol with the MiSeq Reagent kit v3. The targeted sequence were variable regions 3 and 4 of the gene coding for the 16S rRNA. The raw sequencing data of the 16S rRNA gene fragment was downloaded

from Illumina Basespace (<https://basespace.illumina.com>) in a fastq file format. Two fastq files were downloaded, one which corresponds to the nucleotide sequences in the Forward reading mode, while other corresponds to the sequences in the Reverse method of reading. After downloading, the sequences were loaded in the QIIME 2 (<https://qiime2.org/>) program and the forward and reverse reads were assembled into the complete sequence. Sequence quality was checked using the DADA2 software package which is optimized for the correction of sequencing errors of the Illumina platform. The sequences passing quality filtering were clustered into an operating taxonomic unit (OTU) at the taxonomic level of the species. Taxonomy was assigned to OTUs using a Naive Bayes classifier pretrained on sequences covering variable regions 3 and 4 from the Greengenes 13\_8 99% 99% OTU database. To determine the differences in the presence of individual OTUs between samples or groups of samples, an ANCOM method was developed to take into account the compositional constraints that represent microbial diversity.

MS Excel (Microsoft, Seattle, WA, USA) and SPSS v.22 (SPSS Inc., Chicago, IL) were used for data analysis and processing. Standard descriptive methods (arithmetic mean, median, standard deviation) were used to describe the characteristics of the subjects. The differences in the characteristics between the groups were compared to the independent Student t-test for continuous data, and the hi-squared test for categorical values, respectively. All obtained results are shown as an arithmetic mean and standard deviation for continuous data and as a percentage for categorical variables, respectively. The level of significance was established at  $p < 0.05$  for all performed tests.

## RESULTS AND DISCUSSION

Although the gut microbiota is relatively stable in a healthy individual, it is influenced by many factors. According to recent research, the most important factor influencing the gut microbiota is the diet (Graf et al., 2015). In addition, research has shown that there is a potential difference in the composition of the gut microbiota according to cereal intake. The aim of this research was to determine whether there is a correlation between the gut microbiota composition and the adequate intake of whole grain cereals.

### ***Anthropometric results***

The study involved 40 subjects of both genders, aged 18 to 35. The average age of all subjects was  $25.75 \pm 4-25$  and there was no significant difference between groups in terms of the body mass index (**Table 1**). There were 13 (32.5%) subjects in the underweight group, 10 (25%) subjects in the adequately nourished group, and 17 (42.5%) subjects in the overweight/obese group. In terms of gender, the subjects were equally distributed, 20 subjects of each gender participated in the study. More female subjects were in the underweight (64.5%) and adequately nourished (70.0%) group, while in the overweight/obese group, there were fewer female subjects (29.4%). The analysis of anthropometric parameters revealed a statistically

significant difference in all observed parameters, except for body height, which was expected considering the distribution of subjects according to the degree of the body mass index, which is shown in table 1.

The TANITA DC-430MA body composition analyser, which works on the principle of bioelectrical impedance, was used to measure the body composition of the subjects. The expected significant difference ( $p < 0.001$ ) was obtained for all parameters measured by the TANITA analyser by conducting a statistical analysis between the groups according to their body mass index (**Table 1**).

Also, the subjects had to give a subjective assessment of the type and consistency of their stool. The estimation was made according to the Bristol stool scale, which ranges from type1 to type7, where type1 indicates a hard, dry nut-like stool shape, while type7 indicates a watery-like stool and type4 is considered optimal. Considering the Bristol stool scale, no significant difference ( $p=0.278$ ) in the type of the stool was observed between the studied groups.

**Table 1** General and anthropometric characteristics of the study cohort

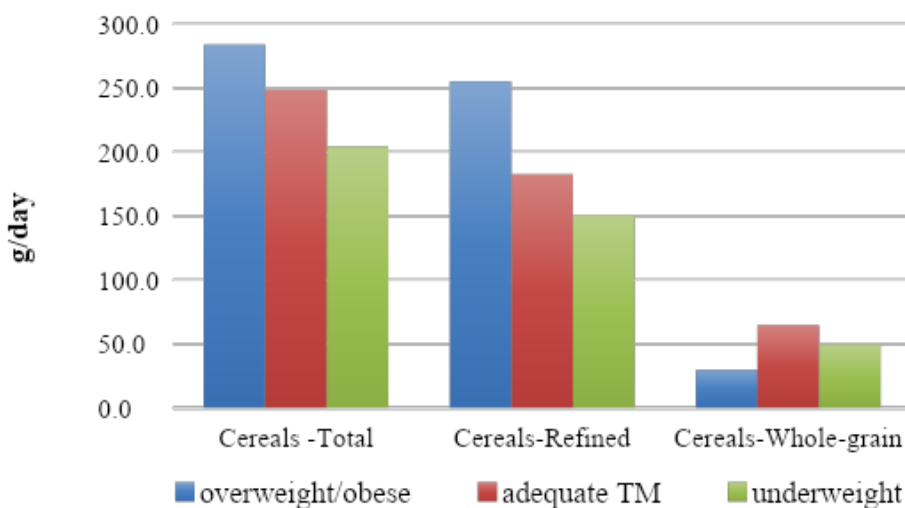
Parameter	Subjects total (n=40)	Underweight subjects (n=13)	Adequately nourished subjects (n=10)	Overweight/ obese subjects (n=17)	$p^*$
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	
Age (year)	25.75 $\pm$ 4.25	24.92 $\pm$ 2.90	24.0 $\pm$ 2.36	27.41 $\pm$ 5.40	0.131
Gender (% female)	50.0	64.5	70.0	29.4	0.077
Body height (cm)	176.88 $\pm$ 11.72	184.31 $\pm$ 10.46	171.64 $\pm$ 9.65	181.91 $\pm$ 12.29	0.053
Body mass (kg)	76.42 $\pm$ 3.86	53.89 $\pm$ 2.18	64.49 $\pm$ 2.45	101.25 $\pm$ 3.75	<0.001
Body fat (kg)	16.34 $\pm$ 1.55	6.52 $\pm$ 0.69	12.36 $\pm$ 0.82	26.18 $\pm$ 1.55	<0.001
Body fat (%)	20.24 $\pm$ 8.69	12.50 $\pm$ 5.63	19.80 $\pm$ 5.01	26.38 $\pm$ 7.64	<0.001
Muscle mass (kg)	57.10 $\pm$ 2.67	44.97 $\pm$ 2.39	48.56 $\pm$ 2.61	71.39 $\pm$ 3.75	<0.001
Fat free mass (kg)	60.08 $\pm$ 2.80	47.37 $\pm$ 2.51	51.13 $\pm$ 2.75	75.07 $\pm$ 3.92	<0.001
Total body water (%)	41.58 $\pm$ 1.94	32.48 $\pm$ 1.72	35.78 $\pm$ 1.84	57.95 $\pm$ 2.71	<0.001
Bone mass (kg)	2.99 $\pm$ 0.13	2.4 $\pm$ 0.12	2.57 $\pm$ 0.13	3.68 $\pm$ 0.18	<0.001
Level of visceral tissue	3.63 $\pm$ 0.52	1.00 $\pm$ 0.00	1.40 $\pm$ 0.27	6.94 $\pm$ 0.62	<0.001
BMI (kg/m <sup>2</sup> )	24.09 $\pm$ 6.11	17.65 $\pm$ 1.01	21.50 $\pm$ 0.94	30.54 $\pm$ 2.76	<0.001



### **Dietary results**

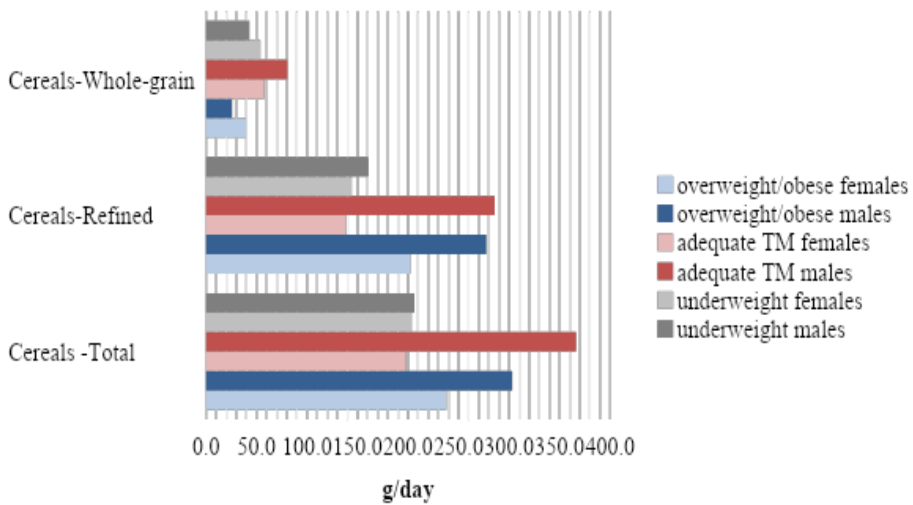
At the beginning of the research, the subjects were asked to fill a three-day food diary in order to examine their average intake of energy and micronutrients, as well as their daily cereal intake and the ratio of the refined and whole grain intake. The cereal intake was later analysed according to the composition of the gut microbiota. In this study, the intake of cereals is particularly important because they are a major source of dietary fibre, which is the main component in the food that affects the composition and activity of the gut microbiota (Ercolini and Fogliano, 2018). It is recommended that at least half of the total cereal intake is derived from whole-grain cereals, as the most active substances are in the shell and the germ of the cereals (Alebić, 2008).

