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ASSESSMENT OF ZINC STATUS IN THE POPULATION OF EASTERN CROATIA

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Summary

The aim of the research is to examine the values of zinc (Zn) concentrations in serum, urine and hair in the population of eastern Croatia and to relate the obtained results to age, sex, previous diseases, professional exposure, place of residence and water consumption. The research was conducted on 601 randomly selected respondents from the patient databases of family medicine offices. The samples were collected in Vladislavci, Dalj, Čepin, Našice, Osijek, Vukovar, Vinkovci, Slavonski Brod and Virovitica. There was a difference in Zn concentrations regarding the sex and age of the subjects. Professionally exposed subjects have significantly higher concentrations of Zn in their hair. There are no differences in zinc concentrations in biological samples between subjects with and those without previous diseases. Higher concentrations of Zn were found in serum in subjects who consumed water from the city water supply, while higher concentrations in hair were found in those consuming local water supply. Higher concentrations of Zn in serum were found in subjects from Vukovar, Vinkovci and Virovitica, urine in subjects from Virovitica; and Zn levels in hair were higher in Dalj, Čepin, Našice and Osijek. Despite the fact that Zn is an essential element, it is also a metal that, in elevated concentrations, is an indicator of anthropogenic pollution. By comparing the reference values of Zn in biological samples and the median values obtained by biomonitoring (urine: 181.2 μgL^{-1}, serum: 438.3 μgL^{-1}, hair: 470.7 μgg^{-1}), it seems that obtained concentrations in analyzed samples were within acceptable ranges.

Keywords: zinc, biological samples, eastern Croatia

Introduction

Zinc is an essential micronutrient in the human body (Rink, 2011). It serves as a cofactor in more than three hundred enzymes in the body and participates in a number of signaling pathways (Hou et al., 2021). Published studies have shown that a minimum of 17% of the

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world's population is insufficient in zinc uptake, especially residents of developing countries (Hess, 2017). Among the zinc-deficient states is neighboring Serbia, where Zn deficiency is considered to be a result of low Zn content in soil and water, resulting in low Zn content if food (Jagodić et al., 2021). Despite the fact that zinc belongs to a group of heavy metals, zinc intoxications are very rare. A much bigger problem is deficit of zinc in the body (Rink, 2011). Although severe Zn deficiency in humans is rare, marginal Zn deficiency is widespread, even in highly developed industrial societies. Researches have shown that optimal Zn intake is important for restoring impaired immune function and reducing the incidence of infectious diseases (Hughes et al., 2004). The recommended daily dose of zinc intake depends on age, sex and race, and for white adult women it is 6.9 mg per day, and for white male adults it is 9.4 mg per day (Briefel et al., 2000).

There are groups that have an increased risk for zinc deficiency such as vegetarians and elderly due to insufficient intake. Zinc deficiency also occurs in those who have an increased need for Zn – women during pregnancy and lactation, patients with allergies and active inflammatory diseases.

Elevated zinc elimination and consequently zinc deficit are found in people with diabetes, renal insufficiency, and in cases of interactions with drugs such as glucocorticoids, antacids, hormonal contraceptives and diuretics.

Decreased zinc absorption is found in people with chronic intestinal diseases such as Crohn's disease and gluten enteropathy due to low intake and increased excretion (Hughes et al., 2004). Zinc intake is declining by aging, and one study conducted in the U.S. in the early 1990s found that only 42.5% of people over the age of 71 have sufficient intake of zinc (Briefel et al., 2000). Zinc deficiency in the elderly increases the risk of hypercholesterolemia, macular degeneration, osteoporosis, hypertension, the onset of type 2 diabetes, worsening of the neuropsychiatric condition and a weaker immune system. The most famous disease caused by zinc definition is acrodermatitis enteropathica, i.e. a genetic disease in which people have a mutation in the ZIP4 zinc transporter that results in the Zn deficiency, starting at infancy, manifested by reduced growth, dysfunction of the immune system, alopecia, dermatitis, diarrhea and cognitive dysfunction. Timely diagnosis, oral zinc supplementation can stop the progression of the disease and sometimes even cure it (Rink, 2011; Prasad, 2013).

In 1974, the National Research Council of the National Academy of Sciences defined zinc as the essential element of the human organism and, for the first time, defined the recommended daily intake of zinc (Briefel et al., 2000).

A major problem in agronomy is the Zn deficiency in soil, which results in crops with low Zn content. In developing countries, basis of nutrition is mainly wheat, and it accounts for more than 50% of ingested calories. This mainly applies to the countries of the Middle East, sub-Saharan Africa and Central and Western Asia (Cakmak, 2008). Since zinc is essential not only for plant growth but also for humans, crop biofortification is carried out with the aim of enriching grains with zinc. Agronomic biofortification is inexpensive and provides a double advantage of increasing yields and improving the concentration of Zn in the grain. There are

several ways of applying zinc, such as soil fertilization, foliar nutrition and seed preparation (Praharaj et al., 2021).
The homeland war in eastern Croatia resulted in environmental contamination resulting in elevation of war related metals and metalloids in population and environment (Vidosavljević et al., 2014).
Elevated concentrations of zinc and other heavy metals can be found in areas of dense traffic. In addition to the possible accumulation of heavy metals in plants, the negative effect of elevated heavy metal concentrations on soil enzyme activity has been demonstrated, potentially having negative environmental effects (Bartkowiak et al., 2017). Drinking water contains 0.01 – 0.1 mg/L^{-1} of Zn (Mills, 1989). Elevated zinc concentrations in the soil can also be a result of industrial pollution. The average zinc concentration in the soil is 10 – 300 mg/kg^{-1}. Also, it is estimated that more than 40% of industrial wastewater is contaminated with heavy metals (Mills, 1989).
Animal meat is the best source of zinc for humans, mainly the red meat. With the development of industrialization, the human diet is less and less dependent on animal meat nutrition and is increasingly based on the diet of cereals and legumes that are rich in indigestible zinc-binding ligands, such as phytates and certain fibers (Mills, 1989). In addition to red meat; oysters, animal liver and cheeses are good source of Zn (Hughes et al., 2004).
The average adult in the body contains a total of 1.4 – 2.3 grams of zinc. Zinc is present in all organs, tissues and secretions of the human body. The highest percentage of zinc is found in skeletal muscles (57%) and bones (29%). Hair and blood plasma contain 0.1% of the total zinc in the body (12). Most of the Zn elimination of zinc from the organism is done through fecal route (70 – 80%) while the remaining part is done by urine and sweat. Fecal elimination increases by increasing zinc intake. Decreased zinc intake also reduces the amount of zinc eliminated by urine and feces (Lee et al., 1993).
Aims of the study were to determine serum, urine and hair zinc concentration values in the population of investigated areas of eastern Croatia and to compare it with similar published studies in order to determine zinc status of the population.

Materials and methods

The study was conducted on 601 randomly selected examinees. Respondents in the study were adult residents of the Osijek-Baranya County, Vukovar-Srijem County, Virovitica-Podravina, County, Požega- Slavonia and Brod- Posavina County namely settlements of Vladislavci, Dalj, Čepin, Našice, Osijek, Vukovar, Vinkovci, Slavonski Brod and Virovitica.
Sample analysis:
Each respondent attached a sample of the first morning urine in a polyethylene urine container (100 ml bottles, Greiner Bio-One, Frickenhausen, Germany). Hair samples (3 cm long and 1 cm wide) were taken from the back of the neck, with the help of stainless-steel scissors and stored in polyethylene bags. Also, one blood test tube was taken out for each respondent. The laboratory technician performed blood sampling using a needle (Vacuette Blood needle, 38 ×

0.9 mm, Greiner Bio-One, Frickenhausen, Germany) and a tube (Vacuette serum gel Tube 3.5 mL, Greiner Bio-One, Frickenhausen, Germany).
Samples were sent to the Institute of Public Health of Osijek-Baranja County, where they were centrifuged to separate the serum, after which the supernatant was transferred to cryo-epruvets (test tubes) and disposed of in the refrigerator of the Institute, at a temperature of - 30 °C. Collected biological samples were sent for ICP-MS analysis of the elements. An analysis of biological samples of subjects (serum, urine, hair) by the ICP-MS method was performed in the laboratory with the procedure in DRC (English for dynamic reaction cell) a mode that serves to remove the interference of the spectacle of individual elements.
ICP-MS analyzes were performed in the Department for Health ecology the Institute for Public Health „dr. Andrija Štampar“ in Zagreb.
The normality of the distribution of continuous variables was tested by Shapiro - Wilk test. Due to the distribution of continuous variables that do not follow normal distribution, continuous data are described by the median and interquartile range. Differences in numerical variables between the two independent groups were tested by Mann Whitney in a test (with a 95% median difference confidence interval), and between three and more groups by the Kruskal Wallis test (Post hoc Conover). All P values were two-sided. The significance level was set to Alpha = 0.05. The statistical program MedCalc® Statistical Software version 20.026 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2022) and SPSS were used for statistical analysis. 23 (IBM Corp.Released 2015. IBM SPSS, Ver. 23.0. Armonk, NY: IBM Corp.).

Results and discussion

The research was conducted on 601 respondents, of whom 321 (53.4%) were men and 280 (46.6%) were women. Regarding age, 408 (67.9%) respondents are under 60 years of age. Most of the respondents, 106 (17.6 %) were from Dalj and 101 (16.8 %) from Virovitica (Table 1).
Alcohol is consumed by 243 (40.4%) respondents, and cigarettes are smoked by 191 (31.8%) respondents. Pre-existing diseases were present in 110 (18.3%) respondents. Exposure at home/professional exposure was recorded in 338 (56.2%) respondents (Table 2).
Medians of zinc in serum, hair and urine are shown in Table 3.
Zinc concentrations in serum were significantly higher in men (Mann Whitney U test, $P < 0.001$), while differences in zinc concentration in hair and urine are not significant according to gender (Table 4).
Subjects under the age of 60 have a significantly higher concentration of zinc in their hair (Mann Whitney U test, $P < 0.001$) compared to those over 60, while those over 60 have a significantly higher concentration of zinc in the serum (Mann Whitney U test, $P = 0.001$) compared to younger respondents (Table 5).
Subjects who were occupationally exposed have significantly lower concentrations of zinc in serum (Mann Whitney U test, $P < 0.001$) and in urine (Mann Whitney U test, $P = 0.004$), while their concentrations are significantly higher in hair (Mann Whitney U test, $P = 0.005$) compared to respondents who were not professionally exposed (Table 6).

Table 1. Basic characteristics of examinees

	Nr. of examinees (%)
Gender	
Male	321 (53.4)
Female	280 (46.6)
Age groups	
>60 yrs.	408 (67.9)
<60 yrs.	188 (31.3)
Location	
Vukovar	51 (8.5)
Slavonski Brod	31 (5.2)
Vinkovci	27 (4.5)
Vladislavci	88 (14.6)
Dalj	106 (17.6)
Čepin	52 (8.7)
Našice	81 (13.5)
Osijek	64 (10.6)
Virovitica	101 (16.8)

Table 2. Alcohol, smoking, co-morbidity and potential Zn exposure in examinees

	No of examinees (%)
Alcohol drinking	243 (40.4)
Cigarette smoking	191 (31.8)
Previous illnesses	110 (18.3)
Possible exposure	338 (56.2)

Table 3. Zinc concentrations in serum (μgL^{-1}), hair (μgg^{-1}) and urine (μgL^{-1})

	Median (interquartile range)
Zinc in serum	438.3 (139.5 – 783.4)
Zinc in hair	473.7 (154.1 – 812.2)
Zinc in urine	181.2 (97.5 – 909.3)

Table 4. Differences in zinc concentrations in serum (μgL^{-1}), hair (μgg^{-1}) and urine (μgL^{-1}) with respect to gender

	Median (IQR) – sex		Differ.	95% confidence interval (CI)		*P**
	Male	Female		From	To	
Zn in serum	559.2 (186.8 – 847.4)	327.1 (100.6 – 705.8)	-122.9	-184.8	-70.6	<0.001
Zn in hair	529.1 (149.7 – 857.3)	457,4 (157.5 - 786)	-11.7	-56.9	28.7	0.57
Zn in urine	195,7 (101.6 – 933.4)	167,7 (88.6 – 871.1)	-15	-43.5	11.9	0.27

IQR – interquartile range; *Mann Whitney U test (Hodges-Lehmann difference)

Table 5. Differences in zinc concentrations in serum (μgL^{-1}), hair (μgg^{-1}) and urine ($\mu g\ L^{-1}$) with respect to age

	Median (IQR) age		Differ.	95% confidence interval (CI)		*P**
	> 60 Years	<60 years		From	To	
Zn in serum	381.8 (108.5 – 758.7)	603.9 (211 – 823.1)	94.6	40.8	155.2	0.001
Zn in hair	594.5 (189.1 – 859.4)	307.4 (115.4 – 751.1)	-100.8	-163.5	-51.3	<0.001
Zn in urine	158.1 (97.7 - 885)	292.2 (95.8 – 978.5)	11.9	-19.2	52.6	0.45

IQR – interquartile range; *Mann Whitney U test (Hodges-Lehmann difference)

Table 6. Differences in zinc concentrations in serum (μgL^{-1}), hair (μgg^{-1}) and urine (μgL^{-1}) with respect to occupational exposure

	Median (IQR) professional exposure		Differ.	95% confidence interval (CI)		*P**
	Non-	Exposed		From	To	
Zn in serum	555.5 (196.6 - 836)	349.6 (111,1 – 741.2)	-105.1	-166.2	-49.5	<0.001
Zn in hair	382.1 (122.6 - 787)	551.9 (188.2 – 841.1)	59.9	19.4	108.7	0.005
Zn in urine	345.7 (109 – 972.4)	146.9 (89.1 – 733.5)	-45.3	-91.5	-13.5	0.004

IQR – interquartile range; *Mann Whitney U test (Hodges-Lehmann difference)

The normal range of serum zinc concentrations is 800 – 1200 $\mu g/L^{-1}$ (Jagodić et al., 2021). By comparing the reference values and median serum zinc concentrations, we can conclude that a minority of the surveyed settlements were within the reference values for serum concentrations (Vukovar, Vinkovci and Virovitica), and the rest of the settlement have showed zinc concentrations below the reference values (Slavonski Brod, Vladislavci, Dalj, Čepin, Našice, Osijek and Virovitica). This study showed that highest serum zinc concentrations were measured in Vukovar, Vinkovci and Virovitica, and the lowest were in Vladislavci (Table 7). It is difficult to explain the significantly higher concentration of zinc in these settlements. One study showed that, according to national legislation, the values of individual heavy metals tested in Vukovar and Vinkovci area were within the reference values for the tested elements in the soil. (Bijelić, 2020). In previous study, areas of high intensity of combat activity showed elevated concentrations of arsenic, mercury, and lead in samples. Some of the settlements defined in the same study as an area exposed to high-intensity combat activities were Vladislavci, Čepin, Dalj and Osijek (Dziedzic et al., 2022). It is interesting that this research showed that the concentration of zinc in hair is significantly higher in Dalj, Čepin, Našice and Osijek compared to other settlements. Hair sample analysis has several advantages over serum samples. The Zn concentration in hair sample is about 100 times higher than in the serum and maintains concentrations more stable than the serum, making it perfect for

assessing long-term exposure. In addition, the hair sample better reflects recent overexposure to metals, as cations are rapidly transferred from blood to tissue storage (King et al., 2015). However, the reference values for zinc concentration in hair have not yet been defined (Cunnane, 1988). The concentration of zinc in urine proved to be significantly higher in Virovitica, and lower in Našice compared to other settlements. In addition to significantly higher zinc concentrations in the hair, Vukovar and Vinkovci also showed significantly more serum zinc concentrations. Zinc in urine is a good indicator of increased zinc exposure if zinc intake basal values are met (Cunnane, 1988). Urinary zinc reference values were 150 – 650 μg^{-1} (Jurić et al., 2017), and when comparison of measured median urinary zinc concentrations and reference values is made than it is visible that the measured concentrations in Virovitica are above that range, and in Našice under the lower referral values. This study therefore shows a somewhat paradoxical finding, where the concentration of zinc in urine is significantly lower compared to the rest of the settlement, and the concentration of zinc in hair is significantly higher as in Dalj, Čepin and Osijek.

Table 7. Differences in zinc concentrations in serum (μgL^{-1}), hair (μgg^{-1}) and urine (μgL^{-1}) according to sampling site

	Zn serum	P*	Zn hair	P*	Zn urine	P*
Vukovar	856 (776.3 - 928.8)		121.4 (106.2 - 162.6)		343.6 (201.1 - 503.3)	
Slavonski Brod	700.9 (607.6 - 801.4)		165.3 (135.5 - 188.4)		282.8 (131.7 - 429.6)	
Vinkovci	960.9 (858.5 - 1058.5)		153.4 (124.4 - 199.2)		418.2 (168.7 - 825.9)	
Vladislavci	142.9 (72.8 - 356.6)		645.3 (353.5 - 868.7)		129.4 (72.2 - 500.1)	
Dalj	151.2 (79.2 - 324.8)	**<0.001**†	780.9 (416.9 - 1139)	**<0.001**‡	695.9 (100.8 - 5738.6)	**<0.001**§
Čepin	285.5 (109.4 - 526.7)		788.4 (648.5 - 989.3)		124.1 (91.8 - 220.3)	
Našice	283.6 (104.2 - 455.2)		770.9 (698.3 - 876.9)		97.1 (28 - 119.7)	
Osijek	295.6 (101.1 - 548.7)		746.4 (424 - 895.2)		117.6 (68.7 - 167.1)	
Virovitica	783.1 (671.6 - 891.9)		114.3 (92.8 - 147)		923.4 (801.6 - 1047.3)	

*Kruskal Wallis test (Post hoc Conover)
†at the $P < 0.05$ level, the concentration is significantly higher in Vukovar compared to all other places except Virovitica; the lowest concentration of zinc is significantly in Vladislavci
‡at the $P < 0.05$ level, there is a significantly higher concentration in Dalj, Čepin, Našice and Osijek compared to other settlements
§at the $P < 0.05$ level, the concentration is significantly higher in Virovitica, and lower in Našice compared to other settlements

Some of the possible explanations for the significant difference in concentrations among settlements are the natural composition of the soil, the proximity of industry and the proximity of roads.
Also, research comparing zinc concentrations in cabbage and potatoes from areas not affected by the Homeland War and those affected by the Homeland War shows that there is no significant difference in zinc concentrations between vegetables of these two areas (Dziedzic et al., 2022). Such a finding is explained by the fact that the population was significantly reduced during the war, and agricultural and industrial production were completely stopped (Dziedzic et al., 2022). Measurement of zinc concentration in soil in the vicinity of Zagreb showed more concentration in soil along the Sava River and near the airport and the main industrial district (Vitale et al., 2007). Nevertheless, another study showed that the soil along the Sava River in northwestern Croatia has zinc concentrations comparable to other soils, according to the literature. Measurements of soil composition along the Drava River in northwestern Croatia showed significantly high zinc concentrations (Romić and Romić, 2003). It is assumed that the significantly high concentration of zinc, lead and cadmium along the Drava is due to the activities of the metal industry in Austria and Slovenia, which are upstream (Halamić, 2003). Higher zinc concentrations were found in the soils along the Danube River near one industrial city in Hungary (Kovacs-Bokor et al., 2021). The concentration of zinc in soil is higher than allowed in Hungary. The measured concentration of zinc in peas grown on such soil has an elevated health risk index for children (Kovacs-Bokor et al., 2021).
Although the environment burdening with heavy metals represent an important public health problem, for zinc we can say that zinc deficit is a much bigger global problem. A neighboring country, Serbia, has the population at high risk of zinc deficit. Serum zinc concentrations of the Serbian population were twice as lower than other world population. Given the serum zinc reference value (800 – 1200 $\mu g/L^{-1}$), the measured mean serum zinc concentrations show that countries such as Poland, Germany, France and United States find themselves within the referral range, while Serbia, Spain, Italy and Greece are below the defined values (Jagodić et al., 2021).
With respect to gender, there are significantly higher zinc concentrations in serum of male respondents in this study, while differences in zinc concentration in hair and urine were not different among male and female respondents. Numerous studies have come to the same results, but the results vary. An explanation for the higher serum zinc concentration in men is that the high concentration of zinc in prostate and semen are necessary to maintain normal reproductive physiology (Barman et al., 2020). Therefore, the recommended daily zinc intake is higher for men than for women (Hughes et al., 2004).
Subjects under 60 years of age in this study have significantly higher concentrations of zinc in hair compared to those over 60 years of age, while those over 60 have a significantly higher serum zinc concentration compared to younger subjects. The results of other studies show diverse and contradictory results of serum zinc concentrations relative to age (Ha et al., 2019). Studies show that the concentration of zinc in hair increases linearly to the age of 30 years (Folin et al., 1991), and after 30 the concentration of zinc in hair decreases linearly (Folin et al., 1991).
Older people are often subject to zinc deficit and it is assumed that only 42.5% of those over 71 take adequate amounts of zinc. The reasons for this are difficult food processing,

psychosocial factors, drug interactions, and the competencies of zinc and other divalent minerals for transmission through membranes (Rink, 2011).
Due to occupational exposure, subjects professionally exposed in this study have significantly lower serum and urine zinc concentrations, while their hair concentrations were significantly higher than those who are not professionally exposed. By professional exposure in the study-authors have covered working in industrial plants, metal industry, landfills, work on large roads and work on the gas stations. In another study, elevated zinc concentrations in hair were measured in a tractor factory worker. However, the assessment of exposure to metals in the workplace using a metal concentration in hair should be done in parallel with the measured metal concentrations in the air. The content of the metal in hair depends on the purity of the hair and the frequency of hair washing, cigarette smoking and is a reflection of the diet of the subjects (Vinnikov et al., 2018). Limitation of this study is that we did not examine possible additional Zn uptake from supplements, especially in COVID-19 era, due to favorable effect Zn has reported to have had on the COVID outcome (Ben Abdallah et al., 2023).

Conclusion

Based on the conducted research and the obtained results, the following conclusions can be drawn:
•Men have significantly higher serum zinc concentrations, while differences in zinc concentration in hair and urine are not significant by sex
•Subjects up to 60 years of age have significantly higher zinc concentrations in their hair, while those over 60 have a significantly higher serum zinc concentration compared to younger subjects
•Professionally exposed subjects have significantly lower serum and urine zinc concentrations, while their hair concentrations are significantly higher compared to subjects who are not professionally exposed
•There were no significant differences in serum, hair and urine zinc concentrations compared to previous diseases
•Significantly, the highest concentration of zinc in serum in Vukovar and Vinkovci is compared to all other places except Virovitica, and significantly the lowest in Vladislavci
•The concentration of zinc in hair is higher in Dalj, Čepin, Našice and Osijek compared to other settlements
•The concentration of zinc in urine were significantly higher in Virovitica and lower in Našice
Further extended research should be done.

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Proceedings

MICROBIOLOGICAL STATUS OF SANDWICHES FROM FAST FOOD ESTABLISHMENTS IN THE AREA OF ZENICA-DOBOJ CANTON

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original scientific paper

Summary

Sandwich is very popular meal (or snack), often consumed outside the home. Sandwiches represent a combination of different ingredients, including meat and salad, cheese, sauce, which makes them potentially risky. The aim of this article is to provide an overview of research on the microbiological status of sandwiches with meat and salad in order to consider the potential danger to the health of consumers. Total of 196 samples (n=196) of sandwiches with meat, cheese and salad were analysed using standard ISO methods. The research was carried out in fast food restaurants, and it showed that the most common parameter that does not comply with the reference values of the Silent and Quiet Guidelines are species from the genus *Enterobacteriaceae*. Similar results were obtained in other studies that dealt with the microbiological status of sandwiches. Out of the total number of samples, 20% of sandwiches had an increased number of *Enterobacteriaceae*. Many factors can affect the microbiological status of sandwiches, including: storage temperature of raw materials, hygienic practices during preparation and contamination of raw materials, environmental hygiene such as contamination of work surfaces, utensils and hands of employees also significantly contribute to the deteriorating microbiological status of sandwiches. The microbiological status of sandwiches with meat and salad, cheese and sauce represent an important issue for public health. Although this research did not prove the presence of pathogenic microorganisms, the increased number of Enterobacteriaceae indicates the existing danger of the presence of pathogenic species. Therefore, it is necessary to adhere to proper hygiene practices during preparation.