The average daily intake of cereals, refined as well as whole-grain, was calculated from the data obtained from the three-day food diary record and was compared between groups according to their body mass index (**Figure 1**). In this study, the average intake of whole-grain cereals was lower than the recommendations (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). In the underweight group, 24.21% of the total cereal intake was whole-grain cereals, while in the adequately nourished group, 25.98% of the total cereal intake was whole-grain cereals. In the overweight/obese group, the intake of whole-grain cereals was only 10.52% of the total cereal intake (**Figure 1**).



**Figure 1** Cereal intake among the study cohort.

When subjects were separated based on gender and BMI, it was noticed that both genders consume more refined than whole-grain cereals, while men in the adequate and overweight/obese groups consume considerably more refined cereals than women. (**Figure 2**).

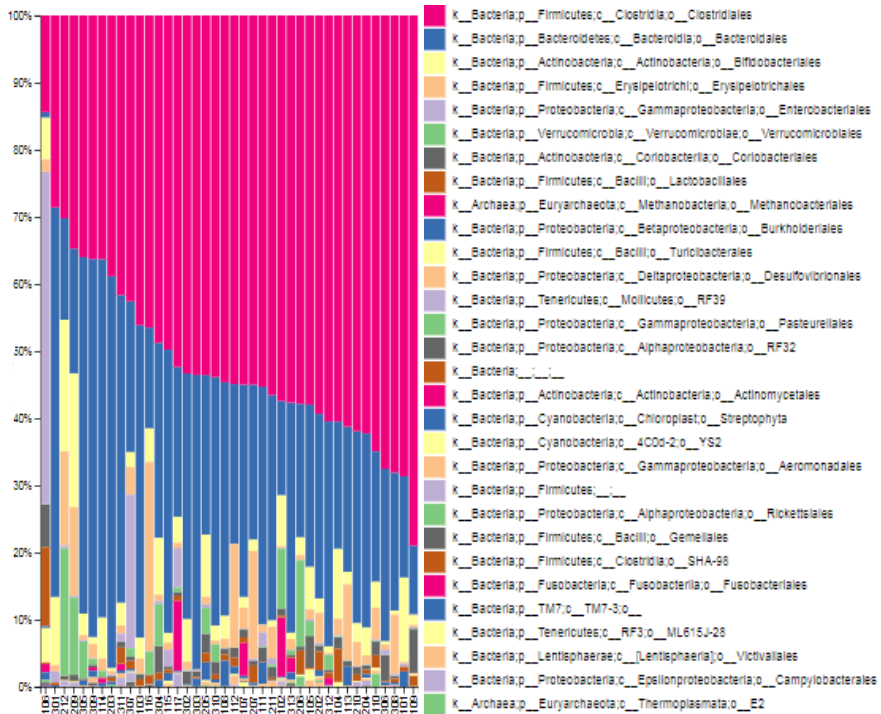


**Figure 2** Cereal intake considering the body mass index and gender.

### **Genomic results**

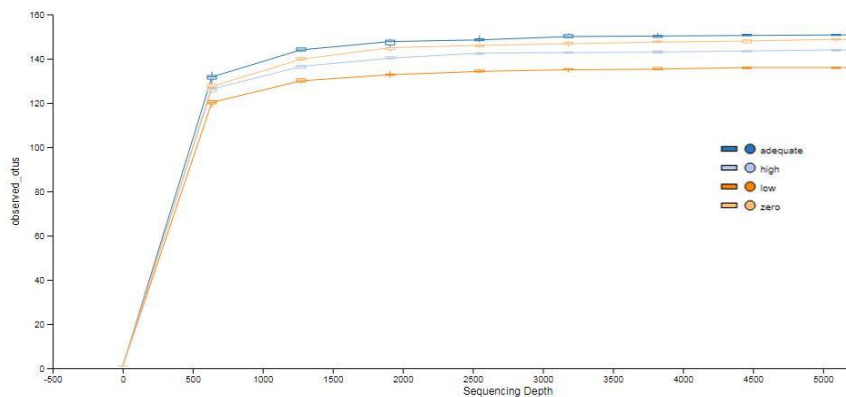
The gut microbiota is characterized by a high biodiversity of microorganisms. So far, out of 52 currently recognized bacterial phyla, five to seven phyla are known to inhabit the gastrointestinal tract of mammals. In general, the gut microbial community is dominated by the phyla *Firmicutes* and *Bacteroidetes*, while members of the phyla *Proteobacteria*, *Actinobacter*, and *Verrucomicrobia* are less abundant (Shin et al., 2015).

The taxonomic composition of a subject’s gut microbiota is shown in the form of a bar chart (**Figure 3**). Each bar is representing the entirety of the subject’s microbiota, here shown at the taxonomic level of order.I. The taxonomic classification was obtained by aligning a representative sequence of each OTU with the Greengenes 16S rRNA database.



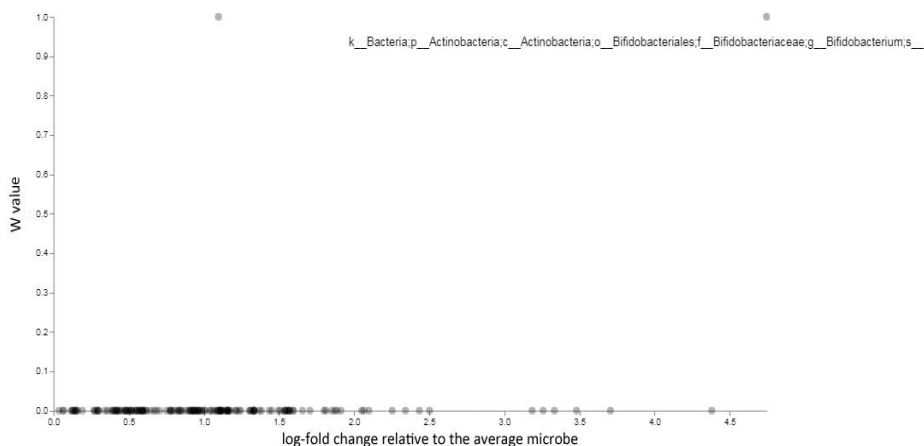
**Figure 3** Taxonomic diversity of the gut microbiota obtained by the analysis of stool samples

Alpha diversity, representing the diversity of each individual sample, has shown highest bacterial abundance in the subject with the adequate intake of whole-grain cereals and the lowest in the subjects having lower than recommended daily intake of whole-grain cereals. A visual display of the results of alpha diversity is shown in **Figure 4**, where the x-axis represents the sequencing depths while the y-axis represents the number of the OTU.



**Figure 4** Alpha diversity of gut microbiota - subjects grouped based on the recommended daily intake of whole-grain cereals.

Based on the ANCOM analysis, identifying differentially abundant bacterial species based on whole-grain cereal intake, only the unidentified bacteria from the genus *Bifidobacterium* had an increased abundance in the group of subjects with the adequate intake of whole-grain cereals.



**Figure 5** Bacterial species with significantly different abundance in subjects, based on whole-grain cereal intake

## CONCLUSIONS

The results showed an increase of gut microbiota diversity in subjects achieving the recommended daily intake of whole grain cereals. Based on the alpha diversity analysis, the ratio of whole grain to refined cereals seems to be important for the biodiversity of microbiota. In the group with the adequate intake, an increase in the abundance of the *Bifidoibacterium* genus was observed.

Future research should involve a larger number of subjects with greater differences in dietary habits to determine the statistically significant influence of the diet on the gut microbiota composition according to the degree of the body mass index.

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## WHEY PROTEINS AS A VALUABLE INGREDIENT TO BAKERY PRODUCTS

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### SUMMARY

Whey protein fractions represent 18 - 20 % of total milk nitrogen content. They can be used as an ingredient to various food products due to their unique functional characteristics, such as emulsifying, gelation, thickening, foaming, and water binding ability. In addition to the desirable functional characteristics, they also have the advantage of their high nutritional value. Biological value, as well as other nutritional value indicators, are due to a more favourable amino acid composition, mostly thanks to the most valuable  $\alpha$ -lactalbumin, the amino acid composition of which is close to the biological optimum. Whey proteins, as ingredients on the market, can be obtained in several different forms, most commonly as whey protein isolate (WPI) and whey protein concentrate (WPC). Whey proteins can be considered as potential ingredients for the bakery industry in view of their desirable functional characteristics and nutritive value. When used in baking, whey ingredients can emulsify, thicken, brown, and foam products. Whey powder also increases solubility, gelation, water binding, and nutritional fortification. However, the effect depends on the type and level of the whey protein concentrate (WPC). WPC is best for egg replacement in baked goods and has a high level of protein. It can help replace gluten levels, because its structure and gas-trapping properties mimic gluten. It can also help reduce fat and carbohydrates while increasing the nutritional benefits of the product. This paper presents the effect of whey proteins on the physicochemical, textural, and nutritional properties of bakery products.

**Keywords:** bakery products, ingredient, whey proteins

### INTRODUCTION

Whey is the main secondary raw material for modern cheese (95%) and casein (5%) industry. Annually, 190 million tons of whey is produced, with a projected tendency of growth of up to 2% per year (European Dairy Association, 2019). Despite the high potential of this very valuable resource, only 70% of the sum total of the produced whey is made into various food products or used in the fermentative industry. The leading manufacturers and refiners of whey are located in Europe and the United States of America, and they make up around 70% of the global production of whey

(Božanić et al., 2014). Annually, they make around 7.7 million tons of powdered whey and whey proteins (Matijević, 2018).

Due to their nutritional value and functional properties, proteins are the most valuable component of whey (Skryplonek, 2018). The nutritional value of whey proteins depends on the favourable ratio of amino acids, especially essential amino acids. Cysteine, lysine, and tryptophan proteins of whey have a better biological value and a favourable ratio of cysteine/methionine which has a better biological availability in the organism when compared to other meat-based or plant-based proteins. Aside from their nutritional value, whey proteins also have good functional properties, such as: high solubility, viscosity, foaming, gelation, and emulsification, as well as water binding properties, which makes them good enriching supplements for various food products, and they help in improving their sensory properties. The usage of whey proteins is very varied in the food industry, more specifically in the production of highly functional food enriched with high value proteins (Bierzuńska and Cais-Sokolińska, 2018). However, these proteins can also be used as a substitute for fat or carbohydrates, or they can be used to improve the product's protein value (Tratnik and Božanić, 2012; Gupta et al., 2016).

Modern technology enables the extraction of whey proteins and the production of various products, from permeates, powder protein concentrates of varying purity, to isolate and hydrolysate powdered proteins. This makes whey proteins easily accessible as resources for numerous products (Deeth and Bansal, 2019).

In bakery, whey proteins are the best substitute for eggs in pastries. They can help in substituting and lowering gluten usage, due to the fact that their structure and gas retention properties maintain the technical properties similar to gluten. They can also improve the nutritional value of baked goods and they can improve certain sensory values such as the aroma. This research intends to show the impact of whey proteins on the physicochemical, textural, and nutritional properties of baked goods.

### **WHEY PROTEIN-BASED PRODUCTS**

Due to their nutritional value and valuable functional properties, whey proteins have invoked researcher interest since the late 80s. The development of membrane techniques, including ultrafiltration, reverse osmosis, and microfiltration, has allowed the production of various whey-based products, such as powdered whey, whey protein concentrate (WPC), whey protein isolate (WPI), whey protein hydrolysate, and pure  $\alpha$ -lactoglobulin and  $\beta$ -lactalbumin.

#### ***Powdered whey***

Liquid whey easily spoils and it needs to be processed as soon as possible. Drying is a process which is most often used in the treatment of whey. Up to 70% of processed whey is made as powdered whey. Outside of that, drying is a part of the technological procedure for making whey powder concentrate (WPC) and whey powder isolate (WPI) (Matijević, 2018).

By drying whey, there is no loss of secondary products and the powder can be stored up to 18 months, which is the base advantage of this process. The high nutritional value of whey makes it a valuable resource in the food industry for various products. It is especially useful in the baking industry. Powdered whey affects various properties of food products such as: a) it enhances the product's sensory properties, b) it enhances the physical properties and regulates the acidity level, and c) it is used as an adsorbent and a fat and oil carrier. However, the high mineral content of whey can make powdered whey an undesirable product due to its pronounced salty flavour. Therefore, before drying, it is necessary to conduct the demineralization of whey (by ion exchange, electrodialysis, or nanofiltration) (Božanić et al., 2014; Matijević, 2018).

### **Whey protein concentrate (WPC)**

Whey protein concentrate (WPC) is a powder made by drying the retentate or concentrate of whey, which is made through the process of ultrafiltration. Whey powder concentrate contains up to 60% of whey protein in dry matter, which is made exclusively through ultrafiltration, while a larger percentage of protein in the dry matter of WPC can be achieved through the process of diafiltration. Proteins can be concentrated up to 80% in dry matter (Tratnik, 2003).

Economically speaking, further concentration can only be justified in the case of the implementation of this product into a final product of a high market value, such as dietary, pharmaceutical, and similar products.

The first generation of proteins concentrated from powdered whey contained about 30-40% of proteins and had significant quantities of lactose, fat, and denatured proteins. It was called a "concentrate" ("Whey Protein Concentrate", or WPC for short). These whey proteins were initially extracted through the various processes of sedimentation (most commonly done with the use of carb-oxy-methyl-cellulose), the product of which was a denatured whey protein with high nutritional value, but lower functional properties, which was often the effect of thermal treatments which were applied during their extraction. With the advent of the membrane separation technique, the production of non-denatured whey protein concentrates, which can further be fractioned into their various components, was enabled (Macwan et al., 2016).

Ultrafiltration of whey is one of the most prospective technologies used to concentrate and extract the native proteins of whey. The technological process of acquiring whey proteins through ultrafiltration is very simple. It begins with a centrifugal separator through which the fat and cheese particles are separated. After the separation, it is pasteurized, cooled, and kept at a certain temperature, after which it is either ultra-filtered or diafiltered and dried. The whey protein concentrate made using this procedure can contain up to 70-80% (in some occasions even more) dry mass protein with a reduced amount of lactose and fat.

It is estimated that WPC (as a finished product) is inferior to whey protein isolates (WPI), but that is not necessarily the case. Even though WPC contains less proteins



in "gram per gram" ratio than the isolate, a high-quality concentrate contains various interesting ingredients that are rarely found in isolates (due to the various procedural solutions) (Herceg and Režek, 2006).

#### ***Whey protein isolate (WPI)***

Whey protein isolate (WPI) is often made through the application of procedures revolving around ionic exchange, due to which it contains more than 90% of proteins in dry matter. This concentration of proteins in dry matter has become the basis on which the isolates are separated from the concentrates, even though, technically speaking, the term "isolate" has been, for the longest time, associated exclusively with the process of ionic exchange. WPI contains minimal amounts of lactose and almost no fat. The advantage of a well-made WPI lies in the fact that it contains more proteins with less lactose, fat, and mineral substances per gram than WPC (Macwan et al., 2016). Whey protein isolates are used in products where their determining factors are their gel firmness, viscosity, water binding ability, and solubility, in which case, the whey protein isolates can significantly improve the texture and flavour of the finished product (Herceg and Režek, 2006; Tratnik and Božanić, 2012).

#### ***Whey protein hydrolysate (WPH)***

Whey protein hydrolysates (WPH) are proteins of the concentrate (WPC) or the isolate (WPI) which are subjected to the enzymatic hydrolysis of the peptide bonds and higher temperature, in order to inactivate any additional enzymes (Smith et al., 2016). Hydrolysis improves the digestibility of the proteins and changes their functional properties, including solubility and heat stability (Nongonierma and FitzGerald, 2015). Despite their health benefits, the usage of whey protein hydrolysates is limited due to their intense bitter flavour, which is a by-product of hydrolysis and the usage of certain enzymes. Small peptides, from 600 to 4140 Da, which are made in the process of hydrolysis, will give off a more pronounced bitter flavour (Leksrisompong et al., 2012). Apart from the small peptides, during the process of hydrolysis, certain sulfuric compounds are made, such as di-methyl-sulphate (which gives it a cabbage like aroma) and methional (which gives it a potato like aroma).

#### **WHEY PROTEINS AS A SUBSTITUTE FOR EGGS**

Eggs belong to one of the basic ingredients in the preparation of dough, and can be added as egg whites or as a whole egg. During the beating process, eggs have the ability to incorporate large quantities of air. When flour is added to this foam, the dough has a light and airy texture. Egg-white proteins (albumins) allow the stability of the net-like structure of the dough during the baking process, once the dough begins to release its incorporated air. The yolk affects the flavour and the colour, and it works as an emulsifier (lecithin). Egg proteins are some of the most valuable proteins since they contain all of the essential amino acids, so the addition of this ingredient improves the nutritional value of baked goods (Asghar and Abbas, 2012).

On the other hand, eggs are also some of the most expensive ingredients, and the baked goods to which they are added can contain a higher level of cholesterol. The search for an alternative to eggs in bakery has presented an interesting challenge, especially if the said substitute could give similar technical and sensory properties to the baked goods as the eggs.

Whey proteins have proven to be structurally, and functionally, similar to egg proteins, so they are considered as a possible alternative. However, if one were to substitute egg proteins for whey proteins, one should consider a multitude of other factors as well (for example, are they substituting fresh eggs or powdered eggs; the amount of egg proteins which they wish to replace with whey protein; how will the whey proteins affect the technical and sensory properties of the product, and similar).

The most represented component of eggs is water, followed by proteins and fat (Asghar and Abbas, 2012). Whey proteins, as an egg substitute, can make up for the protein component (about 12%), but they need water and fat (**Table 1**). The main components of fresh eggs can be replaced by a mixture of 15% whey protein concentrate (WPC 80, with an 80% concentration of proteins), 10% oil, and 75% water. If you want to replace powdered eggs with whey-based proteins, which will change the protein component, it is necessary to add oil. Similar chemical composition to the one found in powdered eggs can be made with a mixture of 60% WPC 80 and 40% oil. As a substitute for WPC 80, WPC 35 (with a 35% concentration of proteins) can be used, but at a 1.4 greater quantity than with powdered eggs. Whey proteins can substitute egg-whites with WPC 80 or WPI (Królczyk et al., 2016).