Keywords: fast food, public health, microbiology, pathogens, sandwich

Introduction

Sandwiches are popular meals often consumed outside the home. They represent a combination of various ingredients, including meat and salad, cheese, sauce, which can make them potentially risky.

Potential risks are:

1. Preparation hygiene: If sandwiches are prepared in unclean conditions or food handlers do not adhere to basic hygiene standards, there is a risk of contamination. Bacteria such as Salmonella or E. coli can cause serious digestive problems.

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2. Raw or undercooked ingredients: If sandwiches contain raw ingredients such as raw meat or raw eggs, there is a risk of bacterial infection, such as Salmonella. Also, inadequately cooked meat can pose a risk of illness.
3. Inadequate cooling: Sandwiches containing cold ingredients like meat, cheese, or mayonnaise must be properly stored at low temperatures to prevent the proliferation of bacteria. If sandwiches are left at room temperature for too long, it can lead to the growth of pathogenic microorganisms.
4. Allergens: Sandwiches often contain various ingredients, including nuts, gluten, dairy products, or other potential allergens. Individuals with allergies should exercise caution when consuming sandwiches outside the home and carefully read labels or ask staff about ingredients.
5. Cross-contamination: If sandwiches are prepared on the same surfaces or with the same utensils as potentially contaminated food (e.g., raw meat), there is a risk of cross-contamination, which can lead to illness.

Thus, there are potential health risks associated with initial contamination of raw foods with pathogenic bacteria as well as subsequent contamination by vendors during preparation and through post-cooking handling and cross contamination (Angelidis et al., 2006).

Research and results

Sampling

Total of 196 samples (n=196) of sandwiches with meat, cheese and salad were analysed using standard ISO methods. The sandwiches contained different ingredient combinations: cooked ham, raw ham, smoked cheese and tomatoes, cucumber, cabbage, chicken and tomato, ham and cheese, tomato and cheese, tuna and vegetables, turkey and vegetables, cooked ham and mushrooms. The ingredients and the expiry date were recorded to ensure that the samples were within their shelf-life period at the end of the study.

Microbiological Analysis

All types of food samples for microbiological testing are prepared under aseptic conditions to prevent contamination of the samples with microorganisms from the environment.

Microbiological determination of Salmonella spp.

Preparation of samples for analysis on *Salmonella* spp. is carried out according to the valid editions of BAS EN ISO STANDARDS 6887-1, 6887-2, 6887-3, 6887-4, 6887-5 and 8261 (www.iso.org).

To prepare the stock sample solution, use the primary BPW enrichment solution (weigh 25 g of sample and pour over 225 ml of BPW or 10 g, and pour over with 90 ml of BPW). The

standard was validated for a test portion of a 25 g sample. Smaller test portions can be analysed without the need for additional validation/verification. Larger test portions of the sample of 25 g. require initial validation/verification, and can be analysed by this method if the study shows that there are no negative effects on the detection of *Salmonella* spp. After the prepared basic dilution, incubate the solution at 34 to 38 °C (37±1 °C), for 18h ± 2 h. It is allowed to store the basic dilution (pre-enrichment), after incubation and before switching to selective enrichments, in a refrigerator at 5 °C and 3 °C for a maximum of 72 hours (AOAC, 2000).

Microbiological determination of Enterobacteriaceae

Preparation of samples - crushing and homogenization, and preparation of basic and decimal dilutions of samples is carried out according to valid editions of BAS EN ISO 6887-1, 6887-2, 6887-3, 6887-4, 6887-5 and 8261 (www.iso.org).
To prepare the basic solution of the sample, use the primary solution for enriching BPW (weigh 25 g of the sample and pour over 225 ml of BPW).
Inoculation and incubation – use a sterile pipette to pipette 1 ml of the sample if it has a liquid content or 1 ml of the initial suspension for other samples. Repeat the procedure with subsequent dilutions each time using a new sterile micropipette tip. If only one dilution is used, inoculate two petri plates of that dilution (ISO 7218-www.iso.org).
Add to each Petri dish approximately 15 ml of VRBG agar, previously prepared and cooled (in a water bath) to 47 °C to 50 °C. The time between the inoculation of the Petri plates and the moment of media pouring (VRBG) should not be longer than 15 minutes.
Carefully mix the inoculum with the medium with horizontal movements and leave it at room temperature, until the sample with the substrate has a solid consistency.
When the substrate is completely solid, add another layer of approximately 5 to 10 ml of VRBG agar prepared and cooled to 47 °C to 50 °C to prevent growth spread and provide facultatively anaerobic conditions.
Turnover and place the plates in an incubator at 37 °C for 24 h ± 2 h.
Counting and selection of colonies for confirmation - characteristic colonies are pink to red or purple (with or without a precipitation halo).
Choose Petri plates that have less than 150 characteristic colonies; count these colonies. Pick five characteristic colonies from each Petri plate for subcultivation. If there are less than 5 colonies on the plate, subculture all suspicious colonies.
Expanded colonies (merged colonies) will be considered (counted) as one colony. If less than one quarter of the Petri plate has grown by spreading, count the colonies on the remaining three quarters and extrapolate to calculate the theoretical number of colonies of the entire plate. If more than one quarter of the plate is overgrown by spreading colonies, discard the count.
Certain Enterobacteriaceae can cause decolorization of their colonies or media. Retrospectively, if no characteristic colonies are present, choose five whitish colonies for confirmation.

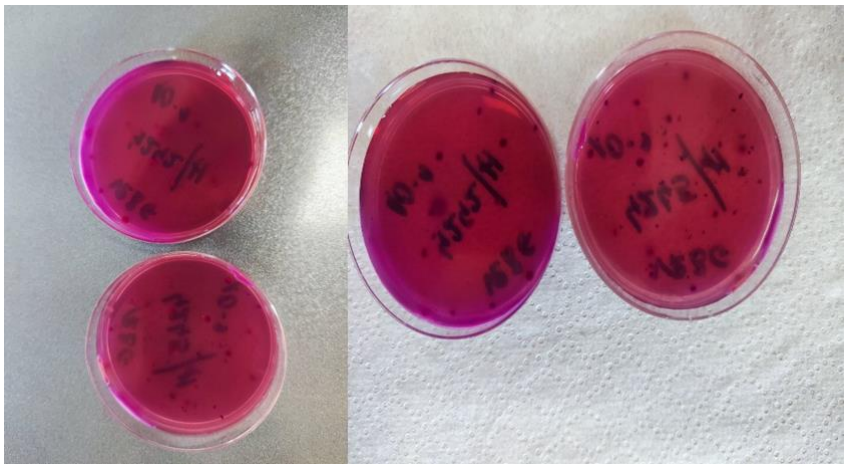

Figure 1. Petri plates with Enterobacteriaceae grown on VRBG agar

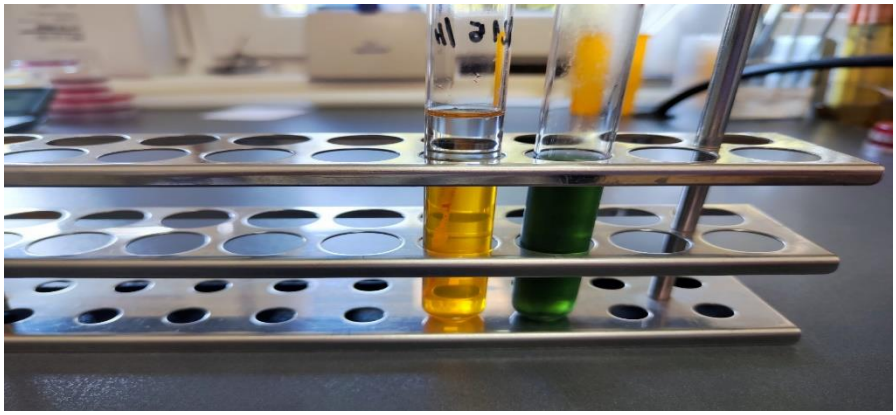

Figure 2. Oxidative-Fermentation Test (O/F Test): Positive: Yellow color/Negative: Green color

Microbiological determination of Staphylococcus aureus

Preparation of samples for analysis is carried out according to valid editions of BAS EN ISO standards 6887-1, 6887-2, 6887-3, 6887-4, 6887-5 and 8261 (www.iso.org).
Using a sterile pipette, transfer 0.1 ml of the liquid sample to one Petri plate with agar (ISO 7218:2007/Amd1:2013) or 0.1 ml of the basic dilution (10-1), if the sample is solid. Repeat the procedure with the next dilution (10-2), and if necessary, with additional dilutions.

If a small number of coagulase-positive staphylococci is expected in the tested sample, the detection limit can be corrected by a factor of 10, which means pipetting 1 ml of the liquid sample or 1 ml of the basic dilution (if the sample is solid) into a large Petri plate with agar (diameter 140 mm or three plates with a diameter of 90 mm).
Carefully distribute (spray) the inoculum, as quickly as possible, over the surface of the agar plate, trying not to touch the edges of the Petri dish. Leave the plates for about 15 minutes at room temperature, then incubate at 37 °C for 24 ± 2 h, and re-incubate for an additional 24 ± 2 h.
After incubation for 24±2 h, mark the typical colonies at the bottom of the plate (Note 1), and after re-incubation, mark the typical and atypical colonies again (Note 2). For counting, use plates containing a maximum of 300 colonies with 150 typical and/or atypical colonies on two consecutive dilutions. One of the plates should have a minimum of 15 colonies.
For confirmation, select 5 colonies (5 typical colonies, if only typical colonies are present or 5 atypical colonies if only atypical colonies are present, or 5 typical and 5 atypical colonies if both types of colonies are present from each plate).
If fewer than 15 typical and/or atypical colonies are present on plates inoculated with undiluted sample (for liquid samples) or with stock dilution, it is possible to determine the estimated number or low number of coagulase-positive staphylococci by retaining all plates containing any typical or atypical colonies. Select such colonies for confirmation.
Note 1: typical colonies are black or grey, shiny and convex (after 24 h incubation they are 1-1.5 mm in diameter, and after 48 h 1.5-2.5 mm) and surrounded by a clear zone (cleaning zone). After 24 hours, an opalescent ring may appear, directly in contact with the colonies, it may appear in the clean zone.
Atypical colonies can appear in one of the following appearances (morphology): (*i*) glossy black with or without a narrow white border, clear zone absent or barely visible and opalescent zone absent or barely visible or (*ii*) grey colonies without clear zone.
Atypical colonies are mainly formed with contaminating strains of coagulase-positive staphylococci, egg dairy products, offal, etc. They are less often formed by strains of coagulase-positive staphylococci that contaminate other products.
Note 2: Bacteria belonging to other genera (not the genus *Staphylococcus*) can have colonies that look like staphylococci. Microscopic examination of the Gram strain, before confirmation, will make it impossible to distinguish other genera from staphylococci.
Pick the labelled suspicious colonies with sterile gauze and transfer them to Brain-Heart broth. Incubate the broth at 37 ºC for 24 ± 2 h.
After a certain period of incubation, transfer 0.1 ml of the sample from the BHI broth to a test tube with 0.3 ml of rabbit plasma (unless the manufacturer has defined other quantities) with a micropipette under sterile conditions. Incubate at 37 °C for 4 to 6 hours. Re-incubate the negatives additionally at 37 ºC for 24 hours (unless the manufacturer has specified the incubation time differently). After incubation, check the presence of a clot by tilting the test tube, if the clot occupies more than 50% of the original liquid volume, it is a sign of a positive reaction, i.e., the presence of coagulase-positive staphylococci. As a negative control, take 0.1 ml of sterile BH broth and transfer it to 0.3 ml of rabbit plasma. After a certain period of

incubation, if the liquid consistency remains, without the presence of coagulum, it is a sign of a valid test.

Microbiological determination of sulphite-reducing Clostridia spp.

Shredding and homogenization, as well as the preparation of basic and decimal dilutions of samples is done according to the valid editions of BAS EN ISO 6887-1, 6887-2, 6887-3, 6887-4, 6887-5 (www.iso.org).
Before the seeding process itself, it may be necessary to heat the basic solution of the sample (to 75 °C, for 20 minutes) in order to remove the vegetative forms of bacteria. If heating is applied, the results must be reported as the number of spores of sulphite-reducing bacteria ("Spores of sulphite-reducing clostridia").
Prepare sterile petri plates, and transfer with a sterile pipette 1 ml of the sample (if it is liquid) or the basic sample solution (for other samples) and 1 ml of the next dilution. Repeat the procedure with additional dilutions using always a sterile pipette tip.
If necessary, heat the basic solution in a water bath, and transfer 1 ml of the basic sample solution and 1 ml of the subsequent dilution to the Petri plates with a sterile pipette. Repeat the procedure with additional dilutions using always a sterile pipette tip. In case there is a need to remove vegetative forms (heating), it is necessary to write it on the work list.
Cover the petri plates with 15 ml of iron sulphite agar, previously dissolved and cooled to 44 to 47 °C in a water bath. The period between the inoculation of the plates and the addition of the nutrient medium should not exceed 15 minutes. Mix the inoculum and substrate carefully with horizontal movements, leave to harden. After the medium has a solid consistency, pour over the samples with 5 to 10 ml of the same medium, put them in anaerobic pots, insert commercially available bags to create anaerobic conditions and put them for incubation at 37 °C for 24 h to 48 h. An anaerobic indicator must also be placed in each pot.
Instead of plates, it is possible to use test tubes (tubes) which, after adding agar and mixing, are placed in cold water so that the agar hardens. After the agar has hardened, add 2-3 ml of the same medium to each test tube. If test tubes are used, it is not necessary to put them in anaerobic conditions. Incubate at 37 ºC for 24 h to 48 h (Krause, 2016).
Note: if thermophilic bacteria are expected, prepare another set of petri plates with the sample, and incubate them at 50 ± 1 °C.

Colony counting

After 24 h and 48 h of incubation, the typical colonies are counted (depending on the degree of black colour and the growth rate of microorganisms). Colonies of black colour, with a possible black environment (black colour is a sign of H2S production) should be counted as sulphite-reducing bacteria (Aldred, 2014).
Note: Diffuse, non-specific blackening of the substrate may occur, especially when the inoculation was done in test tubes instead of Petri dishes. The growth of anaerobic bacteria,

which only produce hydrogen (not H_2S), can reduce the presence of sulphite and lead to a general blackening of the medium.
Count colonies on each plate containing no more than 150 typical colonies and no more than 300 total colonies.
When the number of colonies is high, it will not be possible to use some tubes for counting. In this case, only tubes containing clearly separated colonies should be used for counting.
Note: This International Standard can be used to count clostridia only. After obtaining characteristic colonies, take 5 of them, from each dish, and confirm the genus Clostridium with confirmatory tests (e.g., respiratory test, spore-forming test).

Results and discussion

Enterobacteriaceae (30 samples): The presence of Enterobacteriaceae in 30 out of the 196 sandwich samples is a concerning finding. *Enterobacteriaceae* is a family of bacteria that includes some well-known pathogens like *Escherichia coli* and *Salmonella*. These bacteria can cause foodborne illnesses when consumed in sufficient quantities. Finding *Enterobacteriaceae* spp. in sandwiches may suggest several issues:
Cross-contamination: The bacteria could have been introduced through contact with contaminated surfaces, utensils, or hands during preparation.
Enterobacteriaceae can multiply rapidly at temperatures within the danger zone (typically between 40 °F/4 °C and 140 °F/60 °C) (Davis, 1987). If sandwiches were stored or handled improperly, such as not being kept at the correct temperature, this could facilitate bacterial growth.
Improper handwashing or glove use by food handlers can introduce Enterobacteriaceae to the sandwiches.
Sulphite Reducing Clostridium (7 samples): The presence of Sulphite Reducing Clostridium in 7 samples raises specific concerns. Clostridium species, including some types of Clostridium perfringens, are anaerobic bacteria that can produce toxins. These toxins can lead to foodborne illnesses, often associated with improperly cooked or stored foods. Possible explanations for the presence of Sulphite Reducing Clostridium include:
These bacteria thrive in environments with little or no oxygen. If sandwiches were prepared or stored in airtight containers or without adequate ventilation, it might have created conditions favourable for Clostridium growth.
Failure to cook or cool sandwich ingredients properly can allow Clostridium bacteria to survive and multiply.
Aerobic Mesophiles (2 samples): Finding aerobic mesophilic bacteria in 2 samples suggests that these sandwiches had an elevated level of general bacteria that grow at moderate temperatures. While most of these bacteria are not harmful, their presence can indicate issues with food handling and storage:
Inadequate temperature control during storage or transport may have allowed these bacteria to proliferate (Appendini, 2002).

Cross-contamination or poor hygiene practices can introduce these bacteria.
In summary, the results indicate that a notable portion of the sandwich samples had microbiological issues, potentially posing health risks to consumers. The specific causes of these issues are multifaceted and could involve temperature control, hygiene practices, and food handling methods (Byrne, 2002). Addressing these concerns would involve implementing stricter food safety measures, improved hygiene training for staff, and better control over temperature and storage conditions to ensure the safety of sandwiches served to consumers.

Conclusion

The conclusion drawn from the results of the microbiological testing on the sandwich samples is that there are significant food safety concerns associated with these sandwiches. The findings indicate that a substantial proportion of the sandwich samples, specifically 30 out of 196, contained elevated levels of Enterobacteriaceae, 7 samples showed the presence of Sulphite Reducing Clostridium, and 2 samples had elevated counts of aerobic mesophilic bacteria. These results signal potential food safety hazards, as these microorganisms can cause foodborne illnesses when consumed in sufficient quantities. The presence of Enterobacteriaceae suggests potential issues with cross-contamination, temperature control, and hygiene practices during the preparation and handling of the sandwiches. The presence of Sulphite Reducing Clostridium highlights concerns about anaerobic conditions and proper cooking or cooling procedures. The presence of aerobic mesophiles suggests potential problems with temperature control and hygiene.
In light of these findings, it is crucial for food businesses, especially those serving sandwiches, to take immediate action to address these hazards. This includes:

1. Establishing and enforcing comprehensive food safety protocols to minimize the risk of contamination during sandwich preparation.
2. Providing thorough food safety and hygiene training to all employees involved in sandwich preparation to ensure they understand and adhere to proper practices.
3. Ensuring that sandwiches are stored and transported at safe temperatures to prevent bacterial growth.
4. Emphasizing the importance of handwashing, glove use, and the cleanliness of work surfaces and utensils to minimize the risk of cross-contamination.
5. Conducting regular microbiological testing of sandwiches and food preparation areas to identify and rectify any issues promptly.
6. Offering consumers accurate and appropriate information on the safe handling and consumption of sandwiches.

The results underscore the critical importance of maintaining stringent food safety standards in the preparation and handling of sandwiches. Addressing these issues is paramount to ensuring the safety and well-being of consumers who enjoy these food products.

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PROBIOTICI KAO TERAPIJSKA OPCIJA ZA FUNKCIONALNU KONSTIPACIJU U OSOBA STARIJE ŽIVOTNE DOBI: REZULTATI RANDOMIZIRANE KLINIČKE STUDIJE

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Sažetak

Starenje stanovništva postalo je jedno od najvažnijih pitanja budućeg društvenog razvoja. Istraživanja potvrđuju da probiotici mogu poboljšati stanje osoba koje pate od konstipacije. Funkcionalna konstipacija čest je poremećaj u starijoj životnoj dobi. Trenutno se provodi mnogo istraživanja usmjerenih na pronalaženje učinkovitijih terapija za liječenje funkcionalne konstipacije, što uključuje ispitivanje farmakoterapijskih opcija, ali i dodataka prehrani koji bi mogli poslužiti kao dopuna konvencionalnoj terapiji. Ovo istraživanje ispitivalo je utjecaj više sojeva probiotika na funkcionalnu konstipaciju i na općenito poboljšanje kvalitete života osoba starije životne dobi smještenih u domu za starije i nemoćne. Ukupno 60 korisnika domova za njegu, starijih od 65 godina, s funkcionalnom konstipacijom definiranom prema kriterijima Rimske IV klasifikacije i sposobnih za razumijevanje postupka, bilo je uključeno u studiju. Sudionici u studiji randomizirani su u dvije skupine kroz 12 tjedana intervencije s probiotičkim sojevima *Bifidobacterium animalis* subsp.*lactis* BLC1, *Lactobacillus acidophilus* LA 3, *Lactobacillus casei* BGP93 ili placebom, kao dodatak njihovoj uobičajenoj prehrani i lijekovima. Primarni ishod studije bio je kumulativni broj stolica zabilježen tijekom razdoblja studije. Šezdeset sudionika (42 žene i 18 muškaraca), prosječne dobi 77,9 godina (65-98), s funkcionalnom konstipacijom, koji su ispunjavali kriterije za uključenje, završilo je studiju. Sudionici su randomizirani u skupinu koja je dobivala placebo (N = 32) ili probiotike (N = 28). Od početka do 12. tjedna suplementacije odabranim probioticima dobiven je rezultat povećanja kumulativne učestalosti stolica u usporedbi s placebom. Suplementacija novim i originalnim tekućim pripravkom koji sadrži *Bifidobacterium animalis* subsp. *lactis* BLC1, *Lactobacillus acidophilus* LA3 i *Lactobacillus casei* BGP93 pokazala se dobro podnošljivom, sigurnom i učinkovitom.

Ključne riječi: probiotici, mikrobiota, osobe starije životne dobi, funkcionalna konstipacija

Uvod

Prema Svjetskoj zdravstvenoj organizaciji u stariju životnu dob svrstavamo osobe starije od 65 godina (Hongtao, 2022). Stanovništvo starije životne dobi definirano je udjelom osoba starijih od 65 godina većim od 7 %. Popisom stanovništva iz 2021. godine utvrđeno je da je u Hrvatskoj udio starog stanovništva u odnosu na ukupnu populaciju 22,45 %, a predviđa se da će do kraja

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2050. godine taj udio porasti do 30 % (Državni zavod za statistiku Republike Hrvatske, 2021). Prema *United Nation's World Population Prospects* broj starijih od 60 godina premašit će broj mladih te se predviđa da će 2050. godine dosegnuti 2,1 milijardu. U Europi, trenutačno, većina je starijih osoba (19,2 %), a slični trendovi prevladavaju i u Hrvatskoj (World Population Prospects, 2021). Procjenjuje se da će starije osobe činiti 25 % ukupne europske populacije uz koju će se vezati 50 % ukupne potrošnje lijekova (Eurostat, 2022). U starijoj dobi teži se individualnom propisivanju lijekova. Osobe starije životne dobi boluju od niza kroničnih bolesti, što zahtijeva istovremenu primjenu više lijekova. Prema istraživanju, koje je provedeno u Hrvatskoj, prosječan broj lijekova koji se propiše osobama iznad 70 godina jest sedam recepata (Germin Petrović, Vlahović-Palačevski, 2011). Istodobno korištenje većeg broja lijekova povećava vjerojatnost interakcija i nuspojava lijekova.

Starenje stanovništva postalo je jedno od najvažnijih pitanja budućeg društvenog razvoja. Posljedice relativno brzog povećanja stare populacije u društvu ogledaju se u najvažnijim sektorima, u koje pripada i zdravstvo. Podaci pokazuju da će se i sustavi zdravstvene skrbi u budućnosti morati prilagoditi demografskim promjenama. Visoki udio vulnerabilnog stanovništva starije dobi zahtijevat će drugačiji pristup liječenju, što ukazuje na potrebu dodatne edukacije zdravstvenih djelatnika iz područja gerijatrije (FIP, 2022).