**Table 1** The basic chemical composition of fresh eggs and powdered eggs, whey protein concentrate (WPC 35 and WPC 80), and whey protein isolate (WPI) (USA Dairy Export Council, 2008)

Compound	Fresh eggs	Powdered eggs	WPC 34	WPC 80	WPI
Protein (%)	12.0	47.4	34.0	81.0	91.5
Fat (%)	10.2	41.0	2.1	7.2	0.5
Carbohydrate (%)	1.1	5.0	46.5	3.5	0.8
Water (%)	75.8	3.1	4.6	4.0	3.7

Whey proteins can partially and fully substitute eggs. However, the quality of the baked product with the added whey is not better or comparable to the egg based product. In the research on the full substitution with whey proteins in the recipe for cakes, cakes made with whey proteins were smaller in volume due to the less stable dough and the easier evacuation of gas (Pernell et al., 2002). Partial replacement of eggs with whey protein isolate has also produced a cake of lesser quality, with a smaller volume and a coarser bubble structure. This can be connected with the higher temperature needed for the denaturation of the whey protein and the

excessive expansion of gas in the cake batter (Arunepanlop et al., 1996). Berry et al. (2009) have found similar results. They have proven that the net-like structure of the cake made out of whey protein is not stable and it contains larger gas bubbles. Outside of the structure of the cake, substitution of eggs with whey proteins affects the consistency and the mouthfeel. In order to get a cake with a desirable consistency and a pleasurable mouthfeel, saccharose was added to the whey protein. Berry et al. (2009) have proven that the saccharose does not enhance the quality of the cake baked with whey proteins. Yang and Foegeding (2010) have shown that the addition of saccharose does increase the volume of the cake when it is made with the foam made of whey proteins, but the structure of the dough becomes rough and it cracks easily. Their research has shown the poor stability of the whey proteins in the transformation from fresh foam to a dry form in the cake. With the addition of saccharose, the functional properties of the whey proteins can be enhanced with partial hydrolysis with pepsin and with the addition of polyphosphates. The application of thermic processing of the whey proteins (Pernell et al., 2002) or the addition of high pressure of up to 1.5 bar in the oven during the baking process (Morr et al., 2003) have not given the satisfactory quality of the cake in which eggs were replaced by whey proteins.

Numerous research studies cite that, with the usage of ultrasound, the structural and functional properties of whey proteins could be enhanced (Jambrak et al., 2008). Therefore, Tan et al. (2014) have researched the effects of ultrasound on the quality of cakes with whey proteins treated with ultrasound. Those cakes did have a larger volume, density, and a better composition of gaseous particles, but they had poorer consistency and firmness.

#### **THE EFFECTS OF WHEY PROTEINS ON THE PHYSICAL-CHEMICAL, RHEOLOGICAL, AND SENSORY PROPERTIES OF BAKED GOODS**

Whey proteins, in their original form, have a low water binding ability. However, if they are subjected to heat they become denaturized, and during precipitation (gelation) they become hydrated and they bind water. Denaturized whey proteins bind over 10 grams of water for every 1 gram of protein, while in their original form they only bind 0.2 grams of water per 1 gram of protein (Krupa-Kozak et al., 2013). The ability of whey proteins to form gels which can contain water, lipids, and other ingredients, and to enhance the texture properties of the finished product is very important for their use in the production of baked goods. Two types of gels with whey proteins have been thoroughly researched; heat induced gels and gels induced in room temperatures (Jovanović et al., 2005). Generally, the process of gelation has two steps: the initial development of protein structures and the unification of denaturized polypeptides through various interactions. Gels produced with WPC contain a couple of different interactions, such as: hydrophobic, electrostatic, and disulphide interactions, and hydrogen-bridged interactions. Whey protein concentrates have a different ability of gelation. WPC products, in general, can gelate on the temperature of 60-90 °C, present in concentrations of 89-120 g/L. The

gelation process is influenced by the temperature and the duration of the heating process, pH balance, ionic strength, and the concentration of salt, proteins, sugars, and lipids (Jovanović et al., 2005).

Shon et al. (2009) have researched the effect of whey proteins in the production of bread made with frozen dough. They had made a control sample of dough and dough with whey proteins added to it, with the addition of  $\kappa$ -carrageenan and sodium alginate, then they froze the dough and kept it frozen for eight weeks. After the eight weeks had passed, the bread with whey proteins added to it had managed to keep a higher moisture percentage than the control sample. The conducted research has proven that the addition of whey proteins increases moisture retention in bread. This research confirms the results of earlier research done by Erdogdu-Arnoczky et al. (1996).

The second research has used the thermally treated WPC 75 (with a 75% concentration of proteins) which was added to the dough in order to prevent the gluten structure from weakening. The WPC was previously dissolved in distilled water, in the quantity of 5%, and thermally treated at the temperature of 82 and 84 °C in order to decrease its solubility and the negative effects on the structure of the dough. The dough was made from wheat flour, dry yeast, fat, and with the WPC 75 solution added to it, and was frozen. The same was done to the dough made without WPC 75, as a control sample, and with the dough made from the untreated WPC 75. The results of this research have shown that the untreated WPC had a negative effect on the properties of the dough and its baking, however, once the WPC is heated, the negative effects become resolved (Kenny et al, 2001). Also, the optimal temperature of the WPC 75 denaturation has been found in this research (72 °C), since that temperature has given the best results. However, the thermally processed WPC 75 has not increased the baking of the dough when compared to the control sample. Based on the results of this research, researchers recommend replacing the mixture of 5% flour with thermally processed WPC, but not with unprocessed WPC, since it could damage the gluten network and result in lower bread quality.

In order to assess the results of the addition of WPC to the baked goods, a research has been conducted concerning the analysis of the effects of WPC on cookies. Cookies have been chosen for this research because they have a longer shelf life, which makes them more practical for the research. Also, cookies are considered "junk food" due to their high concentration of fats and sugars. Enriching cookies with whey proteins would help increase their nutritional value (through the supplementation of essential amino acids such as lysine and tryptophan) (Conforti and Lupano, 2004). The researchers have made dough with 0.5, 11, and 25% WPC 40 (with a 36.4% protein concentration) or WPC 80 (with a 68% protein concentration). They measured the pH balance, firmness, consistency, adhesiveness, and the cohesion of the dough. The baked cookies were different according their content since WPC 80 had more lactose and around 50% more proteins than WPC 40. The dough that contained 5% of the mixture of WPC 80 had a lower dough firmness and

consistency, but increased cohesion. Adding WPC 40 and WPC 80 to the dough lowered its elasticity, since whey proteins disturb the formation of the gluten structure and the development of the dough (Conforti and Lupano, 2004). The results of this research have shown that WPC can be an acceptable as an additive in the production of baked goods, when we consider dough properties. The sensory properties of whey protein-based products influence the decision of their addition to baked goods and, by approximation, their acceptability with consumers. A panel group of sensory analysts has conducted research on the samples of WPC and WPI in which they wanted to find out the differences between these two types of whey protein-based products. The panellists have given the WPC better grades for flavour when compared to the WPI sample, due to its more intense milky flavour and sweet, caramel-like, flavour (Mortenson et al., 2008). The sweet flavour of WPC partially comes from a higher concentration of lactose in WPC and its milkier flavour/texture can be attributed to the higher concentration of milk fat in it (Mortenson et al., 2008). Besides that, the WPC samples had a bitter-sour note which is a direct consequence of a higher acidity level of whey. The panellists have noticed that there is no difference between the sensory properties of WPC and WPI, even though certain processing procedures are used, such as instantiation, and they consider them an acceptable addition to food products.

The sensory properties of rye bread enriched with WPI or WPH and their acceptability with consumers have been researched by Song et al. (2018). A panel of sensory analysts has confirmed that WPH gives a bitter taste to the rye bread because of the more pronounced Maillard reactions and a higher percentage of bitter peptides (Torri and Salini, 2016). However, even though the research had proven that the consumers have marked this bitterness as a negative, a smaller group of consumers was attracted to the bitterness. The reason for this could be the fact that rye bread has a naturally bitter flavour (Heiniö et al, 2003). WPH had also given a sour taste to the bread, which the consumers marked as a positive. Along with the bitter and sour taste, due to its free amino acids, WPH has given the rye bread an umami flavour as well (Fu et al., 2018). The addition of WPH has changed the texture of rye bread, increasing its firmness and elasticity, which was a direct consequence of the aggregation of whey proteins (Havea et al., 2001). The foaming property of WPI has increased the volume of the rye bread, but, due to its water binding property, it increased the bread's rigidity and dryness (Foegeding et al. 2002). Rye bread enriched with WPI had less pronounced bitter taste in comparison to WPH. However, the texture and/or mouth feel limit the application of WPI in the production of rye bread. The dryness of the bread with WPI is probably the main cause behind the fact that half of the participants in this research reject this bread.

### **NUTRITIVE ADVANTAGES OF WHEY PROTEINS AS ADDITIVES IN BAKING**

Modern food trends give more attention to products with a lower fat and sugar content and to products enriched with proteins that have an added health benefit for its consumers. Following this trend is a great challenge for the baking industry, since traditional baked goods are rich in carbohydrates and fats. Besides, wheat proteins have a lower biological value than animal-based proteins, and they can cause certain health issues in their consumers such as: allergies, celiac disease, and gluten intolerance (Flambeau and Respondek, 2017). Science and technology are attempting to produce baked goods that will satisfy modern day consumers and whey proteins, due to their nutritional value and functional properties, could be an acceptable alternative to carbohydrates and fats, and they could be a highly valued protein component in gluten-free baked goods.

#### ***Whey proteins as a substitute for carbohydrates***

The sum total of carbohydrates in baked goods can vary from 37% in white bread all the way up to 40% in fruity cakes or 45% in iced biscuits. The sum total of carbohydrates depends not only on the basic baked good, but also on the carbohydrates in the glaze, chocolate icing, fillings, and similar, which can increase it significantly (Abdellatif et al., 2006).

The replacement of carbohydrates in baked goods requires a systemic approach, because it isn't enough to lower the carbohydrate ratio in baked goods, it is also important to increase the protein ratio as well. The addition of whey proteins, such as WPC 80 or WPI, in combination with sugar alcohols, fibres, and artificial sweeteners, can contribute to the lowering of the carbohydrate content in baked goods. Most baked goods contain 2 grams, or less, of protein per serving. In order to retain the characteristics of the product, it is realistic to increase the protein content to 4-5 grams per serving. In some biscuits, WPC can replace 10-20% of the wheat flour (Puranik, 2003).

#### ***Whey proteins as fat substitutes***

There are plenty of "fat substitutes" which lower the energy value of the product and the product retains similar sensory properties. These substitutes can be a replacement for the fat or can be used as fat mimetics, and they have a lower energy value and can partially or fully replace the fat. Whey proteins fall under the fat mimetic group of ingredients in baked goods and can be used to partially substitute fat (Colla et al., 2018).

The ability of whey proteins to mimic fat is based on their water binding properties. Usually, whey protein concentrate (WPC 34) with 34% of whey proteins is used for fat mimicking purposes. WPC binds the added water in the mixture for the baked product and gives a soft texture to the product, similar to a product that contains fat. WPC functions very well as a fat mimetic in baked goods, with a higher moisture level. Using WPC can reduce up to 50% of fat in baked goods (Zoulias et al., 2002; Basman et al., 2008). However, whey proteins are not an ideal fat substitute, since

they can worsen the flavour of the product and lower its acceptability for consumers (Colla et al., 2018).

### **Whey proteins as gluten substitutes**

Whey proteins are being used more prominently in the production of gluten free baked goods because they have good nutritional and functional properties. These proteins have improved gluten free products, such as bread, in terms of preference and the increase in bread volume, when compared to the bread made out of control rice flour (Gallagher et al., 2004). The most widespread whey protein-based products used in the formation of gluten free baked goods are the whey protein concentrate (van Riemsdijk et al., 2011b) and the whey protein isolate (Gallagher et al., 2004). It has become popular to add different hydrocolloids with whey protein in the production of gluten free products (Lazaridou et al., 2007). Even though there is a significant possibility for the usage of whey proteins in gluten free products (**Table 2**), there are certain limitations to their use. One of such limitations can be seen in people who suffer from the inflammation of the small intestine (Crohn's disease). They can have lactose intolerance tied to their disease, which can make them intolerant to WPC (which contains lactose). Secondly, whey proteins can be triggers for certain allergic reactions (Tratnik and Božanić, 2012).

Whey protein concentrates can be used to overcome the unwanted weakening of the gluten network in frozen baked goods (Asghar et al., 2009; Deora et al., 2014). The addition of WPC can change the rheological attributes of empanada paste (a traditional Argentinian dish) by increasing its elasticity and lowering the hardness of the dough (Lupano, 2003). Outside of their positive role for the improvement of the nutritive properties of a product, whey proteins have been shown as great ingredients for uniting the structure in the development of gluten free products. (Krupa-Kozak et al, 2013; Marti et al., 2014; Matos et al., 2014). In a recent study, the mesostructured particles of whey proteins were used in the baking process of gluten free bread (van Riemsdijk et al., 2011b). The idea of using the mesostructured particles of whey proteins is based on the fact that the gluten structure, present in the developed wheat dough, has the particle structure on a mesoscopic scale with the length of 100 nm to 100 μm. Previous research confirms the hypothesis which claims that the behaviour of suspended particles can be regulated with the mesoscopic properties of the particle network and the network will remain strongly elastic on a low strain value (van Riemsdijk et al., 2011a). This research proposes an alternative concept of product development based on whey proteins on a mesoscopic level. The key property of this formulation is that whey proteins and hydrocolloids from bean legumes form a mesoscopic suspension of whey protein particles. The change of proteins on a mesoscopic scale is a relatively recent concept. On this level, there is a high degree of interactions present in the whey protein suspension that can form a network. Therefore, the usage of a mesoscopic network of whey protein particles presents an innovative concept for the development of a new, gluten-free generation of products (Deora et al., 2014).

**Table 2** Application of whey proteins in gluten-free product development (Deora et al., 2014)

<b>Protein source used</b>	<b>Gluten-free product</b>	<b>Key observations</b>	<b>References</b>
Casein and albumin protein isolates	Bread	Improved crumb texture and better specific volume	Storck et al. (2013)
Milk protein isolate and novel rice starch	Bread	Dairy protein and rice starch addition increased loaf volume	Gallagher et al. (2003)
Whey protein particle system	Bread	Improved specific volume and a bubble size comparable with anormal wheat bread	van Riemsdijk et al. (2011b)
Pea, soybean, egg albumen, and whey proteins	Bakery products	Pea, soybean, and whey proteins decreased the final viscosity in addition to the peak viscosity	Marco and Rosell (2008)
Whey protein particles	Bread	Improved bread properties by the ability to form disulphide bonds. Ability to form viscoelastic properties. Mesoscopic protein particle networks can imitate gluten properties	van Riemsdijk et al. (2011a)
Sodium caseinate, milk protein isolate, whey protein isolates	Bread	Whey proteins increased specific volume and decreased hardness over time	Nunes et al. (2009)

The addition of whey proteins in a 5% w/w ratio per the amount of bread has influenced the adhesive and rheological properties of the dough (Marco and Rosell, 2008). This research has confirmed the fact that whey proteins, in combination with rice prolamin, lower the peak and final viscosity of the dough.

The addition of transglutaminase (TG) with whey proteins can be used to form the protein network and can modify the rheological properties of rice dough (Gujral and Rosell, 2004; Marco and Rosell, 2008). A research was conducted, which studied the effect of different protein isolates (peas, soy, egg albumins, and whey proteins) and TG on the properties of rice flour. The results have shown that different sources of protein affect the elasticity of the dough. Pea proteins and soy proteins have increased the elasticity, while egg albumins and whey proteins have lowered it.



Also, another research has shown that by including whey proteins into gluten free bread, with time, it induced a larger volume and lowered its toughness (Nunes et al., 2009). The study showed that the implementation of whey proteins has increased the nutritional values, as well as the characteristics of gluten free baked bread.

If we take all of the above into consideration, we can see that whey proteins offer a significant and interesting approach to the development of gluten-free products since they increase the nutritional value and the quality of the product.

## CONCLUSIONS

Whey proteins are, by all means, a resource which can find its most significant use in the baking industry, due to its functional and nutritional properties. With a good nutritional value, whey proteins, as an additive, can enhance baked goods and give them additional value. Outside of that, they can replace carbohydrates, fats, and/or gluten to a certain extent, which can make these baked goods acceptable regarding the demands of a modern consumer in search for healthier alternatives. Additionally, when added to traditional recipes, they can replace more expensive resources, such as eggs.

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## IMPACT OF CRICKET (*ACHETA DOMESTICUS*) FLOUR ADDITION ON THE VISCOMETRIC PROFILES OF FLOUR MIXTURES

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### SUMMARY

Cricket flour from *Acheta domesticus* can be an important source of protein and is perceived as a food product ingredient. The main constituents of the cricket body are protein, chitin and fat (Grabowski et al., 2008). Crickets usually contain about 71-73% of water, therefore, in dried mass the percentage of those important constituents is changing significantly. As protein, fat, and chitin are perceived as ingredients impacting the viscometric behaviour of flour mixtures, such characteristic should be taken into account when planning their wider usage in food products. Commercially available cricket flour contains 69.1% of protein, 18.5% of fat and 7.7% of fibre, 0.7% of other carbohydrates and 1.03% of salt. Such high content of proteins and fat can significantly impact the viscosity of gels made from flour mixtures.

The main objective of this study was to investigate the viscosity changes by the viscometric profile obtained with an RVA standard 1 method of flour mixtures composed of rice flour and house cricket flour. Both flours were purchased commercially and six mixtures were prepared to contain 5%, 10%, 15%, 20%, 25% and 30% of cricket flour. The pasting properties were taken both in water and in 0.01 M AgNO<sub>3</sub> to investigate the enzyme activity impact on total viscometric profile. The progressing addition of cricket flour gradually lowered the peak viscosity (PV) of mixtures. However, the samples measured in 0.01 M AgNO<sub>3</sub> revealed higher PV values for mixtures with higher cricket flour addition. The pasting temperature was constantly rising with increasing amount of cricket flour in mixtures, while the final viscosity differences were lower between samples indicating the additional activity present in samples.