Starenje se povezuje s generalnim porastom učestalosti upala koji odgovara povećanju razine reaktivnog C-proteina (CRP) i pro-upalnih citokina. Taj fenomen, poznat pod nazivom „inflammaging“, karakteriziran je kroničnim upalnim stanjima koji vode do trajnih oštećenja organizma (Bartlett i sur., 2012). Pretpostavlja se da promjene crijevne mikroflore, koje nastaju starenjem, pridonose razvoju sistemske upale organizma. Istraživanje povezuje starenje s povećanom incidencijom upalnih bolesti crijeva u istoj populaciji. Starenje negativno utječe i na ravnotežu mikrobiote, što se dovodi u direktnu vezu s konstipacijom (Ostan i sur., 2016). Terapijskom manipulacijom mikrobioma selektivno se mijenja odnos dobrih i štetnih bakterija koji mogu usporiti inflamatorni odgovor (Hooper i sur., 2012; Ganesh i Versalović, 2015). Promjene raznolikosti crijevnih mikrobiota snažno su povezane sa starenjem, ali mogu utjecati i na pad općeg zdravstvenog stanja, pothranjenost i povećanu potrebu za lijekovima koji se često javljaju u starijih osoba. Utvrđene su razlike u sastavu profila crijevne mikroflore u usporedbi sa zdravim starijim pojedincima i hospitaliziranim ili institucionaliziranim starijim pacijentima (Rondanelli, 2015).

Funkcionalna konstipacija jedan je od kroničnih zdravstvenih problema koji značajno smanjuje kvalitetu života. Zahvaća 16 % svjetske populacije, posebice osobe starije dobi s incidencijom od 26 % kod muškaraca i 34 % kod žena (Leung i sur., 2011). Prevalencija konstipacije raste do 80 % kod ljudi koji su smješteni u domove za starije i nemoćne te kod hospitaliziranih osoba. Uzroci su smanjeno kretanje, dehidracija, kronična medicinska stanja (dijabetes, hipertenzija, demencija, Parkinskonova bolest, neuropatija) te lijekovi koji se primjenjuju (antikolinergici, antidepresivi, antiepileptici, opoidni analgetici, nesteroidni antiinflamatorni lijekovi) (Gustafsson i sur., 2019; Takaoka i sur., 2020).

Probiotici pokazali su se korisnim u olakšavanju problema s konstipacijom jer je nepravilno pražnjenje crijeva često posljedica narušene crijevne mikroflore. Na razini reda i taksonomskom

nivou različitost je velika u odrasloj dobi i karakterizirana je interindividualnom različitosti. Promjene životnog stila, prehrane i lijekovi imaju značajan učinak na mikrobiotu osoba starije životne dobi (Ostan i sur., 2016). Postoje dokazi da su upalni procesi, oksidacijski stres i crijevna mikrobiota u izravnoj vezi s prehranom te da je moguće odgoditi promjene koje nastaju starenjem ako se intervenira pravilnom prehranom i/ili nadopunom pojedinih nutrijenata. Crijevna flora mijenja se starenjem, a uglavnom dolazi do smanjenja broja bifidobakterija. Iako još uvijek nije dokazano da je to razlog nastanka konstipacije, uočeno je da promjene crijevne flore dovode do smanjenja intestinalne pokretljivosti te da kratkolančane masne kiseline, kao produkt bifidobakterija, imaju važnu ulogu u skraćivanju vremena prolaska stolice (Hooper i sur., 2012). Mikrobiom starije životne dobi pokazuje veće interindividualne varijacije u usporedbi sa zdravom odraslom populacijom. Također, uočene su poveznice s mikrobiotom i prehranom te životom u zajednici, obitelji ili domu za starije i nemoćne (Ganesh i Versalović, 2015).

Materijal i metode

Od korisnika doma za stare i nemoćne, koji pate od funkcionalne konstipacije, odabrani su oni koji su bili podobni za uključivanje u studiju. Zainteresirani korisnici bili su upućeni u detalje protokola studije te su dobili uvid u Informirani pristanak. Nakon što su im objašnjeni ciljevi te moguće koristi i rizici istraživanja, dobrovoljno sudjelovanje potvrdili su potpisivanjem Informiranog pristanka. Nakon toga provedena je vizita probira na koju su potencijalni ispitanici došli ujutro natašte te im je izvađena krv. Učinjena su im antropometrijska mjerenja, uzet uzorak krvi iz periferne vene podlaktice te im je izmjeren arterijski tlak. Ispitanicima je uzeta detaljna povijest bolesti uključujući medikamentoznu terapiju, OTC pripravke, ostale dodatke prehrani i navike (pušenje cigareta, konzumiranje alkohola, prehrambene navike). Preporučeno im je da tijekom istraživanja nastave uzimati dosadašnju terapiju budući da je pripravak probiotika samo dopuna konvencionalnoj terapiji. Zamoljeni su da o svakoj promjeni stanja, uzimanju dodatnih lijekova ili dodataka prehrane obavijeste medicinsko osoblje doma i ljekarnika koji provode istraživanje. Pri uključivanju, kao i pri svakoj izmjeni terapije, za svakog ispitanika bilježi se zaštićeno ime lijeka, djelatna tvar i ukupna doza. Prilikom statističke analize uzeta je u obzir mogućnost izazivanja konstipacije od strane lijekova za koje je učinak definiran u Sažetku opisa svojstava proizvoda.
Istraživanje je bilo dizajnirano kao randomizirana, dvostruko slijepa studija u trajanju od 12 tjedana. Studija je registrirana na Clinical Trails.gov pod brojem NCT04506801. Pozitivno mišljenje o predloženom protokolu studije dalo je Etičko povjerenstvo doma za starije i nemoćne „Sv. Kamilo de Lellis“ i Povjerenstvo za etičnost eksperimentalnog rada Farmaceutsko-biokemijskog fakulteta Sveučilišta u Zagrebu (Klasa:643-03/17-01/01; Ur.broj: 251-62-03-17-21, Zagreb, 2. listopada 2017.)
Ispitivani (aktivni) tretman: probiotik (*Lactobacillus acidophilus* LA3;$1\cdot10^{11}$ cfu/g, *Bifidobacterium animalis* subsp. lactis BLC1; $1{,}5\cdot10^{11}$ cfu/g i *Lactobacillus casei* BGP93 $2\cdot10^{11}$cfu/g**)** u obliku tekuće oralne formulacije, proizvođača Pharmas d.o.o. Formulacija probiotika prilagođena je osobama starije životne dobi s obzirom na to da je u tekućem obliku

najlakša za oralnu primjenu uz jelo. Aktivni tretman sponzorirala je farmaceutska kompanija Pharmas d.o.o. i pripremljen je isključivo za potrebe istraživanja.
Kontrolni tretman (placebo): srednjelančano trigliceridno ulje (frakcionirano ulje dobiveno iz kokosovog ili palminog ulja s pretežitim sadržajem triglicerida kaprilne (C8) i kaprinske kiseline (C10) + silicijev dioksid); istog je izgleda, farmaceutskog oblika te ima isti način primjene kao i aktivni tretman; razlika između proizvoda i placeba samo je u probiotičkim kulturama. Kontrolni tretman sponzorirala je farmacetuska kompanija Pharmas d.o.o. i pripremljen je isključivo za potrebe istraživanja.
Uključni kriteriji (svi moraju biti zadovoljeni): a) informirani pristanak – osobno; b) funkcionalna opstipacija definirana prema Rimskim IV kriterijima – na početku uvodnog dijela na temelju povijesti bolesti, na kraju temeljem četverotjedne opservacije; c) dob > 65 godina; d) očekivani životni vijek > 12 mjeseci; e) sposobnost da se razumije operativni dio pokusa i redovita primjena alociranog tretmana; f) bez kliničkih znakova akutne upale (prema mišljenju osoblja koje je s ispitanicima u svakodnevnom kontaktu).
Kriteriji za neuključivanje: a) sumnja na opstruktivni ileus ili prethodni opstruktivni ileus; b) sumnja ili potvrđena dijagnoza: sindroma iritabilnog kolona, ulceroznog kolitisa, Crohnove bolesti, maligne bolesti probavnog trakta; c) dijareja bilo kojeg uzroka unutar zadnjih mjesec dana; d) akutna infektivna bolest unutar zadnjih mjesec dana, što isključuje osobe koje koriste antibiotike, e) osobe koje u farmakoterapiji imaju opoidne analgetike.

Rezultati i rasprava

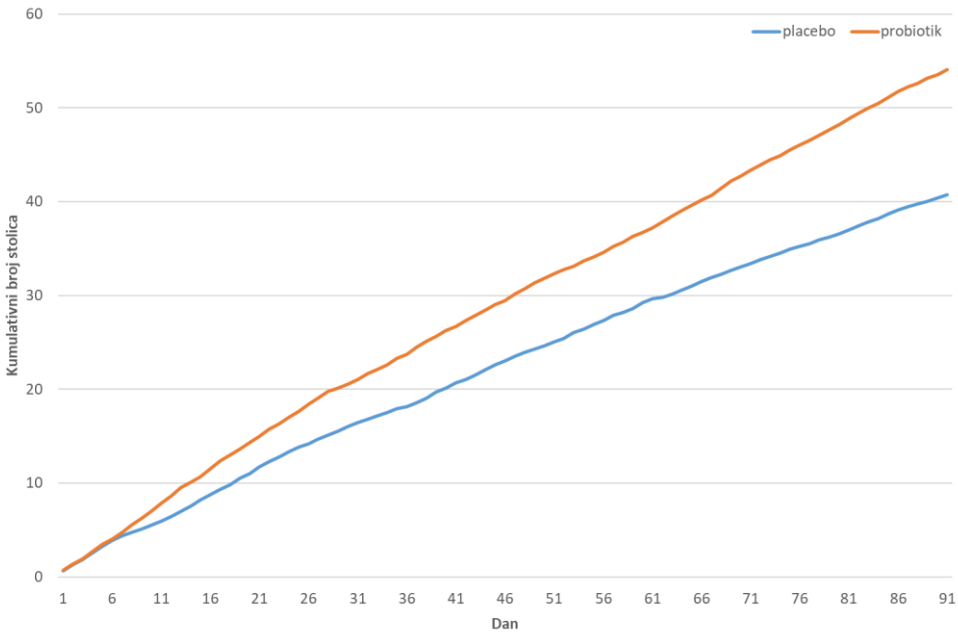
Podaci u ovom istraživanju prikupljeni su od 60 korisnika doma za starije i nemoćne. Od ukupnog broja ispitanika 42 bilo je ženskog spola i 18 muškog spola. Srednja dob ispitanika bila je 77,9 godina. Takvi podaci u skladu su s podacima Hrvatskog zavoda za javno zdravstvo iz 2011. godine, gdje je udio žena starijih od 65 godina iznosio 60,96 %, a udio muškaraca 39,04 % (HZJZ, 2011). Od uključenih osoba 28 primalo je probiotik, dok su preostala 32 ispitanika dobivala placebo. Deskriptivni podaci za dob ispitanika prikazani su u Tablici 1.

Tablica 1. Deskriptivni podaci ispitanika
Table 1. Descriptive data of study participants

	N*	M	- 95 % IP	+ 95 % IP	Med	Min	Maks	DK	GK	SD
Dob	60	77,9	75,7	80,2	79,7	56,1	96,7	72,8	84,0	8,84
Broj dijagnoza	60	4,3	3,8	4,9	5	0	9	3	6	2,3
ITM	60	27,4	25,8	29,0	27,1	15,9	47,8	22,6	31,2	6,05

*N = broj ispitanika; M = srednja vrijednost; -95%/+95% IP = intervali pouzdanosti; Med = medijan; Min/Maks = najniža i najviša vrijednost; DK/GK = donji i gornji kvartil; SD = standardna devijacija

Proporcija ispitanika s normalnim pražnjenjem crijeva prikazana je kao srednji broj stolica prema danu sudjelovanja u istraživanju. Srednji broj stolica kod ispitanika koji su koristili probiotik jest 54, a u skupini placebo 41 (Slika 1).



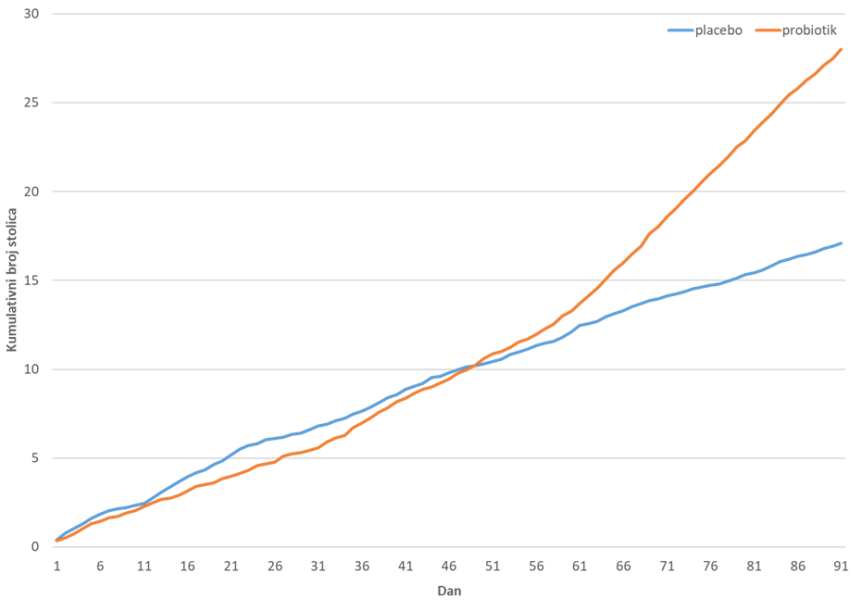
Slika 1. Kumulativni broj stolica
Figure 1. Cumulative numbers of stools

Upotreba probiotika u ovoj studiji pokazala se sigurnom, što su i dokazale mnoge recentne studije (Arboleya i sur., 2016; Hamilton-Miller, 2004; Claesson i sur., 2012). Tijekom studije nije prijavljena nijedna nuspojava. Dodatak probiotika na konvencionalnu i alternativnu terapiju korisnika doma pokazalo se učinkovitim za poboljšanje pražnjenja crijeva, odnosno za funkcionalnu konstipaciju, što je bio i opći cilj ovog istraživanja.
Primarni cilj istraživanja bio je ispitati učinak probiotičkih sojeva na broj dnevnih stolica. Prvi put ispitivani su sojevi *Lactobacillus acidophilus* LA3;$1 \cdot 10^{11}$ cfu/g, *Bifidobacterium animalis* subsp. *lactis* BLC1; $1{,}5 \cdot 10^{11}$ cfu/g i *Lactobacillus casei* BGP93 $2 \cdot 10^{11}$cfu/g na funkcionalnu konstipaciju osoba smještenih u dom za starije i nemoćne. Dokazano je da se starenjem smanjuje broj bifidobakterija koje izravno utječu na vrijeme tranzita kroz crijeva (Didari i sur., 2014) što bi mogao biti jedan od uzroka konstipacije.

Iako je upotreba laksativa kod većine pacijenata dobrog učinka, kod nekih pacijenata koji pate od konstipacije ne daje zadovoljavajuće rezultate (Snydman, 2008). Probiotici upotrebljavaju se pri rješavanju problema konstipacije, što potvrđuju i istraživanja koja su do sada provedena. *Lactobacillus* i *Bifidobacterium* čine probiotičku floru u probavnom sustavu te štite od patogena i održavaju zdravlje mukoze. Istraživanja su pokazala da, kod osoba koje pate od konstipacije, obje vrste probiotika nedostaju u crijevnoj flori, što nam daje prostor za istraživanja (Sanders, 2010). Najispitivaniji probiotički sojevi roda su *Bifidobacterium i Lactobacillus* i upravo ti rodovi korišteni su u ovoj studiji. Studije su pokazale da *Bifidobacterium lactis* poboljšava vrijeme tranzita (Snydman, 2008), što se pokazalo točnim i u ovom istraživanju. *Lactobacillus acidophilus* LA-5 *i Bifidobacterium animalis* subsp.*lactis* BB-12 smanjuju konstipaciju kod starijih pacijenata te poboljšavaju frekvenciju pražnjenja (Sanders, 2010; Mangiola i sur., 2018). Također, studije su pokazale da povoljan učinak na konstipaciju ima i soj *Lactobacillus casei Shirota*. U ovom istraživanju korišteni su *Lactobacillus acidophilus* LA3;$1 \cdot 10^{11}$ cfu/g, *Bifidobacterium animalis* subsp. *lactis* BLC1; $1 \cdot 10^{11}$ cfu/g i *Lactobacillus casei* BGP93 $1 \cdot 10^{11}$cfu/g.

Broj stolica u grupi koja je primala probiotik veći je u usporedbi s placebom, ali nema statističke značajnosti između ispitivanih grupa. Ipak, uviđa se pozitivan trend povišenja razlike između grupa. P-vrijednosti na početku studije bile su 0,994 (prvi tjedan), a 0,090 (zadnji tjedan). Može se zaključiti da je za potpuni učinak potrebno više od 90 dana upotrebe kako bi se postigla statistička značajnost. Neka dvostruko slijepa i randomizirana istraživanja pokazala su učinak nakon 152 dana na poboljšanje frekvencije defekacije gdje su u ispitivanju korišteni probiotici roda *B. lactis* (Bongers i sur., 2009). U studiji koja je koristila kombinaciju probiotičkih sojeva *Lactobacillus* i *Bifidobacterium* u znatno kraćem vremenu (45 dana) postiže se učinak na smanjenje korištenja laksativa za čak 10 % (Yamashiro, 2017). Rezultati navedenih studija sugeriraju da primjena probiotika ima mali, ali značajan učinak na konstipaciju kod osoba starije životne dobi. Da bi se na kraju postigla statistički značajna razlika između grupa u ovoj studiji, bilo bi potrebno produžiti vrijeme korištenja probiotika. Rezultati su u skladu sa sličnim studijama koje su ispitivale frekvenciju pražnjenja crijeva (Agrawal i sur., 2009; Bacteriotherapy, 2021).

Nakon izuzimanja podataka onih dana kada su sudionici koristili laksative, kumulativni broj stolica u skupinama ponovno je uspoređen. Iz rezultata prikazanih na Slici 2 vidljive su vrlo slične kumulativne vrijednosti broja stolica za obje skupine tijekom prvih šest tjedana. Nakon toga razlika između skupina počinje rasti u korist probiotika i 70-og dana kumulativni broj stolica u ispitanika koji su koristili probiotik jest 18, dok je u placebo skupini 13 te postaje statistički značajan nakon 70. dana tretmana kada su $p < 0,05$. Devedesetog dana tretmana ukupan broj stolica u ispitanika koji su koristili probiotik bio je 28,0 (medijan=23), a u placebo skupini 17,1 (medijan=16).



Slika 2. Razlika u broju stolica u dane kada ispitanici nisu uzimali laksative
Figure 2. Difference in the number of stools on days when participants didn't take laxatives

Pregled terapije ispitanika pokazao je da najviše ispitanika ima dijagnosticiranu bolest krvožilog sustava (N=47) te mentalnih poremećaja (N=43). Bolesti mišićno-koštanog sustava i vezivnog tkiva, zatim endokrinih, nuticijskih i metaboličkih bolesti te bolesti probavnog sutava dijagnosticirane su kod upola manje ispitanika. Ostale bolesti bile su dijagnosticirane pojedinačno kod pet ili manje ispitanika. Uvidom u terapiju ispitanika napravljen je i pregled djelatnih tvari te je utvrđena njihova učestalost u primjeni. Najpropisivaniji je kalijev klorid (N=23), pantoprazol (N=22), tremadol/paracetamol (N=18) i furosemid (N=14). Ukupno je identificiran 141 terapijski problem na ukupnom broju od 60 ispitanika. Gotovo svi ispitanici imali su barem jedan terapijski problem. Terapijski problem, povezan s konstipacijom, neželjeni je učinak lijeka tramadol i opoidnih analgetika.

Zaključak

Primjena probiotičkih sojeva *Lactobacillus acidophilus* LA3, *Bifidobacterium animalis* subsp. *lactis* BLC1 i *Lactobacillus casei* BGP93 sigurna je. Tijekom istraživanja nisu zabilježene nuspojave ni neželjeni štetni događaji te probiotici pokazuju povoljan učinak na osobe koje pate od funkcionalne konstipacije. Ovo istraživanje ukazalo je na važnu ulogu ljekarnika u zdravstvenoj skrbi za korisnike domova za starije i nemoćne osobe.

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Proceedings

PROBIOTICS AS A THERAPEUTIC OPTION FOR FUNCTIONAL CONSTIPATION IN THE ELDERLY: RESULTS OF A RANDOMIZED CLINICAL TRIAL

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original scientific paper

Summary

The absolute and proportionate increases in older populations has become an outstanding demographic trend and a serious public health issue globally, with manifold social consequences that require early planning of optimal care for older adults. Constipation is one of the most common gastrointestinal conditions, particularly among older individuals. Therefore, today a lot of research is focused on finding more effective therapies for treating functional constipation, which includes not only examining pharmacotherapeutic options, but also dietary supplements that could serve as a supplement to conventional therapy. Clinical research to date suggests that probiotics may improve the condition of people suffering from constipation. Therefore, the aim of this study was to examine the impact of multiple strains of probiotics on the functional constipation of elderly people housed in a nursing home, using a randomized, double-blind, placebo-controlled, parallel study design. A total of 60 elderly nursing home residents, aged 65 years or more, with functional constipation defined according to the Rome IV criteria and able to understand the procedure, were eligible for inclusion in the study. Using a double-blind, placebo-controlled, parallel design, each participant was randomized to either the selected mixture of probiotics (*Bifidobacterium animalis* subsp. *lactis* BLC1, *Lactobacillus acidophilus* LA3, *Lactobacillus casei* BGP93) or placebo for 12 weeks as an adjunct to their usual diet and medications. The primary outcome of the study was the cumulative number of stools recorded over the study period. Sixty participants (42 females and 18 males), aged 77.9 (65- 98), with functional constipation, who met the eligibility criteria, completed the study. Subjects were randomly assigned to either placebo (N = 32) or probiotic (N = 28) group. From baseline to 12 weeks supplementation of selected probiotics resulted in no significant increase of the cumulative stool frequency in comparison with placebo. The supplementation with a new and original liquid formulation containing *Bifidobacterium animalis subsp. lactis* BLC1, *Lactobacillus acidophilu s*LA3 and *L. casei* BGP93 has been proven as well tolerated, safe and efficacious.

Keywords: probiotics, microbiota, elderly, constipation

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Proceedings

CONTENT OF AFLATOXIN B1, CHLORIDE, NITRITE AND HEAVY METALS IN MEAT PRODUCTS IN THE AREA OF ZENICA-DOBOJ CANTON, BOSNIA AND HERZEGOVINA

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original scientific paper

Summary

Many additives are of essential importance for the taste and quality of products, however, many of them pose a danger to human health, and are subject of daily routine quality control of products intended for human consumption. In addition to additives, residues represent a major challenge as possible contaminants of products from the field to the dining table. Since meat is one of the most significant food sources, our research aimed to examine the quality of meat and meat products in the Zenica-Doboj Canton market. Through the research, 85 samples of meat products (salami, sausages, dried meat products) were processed and all were analyzed for the presence of aflatoxin B1 (AFL B1), as well as for chlorides, nitrites and heavy metals (cadmium and lead). The average content of AFL B1 in the meat samples was 0.048 µg/kg (with a range of 0.019 to 0.105 µg/kg). Chloride content was detected in the range from 0.000 to 9.955 %m/m (average concentration 2.377 %m/m). The average nitrite content was 8.330 mg/kg (min. 0.550 – max. 45.705 mg/kg). Among the 85 processed samples, lead and cadmium were detected in 79 and 29 products (92.94% and 34.11%), with an average of 0.136 and 0.042 mg/kg, respectively. Lead was detected in the range from 0.000 to 3.474 mg/kg, and cadmium from 0.000 to 2.544 mg/kg. The results show the need for continuous monitoring of the amount of additives in meet products on the market, and regular monitoring of residues in products intended for human consumption. It is necessary to stick to proper hygienic practices during the preparation of the product at all stages from the field to the dining table.