**Keywords:** pasting properties, edible insects, rice flour, *Acheta domesticus*

## **INTRODUCTION**

Nowadays, the new protein source search constitutes emerging trends in food industry. The climate change and biodiversity conservancy force new directions in food production. Food and feed safety is expected to experience great stress in the coming decades due to the rapid growth of the world population and the resulting increase in demand for animal protein. In this context, the need for new sources of nutrients is becoming apparent (Probst et al., 2015).

The European Commission's 2015 Sustainable, Safe and Nutritious Food document, produced by the Business Innovation Observatory under the auspices of the Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs, Directorate F "Innovations and Advanced Manufacturing Methods" Innovation, Unit F1 "Innovation Policy and Development Investment" identified two promising sources of solutions to the growing problem of food shortage. These are edible insects and algae/water algae (Probst et al., 2015). Insect consumption, known as entomophagia, has many benefits - both nutritional, including a high content of protein, minerals and vitamins, as well as economic and environmental benefits, such as a reduction in greenhouse gas emissions, low cost consumption, and a lack of by-products (Probst et al., 2015).

It is estimated that by year 2054 the alternative proteins will account for 33% (i.e. 311 million metric tons, MMT) of world protein intake, with insects accounting for about 11% of the alternative protein market. This means that around 34 MMT of insects will be consumed worldwide by 2054. The edible insect industry alone could become a €320 million company in the US and Europe over the next decade (Probst et al., 2015).

Insect consumption is a foreign concept in Western culture, but is estimated to be an integral part of the diet of at least 2 billion people in Asia, Latin America and Africa. According to Lux Research's report, the current dominance of animal proteins will decrease over the next decades. In contrast, since the world's protein intake is estimated to reach 944 million metric tons (MMT) by 2054, the intake of alternative protein sources is predicted to grow at a rate of 9% per year (Lux Research, 2014)

The main factor that will ensure the integration of alternative protein sources into Western diets is increasing health and environmental awareness. A number of initiatives have been recently launched to sensitise customers and other stakeholders to the environmental burden of animal husbandry and to increase their interest in alternative proteins (Halloran et al., 2018).

One of the industrial sectors which especially needs protein supply is gluten-free bakery production. The lack of gluten leads not only to the techno-functional problems during bakery production but also to insufficient nutrients balance (Vici et al., 2016). Several different protein sources have been studied as supplementation for gluten-free breads, like soy protein isolate, albumin from white egg, pea protein, collagen, lupine protein leading to important changes in the rheological properties of bread dough, resulting bread volume, porosity of crumb and staling characteristics (Crockett et al., 2011; Ziobro et al., 2013).

Cricket (*Acheta domestica*) powder as food ingredient can provide a substantial source of protein into food formulations. The cricket body is mainly built from protein, chitin and fat, containing about 71-73% of water. While protein, fat and chitin are ingredients impacting the rheological behaviour of flour mixtures, their viscometric characteristic should be analyzed when composing the receipt.

The main objective of this study was to investigate the viscosity changes of flour mixtures composed of rice flour and house cricket flour by the viscometric profile.

## MATERIALS AND METHODS

The ingredients, rice flour (Melvit, Poland) and cricket flour (Crunchy critters, UK), were purchased from the local market. Viscometric profile was obtained using AACC International Method 76-21.01, ICC Standard No. 162 method Standard 1 on RVA Starch Master2, Newport Scientific, Australia.

The analysis procedure for all samples was as follows: the 2.5 g sample recalculated on dry basis was placed in the RVA container with the solvent amount adjusted to the total weight of 28.5 g. The stirring speed was set for 960 rpm for 10 seconds and then maintained at 160 rpm for the rest of a test time of 13 min. The initial 50 °C temperature was held for 1 min, ramped up to 95 °C at 4 min and 42 s, held at 95 °C for 3.5 min, then ramped down to 50 °C at 3.5 min and finally held at 50 °C for the last 2 min. The pasting profile parameters were extracted from each run as peak viscosity (PV), hold viscosity (HV), final viscosity, breakdown and setback (BV and SV) viscosities and peak temperature (PT). Two different solvents, distilled water and AgNO<sub>3</sub> 0.01 M solution, were used for viscometric profile analysis. The flour blends were prepared in proportion of 5%, 10%, 15%, 20%, 25% and 30% of cricket flour vs. rice flour.

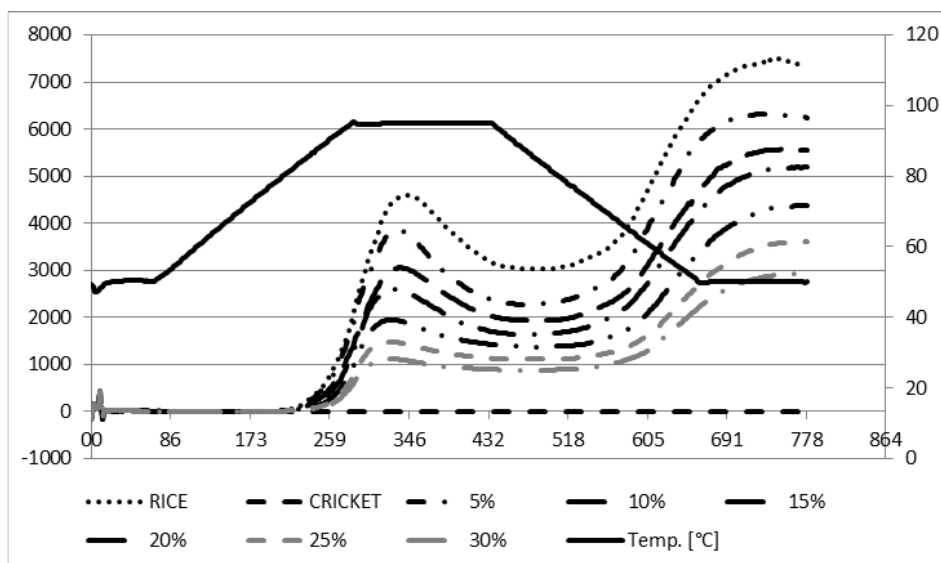
## RESULTS AND DISCUSSION

The pasting curve of rice flour and cricket flour blends analyzed in distilled water are presented in **Figure 1**. The typical pasting curve profile of rice flour is strongly maintained. Specific form of the curve is gradually smoothed with increased addition of cricket flour.

The pasting parameters of rice flour and cricket flour blends are presented in **Table 1**. The peak viscosity was significantly lowered in each blend regarding the initial 100% rice flour. The equilibrium point between starch granule swelling and polymer (amylose) leaching results in the highest viscosity of the sample registered as peak viscosity. This parameter also indicates the water-binding capacity of the analyzed blend and provides suggestion about the highest viscosity value obtained during the hot processing. Moreover, hold viscosity and final viscosity values also showed the similar behaviour profile being significantly lowered according to the increasing amount of cricket flour in the blends. The similar behaviour was observed by Saleh et al. (2014) while studying the pasting properties of different composite blends based on long grain rice flour substituted with 10% and 15% (right) of defatted rice bran, rice bran fiber, rice bran proteins and stabilized rice bran. Both rice bran fiber



and rice bran protein addition smoothed the pasting curves and lowered the pasting parameters values and such behaviour was strongly dependant on the dose. Taking into account that cricket flour consists mainly of protein and fiber, the obtained results are consistent with existing literature. The pasting temperature was observed to be risen with the increased amount of cricket flour addition. Such behaviour is usually related to the fat content increase, even in the native rice samples as demonstrated by Kraithong et al. (2017) who investigated the pasting properties of different Thai rice cultivars and obtained the highest pasting temperature of 87.7 °C for Riceberry, brown Jasmine rice with the fat content of 3.60%.

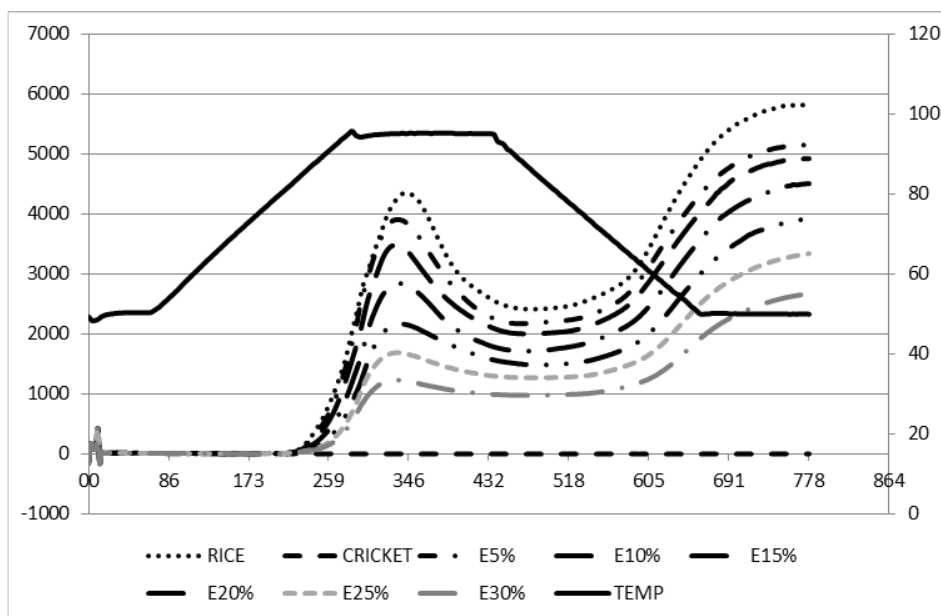


**Figure 1** Pasting curve of rice flour: cricket flour blends in distilled water

**Table 1** Pasting parameters of rice flour: cricket flour blends in distilled water

	PV [mPa*s]	HV [mPa*s]	FV [mPa*s]	BD [mPa*s]	SB [mPa*s]	PT [°C]
<b>R</b>	4617.0 g	3026.5 g	7370.5 g	1590.5 d	4344.0 f	84.4 a
<b>5 %</b>	3852.0 f	2274.5 f	6245.0 f	1577.5 d	3970.5 c	84.7 a
<b>10 %</b>	3055.5 e	1925.0 e	5546.0 e	1130.5 c	3621.0 d	86.6 b
<b>15 %</b>	2611.0 d	1641.5 d	5184.5 d	969.5 c	3543.0 d	87.7 c
<b>20 %</b>	1945.0 c	1374.5 c	4372.5 c	570.5 b	2998.0 c	89.3 d
<b>25 %</b>	1486.0 b	1107.5 b	3616.5 b	378.5 ab	2509.0 b	90.3 e
<b>30 %</b>	1123.0 a	878.0 a	2910.5 a	245.0 a	2032.5 a	91.5 f

The native activity of enzymes, mainly  $\alpha$ -amylase in flours, can strongly impact the resulting viscosity of gels and doughs/batters. To properly assess the impact of water binding compound on viscometric profiles of any flour blends, it is recommended to evaluate the pasting profile in enzyme inhibiting solvent. One of the most useful are heavy metal salts, mainly silver salts such as  $\text{AgNO}_3$  solutions. Inhibiting the activity of endogenous enzymes allows demonstrating more clearly the specific contribution of rising amount of ingredients added to the composite blends of flours. The pasting curves of rice flour vs. cricket flour blends in 0.01 M  $\text{AgNO}_3$  solution are shown in **Figure 2**.



**Figure 2** Pasting curve of rice flour vs. cricket flour blends in 0,01M  $\text{AgNO}_3$  solution

The profiles obtained from viscometric analysis of rice flour and cricket flour blends in  $\text{AgNO}_3$  solutions demonstrate better separation one from another indicating different behaviour capacity of each blend. The pasting parameters changes are shown in **Table 2**.

The peak viscosity (PV) for each blend sample was significantly lower with increasing the amount of cricket flour confirming the behaviour observed in distilled water solutions. The impact of  $\alpha$ -amylase activity has been shown comparing PV, HV and FV values with samples measure in distilled water. The pasting temperatures have not been significantly changed after addition of 0.01 M  $\text{AgNO}_3$  solution which suggests that they mainly depend on the starch content in blend.

**Table 3** Pasting parameters of rice flour: cricket flour blends in 0,01M AgNO<sub>3</sub> solution

	PV [mPa·s]	TV [mPa·s]	FV [mPa·s]	BD [mPa·s]	SB [mPa·s]	PT [°C]
R	4355.5 g	2412.5 g	5819 g	1943 g	3406.5 g	84.2 a
5 %	3908 f	2167 f	5152 f	1741 f	2985 f	84.55 ab
10 %	3491.5 e	1998.5 e	4928.5 e	1493 e	2930 ef	85.15 b
15 %	2838.5 d	1709 d	4504 d	1129.5 d	2795 d	87.15 c
20 %	2172 c	1483.5 c	3933.5 c	688,5 c	2450 c	89.35 d
25 %	1687 b	1269.5 b	3346 b	417.5 b	2076.5 b	90.55 e
30 %	1230.5 a	973 a	2673 a	257.5 a	1700 a	91.4 f

### CONCLUSIONS

All the analyses carried out in the investigated blends confirmed the suitability of cricket flour to be incorporated into rice flour blends without losing the paste forming abilities. The high protein and lipid content of cricket flour makes it suitable for human consumption. The ability to stabilize the viscosity changes during heating-cooling cycle opens the opportunity to obtain cricket flour/rice flour gels stable to temperature changes. It means that direct application in food products, especially those undergoing changing temperature conditions, is possible. Such viscometric behaviour also suggests the suitability of investigated blends for bread-making purposes.

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## THE INNOVATIVE SOLUTIONS AND THE DEVELOPMENT OF A LOW-ENERGY AIR DRYER

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*Professional Article*

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### SUMMARY

Drying is one of the most energy-intensive industrial processes, regardless of the processing material. In the industrial food production, drying can be used as the only step for food preservation and/or as a preparation stage in food processing. However, the quality of final dried product is a priority that needs to be accomplished. This study presents the innovative solution and the development of a low-energy air dryer based on heat pump for application in food drying with a closed circulation of relatively low temperature air (up to 70 °C).

**Keywords:** low-energy air dryer, energy efficiency, food

### INTRODUCTION

The tasks of the drying technique and technology are primarily to preserve the quality of agricultural products through the drying process with minimum energy inputs and environmental impacts. Drying is an energy-intensive process, and for cost-effectiveness, it needs to be carried out in the shortest possible time with as less energy consumption as possible. The complexity of the drying process is reflected throughout the simultaneous transfer of heat and matter (moisture), and in changes in the physical and structural properties of the material being dried. That is particularly evident in the drying of food materials that are mutually significantly different in composition and structure. Dried fruits and vegetables of high quality can only be obtained by carefully selecting the variety within each species.

Drying can take place as a natural process or as an industrial unit operation. The natural process cannot be controlled while the industrial process is more or less controlled. The natural process depends on the season and applied conditions in terms of temperature and air humidity that agricultural products are exposed to. However, such dried products have a relatively high final moisture content (Tiwari et al., 2016.). Industrial processes are mostly based on the indirect removal of moisture

by hot air, which flows over the foodstuff, after which it is released into the environment with high relative humidity and lower temperature compared to the beginning of the process.

In order to analyse the drying process and determine the design parameters of the dryers, it is necessary to know the static and kinetics of drying process (Budin and Mihelić-Bogdanić, 2013). Drying statics implies the correlation of the initial and final parameters of the material and the drying agent based on the balance of matter and energy, while the drying kinetics implies a relationship between the change in material humidity with time and the process parameters.

Improved conditions of the drying process and better utilization of air heat can be achieved by different methods that simultaneously fulfil the material properties and are more energy efficient. The combination of heat regeneration and circulation of drying air is applied in industrial driers to achieve better results and greater usability. The workflow needs to be managed in such a way as to increase productivity and reduce energy consumption. In order to reduce energy consumption, an optimization needs to be carried out, which involves a combination of recirculation (air circulation) and heat regeneration. In that way, the drying air returns its efficiency and it can be reused for drying without discharging into the environment (Minea, 2013; Moses et al., 2014).

This study presents the innovative solution and the development of a low-energy air dryer based on heat pump for application (Budžaki et al., 2019) in food drying with the heat regeneration and circulation of drying hot air.

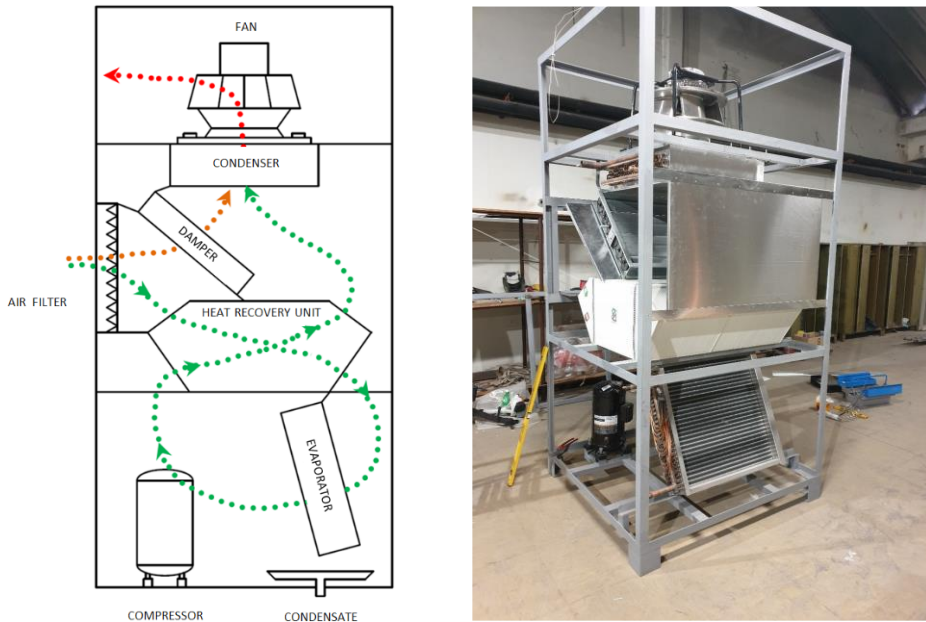
### ***Design and development of a lower-energy air dryer***

The dryer is a machine with the purpose of drying that is, reducing the moisture of raw material to a desired value. This can be achieved by means of hot air circulating through the dryer.

Low-energy condensing dryers *SINAI* and *THAR* spend 3-4 times less energy than conventional dryers. With regard to the technology used, they provide better controllability of the drying process, which is particularly important when drying agricultural products.