Keywords: residues, additives, lead, cadmium, public health

Introduction

It has been recognized for centuries that food serves as the cornerstone of human health. Dietary habits and human nutritional requirements have evolved in tandem with technological and industrial advancements throughout history. The journey from field to table was considerably shorter, as food was prepared and consumed at home within a shorter time frame. However, this trajectory has substantially elongated, amplifying the number of risks that impact the safety of our food (Barnes et al., 2020).

One such risk is the potential contamination by various types of toxins, including mycotoxins, heavy metals, pesticides, etc. Such contamination can occur during the production, storage, and distribution of food products, with the source of these toxins emanating from the environment,

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raw materials, or arising during the food processing itself (Abdolshahi and Shokrollahi Yancheshmeh, 2020). Another risk associated with the extension of the field-to-table journey is the use of food additives. In some instances, their use remains unregulated and may have adverse implications for human health.

Mycotoxins are secondary metabolites synthesized by molds during their growth on substrates of both plant and animal origin. Mycotoxins pose a significant challenge to both human and animal health, as per estimates by the Food and Agriculture Organization (FAO), approximately 25% of the global food supply is contaminated by mycotoxins (Chaytor et al., 2011). The occurrence of mycotoxins is contingent upon factors such as the specific mold species, climatic conditions, as well as various physicochemical elements including temperature, moisture content in food, gas concentrations in the atmosphere, and others. Among the aflatoxins identified, AFL B1 is the most widely distributed and most harmful substance, which is the most toxic and carcinogenic substance (Dai et al., 2017).This mycotoxin is of significant concern in the field of food safety and public health. Its toxicity is associated with its ability to cause DNA damage, leading to mutations and ultimately contributing to the development of liver cancer (Bedard and Massey, 2006).

Heavy metals are defined as metals with a density greater than 5 g/cm³. They gradually accumulate in the food chain and have adverse effects on human health. Their toxicity depends on several factors, including dosage, exposure route, chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals (Zhushan and Shuhua 2020). Due to their high degree of toxicity, cadmium, lead, arsenic, chromium, and mercury are classified as priority metals of public health concern. These metallic elements are considered systemic toxicants known to cause organ damage even at lower levels of exposure. They are also classified as human carcinogens (Dilek and Kadiriye, 2006). Lead affects the function and structure of the kidneys, bones, the central nervous system, and results in harmful biochemical, histopathological, neuropsychological, fetotoxic, and reproductive effects. Cadmium is considered one of the most toxic metals (Amani and Lamia, 2012). High concentrations in humans can lead to skeletal deformities, kidney lesions, lung damage, and may have carcinogenic and mutagenic effects (Evis et al., 1987).

Additives are substances or mixtures of substances added to meat and other food products during production, processing, packaging, storage, or transportation to enhance their qualitative characteristics, including appearance, taste, smell, color, texture, and shelf life (Sambu et al., 2022). Additives are categorized into various groups, including preservatives, antioxidants and synergists of antioxidants, flavor enhancers, emulsifiers, thickeners, binding agents, gelling agents, colorants, sweeteners, acid regulators, enzyme preparations, and other additives (Schröder, 2003). It's important to distinguish additives from spices, such as common table salt (NaCl) and herbs, which are not considered additives. In the meat industry, nitrates have found widespread application as additives, while salt is utilized as a seasoning or an ingredient in brine mixtures for curing. Nitrates in meat and meat products belong to the group of preservatives. They improve the quality, shelf life, and safety of products, primarily by inhibiting the growth and reproduction of bacteria like *Staphylococcus aureus* and *Clostridium botulinum.* Nitrates also influence the color, smell, taste, and texture of meat products. Despite their beneficial characteristics as preservatives, their use has a detrimental impact on human health, and efforts are made to minimize their usage (Honikel, 2008). Additionally, kitchen salt, aside

from enhancing meat flavor, inhibits the growth and reproduction of bacteria, reduces water activity in meat, and affects water-binding capacity (Tobin et al., 2012).
Considering that all the mentioned factors have a significant impact on human health, this research aimed to examine the quality of meat regarding the presence of mycotoxins, heavy metals, and additives and determine whether it complies with the prescribed limits.

Materials and methods

The research was conducted on 85 samples of meat and meat products collected from the Zenica-Doboj Canton. The collected samples are representative and prepared in a consistent and standardized manner, which is crucial for obtaining reliable and meaningful analytical results.

Aflatoxin B1

Determination of aflatoxin B1 was performed using the immunoenzymatic method on an ELISA device (LABTRONE LMPR-A20) in accordance with the manufacturer's specification. The homogenized meat sample (2±0.05 g) was put into a 50 ml centrifuge tube, then added 8 ml of ethyl acetate, oscilated for 5 min, centrifuged at room temperature (4000xg, 10 min). After that 2 ml supernatant was taken, dried with water bath at 50 °C. The residual was dissolved with 2 ml of N-hexane, oscilated for 1 min, added 1 ml of Sample Diluent A, and centrifuged at room temperature (4000xg, 5 min). After that 50µl of the lower liquid was taken, and the content of AFL B1 was determined by ELISA kit (Art. No. E-TO-E017, Elabscience Biotechnology).

Lead and Cadmium

The determination of lead and cadmium was carried out using a standard method, EN 14084: 2023, IDT. The homogenized meat sample (0.5 g) was put into a 70 ml vessel, then added 1 ml of deionized water, 8 ml of nitric acid (not less than 65% mass fraction) and 1 ml of hydrochloric acid. The vesseles were sealed and samles were digested as prescribed by manufacturer (Anton Paar Multiwave ECO). The graphite technique method was used for determination, and examples of wavelength, gas mixture/temperature programmes and other instrumental parameters appropriate for each metal are found in manuals provided with the instrument (GBC SavantAA Z enduro).

Nitrite

The nitrite content determined according to the procedure described in International standard ISO 2918-1975 and expressed as milligrams of sodium nitrite per kilogram. The method's principle is extraction of test portion with hot water, precipitation of the proteins and filtration, in the presence of nitrite development of a red colour by addition of sulphanilamide and N-1- naphthyl ethylenediamine dihydrochloride to the filtrate and photometric measurement at wavelength of 538 nm. For the photometric measurement was used Shimadzu spectrofotometer UV-2600.

Chloride

Determination of chloride content was according to standard ISO 1841-1:1996 based on Volhard method. The method's principle is extraction of test portion with hot water and precipitation of the proteins, after filtration and acidification, addition of an exces of silver nitrate solution to the extract, and titration of this excess with potassium thiocyanate solutin.

Results and discussion

The average content of AFL B1 in the samples was 0.048 µg/kg (with a range of 0.019 to 0.105 µg/kg). By classifying processed samples according to the type of meat, we obtained the following data: Results of AFL B1 residue levels ranged from 0.019 to 0.105 with an average value of 0.051 µg/kg for beef products; 0.019 to 0.090 with an average value of 0.047 µg/kg for chicken products; 0.032 to 0.105 with an average value of 0.048 µg/kg for turkey products; and 0.034 to 0.042 with an average value of 0.038 µg/kg for pork product. The highest average concentration of AFL B1 is present in beef products, while the lowest average concentracion is in pork products, with very little difference between them (Figure 1). The reason for this could be animal feed that is also contaminated with mycotoxins, as well as similar meat processing procedures.

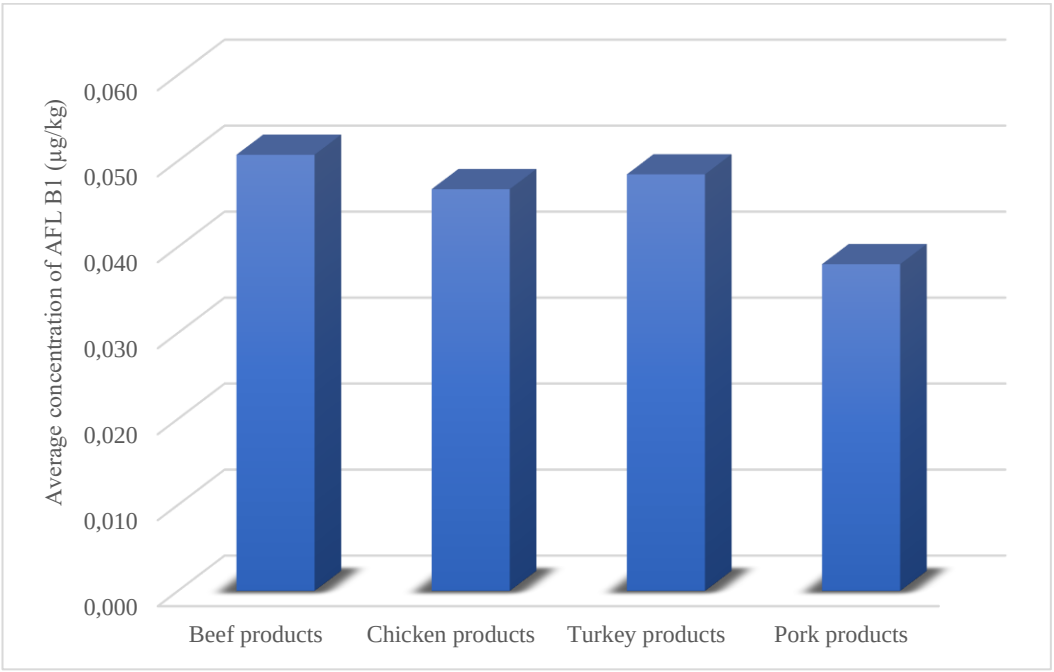

Figure 1. Average concentration of AFL B1 in different kinds of meat products

Similar research was obtained by Algahtani et al. (2020). Their results of Aflatoxin B1 residue levels ranged from ND to 13 µg/kg, and are much higher than results in our study, which may be due to the use of meat additives previously contaminated with aflatoxins.
The results for heavy metals show a large variability of lead and cadmium concentrations in some of the meat and meat products groups. However, this variability in biological samples is considered to be normal since the sources of this metal are numerous. Furthermore, lead and cadmium concentrations, also, depend on the environmental conditions and the food production methods. Even within a certain type of food, concentration variations can be large produced by heterogeneities in the distribution of the metal (Reilly, 1991).
Among the 85 processed samples, lead was detected in 79 and cadmium was detected in 29 products (92.94% and 34.11%), with an average of 0.136 and 0.042 mg/kg. Lead was detected in the range from 0.000 to 3.474 mg/kg, and cadmium from 0.000 to 2.544 mg/kg.
According to all results, the lead content exceeded the maximum residue limit (MRL) for meat (0.1 mg/kg) in 24% of samples (Figure 2), while the cadmium content exceeded the MRL for meat (0.05 mg/kg) in 6% of samples (Figure 3). These are highly concerning results that can have a significant impact on human health.

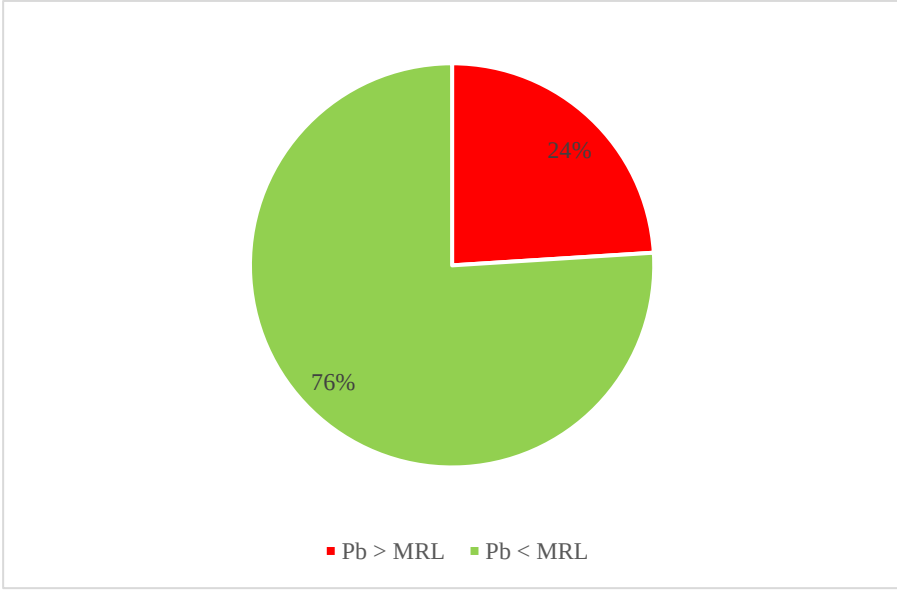

Figure 2. The lead content in relation to MRL

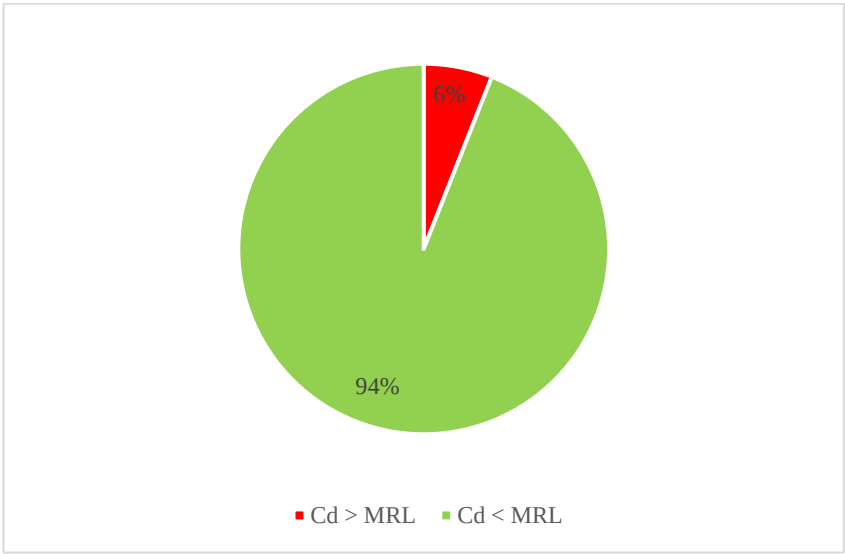

Figure 3. The Cadmium content in relation to MRL

Regarding the analysed meat product samples (Table 1), both the lead and cadmium concentrations measured in this study were well above the values found in recent literature. These results indicate the need for more frequent monitoring, as well as stricter legal regulations that apply to all industries that are potential environmental pollutants with heavy metals.

Table 1. Concentration of lead and cadmium in meat products (µg/kg) reported in Spain and in this study

	Gonzalez-Weller et al., 2011		This study	
Type of meat product	**Pb**	**Cd**	**Pb**	**Cd**
Chicken products	3.16	4.15	78.04	7.05
Beef products	6.72	4.76	234.03	95.85
Turkey products	9.12	5.98	23.78	7.56
Pork products	4.89	6.50	68.50	N.D.

Regarding the analysed pork meat product samples, both the lead and cadmium concentrations measured in this study were similar with the values found in Romania (Hoha et al., 2014).
The average nitrite content was 8.330 mg/kg (min. 0.550 – max. 45.705 mg/kg). Figure 4 shows average concentracion of nitrites in different meat categories, depending on their thermal proccesing. The smoked meat samples have the highest concentracion of nitrites, however, a

surprisingly high concentration of nitrites was found in fresh meat. The higher concentration of nitrites in fresh meat can be explained by their effect on the characteristic color of the meat, inhibition of bacterial growth, and preservation of specific aroma.

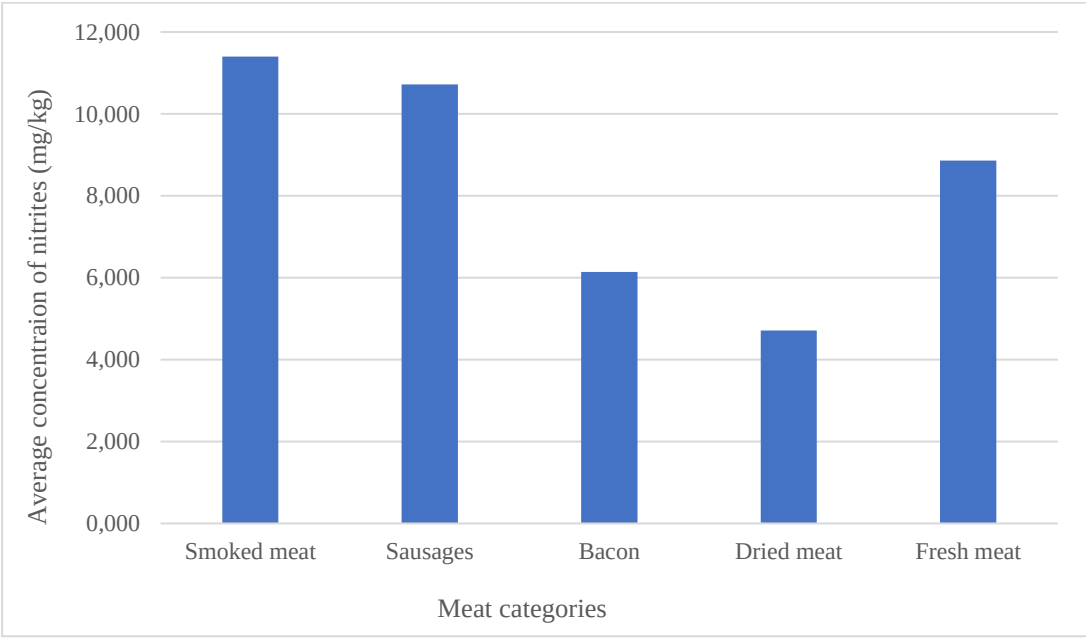

Figure 4. Average concentracion of nitrites in different meat categories (mg/kg)

The average nitrite concentrations were 11.396 mg/kg in smoked meat, 10.721 mg/kg in sausages, 6.145 mg/kg in bacon, 4.712 mg/kg in dried meat, and 8.861 mg/kg in fresh meat. A similar study in Turkey showed concentrations for nitrites in sausages 102.8 mg/kg, and that is much higher than our study resullts (Yalçin and Yalçin, 1998). In the fresh meat, Gozdecka et al. (2021) in Poland measured nitrite content ranging from 1 to 5 mg/kg. It was reported by Dennis et al. (1990) that the mean nitrite content in bacon was 24.0 mg/kg. Thus, differences are more likely to be due to the manufacturing processes and different meat products.
Nitrates and chlorides are often used together in meat preparation or processing procedures, hence it has been beneficial to monitor the concentration of both. Chloride content was detected in the range from 0.000 to 9.955%m/m (average concentration 2.377%m/m). The average chloride concentrations were 1.85% in smoked meat, 2.04% in sausages, 9.96% in bacon, 3.92% in dried meat, and 1.04% in fresh meat. Bacon have the highest concentracion of chlorides (Figure 5).

Aaslyng et al. (2014) when analyzing Danish crushed meat products such as sausage, cooked hams found average salt content of 2.19% and 2.28%, respectively. Capuano et al. (2013) detected average salt content of 1.93 to 2.66%.
The obtained results can be explained by the application of salt due to its multifunctionality, such as shaping taste, enhancing aroma, influencing texture, and increasing cost-effectiveness. However, they cannot be justified in terms of the harmful effects of salt on raising blood pressure in humans and its impact on the kidneys. Excessive salt consumption is also not justified because there are technological processing methods to avoid this.

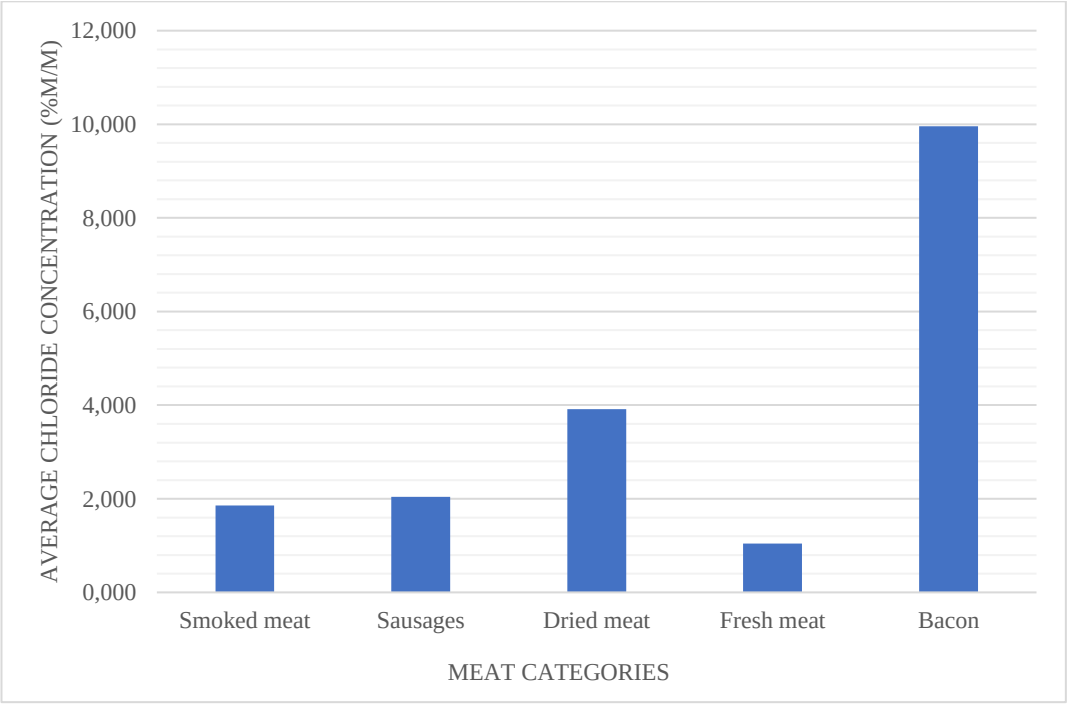

Figure 5. Average concentracion of chloride in different meat categories

Conclusion

In the course of meat processing, it is paramount to take into account the prevailing conditions and potential sources of contamination. Vigilant and frequent monitoring of meat and meat products is indispensable to ensure their safety and quality. Animal feed, constituting a pivotal link within the food chain, necessitates meticulous scrutiny to prevent contamination. Regulatory frameworks, which oversee the quality of meat and the presence of contaminants, ought to define precise limit values for all pertinent parameters.

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TECHNOLOGICAL QUALITY OF WHEAT AFFECTED BY NITROGEN FERTILIZATION

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Summary

Abiotic stress, such as nitrogen (N) deficiency, negatively impacts the yield and quality of wheat (*Triticum aestivum* L.). This study aimed to assess the effect of reduced and standard N supply on the baking quality of three bread wheat cultivars. The N treatment consisted of two fertilization levels: 0 kg N ha^{-1} as reduced N supply and 100 kg N ha^{-1} applied as split top-dressings at two growth stages, corresponding to standard N fertilization practice. The results showed that under reduced nitrogen supply, the protein content (12.1% vs. 13.1%) and wet gluten were lower by 7.8%, and 8.2%, respectively, compared to optimal N rate. Conversely, gluten index and falling number decreased by 2.4% and 16.4%, respectively, under higher N supply.

Keywords: wheat, cultivar, nitrogen fertilization, baking quality

Introduction

Wheat (*Triticum aestivum* L.) is widely used worldwide for producing bread and other products like noodles, pasta, cakes and biscuits. The technological quality of wheat flour depends mainly on protein content, especially gluten proteins, dough rheological parameters (gluten strength and viscoelasticity), hectoliter weight, thousand-kernel weight, and α-amylase activity (Xue et al., 2019). These quality parameters are influenced by genotype, environmental conditions (soil and climate conditions, fertilization, irrigation, etc.), and genotype x environment interaction (Yong et al., 2004; Drezner et al., 2007; Horvat et al., 2012). During all stages of wheat growth, fertilization plays a crucial role in providing mineral nutrients, with N being particularly important due to its positive effect on protein (P) and wet gluten (WG) content (Guerrini et al., 2020; Horvat et al., 2021). However, N deficiency can negatively affect wheat yield and quality (Ivić et al., 2021). Hence, this study aimed to investigate the impact of reduced and standard N supply on the technological quality of bread wheat cultivars.