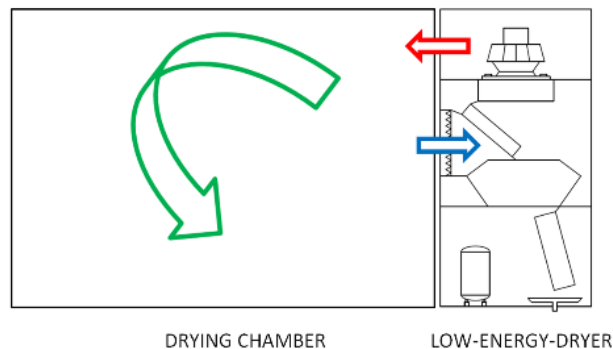
Materials that will be dried (clothes, fruits or vegetables) are placed into the drying chamber. The user selects the desired drying mode (depending on the materials ready to be dried) on the control panel located on the air-drying device. The drying mode has an enormous effect on the speed and quality of drying, regardless of whether the production mode or the modified mode is used. The most important part of this type of a low-energy dryer is the air-drying device which prepares the air (heats it up), after which the heated air enters the drying chamber and passes through the materials being dried. When the air returns into the drying device, air circulation in the system is completed, and the process is repeated until the materials are dry. The drying device is extremely economical and does not deplete the ozone layer because it uses refrigerant R134a.

During running, process parameters can be read via the control panel, including relative air humidity, temperature, air enthalpy, absolute humidity, etc. During the process, there is no contact with fresh air from the outside. The entire process includes the flow of an equal amount of air in a closed system.



**Figure 1** Schematic presentation of a low-energy air dryer

**Figure 1** shows a low-energy dryer for the drying air preparation that consists of an evaporator, compressor, condenser, ventilator and a heat recovery unit. The humid and warm air coming from the drying chamber in the low-energy dryer unit is dewatered and reheated to a drying temperature that depends on the material that needs to be dried (food or non-food). The integration of the low-energy dryer for air preparation with the drying chamber is shown in **Figure 2**.



**Figure 2** Schematic presentation of a low-energy air dryer with a drying chamber

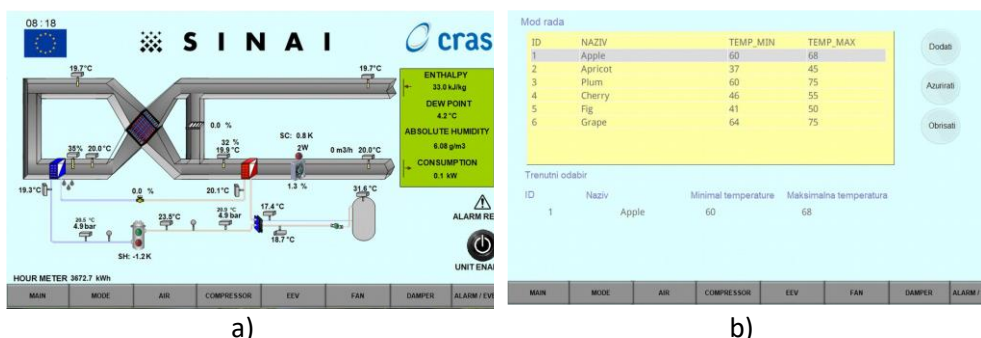


The equipment is designed for drying a variety of materials including clothes and foods (fruits, vegetables and herbs). The use in two sectors, hotel and agro-food industry, is the result of the introduction of a dual name for dryer. The name *THAR* indicates the use in the hotel industry for drying laundry, while the name *SINAI* is a designation for drying agricultural products. The unit is freestanding and consists of two parts, a drying chamber and a condensing unit, which are connected by ducts for supply and exhaust air. Unlike conventional dryers, the *SINAI* and *THAR* condensing dryers use the flow of the same amount of air in a closed system. During the process, there is no mixing of air inside the dryer with fresh air from the outside. This innovative low-energy air dryer can save up to 59-70% of the energy required for conventional hot air drying (Lee and Kim, 2009; Taseri et al., 2018). After designing commercial equipment, tests need to be carried out in an appropriate laboratory scale that simulates commercial operating conditions. In cases where laboratory testing cannot be performed, commercial drying may be based on the experience of the equipment manufacturer as an important source of data. Based on scientific research related to the drying of different materials, a prototype of the dryer was made; the validation was tested in the food drying systems as well as on textile materials. On the prototype model, manufactured by Cras Ltd., the following tests were performed: the modelling of the drying system; moisture transfer testing; air quality testing; checking the content of dry matter particles in and out of the chamber; thermal dissipation of energy from the chamber; testing of primary measuring devices and testing the condensing unit drive group. The fan aspirates the heated and dry air, directing it to flow through the top opening on the left side of the dryer and blow into the dryer chamber where the material for drying was placed. At the inlet to the dryer chambers, the air temperature was measured with a PT1000 probe. The drying chamber contains partitions that take the air flow in the desired direction. By recirculation in the chamber, the air becomes saturated with the moisture it receives from the drying materials. During the drying process, the indoor air does not come in contact with the fresh air outside. The whole process takes place by flowing an equal amount of air in a closed system. Since it is a closed dryer system, the excess heat generated during operation can be used for heating up the sanitation water and/or for unburdening the heating system (such as plant halls), via a fan-convector/heat exchanger unit that requires a circulation pump. The control is designed so that the dew point of the return air is continuously monitored and the control is held for optimum evaporation of the system, taking into account the compressor characteristic for the most efficient separation of moisture from the air. From temperature and humidity, the absolute humidity ( $\text{kg/m}^3$ ) can be calculated as the indication of the amount of moisture before the evaporator and the amount of moisture after the evaporator. The efficiency of individual modes of operation by collecting condensate and measuring the efficiency of moisture separation by changing the operating parameters were tested.

### Experimental measurements and data collection

Prior to commissioning, an electrical control circuit for the automatic control of the system was created. When designing the wiring diagram, a Freon circuit diagram was used to select the number and type of sensor to be monitored in the operation of the device. In the frame of automatic control, there are executive parts: low pressure switch (LP) - low pressure switch on suction in the compressor; high pressure switch (HP) - high pressure switch at the outlet of the compressor; compressor thermal protection (COMPRESSOR\_TP) - compressor motor protection switch; fan alarm (FAN\_AL) - air circulation fan error. In case that one of the conditions is not fulfilled, the compressor does not have the signal to start operation. Thermocouples mounted inside the dryer, 10 points measure the air temperature (inlet and return flow from the drying chamber, inlet and outlet of the evaporator - after the recuperator, in the outlet tube of the evaporator and condenser, the inlet and return temperature of the Freon-water exchanger and the outlet temperature from the compressor).

Operations of the dryer are simply regulated via automatic control. The regulation of operation in the drying program is designed in such a way that the user selects the foods, and at the same time chooses the recommended mode of operation for the specified material, all based on an experimental tests conducted on the prototype. **Figure 3** shows the interface for automatic control of the dryer operation.



**Figure 3** Interface for automatic control of the dryer operation: display of process diagram (a) and drying process menu (b)

Drying is carried out from an initial humidity to a final humidity, which is lower than 10%. In condensers, the drying air temperature should be about 50 °C and the inlet temperature 5-10 ° lower at air flow rates of about 3 m/s. The process lasts until the humidity is reduced to the desired level (when it evaporates about 80% of the balance from the initial one). A prototype low-energy dryer designed for drying materials and raw materials is intended for two areas, the hotel industry (laundry) and the agro-food industry (fruits, vegetables and herbs). Considering the two applications, the prototype of the low-energy dryer was tested during two drying cycles in the period 9/2018 to 6/2019. The evaluation of the functionality of the low-energy dryer in the first test cycle was performed on the drying of linen and sheets, provided by Hotel Osijek from Osijek. In the second test cycle, the functionality of

the prototype low-energy dryer was evaluated during the drying of agro-food products: plums (with and without pits), pumpkin seeds (ordinary and hull-less), red spice pepper, pomegranate and aronia (berry and cake left over after juice production from aronia). Agro-food materials were supplied by the producers from Osijek-Baranja County. The data collected during drying, in both cycles, were used to create programs that, as the part of the developed and constructed prototype of the low-energy dryer, enable potential customers to select the appropriate drying program for the material they wish to dry when commissioning the dryer, with the option of entering drying parameters independently. The testing was conducted by the Cras Ltd.'s R&D team, in cooperation with the Faculty of Food Technology Osijek. By collecting data during textile and food drying the results showed that the amount of energy consumption ranged from 44.38 to 59.17 kWh/t evaporated water. Compared to the consumption of a conventional hot air dryer (900 - 1400 kWh/t evaporated water) and/or superheated steam dryer (170 to 400 kWh/ evaporated water), the constructed dryer shows significantly lower energy consumption, up to 3.17%.

## CONCLUSIONS

The prototype of the *SINAI* and *THAR* low-energy dryer has been successfully designed, constructed, tested and automatized. In addition, within the framework of the implemented project, the final goal was achieved, i.e. the commercialization of a low-energy dryer that can be used for the drying of textile and agro-food materials.

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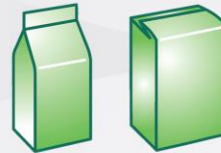
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