Materials and methods

Materials and N rates

During the 2019/2020 growth season, three winter wheat cultivars were grown at the Agricultural Institute Osijek under two N fertilization levels. The experiment followed a split-plot factorial design with plot sizes of 7.56 m^2 and a sowing rate of 350 kernels m^{-2}. Basic and

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pre-sowing fertilizations of 74 kg N ha^{-1} were applied using 100 kg ha^{-1} of urea (46% N) and 400 kg ha^{-1} of NPK (7:20:30). The N treatment included two levels: 0 kg N ha^{-1} as reduced (N_0) and 100 kg N ha^{-1} as standard N fertilization practice (N_{100}). N_{100} plots received 50 kg N ha^{-1} in tillering and another 50 kg N ha^{-1} in stem extension growth stages. Standard practices of wheat production in the Slavonia region were followed for herbicide, insecticide, and fungicide applications.

Analysis of technological quality traits

Wheat grains were milled using an experimental mill (Quadrumat Senior mill, Brabender). Near infrared transmission (NIT) with the Infratec 1241 analyzer (Foss) was used to determine grain P content following the method HRN EN ISO 20483:2014. WG content and gluten index (GI) were determined using Glutomatic system (Perten) following the method HRN EN ISO 21415-2:2015. Falling number (FN) was determined using the Falling Number Perten Instruments and the HRN EN ISO 3093:2010 method on milled grains passed through a 0.75 mm sieve.

Data analysis

Analysis of variance (ANOVA) and Tukey's HSD test were conducted to identify statistically significant differences ($p < 0.05$) in the analyzed quality traits under the influence of genotype, nitrogen, and their interactions.

Results and discussion

Wheat technological quality

The average grain P concentration was 12.6%, and this trait was influenced by genotype and N factors (Table 1). Increasing N rate from N_0 to N_{100} enhanced the grain P concentration by 7.8% (Table 2), which is consistent with previous studies (Guerrini et al., 2020; Horvat et al., 2021). Among the cultivars, Srpanjka produced grains with the lowest P concentration at both N rates compared to cultivars OS Olimpija and Kraljica. The response of cultivars to N treatment was specific, and only under N_{100} rate, the cultivar Olimpija showed an increase in P content from 12.9% to 14.5% (Table 2), thus classified into a higher quality group according to Croatian Regulation on contractual relations for wheat purchase (NN 62/19).

Table 1. Summary of the analysis of variance

Source of variation	df	Mean Square			
		P	WG	GI	FN
Genotype (G)	2	6.630*	45.691*	15.2*	3278*
Nitrogen rate (N)	1	2.490*	19.507*	12.0*	11285*
G X N	2	0.341*	19.00*	3.308*	0.3^{ns}
Error	6	0.006^{ns}	0.354^{ns}	1.2^{ns}	121^{ns}

df = degrees of freedom; P = protein content; WG = wet gluten content; GI = gluten index; FN = falling number.
* Significant at $p < 0.05$.

The average WG concentration was 27.3%. Cultivar OS Olimpija produced grains with the highest WG (30.4%), while the lowest values were observed for the genotype Srpanjka (23.7%). Both genotype and N fertilization significantly influenced WG content, and under N_{100} WG increased by 8.2% (Table 1 and 2), what is consistent with Guerrini et al. (2020) and Horvat et al. (2021).

Table 2. Technological quality of wheat cultivars

CULTIVAR	N RATE	P (%)	WG (%)	GI	FN (s)
KRALJICA	N_0	12.9^{c}	26.7^{bc}	97^{ab}	412^{b}
	N_{100}	13.2^{c}	28.9^{cd}	95^{a}	395^{b}
OLIMPIJA	N_0	12.9^{c}	29.9^{d}	97^{ab}	429^{b}
	N_{100}	14.4^{d}	30.8^{d}	95^{a}	320^{a}
SRPANJKA	N_0	10.7^{a}	21.4^{a}	100^{b}	478^{c}
	N_{100}	11.7^{b}	25.9^{b}	99^{b}	386^{b}
MEAN		**12.6**	**27.3**	**97**	**403**
N_0		12.1^{a}	26.1^{a}	99^{b}	436^{b}
N_{100}		13.1^{b}	28.4^{b}	96^{a}	375^{a}
DIFF. N_{100} vs. N_0 (%)		**7.8**	**8.2**	**-2.4**	**-16.4**

P = protein content; WG = wet gluten content; GI = gluten index; FN = falling number. * Significant at $p < 0.05$. Means followed by the same letters in the column belong to the same cluster.

Regarding gluten strength measured by GI, all factors affected it. Unlike P and WG, the GI values decreased with increasing N level (from 99 to 96), what is in agreement with Gagliardi et al. (2020). Nevertheless, all analyzed cultivars showed high gluten strength under both N rates (Table 2). Horvat et al. (2021) also observed high gluten strength with an average GI value of 95 in their analysis of 16 wheat cultivars grown under low N levels.
The FN of wheat averaged 403 s (Table 2), and it was influenced by both genotype and N fertilization (Table 1). Specifically, the application of N_{100} favored a decrease in FN (436 s vs. 375 s). Grains with FN values below 220 s are considered inferior due to the risk of pre-harvest sprouting, which often leads to a lower price in trade (Kiszonas et al., 2018). In the absence of lodging, N supply often increases falling number (Kindred et al., 2005), but this effect depends on cultivar, year and location (Clarke et al., 2004). On the other hand, grains with insufficient α-amylase, indicated by FNs above 400 s, can result in bread with a low volume unless α-amylase is added, either in the form of malt flour or modern commercial enzyme preparations (Ral et al., 2016).

Conclusion

The analysis revealed significant differences among the analyzed quality traits, depending on the cultivars and N rates. Increasing N rates enhanced grain P and WG content. The cultivars'

response to the increase in N rate is specific, but due to the increase in P under higher N rate, only the cultivar Olimpija has been classified into a higher quality class. With increasing N, the gluten strength decreased, but to a lesser extent compared to the FN.

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THE ROLE, SIGNIFICANCE, AND PREVENTION OF VITAMIN B9 DEFICIENCY IN UNA-SANA CANTON CONSUMERS

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original scientific paper

Summary

Vitamin B9 comes in two forms; folic acid and folates. These two forms of vitamin B9 differ from each other in terms of bioavailability in the body, but their metabolic pathway is similar and they have the same biological activity. In the human body, folic acid is absorbed much better than folate. Folates represent the natural form of this vitamin that can be found in food, while folic acid is a synthetic form of the vitamin used for the production of supplements or the enrichment of foods with vitamin B9. Vitamin B9 plays a role in numerous cellular reactions that include amino acid metabolism, purine and pyrimidine biosynthesis, and DNA methylation. Folate deficiency affects the rate of division of cells that have a great need for purine and pyrimidine bases, resulting in megaloblastic anemia and hyperhomocysteinemia, which is characterized as an important risk factor for the development of cardiovascular diseases. The most common birth defects associated with folic acid deficiency in the periconceptional period are neural tube defects (NTD). The aim of this paper was to examine and analyze the attitudes and knowledge of Una-Sana Canton consumers towards vitamin B9, and to examine the frequency of use of this vitamin supplements among the respondents. The most significant methods used in the work are the sample method, surveying and statistical method, and the research instrument was an anonymous closed questionnaire. The 136 respondents of both sexes were included in the research, and the results showed that 31% of them had never heard of the term "folic acid" or "folates", which mostly applied to men. 61% of the respondents had once used folic acid supplements, while only 33% of the respondents received a recommendation from a doctor to consume these supplements.

Keywords: vitamin B9, folic acid, folates, periconception, neural tube defects (NTD)

Introduction

Folic acid is a vitamin that belongs to the B group of vitamins, and is often referred to as vitamin B9. This is a group of twelve different vitamins that are numbered according to the order of discovery in science: B1, B2, B3, etc. Although each of these vitamins has a different chemical structure, they are classified into one group due to their common content in many foods, but also their common role in metabolism, immune response and normal function of the nervous system (Bundalo et al., 2013). The population of women of reproductive age is most affected by vitamin B9 deficiency because they have a greater need for folates in the periconceptional,

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prenatal and natal period. If they do not meet these increased needs for vitamin B9, miscarriages, premature births and congenital malformations can occur as a consequence, among which the two most common ones stand out; neural tube defect and anencephaly. Pregnancy planning strategies, promotion of vitamin B9 supplementation, compulsory fortification of foods with folic acid in certain countries and similar initiatives have led to a reduction in the incidence of diseases caused by folate deficiency and congenital disorders (Crider et al., 2011). Folic acid and folates are not one and the same, so they are two different forms of vitamin B9. Folates represent the natural form of this vitamin that can be found in food and in the body, while folic acid is a synthetic form of the vitamin used for the production of supplements of this vitamin or food fortification. An interesting fact is that folic acid is much better absorbed in the human body than folate (Barasi, 2003).

Socioeconomic conditions are an important factor that hinders the availability of folate-rich foods in the daily diet of the general population (Miyaki et al., 2013). However, despite this, the recommended daily intake of 0.4 mg per day is certainly difficult to achieve through nutrition, so the idea came up that everyone would benefit from folate-enriched food. In order to reduce the incidence of neural tube defects, the US Food and Drug Administration (FDA) mandated that as of January 1, 1998, all cereal products must be fortified with 0.14 mg of folic acid per serving. 100 g of grain (Food and Drug Administration 1996). It has been estimated that this measure could enrich the daily intake of folate in adults by an average of 0.1 mg (Shojania and Kauster, 2010). Mandatory fortification of cereal products, such as wheat flour and bread made from wheat flour, is carried out in 72 countries around the world. In countries with mandatory flour fortification, folate status improved significantly and the rate of neural tube defects dropped by almost 80%. However, many countries do not implement mandatory fortification of cereals and their products (Huđek, 2015). The countries of the European Union have not agreed on the issue of food fortification with folic acid, but fortification is carried out in some countries such as Great Britain (Babić, Božović and Vraneković, 2014). In Bosnia and Herzegovina, no law has been passed that would require employers in the food industry, who are engaged in the production or processing of cereals and cereal products, to be fortified with folic acid. However, on the BiH market, you can find foods enriched with folic acid, and these are mainly imported products, and these are most often various types of cereals intended for breakfast (cereal flakes or muesli). As far as flour is concerned, domestic products of BiH producers, which are not enriched with folic acid, are predominantly represented on the market. A new technology of triple fortification of salt with iodine, iron and folic acid is also under development. According to the given study, the costs for adding these micronutrients to salt would be about $27 per person per year, which makes this technology profitable in the fight against the consequences of the deficit of the mentioned micronutrients (Modupe et al., 2021). The subject of this research is the role of vitamin B9 in the human body, and the importance of its intake through nutrition in disease prevention. The particularly important role of this vitamin is in the prevention of congenital malformations, which again indicates the importance of its sufficient intake for all women of reproductive age. There is a scientifically proven correlation between insufficient intake of vitamin B9 (folic acid and folate) on the one hand and an increase in the incidence of neural

tube defects on the other hand, which result in severe diseases of newborns. Taking into account this scientific evidence and the eating habits of modern society, the recommended daily intake (RDA) of 400 mcg (Institute of Medicine. Food and Nutrition Board, 1998) for each adult is almost impossible to achieve through diet alone, and therefore fortification of foods with folic acid with appropriate supplementation is considered necessary in every society. Unfortunately, many countries do not have a system of mandatory fortification of foods with folic acid, nor a developed public health system for promoting the importance of folic acid supplementation, especially in pregnant and lactating women as the most vulnerable population in this context.

Materials and methods

According to this problem, the following research questions were defined:

- Are the citizens of Una-Sana Canton familiar with the term "folic acid"?
- How often do doctors recommend folic acid supplements to patients?
- How often do citizens consume folic acid supplements or other dietary supplements that contain folic acid (eg B complex vitamins, multivitamins, etc.)?
- What is the attitude of citizens towards folic acid; do they think that its intake is beneficial or harmful for people's health, especially pregnant and lactating women?
- What do citizens know about food fortification with folic acid and the need for folic acid supplements?
- What do citizens think about promoting folic acid supplementation; is it useful for citizens to educate themselves or only for pharmaceutical companies as a marketing tool?
- Based on the presented problem and defined research questions, the following research goal was set: The goal of this research was to examine and analyze the knowledge and attitudes of the consumers of the Una - Sana Canton towards vitamin B9.

Research methods

The following scientific methods were used in the work: synthesis method, analysis method, deductive method, inductive methods (incomplete, predicative and causal induction), concretization method, generalization method, specialization method, classification method, description method, compilation method, empirical-non-experimental (survey) method, statistical method, sample method and survey method (Radeka, 2018). In addition to these main ones, other scientific methods were used in the work. It should also be mentioned the limitations when collecting data that related to statistical data on the number of children born with any malformations in general in any area of Bosnia and Herzegovina (BiH). Thus, data on the number of cases of neural tube defects are unavailable on important information systems (Institute of Public Health at any administrative level, Federal Statistical Office, BiH Statistics Agency, etc.), and no scientific research papers published in domestic or foreign journals were found containing such information.

Research techniques and instruments

In this research work, the following techniques were used: survey technique and scaling technique. The instrument that was used to apply the survey technique was an online survey questionnaire compiled and conducted using an online tool, i.e. software for survey administration - "Google Forms". The survey questionnaire was anonymous and contained all closed-ended questions (see attachment 1), and was open for filling in for a period of 26 days (October 18, 2022 – November 12, 2022). It consisted of three parts: the first part with demographic data (a total of 4 questions; age, gender, education and place of residence) in which a nominal and interval scale was used. The second part consisted of three questions related to the research problem, where a nominal scale was used. The third part of the questionnaire related to the scales for measuring attitudes (consisting of four statements, each of which 23 statements has its contradictory counterpart, which checks the comprehensibility and sincerity of the answers; therefore, a total of 8 statements for scaling). The instrument for measuring attitudes used in this part of the questionnaire was a Likert scale. The tools used in the research work are: an electronic computer with software packages for word processing - MS Word, a software package for tabular calculations and creating graphs - MS Excel, online software for creating and conducting surveys - Google Forms and a calculator.

The sample (respondents) in the research

A total of 136 respondents participated in the research. According to the aim of the research, all respondents are from the area of the Una-Sana Canton. The number of respondents by municipality was determined in such a way as to achieve representativeness of the sample, i.e. it was correlated according to the actual share of the population. The estimate of the total number of inhabitants in USK for the year 2020 was 266,535 inhabitants (Federal Bureau of Statistics, 2016; Una-Sana Canton in numbers, 2021), (Federal Bureau of Statistics, 2016). The number of respondents by municipality, as well as the estimated number of inhabitants, is shown in Table 1.

Table 1. Structure of respondents and residents according to municipalities in USK

Municipality	Number of respondents	Percentage of respondents (%)	Number of inhabitants (in 2020)	Percentage of population (%)
Cazin	30	22 %	65239	24 %
Bihać	32	23 %	55805	21 %
Velika Kladuša	15	11 %	39994	15 %
Sanski Most	27	20 %	39651	15 %
Bosanska Krupa	10	7 %	24587	9 %
Bužim	9	7 %	19240	7 %
Ključ	7	5 %	15674	6 %
Bosanski Petrovac	6	4 %	6345	2 %
In total	136	100 %	266535	100 %

Results and discussion

From the attached table, it can be seen that the percentage of respondents from individual municipalities meets the representativeness of the sample. As for the gender structure of USK residents, the ratio of female to male population is balanced, with a slightly higher number of women (49.5% men: 50.5% women) (Federal Bureau of Statistics, 2020). When it comes to gender structure of the respondents, a much larger number of women were present. The survey questionnaire is structured in such a way that it classifies all respondents based on age into three groups: young age (from 15 to 44 years old), middle age (45-59 years old) and old age (60 and older). Out of a total of 136 respondents, 104 belong to the youth group, 19 to the middle age group and 13 to the elderly group. In real conditions, the youthful population is also dominant in USK, the middle-aged population is less numerous and the elderly population is the least numerous, which is also comparatively shown in the percentage shares. In the same way, a comparison was made between the structure of the respondents according to qualifications and the real conditions regarding professional training in USC.
When the benefit of folic acid for the health of people, especially pregnant and lactating women, has been established, the next focus of scientific research is the understanding of its importance by the general public. As the emphasis was placed on the special importance of folic acid for the prevention of neural tube defects (NTD), most of the conducted surveys of attitudes and knowledge about folic acid in the general public were conducted precisely on the target population of women, both in the world and in our country. The tests are mainly based on women's general knowledge about folic acid, and then on its proper application, frequency of use, and which factors influence these attitudes; the correlation with socio-economic and demographic factors was most often examined. This research is somewhat different compared to previously conducted studies of attitudes about folic acid in our region, and it differs in that the sample was taken from the general population and includes men. Thus, according to the results of this research, it can be seen that men have encountered the term folic acid much less and have less knowledge about its benefits for health compared to women. Out of a total of 69% of respondents who answered that they had heard of the term "folic acid" or "folate", as many as 83% of them were women, while the remaining 17% referred to men. Of the total number of respondents, 61% of them stated that they consumed very often folic acid supplements or some other dietary supplements that contained folic acid (e.g. vitamins of the B complex, multivitamins, etc.), and only 33% of the respondents received a doctor's advice to consume folic acid supplements. From the above, it can be assumed that most respondents took folic acid supplements on their own initiative or on the recommendation of someone else who is not a professional. 50% of respondents stated that folic acid is beneficial for human health, and 48% of respondents believe that insufficient intake of folic acid can have negative health consequences. On the other hand, a less than half number of respondents (40%) expressed a neutral attitude towards the claim that folic acid is beneficial for human health, and 42% of them also expressed a neutral attitude towards the negative consequences caused by the deficiency of this vitamin. This may indicate ignorance or an insufficient level of knowledge

of USK citizens on this issue. 41% of respondents stated that folic acid should be used by pregnant and lactating women for the sake of normal development of the fetus (fetus, child), 17% of respondents denied this claim, while 42% of respondents expressed a neutral attitude. According to this relationship of respondents, the majority of USC citizens are not aware of the benefits of folic acid for normal fetal development; of the fetus; of a child. 22% of respondents believe that folic acid is artificially added to some foods that USC citizens buy and consume every day, and the same percentage (22%) of respondents believe that folic acid is not added to foods. The remaining 56% of respondents expressed a neutral attitude. Based on this ratio of positive, negative and neutral attitudes, it can be concluded that USC citizens are completely uneducated about the possible content of folic acid in the foods they buy every day, which can roughly indicate how much USC consumers read the declaration on food products and foodstuffs when shopping. 38% of respondents believe that promoting folic acid supplements is useful for all citizens and that more such promotions should be done. The same percentage (38% of respondents) denies the claim that only pharmaceutical companies benefit from the promotion of folic acid supplements and that their goal is only to make people buy more of their products. However, by a whole 10% more, that is, 48% of them expressed a neutral attitude towards the first statement about promoting folic acid supplements, and 33% of respondents were neutral towards the second statement; that only pharmaceutical companies benefit from it. A neutral attitude could point to the lack of interest of USC citizens on this topic, but the percentage of 38% of respondents, on the other hand, represents a good potential for initiating the mentioned promotions.

Garcia et al. (2018) conducted a descriptive qualitative survey of attitudes about the benefits of folic acid consumption among Pakistani, Bangladeshi and British mothers on the one hand and health workers on the other. The research instrument was a survey questionnaire, and the study results established similarities in opinions about the benefits of folic acid consumption among all three groups of mothers, regardless of the fact that they do not come from the same country. The study found that very few mothers consumed folic acid before conception, insufficient understanding of the benefits of using folic acid, and only a few respondents knew how to list foods rich in folic acid. On the other hand, health workers widely believed that most mothers used folic acid before conception, 35 of which clearly indicates the wrong perception of health workers about the behavior of women, potential pregnant women. As for similar researches, by reviewing the relevant literature it is possible to find quite a few of them related to this topic, but as stated at the very beginning, mostly women, mothers, and pregnant women were included in the researches. Cui et al. (2021), on the basis of the reduced prevalence of NTDs in China in the period 2000-2017, which was associated with periconceptional supplementation of folic acid, conducted a study assessing the knowledge and actual use of folic acid among Chinese pregnant women, examining the factors that influenced the use of folic acid supplements. 428 pregnant women were included in the research, and the research instrument was an interview. 82% of respondents knew that folic acid can prevent neural tube defects. When asked if they had ever taken folic acid, 95% of the respondents answered yes.

64% of respondents from rural areas did not take folic acid supplements before conception, and 46% of them did.

The probability that housewives and those who had an unplanned pregnancy will not start taking folic acid before pregnancy was also determined. By comparing the results of this research with the results obtained in this paper, the percentage of women who have ever consumed folic acid in China was higher than in the Una-Sana Canton. This is an expected order given that China previously implemented a strategy of periconceptional folic acid supplementation. As for our climate, a review of the relevant literature provides interesting information in an article in the scientific journal Medica Jadertina entitled "Folic acid - what do pregnant women in Zadar County know and how much it is used", which was published in 2011 by Vitale et al. The goal of this research was to determine how familiar mothers in Zadar County are with folic acid, and how much they were taking. The research instrument was an anonymous questionnaire, and the research was conducted in 2007 and lasted seven months. 340 women participated in the study. 80% of respondents answered that they know what folic acid is, and almost 85% of them believe that it is useful. 49% of the respondents received advice to consume folic acid, and the advice was mostly given by a doctor, while 39% of the respondents took some form of folic acid on their own initiative. Logistic regression showed that the main factors for taking folic acid are planned pregnancy and 36 spontaneous termination of pregnancy or stillbirth. Likewise, women with higher education consumed more folic acid compared to respondents with lower education, who are in a multiple disadvantageous position; in addition to insufficient education, they have no initiatives on supplementation either from the health system or from the social network to which they belong. In the Una Sana Canton, according to the results of this research, the same percentage as in the Zadar County, i.e. 83% of women stated that they had heard of the term folic acid. In the Una-Sana Canton, 39% of respondents received advice from doctors to consume folic acid supplements, and in Zadar County, 49% of respondents. So, in Zadar County, doctors recommend the consumption of folic acid to female patients somewhat more often. Similar research was conducted in the Bjelovar-Bilogora County as part of the final thesis (Valentić, 2021) at the Faculty of Medicine of the University of Zagreb. The research was conducted in 2021 and lasted a total of five months, and included 209 pregnant women and new mothers, who filled out the survey questionnaire. The results of this test show that 70% of respondents know what folic acid is, and 88% of them believe that it is beneficial for health. Of the respondents who stated that they took a folic acid preparation before conception, most of them (62%) received advice from a doctor, while a minority (25%) took the preparation on their own initiative. In Bosnia and Herzegovina, a study was conducted on the assessment of folic acid supplementation among pregnant women in BiH, which is the first study of its kind in this country. The research was conducted in 2015 and lasted about a month. The instrument was an online questionnaire that was available on a specialized portal for pregnant women and new mothers, and the main variable was the use of folic acid supplements. The research included 67 respondents. It is relevant to present only the fact that 88% of respondents stated that they

used folic acid supplements at any time during pregnancy, and only 18% of respondents used folic acid supplements before conception (Djedjibegovic et al., 2020).

Conclusion

In the second half of the twentieth century, numerous studies were conducted that confirmed the link between low blood folate levels in women in the periconceptional period with a higher risk of neural tube defects (NTD). According to the research results of this paper, the following conclusion was reached: Citizens of the Una-Sana Canton are not sufficiently aware of the importance of folic acid for human health and its role in disease prevention. Women knew more about folic acid compared to men. Doctors rarely recommend folic acid supplementation to patients in the USC area, which could be particularly unfavorable for women in labor. USC consumers are not familiar with the possibility of fortifying foods with folic acid and do not know that folic acid is artificially added to some of the foods they consume every day. For the USC area, it is not possible to find statistical data on the number of newborns with a neural tube defect or any malformations in general, so it is not possible to question the connection of these cases with a low level of folate in the mother's blood. Recommendations for carrying out further research in the area of USC: First of all, it is necessary to initiate the public health system in this area to keep records of cases of newborn children with any malformations, which would represent a good basis for numerous scientific researches or, if records are already being carried out, to make these data easily available for the scientific research community of this canton. Likewise, keeping records of the frequency of hyperhomocysteinemia among the USC population could be linked to the incidence of cardiovascular diseases, which are considered the leading causes of mortality in the Federation of Bosnia and Herzegovina. Furthermore, it would be good to conduct a similar study on the most vulnerable population, i.e. to include only pregnant women and women giving birth, and to examine the actual use of folic acid by this population, in what doses and whether they know the right time to take folic acid supplements in order to prevent defects neural tubes. Likewise, the low socio-economic status of consumers in this canton could be linked to the frequency of the use of folic acid supplements.

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ANTIFUNGALNI POTENCIJAL MLIJEČNO-KISELIH BAKTERIJA NA ZRNU SOJE

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Sažetak

Gljive nanose velike štete poljoprivrednim proizvođačima kao uzročnici biljnih mikoza. Gljive su, također, često prisutne i u skladišnim uvjetima. Neki rodovi gljiva proizvođači su mikotoksina različite toksičnosti koji zaostaju na sjemenskom materijalu i predstavljaju opasnost za sigurnost hrane. Prevencija gljiva, kao i njihova dekontaminacija, predmet su brojnih istraživanja. Sigurna hrana i hrana za životinje bez agrokemikalija i toksigenih gljiva smatraju se važnim pitanjima, stoga su nužna znanstvena istraživanja u području biokontrolnog potencijala bioloških agenasa, poput mikroorganizama i njihovih metabolita u suzbijanju njihovog rasta. Budući da mliječno-kisele bakterije posjeduju antifungalna svojstva, cilj ovog istraživanja bio je utvrditi biokontrolni potencijal *Lactobacillus brevis* i *Lactobacillus casei* u suzbijanju rasta *Fusarium verticillioides* i *Penicillium chrysogenum* na prirodnom supstratu – sjemenu soje. Rezultatima je utvrđena statistički značajna inhibicija rasta obiju ispitivanih gljiva, pri čemu je inhibicija rasta micelija *F. verticillioides* bila u rasponu od 71 do 79 %, dok je inhibicija *P. chrysogenum* bila od 88 do 91 %. Daljnjim istraživanjima potrebno je detaljnije utvrditi metabolite koji su odgovorni za utvrđenu antifungalnu inhibiciju, kao i utvrditi inhibiciju sinteze mikotoksina te utvrditi učinkovitost bakterija mliječne kiseline na druge fitopatogene gljive.

Ključne riječi: biokontrola, *Lactobacillus, Fusarium verticillioides, Penicillium chrysogenum,* soja

Uvod

Uskladišteni zrnati poljoprivredni proizvodi često su kontaminirani gljivama. Gljive kontaminiraju poljoprivredne kulture u poljskim uvjetima te, nakon žetve, dospijevaju u skladišta, a tipičan predstavnik takve kontaminacije rod je *Fusarium.* Kontaminacija može nastati *in situ* skladišnim plijesnima poput roda *Penicillium* i *Aspergillus*. Kontaminacija rodom *Fusarium* na zrnu soje u Republici Hrvatskoj u razdoblju od 2002. do 2008. godine kretala se u intervalu 4 – 17 % prema istraživanju Ivić i sur. (2009), a *Fusarium verticillioides,* uz *Fusarium sporotrichoides,* najdominantnija je vrsta. Zrno soje kontaminiraju i drugi rodovi: *Peronsospora, Diaporthe/Phomopsis, Cercospora, Cladosporium, Alternaria, Aspergillus* i *Penicillium* (Medić-Pap i sur., 2007; Petrović i sur., 2014). Intenzitet rasta i razvoj gljiva u skladišnim uvjetima ovisi o vlazi zrna, temperaturi i relativnoj vlažnosti (Mohapatra i sur., 2017). Osim fungalne kontaminacije, plijesni su producenti mikotoksina čija ingestija uzrokuje akutne ili kronične simptome i oboljenja ljudi i životinja. *Fusarium* mikotoksini uključuju najdominantnije tri klase: zearalenon, fumonizine i trihotecene, a također su i producenti fuzaproliferina, bovericina, eniatina i monoliformina koji su

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kancerogeni, teratogeni, imunosupresivni, neurotoksični te imaju širok raspon toksičnih učinaka (Esriva i sur., 2015). Rod *Penicillium* sintetizira široki spektar sekundarnih metabolita, uključujući i mikotoksine, a najrašireniji su: ohratoksin A, patulin, citrinini i drugi (Frisvad i sur., 2004). *P. chrysogenum* producent je PR toksina, patulina, kao i penicilinske kiseline koji su imunotoksični, citotoksični, hepatotoksični, s nizom drugih toksičnih učinaka (Frisvad i sur., 2004; Frisvad, 2018).
Kontaminirano uskladišteno zrno u konačnici utječe na smanjenu nutritivnu kvalitetu i kvantitetu uskladištene mase (Mohapatra i sur., 2017).
Smanjenje fungalne kontaminacije i sinteze mikotoksina provodi se mjerama prevencije, a u slučaju pojave mikotoksina u skladišnim uvjetima provode se mjere dekontaminacije. Dekontaminacija zrnatih proizvoda provodi se fizičkim, kemijskim ili biološkim postupcima. Zbog razvoja svijesti i proizvođača i potrošača o štetnim učincima kemijskih tvari, u zaštiti i dekontaminaciji zrnatih proizvoda veliku pažnju privlače biološke metode. Mliječno-kisele bakterije posjeduju antifungalne i antimikotoksikogene osobine i potencijalno se mogu korisiti i u realnim agrikulturnim sustavima (Oliveira I sur., 2014; Fleurat-Lessard, 2017; Lamont i sur., 2017, Kanižai Šarić i sur., 2021). Cilj ovog rada bio je utvditi potencijalne antagonističke osobine roda *Lactobacillus* u inhibiciji rasta roda *Fusarium* i *Penicillium* na zrnu soje u *in vitro* uvjetima.

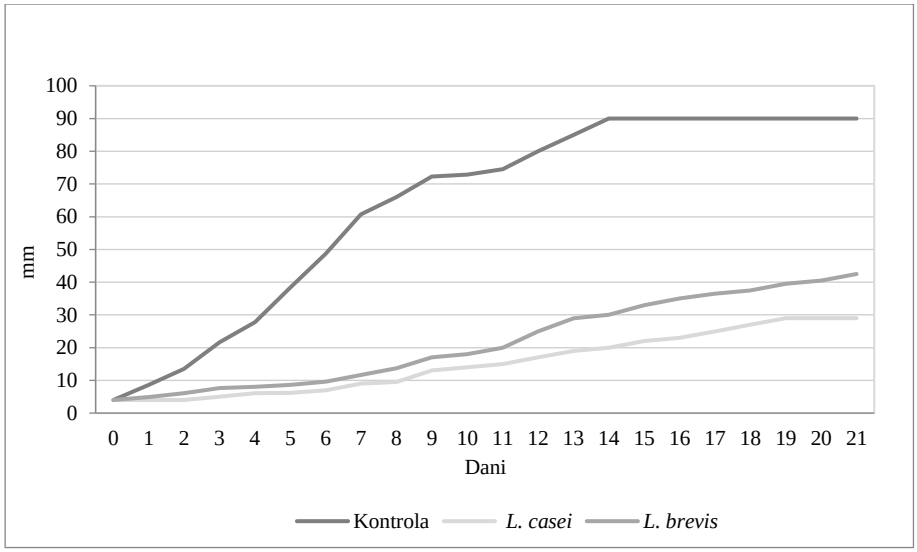
Materijali i metode

U pokusu su korištene čiste kulture *Fusarium verticilloides* M-7075 (Fusarium Research Center, Department of Plant Pathology, Penn State University, SAD) i *Penicillium chrysogenum* iz kolekcije Katedre za mikrobiologiju Fakulteta agrobiotehničkih znanosti Osijek. Čiste kulture gljiva umnožene su na krumpir-dekstroznom agaru (Biolife, Italija) pri temperaturi od 25 °C tijekom sedam dana. Mliječno-kisele bakterije *Lactobacillus casei* i *Lactobacillus brevis* iz kolekcije Katedre za mikrobiologiju i zemljišne resurse Fakulteta agrobiotehničkih znanosti Osijek uzgojene su na MRS (de Man, Rogosa, Sharpe, Merck, Njemačka) tekućoj podlozi pri 37 °C kroz 48 sati. Postavljen je *in vitro* pokus sa zrnom soje iz ekološke proizvodnje (Eko-Jazo d.o.o), koje je korišteno kao prirodan supstrat za uvrđivanje antifungalne djelotvornost, i izvagano je u količini od 10 g i sterilizarno vodenom parom pod tlakom. Sjeme je tretirano s $3x10^9$ stanica po ml mliječno-kiselih bakterija i provedena je inokulacija micelijskim diskom čistih kultura plijesni uzetim s rubova čistih kultura promjera 4 mm. Pokus je postavljen u četiri ponavljanja. Svakodnevno je praćen rast plijesni, u razdoblju od 21-og dana, koji je korišten za računanje stope rasta. Rezultati su statistički analizirani studentovim t-testom. Za statističku analizu podataka korišteni su Microsoft Excel (2019.) i Statistica 14.0.0.15 (Tibco 2020.).

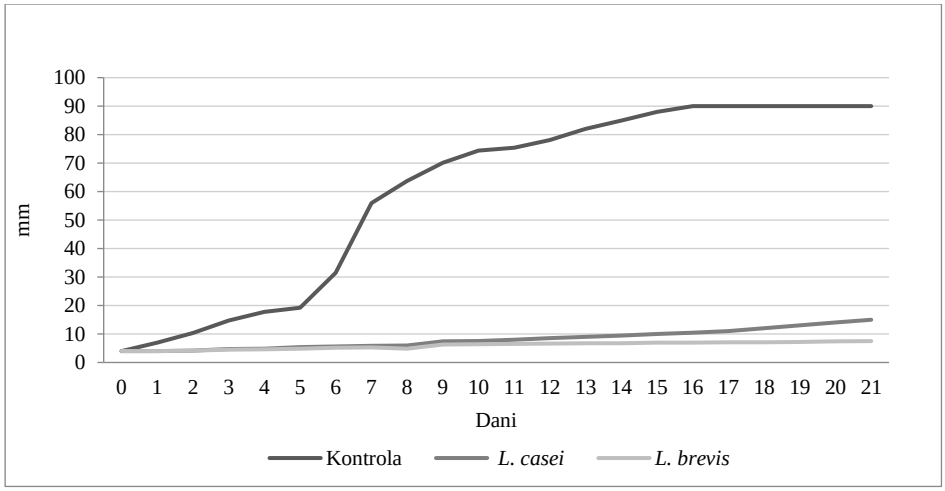
Rezultati i rasprava

U ovom istraživanju utvrđena je inhibicija rasta *F. verticillioides* koja se kretala od 71 do 79 %, pri čemu je *L. casei* imao jače antagonističko djelovanje u usporedbi s *L. brevis* (Slika 1) uz utvrđenu statističku značajnost ($p<0,01$).
Antifungalnu i antimikotoksikogenu aktivnost mliječno-kiselih bakterija utvrdili su i drugi istraživači. Mateo i sur. (2023) u „*dual culture*" pokusu utvrdili su osjetljivost gljiva na mliječno-kisele bakterije koja je rangirana na sljedeći način: *F. oxysporum* > *F. poae* = *F. culmorum* ≥ *F. sporotrichioides* > *F.*

langsethiae > *F. graminearum* > *F. subglutinans* > *F. Verticillioides*, a najučinkovitij je *Leuconostoc mesenteroides* ssp. *mesenteroides*. Isti autori, također, utvrdili su i inhibiciju zearalenona, deoksinivalenola i 3-acetildeoksinivalenola te su zaključili da LAB soj, vrsta gljiva, temperatura i njihove interakcije značajno utječu na antifungalni i antimikotoksikogeni učinak LAB sojeva.



Slika 1. Antifungalna djelotvornost mliječno-kiselih bakterija u inhibiciji rasta *Fusarium verticillioides*
Figure 1. Antifungal LAB efficiency in suppressing growth of *Fusarium verticillioides*



Slika 2. Antifungalna djelotvornost mliječno-kiselih bakterija u inhibiciji rasta *Penicillium chrysogenum*
Figure 2. Antifungal LAB efficiency in suppressing growth of *Penicillium chrysogenum*

Suproniene i sur. (2014) utvrdili su inhibiciju rasta *Fusarium* spp., *Bipolaris sorokiniana* i *Alternaria* spp. na zrnu pšenice djelovanjem *Lactobacillus sakei*, *Pediococcus acidilactici* i *Pediococcus pentosaceus.* Kanižai Šarić i sur. (2021) utvrdili su inhibiciju *F. graminearum* djelovanjem *L. casei* u tekućoj podlozi koja se kretala od 50 % do 88 %, *L. brevis* od 70 % do 82 % i *L. mesenteroides* od 30 % do 79 %. Inhibicija od 83 do 90 % utvrđena je djelovanjem istih bakterija na prirodnom supstratu, odnosno na zrnu pšenice u istom istraživanju.
U ovom istraživanju inhibicija P. *chrysogenum* kretala se od 88 % djelovanjem *L. casei* i 91 % djelovanjem *L. brevis* (Slika 2) uz utvrđenu statističku značajnu razliku u usporedbi s kontrolom ($p<0,01$). Hanfi i sur. (2014) izolirali su *P. pentosaceus*, *Lactobacillus plantarum*, *Lactobacillus graminis*, *Lactobacillus coryniformis* i *Weissella cibaria* i utvrdili su veću ili manju inhibiciju skladišnih plijesni *Penicillium expansum*, *P. chrysogenum*, *Penicillium glabrum*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus carbonarius*, *Fusarium graminearum* i *Alternaria alternata*. U istom istraživanju svi LAB izolati inhibirali su rast *P. chrysogenum.* Autori su zaključili da temperatura, pH i bakterijska biomasa imaju značajan učinak na inhibiciju rasta navedenih gljiva. Slično su utvrdili Chen i sur. (2021) koji su iz kefira izolirali *Lactobacillus kefiri* i *Pediococcus acidilactici* i dokazali antifungalno djelovanje protiv *P. Expansum*, pri čemu je utvrđeno da su glavni antifungalni spojevi organske i karboksilne kiseline u supernatantima obiju vrsta LAB. Inhibicija *P. chrysogenum* djelovanjem *L. casei* u tekućoj hranjivoj podlozi kretala se od 37 % do 76 %, *L. brevis* inhibira rast od 50 % do 79 %, a *L. mesenteroides* reducira rast 69 % na hranjivoj podlozi – potvrdilo je istraživanje Kanižai Šarić i sur. (2021), međutim, nije utvrđena statistički značajna inhibicija, iako je bila visoka i kretala se od 76 do 82 % na zrnu pšenice. Slično su potvrdila i istraživanja Suproniene i sur. (2014) koji su utvrdili kako je utjecaj LAB na klijavost sjemena i na bolesti klijanaca u laboratorijskim i poljskim pokusima u većini slučajeva neznatan, što je rezultat interakcija u kompleksnom sustavu poput tla.
Ovo istraživanje potvrdilo je veliki potencijal *L. brevis* i *L. casei* u biokontroli roda *Fusarium* i *Penicillium* na prirodnom supstratu. U kompleksnim sustavima prisutne su brojne biotske interakcije te je, općenito, učinak mliječno-kiselih bakterija manji u odnosu na istraživanja koja su usredotočena na uzgoj na hranjivim podlogama. Naravno, potrebna su i daljnja istraživanja u kojima je potrebno utvrditi i interakcije između nesterilnog zrna i mliječno-kiselih bakterija i koja bi trebala identificirati spojeve koji su odgovorni za navedeno antagonističko djelovanje roda *Lactobacillus.* Također, potrebno je utvrditi i utjecaj na biosintezu mikotoksina u takvim uvjetima.

Zaključak

U ovom istraživanju utvrđeno je jako antagonisitčko djelovanje *L. casei* i *L. brevis* u inhibiciji radijalnog rasta *F. verticillioidies* i *P. chrysogenum* na sterilnom zrnu soje. Potrebna su daljnja istraživanja koja bi identificirala spojeve koji su odgovorni za tu inhibiciju, kao i interakcije u realnom sustavu na nesterilnom zrnu između prirodne mikroflore i mliječno-kiselih bakterija. Također, daljnja istraživanja trebala bi utvrditi i utjecaj tretmana na biosintezu mikotoksina.

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ANTIFUNGAL POTENTIAL OF LACTIC ACID BACTERIA ON SOYBEAN SEEDS

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original scientific paper

Summary

Fungi, it is well known, cause great damage to agricultural producers as the causative agent of plant mycosis. Fungi are often present in storage conditions as well. Some fungi genera are producers of mycotoxins of different toxicity that remain on the seed material and represent food safety hazards. The fungi prevention as well as their decontamination is the subject of numerous studies. Safe food and animal feed without agrochemicals and toxigenic fungi are considered to be important issues, therefore scientific study in the field of the biocontrol potential of biological agents, such as microorganisms and their metabolites in suppressing the fungi growth are necessary. Lactic acid bacteria possess antifungal properties, and this research aimed to determine the biocontrol potential of *Lactobacillus brevis* and *Lactobacillus casei* in suppressing the mycelial growth of *Fusarium verticillioides* and *Penicillium chrysogenum* on a natural substrate-soybeans seeds. The results determined a statistically significant inhibition of the growth of both tested fungi, with the inhibition of the mycelial growth of *F. verticillioides* ranging from 71 to 79%, while the inhibition of *P. chrysogenum* was 88 to 91%. Further research should determine in more detail the metabolites that are responsible for the established antifungal inhibition, as well as determine the inhibition of mycotoxin synthesis and establish the effectiveness of lactic acid bacteria on other phytopathogenic fungi.

Keywords: biocontrol, *Lactobacillus*, *Fusarium verticillioides*, *Penicillium chrysogenum*, soybean

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MINERAL COMPOSITION OF PUMPKIN AND ITS BY-PRODUCTS

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original scientific paper

Summary

Fruits and vegetables are often rich in minerals because they are important nutrients for plant growth and development. Minerals are stored in different parts of the plant to protect it from environmental stressors such as physical damage. The objective of the present work was to determine the content of minerals in raw and dried pumpkin pulp, peel and seeds. Since the drying process can affect mineral content because some of them are heat sensitive, mineral composition in dried pumpkin was of particular interest. The mineral composition of the samples was determined using inductively coupled plasma mass spectrometry. The results indicate that both, raw and dried pumpkin can be a good source of important minerals such as calcium, magnesium, manganese, zinc, and potassium which are essential for various physiological functions in the body. In the samples studied, the peel was the most mineral-rich part. Thus, the consumption of pumpkin and its by-products can be an effective strategy to obtain these minerals and contribute to a balanced diet that promotes optimal health and well-being. By including pumpkin, especially dried pumpkin, and its by-products in daily meals, individuals can have access to a wide range of minerals important for various physiological functions.

Keywords: pumpkin, by-products, minerals, vacuum drying, ICP-QQQ analysis

Introduction

The pumpkin is a widespread fruit that is mainly grown in tropical and subtropical countries. After harvest, pumpkins have a shelf life of one to three months, after which they become susceptible to microbial spoilage, moisture loss and colour changes. To extend the shelf life of pumpkins, various drying methods (Indiarto et al., 2021; Radojčin et al., 2021; Karlović et al., 2023) can be used to obtain pumpkin powder, which is rich in nutrients and health-promoting substances (Salehi et al., 2019; Kulczyński et al., 2019). Due to its desirable sweet taste and yellow-orange colour, pumpkin powder could be added as a functional ingredient to various foods to increase the nutrient content or improve the taste.

In addition to the raw and dried pulp, the other pumpkin fractions, e.g. the peel and seed, which remain as by-products after separation of the pulp, are also valuable sources of various macro- and micronutrients and bioactive compounds from a nutritional point of view (Kaur et al., 2022; Hussain et al., 2022; Ninčević Grassino et al., 2023a; Ninčević Grassino et al., 2023b). Considering their functional properties and documented benefits to human health (Rolik et al., 2020; Aziz et al., 2023),

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this paper focuses on presenting the mineral composition of raw and dried pumpkin pulp, peel and seeds The objective is to compare these fractions to determine which serves as a more micronutrient-rich source As there is no comprehensive data in the literature on the micronutrient profiles of *Cucurbita* spp. and in particular no data on all vacuum-dried pumpkin fractions, the present study focuses on (*i*) determining the content of essential (macro, trace and ultra-trace) elements in raw and dried pumpkin fractions, (*ii*) to compare the essential element contents in different raw and dried pumpkin fractions to test the influence of vacuum drying, and (*iii*) to answer which of the pumpkin forms (raw, dried or both) would represent a preferred form of essential element intake. In addition to essential elements, the same approach was applied to non-essential elements, which were also determined in raw and dried pumpkin fractions.
Overall, the data presented show the potential use of pumpkin fractions as a source of micronutrient supplements, particularly peel and seeds, the utilisation of which would be in line with global sustainable trends in the use of food by-products.

Materials and methods

Chemicals

All reagents, standards and solvents were of analytical grade. Nitric acid (HNO_3, 65%, supra pure) and hydrofluoric acid (HF, 48%, supra pure) used for microwave-assisted digestion of pumpkin samples were obtained from Kemika (Zagreb, Croatia). HNO_3 (65%, supra pure), used to acidify samples before ICP-QQQ analysis, and In (1 µg/L), used as an internal standard, were supplied by Fluka (Steinheim, Switzerland).
The multi-element standard solution (1-10 µg/L) containing Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Se, Sr, Ti, Tl, V and Zn, and the single-element standard solutions of Sn (1.000±0.002 g/L) and Sb (1.000±0.0002 g/L) were obtained from Analytica (Prague, Czech Republic). The single-element standard solution of U (1.000±0.002 g/L) was provided by Aldrich (Milwaukee, WI, USA). The reference solution containing K (200 mg/L), Mg (400 mg/L) and Na (1000 mg/L) was obtained from Fluka (Glottertal, Germany).
Milli-Q water used for the preparation of reagents, standards and solvents was obtained using the Millipore purification system, resistivity 0.07 µS/cm.
Certified reference materials used include Citrus leave (NCS ZC73018, China National Analysis Center for Iron & Steel) and Apple leave (NIST SRM 1515, The National Institute of Standards and Technology, USA).

Materials

Fresh pumpkin (*Cucurbita moshata*) was purchased from Exotic king (Šulog, Donja Bistra, Croatia). The fruits were washed with tap water and cut in half lengthwise. The seeds and peel were separated from the pulp, and the pulp was then cut into a thick slice. One part of pumpkin fractions (pulp, peel and seeds) was subjected to vacuum drying at temperature of 60 °C and pressure of 100 mbar using a vacuum drying oven with pump (D-91126, Schwabach, Germany), The second part of pumpkin fraction were used as a raw in order to check the efficiency of drying. Before analysis all fractions were milled.

Multielement analysis

Sample preparation

The raw and dried pumpkin samples were liofilized, homogenized in an agate mill and stored at room temperature until use. Prior to ICP-QQQ analysis, subsamples (0.05 g) of pumpkin fractions were subjected to a total digestion in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) with a mixture of 6 mL of HNO_3 (65%, supra pur) and 0.1 mL of HF (48%, supra pur). After completion of the digestion, each digest was transferred to a pre-cleaned volumetric flask and diluted to 100 mL with ultrapure water. Prior to analysis, in samples 2% *v/v* HNO_3 (65%, supra pure) and 1 μg/L of In as an internal standard element was added.

Multi-element determination

Multielement analysis of the digested raw and dried pumpkin samples was performed by ICP-QQQ, a triple quadrupole inductively coupled plasma mass spectrometer Agilent 8900 (USA). Calibration curves were generated by external standardisation using a range of standard solutions, including a blank sample. For the quantification of selected elements separate standard solutions were prepared as follows. The standard solutions for trace element determination were prepared by appropriate dilution of the multi-element standard solution (1-10 μg/L) containing Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Se, Sr, Ti, Tl, V and Zn, and to which individual element standard solutions of Sn (1.000±0.002 g/L), Sb (1.000±0.002 g/L), and U (1.000±0.002 g/L) were added. The concentrations of the major element (Ca, K, Mg, Na) were determined on the basis of standard solutions prepared by appropriate dilution of the multi-element standard solution (Fluka, Steinheim, Switzerland). All solutions of standards and samples were stabilized by adding 2%, *v/v* HNO_3 (65%, supra pure). A 1 μg/L solution of In is used as an internal standard. Blank solutions were prepared in the same way as the sample solutions. All samples were analysed for the total mass fraction of twenty-nine elements, i.e., Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, Tl, U, V, and Zn.
The quality control of the measurements was carried out by simultaneous measurement of procedural blanks and the certified reference materials for plants: (*i*) Citrus leave (NCS ZC73018, China National Analysis Center for Iron & Steel, China) and (*ii*) Apple leave (NIST SRM 1515, National Institute of Standards and Technology, SAD).

Results and discussion

Multi-element ICP-QQQ analysis of raw and dried pumpkin fractions

To determine whether the mineral composition changes after vacuum drying of pumpkin fractions (pulp, peel and seeds), a simultaneous analysis of the raw pumpkin fractions was performed. The results of ICP-QQQ analysis (Figures 1, 2 and 3) showed that K, Mg, Ca and Na were the predominant

macroelements present in higher concentrations in all analysed samples. The mass fractions obtained ranged from 7332 to 40237 mg/kg for K, 373 to 5196 mg/kg for Mg, 974 to 4322 mg/kg for Ca and 4.12 to 62.2 mg/kg for Na, depending on the raw or dried pumpkin fractions. The dried peel contained the highest levels of K (31025 mg/kg) and Ca (3765 mg/kg), while the dried and raw seeds contained the highest levels of Mg, 5018 and 5196 mg/kg, respectively. Although the dried pulp has the highest Na content (412 mg/kg), the raw and dried seeds are also a valuable source with values of 58.3 and 62.2 mg/kg, respectively. The dried fractions appear to be better sources of macroelements than the raw ones. For example, the dried peels and seeds can provide more than 100% of the daily requirement of K and Mg for an adult (19 to 70 years, women and men), considering the recommended daily allowance (RDA) or adequate intake (AI) (Institute of Medicine, 2006; EFSA, 2006) for a 100 g serving.

Yetesha et al. (2023) demonstrated that the macroelement content differs among various pumpkin components. Specifically, potassium (K) values were reported as 29531 mg/kg for pulp, 27806 mg/kg for peel, and 10667 mg/kg for seeds. In addition, Ca predominates in the peel (5268 mg/kg), followed by pulp (3191 mg/kg) and seeds (2919 mg/kg). Mg predominates in the peel (4716 mg/kg), followed by seeds (4668 mg/kg) and pulp (848 mg/kg). The sodium content in their study is higher in the by-products (167 mg/kg and 171 mg/kg for peel and seeds, respectively) than in the pulp (63.1 mg/kg).

This study, along with others, confirmed that by-products are valuable sources of macro essential elements. According to Gade et al. (2022), the seeds contained K (1146.46 mg/100 g), Mg (1012.73 mg/100 g), Na (82.2 mg/100 g) and Ca (75.15 mg/100 g). Jahan et al. (2023) showed that K and Na predominate in the peels (378.81 mg/100 g and 33.92 mg/100 g), while Ca (7.58 mg/100 g) is found in the seeds. Kulczyński et al. (2019) reported that K predominates in the pulp with mass fractions of 4104.3 to 7386.9 mg/100 g, depending on the pumpkin varieties (*C. pepo* and *C. moschata*). The content of Ca (133.29 to 503.56 mg/100 g), Na (217.36 to 346.92 mg/100 g) and Mg (80.78 to 151.25 mg/kg) is also high and depends on the pumpkin varieties. The influence of the pumpkin variety on the content of macroelements is also shown in the work of Czech et al. (2018), who obtained values of 3.03-6.35 g/kg (K), 0.255-0.688 g/kg (Ca), 56.39-197.8 mg/kg (Mg) and 12.52-21.40 mg/kg (Na). Furthermore, it was found that the contents of K, Ca and Mg in *C. maxima* were not influenced by heat treatments (boiling and baking), whereas a reduction was observed in *C. pepo* (Sıçramaz et al., 2023).

Apart from essential macroelements, the peels and seeds are also identified as richer sources of essential trace elements such as copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) than the pulp (Figure 2). This suggests that these by-products have the potential to offer a higher intake of trace elements compared to the pulp. Thus, the dried seeds and peels can provide about 40 to 90% and more than 100% of the daily requirement of Fe for an adult (19 to 70 years old, women and men, respectively), taking into account the RDA or AI (Institute of Medicine, 2006; EFSA, 2006) for a 100 g serving. The raw and dried seed fractions can provide about 60 to 80% or more than 100% of the daily requirement of Zn, Mn and Cu.

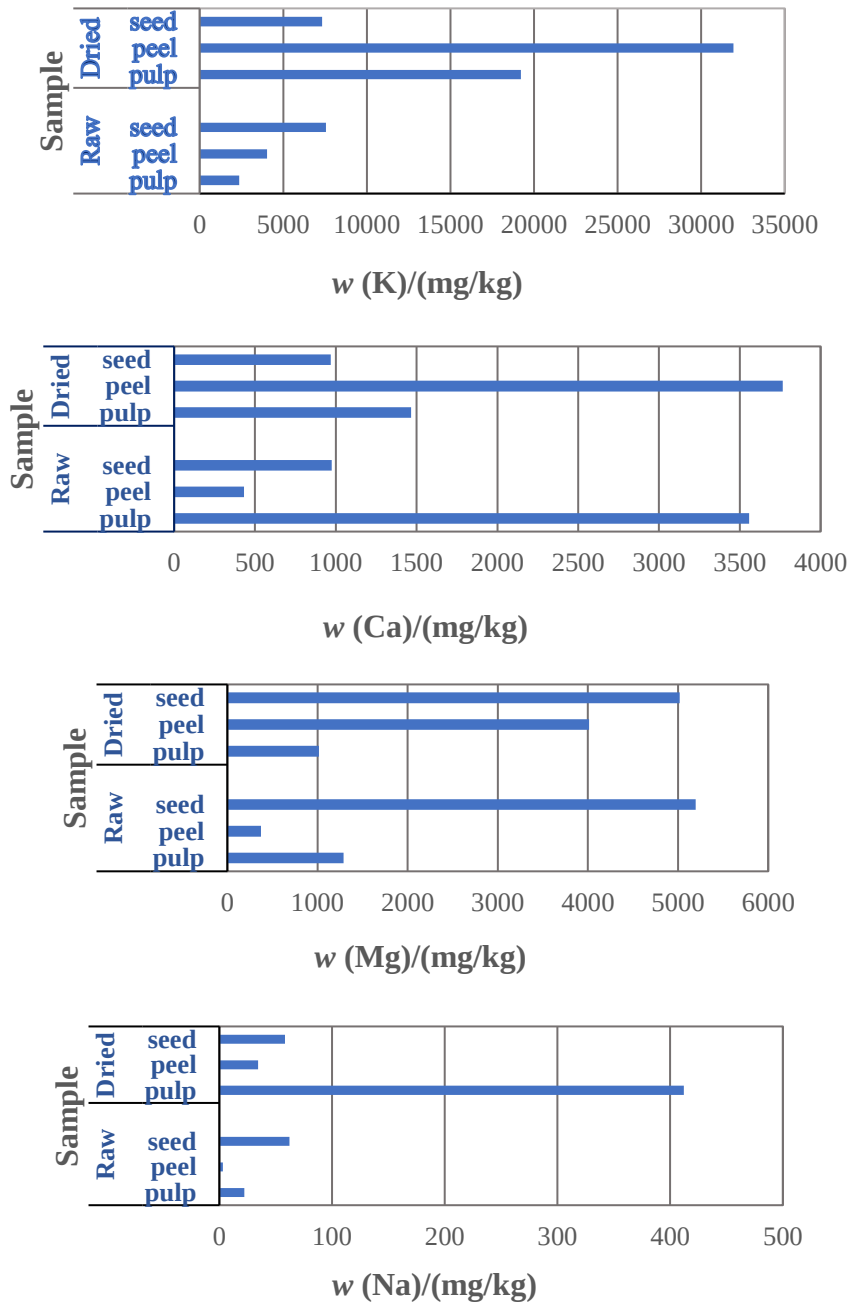

Figure 1. Macroelements content in raw and dried pumpkin fractions determined by ICP-QQQ analysis

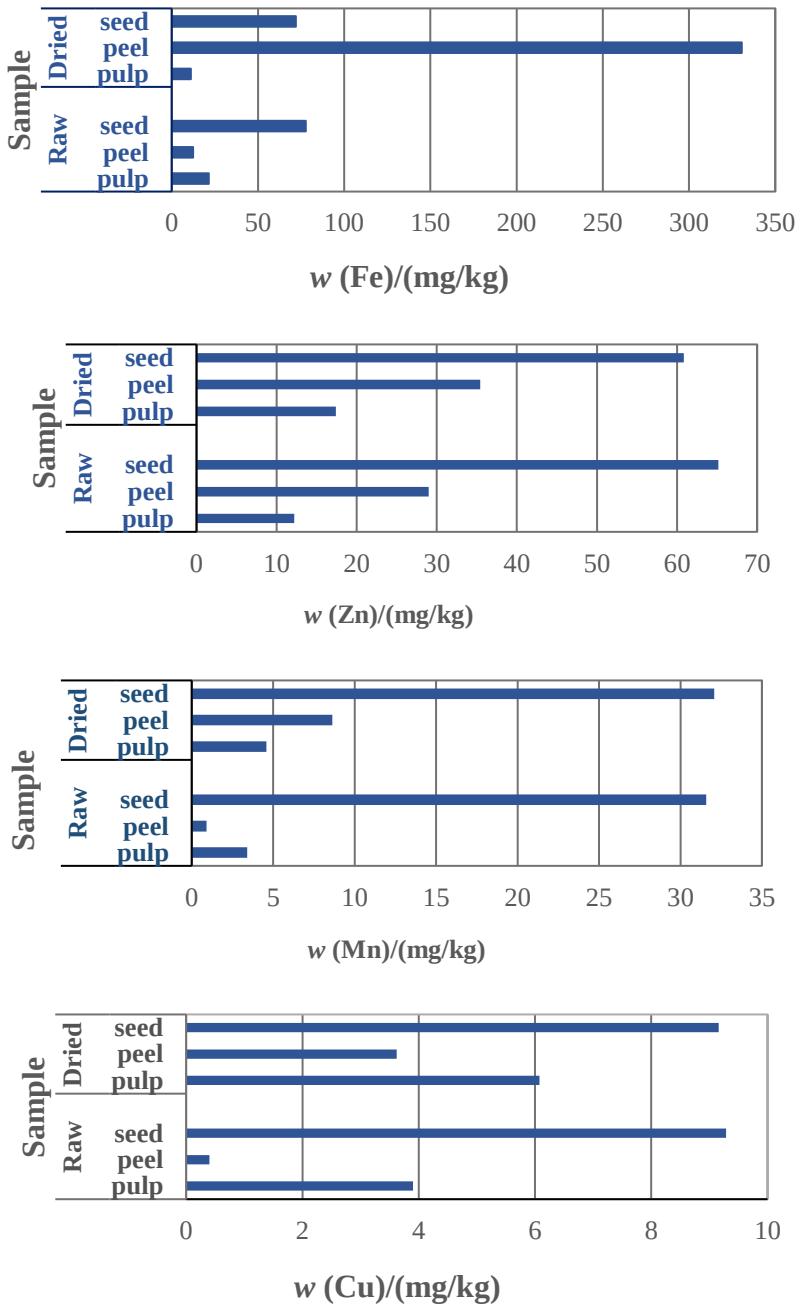

Figure 2. Trace elements content in raw and dried pumpkin fractions determined by ICP-QQQ analysis

Among the trace elements determined, Fe predominates with mass fractions between 11.1-331 mg/kg, depending on the sample analysed. Zinc is found in the range of 2.2-65.2 mg/kg, followed by Mn and Cu with amounts of 3.04-32.1 mg/kg and 3.9-9.29 mg/kg respectively. In comparison to this work, Kulczyński et al. (2019) reported 1.04-2.59 mg/100 g, 0.54-1.31 mg/100 g, 0.28-0.85 mg/100 g and 0.21-0.63 mg/100 g of Fe, Zn, Mn and Cu for raw pumpkin pulp, respectively. Gade et al. (2022) reported values of 20 mg/100 g, 10.25 mg/100 g, 7 mg/100 g and 2.13 mg/100 g for Fe, Zn, Mn and Cu, respectively, for seeds. According to Yetesha et al. (2023), the average mass fractions of Fe, Mn, Zn and Cu are 158 mg/kg, 28.9 mg/kg, 24.2 mg/kg and 8.14 mg/kg, respectively. In raw pulp, they are 111 mg/kg, 15.1 mg/kg, 10.6 mg/kg and 9.63 mg/kg for Fe, Cu, Zn and Mn respectively. The average metal mass fractions in pumpkin seeds are 225 mg/kg, 67.8 mg/kg, 33.4 mg/kg and 10.8 mg/kg for Fe, Zn, Mn and Cu.

Figure 2 shows that vacuum drying has an effect on the content of trace elements, so that higher and slightly lower amounts of Fe, Zn, Mn and Cu were obtained after drying the peels and seeds than in the raw peel and seed fractions. After drying the pulp, the Fe content decreased, while the Cu and Zn content increased and the Mn content remained at the level of the raw pulp. The work by Siçramaz et al. (2023) also shows that after cooking and baking the pulp, the Fe and Mn content remained the same or increased depending on the pumpkin species (*C. maxima* and *C. pepo*). The Cu content was resistant to cooking in *C. maxima*, but in *C. pepo* it was damaged by both heat treatments. The Zn content decreased in *C. pepo* by boiling, but remained the same in *C. maxima*.

In addition to the macro- and trace elements, the raw and dried pumpkin samples are able to cover the daily intake (DI) of essential ultra-trace elements, i.e. Mo, Cr, Co and Se, which were found in amounts between 0.017 and 1.14 mg/kg (Figure 3). The peels and seeds are almost the predominant sources, so the following general trends can be described: dried peels for Cr (DI ~20%), dried seeds for Mo (DI > 100%), dried peels for Co (RDA = not available) and dried and raw seeds for Se (~20%). In comparison to this work, Yetesha et al. (2023) showed amounts of 0.46 mg/kg Cd and 0.36 mg/kg Cr in raw pulp fractions. The average Cr and Cd mass fractions in the peels are 0.28 mg/kg and 0.24 mg/kg, respectively, while in the seeds they are 1.39 mg/kg and 0.69 mg/kg, respectively.

Regarding the non-essential elements (Table 1), the raw and dried pumpkin fractions contained Cd (0.009-0.47 mg/kg), Pb (0.031-0.77 mg/kg) and Sn (0.041-0.162 mg/kg) at levels below the maximum levels set by the EU Commission (2006). According to Regulation No. 1881/2006 (EU Commission, 2006), the maximum levels for cadmium, lead and tin are 0.05-1 mg/kg, 0.02-1.5 mg/kg and 50-200 mg/kg respectively, so that their consumption cannot cause toxic effects. Among the potentially toxic elements, Al is the most abundant (10.9-136 mg/kg), and the highest levels were found in dried peel (136 mg/kg). Generally, the majority of studies have found significant variability in the aluminum concentration of various food items. Specifically, an analysis of vegetables in France revealed that mushrooms, spinach, radish, Swiss chard, lettuce, and corn salad, exhibited the highest aluminum levels, ranging from 5 to 150 mg/kg (EFSA, 2008). Other elements that may have potential toxicity include Ba, Ni, Ti, Sr and Rb at levels of 0.17-3.64 mg/kg), 0.3-3.23 mg/kg, 0.62-9.00 mg/kg, 0.96-7.00 mg/kg and 1.7-14.1 mg/kg (Rb), respectively (Table 1).

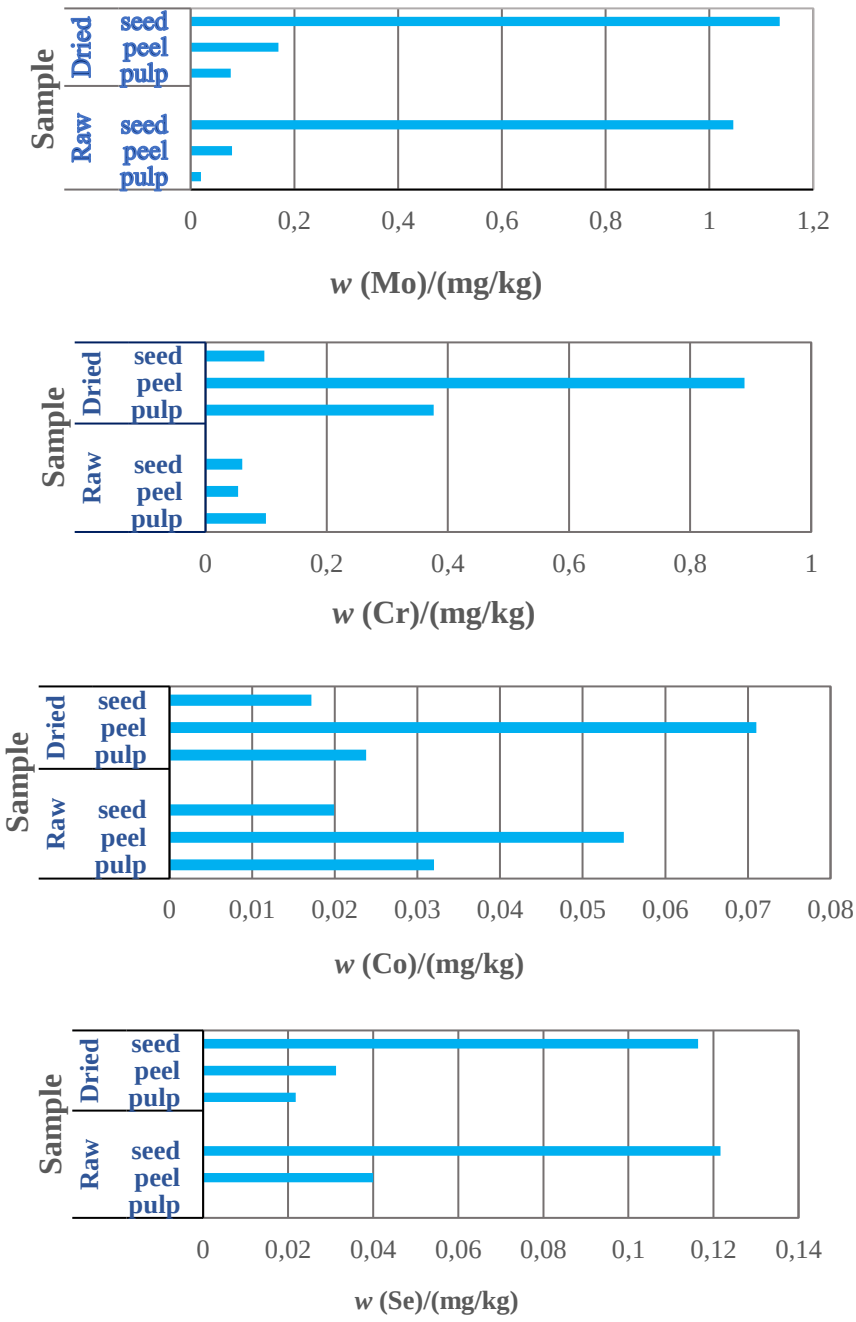

Figure 3. Ultra-trace elements content in raw and dried pumpkin fractions determined by ICP-QQQ analysis

Table 1. Content of potential toxic elements in raw and dried pumpkin fractions determined by ICP-QQQ analysis.

Elements	*w* (element)/(mg/kg)					
	Raw pumpkin			Dried pumpkin		
	Pulp	Peel	Seed	Pulp	Peel	Seed
Al	20.7	16.8	12.5	12.1	136	10.9
As	0.07	0.09	0.07	0.49	0.12	0.09
Ba	1.8	4	0.66	0.17	3.64	0.45
Be	<LOD	0.007	<LOD	<LOD	0.004	<LOD
Bi	<LOD	<LOD	<LOD	0.002	0.002	<LOD
Cd	0.011	0.012	0.009	0.047	0.020	0.011
Li	0.01	0.11	0.00	3.92	0.09	<LOD
Ni	1.1	3.17	3.23	0.14	3.23	2.93
Pb	0.047	0.108	0.032	0.031	0.161	0.032
Rb	7.5	16.7	5.36	6.29	14.1	5.20
Sb	0.002	0.005	0.004	0.024	0.028	0.002
Sn	0.072	0.092	0.062	0.052	0.162	0.041
Sr	4.9	7	1.02	2.68	6.95	0.96
Ti	1.2	9	1.00	1.45	7.42	0.62
Tl	0.009	0.054	<LOD	<LOD	0.045	<LOD
U	0.023	0.023	0.008	0.003	0.006	<LOD
V	0.115	0.35	0.07	0.01	0.18	0.01

LOD-Limit of Detection

Compared to these metals, the amounts of Sb, U, V and Li are low at 0.002-0.005 mg/kg, 0.003-0.023 mg/kg, 0.01-0.07 mg/kg (V) and 0.01-3.92 mg/kg respectively (Table 1). Obviously, the yield of non-essential elements varies between the pumpkin fractions, and the highest amounts are mainly found in the raw and dried peel. Therefore, in order to use them as well as the pulp and seeds as potential food supplements, their health risk must be determined.

Conclusion

Since the drying process can affect the content of minerals because some of them are sensitive to heat, their composition in the dried pumpkin was of particular interest. With the aim of validating the content of minerals in dried pumpkin fractions (pulp, peel and seeds), a comparative study with raw pumpkin was also carried out in this work. The results of high-resolution inductively coupled plasma mass spectrometry (ICP-QQQ analysis) show that both raw and dried pumpkin fractions are a good source of essential macroelements (K, Mg, Ca and Na). Dried peel exhibited the highest levels of K (31025 mg/kg) and Ca (3765 mg/kg), while Mg levels were 5018 mg/kg in dried seeds and 5196 mg/kg in raw seeds. Na content in dried pulp was 412 mg/kg. Among essential trace elements, Fe predominates, ranging from 11.1-331 mg/kg, depending on the sample analysed. The essential elements in the ultra-trace range (Mo, Cr, Co and Se) were present in quantities between 0.017 and 1.14 mg/kg. Thus, by including pumpkin pulp and its by-products in daily meals, individuals can have

access to a wide range of essential elements that are important for optimal health and well-being. However, it is important to note that the content of toxic elements ranges from 0.002-136 mg/kg. Monitoring health risks is therefore essential to ensure compliance with food safety standards.

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Proceedings

PHENOLIC ANTIOXIDANTS FROM ADRIATIC SEA FENNEL WILD-GROWN POPULATIONS

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original scientific paper

Summary

The halophytic plant, sea fennel (*Crithmum maritimum* L.), is widespread along the coastal region of Dalmatia (Croatia). Since ancient times, this plant has been used as a food and for the prevention of various diseases. In this work, the phenolic profile and antioxidant properties of the vegetative (leaves) and generative (flowers) parts of the sea fennel were analysed. The samples were collected during the flowering period at 10 different locations along the Adriatic coast. Total phenolics were determined spectrophotometrically, while chlorogenic acid and its derivatives were analysed by high performance liquid chromatography (HPLC). The antioxidant properties were tested using the Ferric Reducing Antioxidant Power (FRAP) and the radical scavenging test against 2,2-diphenylpicrilhydrazyl radicals (DPPH). The results showed a pronounced phenolic content and antioxidant activity of the samples. Large differences were observed between the results obtained, which were related to the place of sampling and the plant part. A significant correlation was found between the phenolic composition of the extracts and their antioxidant potential. Despite the observed differences, the high content of phenolics, especially the dominance of biologically active chlorogenic acid, and the good antioxidant activity of all samples indicate the great potential of using sea fennel in different areas.

Keywords: Crtihmum maritimum L., phenolic compounds, antioxidants, chlorogenic acid, FRAP, DPPH

Introduction

Plants are a source of various biologically important phytochemicals that can be of great benefit to humans and plants. As for the plant itself, phenolic compounds, as one of the most widespread plant bioactive compounds, play an important role in attracting pollinators, protecting against abiotic and biotic stress, providing mechanical support and defending the plant against various pests (Dai and Mumper, 2010; Cheynier et al., 2013). Apart from the numerous positive effects in the plant, phenolics act as antioxidants, imitate hormones or

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suppress the occurrence of diseases and are thus of great benefit to the human body (Dai and Mumper, 2010; Kumar and Goel, 2019; Rahman et al., 2021).

Among the numerous bioactive compounds, the most important are certainly polyphenolics and phenolic acids. Of the phenolic acids, chlorogenic acid, an ester of caffeic acid and (-)-quinic acid, is particularly noteworthy due to its numerous beneficial effects, the most pronounced of which are its antioxidant, antimicrobial, anti-inflammatory and antitumour properties (Houta et al., 2013; Naveed et al., 2018; Lou et al., 2021; Kraouia et al., 2023; Maoloni et al., 2023). Chlorogenic acid has been reported to inhibit reactive oxygen species and act as a free radical scavenger, which may play an important role in the prevention of oxidative and age-related diseases (Meot-Duros and Magné, 2009; Tajik et al., 2017; Singh et al., 2022). Significant amounts of this compound are found in halophytic plant species growing on coastlines around the world, where they are exposed to various stresses, in particular high soil salinity or droughts (Generalić Mekinić et al., 2018; Pungin et al., 2022; Souid et al., 2022; Kraouia et al., 2023; Politeo et al., 2023b; Veršić Bratinčević et al., 2023).

Therefore, scientific interest has recently focused on halophytes, as they have the potential to grow and develop in locations where other species do not thrive (Meot-Duros and Magné, 2009; Castillo et al., 2022; Martins-Noguerol et al., 2022; Zenobi et al., 2022). One of the interesting facultative halophyte species is *Crithmum maritimum* L., known as sea fennel. This plant is very adaptable to the Mediterranean climate and favours habitats with a lower salt concentration. It can be found everywhere along the coast, on rocks, jetties and sandy beaches. In addition to the salt, the type of habitat largely determines the life of the sea fennel as well as the chemical and nutritional composition of the plant. For example, studies have shown that sea fennel growing on sandy sites contains much higher amounts of phenolic compounds, especially chlorogenic acid (up to 9 times more) (Generalić Mekinic et al., 2018; Souid et al., 2021; Pungin ete al., 2022; Kraouia et al., 2023; Maoloni et al., 2023; Pedreiro et al., 2023).

Since ancient times, sea fennel leaves have been consumed as a food and spice, but they have also been used in folk medicine for their nutritional potential and numerous biologically active compounds. According to scientific studies, this halophyte has great potential for the food industry, both for fermented and non-fermented products, herbal teas, powders, sauces and spices (Renna, 2018; Martins-Noguerol et al., 2022; Kraouia et al., 2023; Pedreiro et al., 2023a). Furthermore, it should not be overlooked that sea fennel is also of great value to the cosmetics industry due to its high content of essential oils and its pleasant and characteristic odour (Generalić Mekinic et al., 2016; Pedreiro et al., 2023; Politeo et al., 2023). Despite the benefits mentioned above, it has not yet been sufficiently researched, as its properties and composition depend on location, growing season, environmental factors, extraction methods and numerous other factors. Although numerous scientific studies have focussed on the leaves of sea fennel, the flower, which is also a rich source of bioactive compounds, should not be neglected. For this reason, the aim of this work was to investigate the influence of location on the chemical composition and biological potential of sea fennel from different locations along the Adriatic coast in order to gain new insights into the benefits of this plant and the possibility of its use in the food industry or other areas.

Materials and methods

Plant material and extraction

The samples of sea fennel were collected in August 2022 at 10 different locations along the Croatian Adriatic coast (Dalmatia region). The locations were from north to south: Krk, Senj, Pag, Šibenik, Split, Drašnice, Korčula, Pelješac, Neretva and Cavtat. Further processing included drying the collected samples (the leaves were freeze-dried and the flowers were air-dried at room temperature. For extraction, 200 mg of the dried and homogenised sample was mixed with 8 mL of 80% methanol in plastic containers, which were then placed in an ultrasonic bath (Bandelin electronic GmbH & Co. KG, Berlin, Germany) for 15 minutes and then mixed in an ES-20 orbital shaker incubator (SIA Biosan, Riga, Latvia) for three hours. The extracts were stored at +4 °C for 24 hours and then filtered (CHROMAFIL Xtra MV 45/25 philtre and BD Discardit II syringe with a volume of 5 mL). The prepared extracts were stored at +4 °C until further analyses.

Determination of total phenolic content and concentration of chlorogenic acid and their derivatives

The spectrophotometric Folin-Ciocalteu method was performed on a UV/Vis spectrophotometer UV-1900i (Shimadzu, Kyoto, Japan) according to the procedure described by Amerine and Ough (1980). In brief: 25 µL of the sample, 1.975 µL of distilled water and 125 µL of Folin-Ciocalteu reagent are pipetted into the cuvette. The solution is stirred and after one minute 375 µL of a 20% sodium carbonate solution (Na_2CO_3) is added. After 2 hours, during which the solution is kept in the dark at room temperature, the absorbance of the samples is measured at 765 nm. The results obtained are expressed as milligrams of gallic acid equivalents per liter of extract (mg GAE/L).
The identification and quantification of chlorogenic acid and its derivatives was determined using a high-performance liquid chromatograph (Shimadzu Nexera LC-40, Shimadzu, Kyoto, Japan) connected to a UV-Vis detector. The reagents used for this method were of HPLC quality and the ultrapure water was from a Millipore system. Standard curves were generated at 6 points by diluting the working standard solution with methanol/water (80:20, v/v). The calibration curve for chlorogenic acid was at concentrations of 5-500 µg/mL, while the concentrations for neochlorogenic acid and cryptochlorogenic acid were 0.1-50 µg/mL. Chromatographic separation was performed at a flow rate of 1 mL/min by gradient elution with solvent A (ultrapure water/85% o-phosphoric acid; 99.8:0.2, v/v) and solvent B (methanol/acetonitrile; 1:1, v/v): 0-16 min, 4-15% B; 16-37.5 min, isocratic 15% B; 37.5-50 min, 15-35% B; 50-60 min, 35% B and 62 min 4% B, with a 3 min re-equilibration. 20 µL of the sample was injected into the HPLC system in duplicate. The results were measured at a wavelength of 220 and 320 nm and processed with LabSolution software (Shimadzu, Kyoto, Japan). They are given in milligrams per liter of extract (mg/L).

Determination of antioxidant activity

Antioxidant activity was measured by two methods using a Sunrise microplate reader (Tecan, Männedorf, Switzerland).
The DPPH method is used to evaluate the free radical scavenging ability of the samples and is based on the reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with antioxidants (Zhong and Shahidi, 2015). The method is based on pipetting 290 µL of the DPPH solution with 10 µL of the sample into the microtiter plate and measuring the absorbance at 517 nm after one hour. The results are expressed as % DPPH radical inhibition.
The FRAP (Ferric Reducing Antioxidant Power) assay measures the reducing capacity of the samples (Zhong and Shahidi, 2015). This method is based on electron transfer, where antioxidants are used to reduce Fe^{3+} to Fe^{2+}, which is visualized as a change in absorbance at 592 nm. Trolox solution was used to create the calibration curve. To test the antioxidant activity, 300 µL of the FRAP reagent solution and 10 µL of the samples were pipetted into a microtitre plate. The change in absorbance is read after 4 minutes and the results are expressed in µM Trolox equivalents (µM TE).

Results and discussion

Total phenolic and chlorogenic acids contents

The results for the total phenolics determined by the Folin-Ciocalteu method in the methanolic extracts of the flowers and leaves of sea fennel are shown in Table 1. From the results obtained, the amount of total phenols in the flower samples was ten times lower than in the leaf extracts, while differences in this ratio along the sampling site were not detected. As for the leaf extracts, the lowest values (151-182 mg GAE/L) were found in the samples from Pag, Split, Drašnice and Cavtat, while the extracts from the southern locations (Korčula and Pelješac) had significantly higher phenol values (313-467 mg GAE/L). The total phenolic content in the flower extracts ranged between 10 and 34 mg GAE/L, with the lowest amounts found in the samples from Split and Cavtat.
As already mentioned, sea fennel accumulates larger amounts of phenolics when growing on sandy sites. This could therefore be one of the explanations for the high phenolic content in the extract from the Neretva, as the plant sample was collected from this site in the river delta itself. Furthermore, we can assume that the reason for the low phenolic content in the samples from Split, Drašnice and Cavtat is the presence of other plant species growing at the sampling sites, especially halophytes such as golden samphire (*Inula crithmoides* L.). It is therefore not to be expected that the sea fennel is exposed to such high salt concentrations as other samples growing in places where no other vegetation was detected.

Table 1. Total phenolic content in sea fennel flowers and leaves

	Total phenolics (mg GAE/L)	
Sample location	**Leaf**	**Flower**
Krk	257 ± 9	30 ± 2
Senj	232 ± 4	34 ± 2
Pag	151 ± 3	23 ± 0
Šibenik	236 ± 8	26 ± 1
Split	160 ± 2	16 ± 0
Drašnice	170 ± 2	29 ± 2
Korčula	469 ± 6	23 ± 1
Pelješac	313 ± 2	22 ± 1
Neretva	231 ± 3	20 ± 1
Cavtat	182 ± 3	15 ± 1

It is difficult to compare the results with those of other studies because different plant parts, harvest times and locations or different extraction and identification methods were used. Meot-Duros et al. (2008) and Houta et al. (2011) have reported that the leaf extracts have a higher phenolic content than the extracts of the sea fennel flowers. Interestingly, Generalić Mekinić et al. (2018) analysed sea fennel in different vegetation periods and concluded that the extracts have the highest total phenolic content before flowering (month of April), then at the beginning of flowering (month of June) and finally in full bloom (month of August). Maleš et al. (2003) also showed that the aerial parts of the sea fennel were richer in phenolics before flowering and at the beginning of flowering than those in the full flowering phase.

The most abundant phenolic acids in the extracts of sea fennel leaves and flowers, chlorogenic acid and its derivatives, were quantified by HPLC. The results are shown in Figs. 1 and 2. Chlorogenic acid was the predominant compound in all analysed leaf extracts, while in the flower extracts, besides chlorogenic acid, criptochlorogenic acid dominated in two samples from the northern Adriatic coast. As with the total phenolic content, the highest concentration was found in the extracts of sea fennel leaves from Korčula, Pelješac and Neretva, while the lowest concentrations were found in the samples from Drašnice, Split and Pag. It is also interesting to note that the proportion of chlorogenic acid in the sum of all detected acids was between 73 and 86% in the leaves and between 26 and 96% in the flowers. The highest concentrations of neochlorogenic and cryptochlorogenic acids were detected in the leaf extracts from Korčula, while cryptochlorogenic acids dominated in the flower samples from Krk and Senj.

Earlier studies also reported chlorogenic acid as the most abundant phenolic compound in fennel leaves (Houta et al., 2013; Generalić Mekinić et al., 2016; Generalić Mekinić et al., 2018; Pungin et al., 2022; Politeo et al., 2023b). Souid et al. (2021) found that the level of neochlorogenic acid was 3.5 times lower (2.03 mg/g) than that of chlorogenic acid (7.25 mg/g). Meot-Duros et al. (2019) showed that plants from sandy habitats develop higher levels of antioxidants such as chlorogenic acid than those growing on cliffs by the sea. Pungin et al. (2022) reported that hydroxycinnamic acid levels increased with soil salinity, but then decreased when the plant was exposed to higher salt stress. All

these statements can be considered as possible causes for the different phenolic content in the analysed samples and for the greater or lesser dominance of a particular phenolic compound. However, the final conclusion considering only one of the listed points cannot be correct, as different abiotic and biotic factors influencing the chemical composition of the plant should be taken into account.

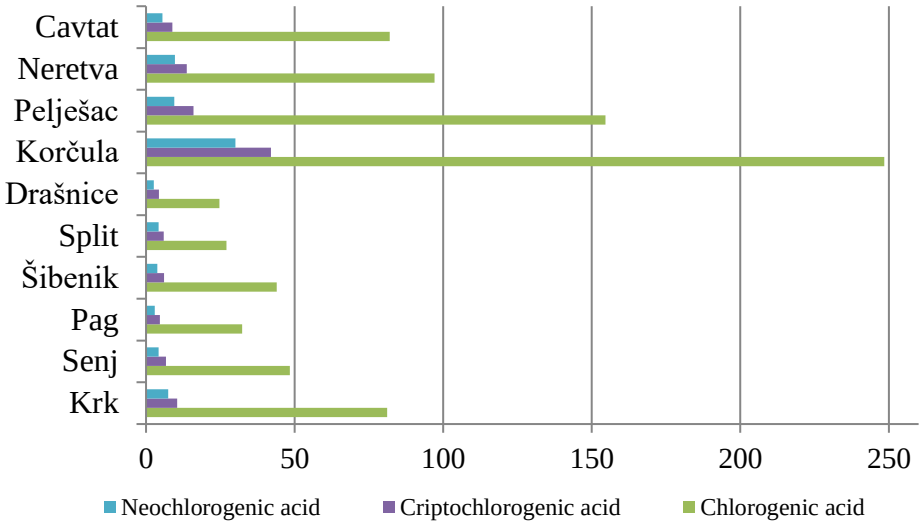

Figure 1. Concentration (mg/L) of chlorogenic acids in leaf samples

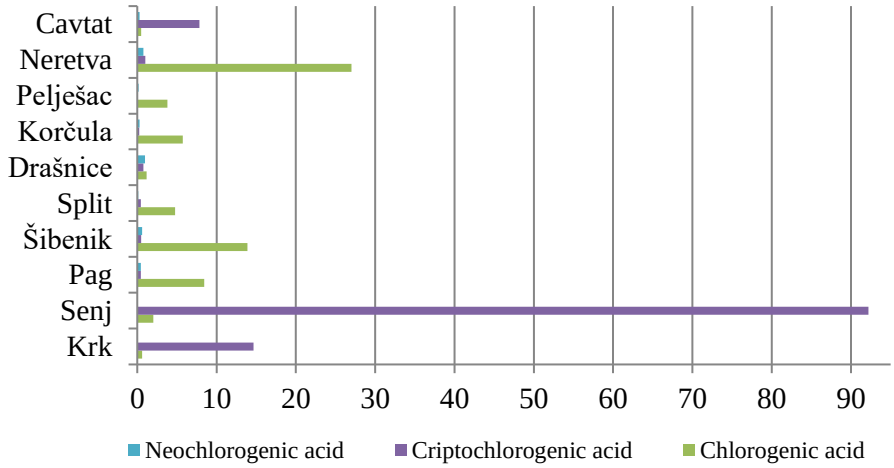

Figure 2. Concentration (mg/L) of chlorogenic acids in flower samples

Antioxidant activity

There are a variety of methods for determining the total antioxidant potential of different samples, including different plant extracts. Due to the different mechanisms of action of certain methods, we used the DPPH method based on the hydrogen atom transfer (HAT) and the FRAP method based on the electron transfer (ET) mechanism in this work, and the results are shown in Table 2. The reducing activity (FRAP) was very good in the leaf extracts from Korčula and Pelješac, two samples that had the highest total phenolic content. Among the flower extracts, the samples from Senj, Krka and Drašnice had the best reducing activity (in the same range as the leaf samples), although their total phenolic content was ten times lower. Generalić Mekinić et al. (2018) determined the reducing capacity of ethanolic extracts of sea fennel in different vegetation periods and reported the highest activity for the leaf extract from April (35.1 mM TE), while the weakest activity was determined for the samples from August and December.
The ability to scavenge DPPH radicals ranged from 24 to 80%, with the highest activity observed in the leaf samples from Korčula and the flower samples from Senj (80 and 64% inhibition, respectively). In addition to the samples from Korčula, the leaf extracts from Pelješac, Neretva and Krka also have good antioxidant potential, while the other leaf extracts showed an inhibition of the DPPH radical of less than 40%. The flower extracts showed very high activity considering their low phenolic content. The percentage of inhibition of the DPPH radical stands out especially in the samples from Krk, Senj, Šibenik and Drašnice with an activity of more than 50%. In the study by Generalić Mekinić et al. (2016), the antioxidant capacity of the ethanolic extract of sea fennel flowers and leaves from August was 61%. In another study, the same group of authors showed a 44.5% DPPH inhibition of the leaf extracts (Generalić Mekinić et al., 2018). Houta et al. (2011) compared the antioxidant capacity of different parts of sea fennel and reported a higher activity of seed and leaf extracts compared to flowers and stems.

Table 2. Antioxidant activity of flowers and leaves of sea fennel extracts

	FRAP (μM TE/L)		DPPH (% inhibition)	
Sample location	**Leaf**	**Flower**	**Leaf**	**Flower**
Krk	549 ± 22	803 ± 1	49 ± 4	55 ± 2
Senj	549 ± 6	1101 ± 2	37 ± 2	64 ± 3
Pag	347 ± 13	679 ± 40	33 ± 2	48 ± 0
Šibenik	517 ± 14	756 ± 32	35 ± 4	53 ± 2
Split	394 ± 5	468 ± 10	25 ± 5	26 ± 3
Drašnice	414 ± 17	885 ± 36	24 ± 1	55 ± 2
Korčula	1509 ± 4	697 ± 9	80 ± 1	45 ± 3
Pelješac	1029 ± 25	589 ± 16	56 ± 6	40 ± 1
Neretva	602 ± 36	570 ± 45	44 ± 3	40 ± 3
Cavtat	537 ± 2	340 ± 4	35 ± 2	31 ± 2

Conclusions

It can be concluded from the results that, in addition to the time of sampling and the vegetation period, the location of sampling also has a significant influence on the chemical composition and biological potential of the sea fennel samples. It can also be stated that the flower extracts showed very good results in terms of phenolic content and antioxidant activity, in some cases better than the leaf extracts. This indicates the extraordinary potential of this plant, which has not yet been sufficiently researched. More attention to this plant would contribute significantly to a better utilisation of this plant for food and other purposes.

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Proceedings

UNSATURATED FATTY ACIDS IMPROVE HEALTH

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review paper

Summary

Meat, as an important part of human diet, belongs to the so-called "functional food", with benefit on physiological processes in humans. After proteins, fats are the most abundant in meat. Meat contains a combination of SFA and UFA. From a health point of view, UFA are particularly important, positively reduce cholesterol in the blood and improve the functioning of the cardiovascular system in the human body. A diet high in SFA increases LDL cholesterol. Both MUFA and PUFA can help lower LDL cholesterol and increase HDL. Lamb and goat meat contains 17% n-$_6$ and 19% n-$_3$ PUFA. Linolenic acid ($C_{18:2n-6}$) and Alpha linolenic acid ($C_{18:3n-3}$) represent essential fatty acids that the human body cannot synthesize on its own and it is necessary to get them through the diet. In order to increase the useful amount of UFA in meat, lately, % of SFA in meat has gradually decreased as a result of the selection of animals for slaughter as well as the application of appropriate modified nutrition that enables the creation of adipose tissue that is rich in UFA. The paper is a review of the literary data on the importance of UFA and SFA on the quality of human health.

Keywords: meat, unsaturated fatty acids, saturated fatty acids, health, functional food

Introduction

The nutritional value of meat in the human diet highlights the fact that in consumer surveys about the quality of food, meat is the product that consumers are most interested in and that they talk about the most. In the diet of a large number of people in the world, especially in developed countries, meat occupies a central position and, in relation to other products, it represents a product with the highest status (Pantič, 2014). Meat is a source of nutrients in a concentrated form and is considered indispensable for proper nutrition (Higgs, 2000). Jimenez-Colmenero et al. (2001) define meat as a functional food, that is, food that can beneficially affect the physiological processes in the human organism, by providing nutrients). Meat itself is a rich source of nutrients. It is high in high-value protein, as well as micronutrients such as iron, selenium, zinc and vitamin B12, while offal is a significant source of vitamin A and folic acid (Biesalski, 2005). As the main source of protein with a favorable amino acid ratio and as a source of well-usable iron, vitamins and minerals, meat occupies an important place in the human diet (Williamson et al., 2005). However, due to the significant portion of fat and the fact that meat contains a relatively high percentage of saturated and a small portion of polyunsaturated fatty acids, in recent decades meat as a product has started to be talked about in a negative context. The composition and amount of fat determine the nutritional value and numerous organoleptic properties of the meat and have a great influence on the firmness

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and sustainability of the meat (Kaić et al., 2013). During the last decades, medicine recommended a reduced intake of saturated fatty acids as a prevention of cardiovascular diseases (Krauss et al., 2000). Kennedy et al. (2009) believe that an excessive intake of saturated fatty acids encourages the development of adipose tissue, which leads to further hypertrophy and cell apoptosis. Such a process would lead to the release of pro-inflammatory substances such as chemokines and cytokines that further induce inflammation and insulin resistance, which increases the risk of cardiovascular and metabolic disorders (Haffner et al., 2006; Ridker et al., 2004).

Chemical composition of meat

Meat is the muscle tissue of the animal. The muscles of most animals contain 75% water, 20% proteins (amino acids), 5% fats, carbohydrates and various minerals and vitamins (Listrat et al., 2016). According to Heinz and Hautzinger (2007), beef consists of 75% water, 22.3% protein, 1.8% fat, 1.2% ash. Lean pork contains 75.1% water, 22.8% protein, 1.2% fat, 1.0% ash. Pork carcass contains 41.1% water, 11.2% protein, 47.0% fat, 0.6% ash. Veal lean meat contains 76.4% water, 21.3% protein, 0.8% fat, 1.2% ash. Poultry meat contains 75% water, 22.8% protein, 0.9% fat and 1.2% ash. According to Junkuszew et al., (2020) lamb meat contains 75.11% water, 20.73% protein, 3.69% fat and 1.11% ash. Goat meat contains 74.2-76% water, 20.6-22.3% protein, 0.6-2.6% fat and 1.1% ash (Devendra, 2007). What sets meat apart from other foods is the large number of essential amino acids. About twenty-nine amino acids are known, of which 20 are essential for protein synthesis (Wu, 2009). Of those 20 essential amino acids, ten amino acids cannot be synthesized by the human body and must be obtained through food. If all the amino acids needed for protein synthesis are not taken in, protein malnutrition may occur (Krauss et al., 2000). In the composition of meat, lipids are found in muscle tissue (intramuscular adipose tissue) and in the corresponding adipose tissue, where triglycerides are found in adipose tissue and in muscle tissue in addition to triglycerides in the membranes of muscle fibers and phospholipids (Karolyi et al., 2007). Lipids are a group of related chemical compounds that share the common property of not being soluble in water. They are divided into simple lipids or fats, complex (eg phospholipids) and pseudolipids (cholesterol). Fats are made of trivalent alcohol glycerol and higher fatty acids (triglycerides) and together with carbohydrates and proteins are one of the three main ingredients of food (Karolyi et al., 2008). The main choice of fats in the human diet are vegetable oils, and animal products are meat, eggs and milk, whose role in biological tissues is multiple, considering that they represent a source of energy, components of biological membranes, precursors for various molecules, as well as transporters of certain vitamins (Petrović et al., 2010). Fatty acids by their composition are carboxylic acids, usually with a long unbranched chain and can be saturated and unsaturated fatty acids. The characteristics of fatty acids depend on the length of the chain, that is, on the number of carbon atoms, the degree of unsaturation and branching of the chain. Short-chain fatty acids are in a liquid aggregate state with a pungent odor and are soluble in water. Fatty acids are built from a carbon chain with a terminal methyl group (CH_3-) at one end and a carboxyl (-COOH) group at the other end of the chain (Karolyi et al., 2007). The classification of fatty acids is carried out according to the length of the carbon chain (which can be from 1 to 30 C atoms), the

position of the first double bond in the carbon chain (omega-9, omega-3, omega-6) as well as the spatial shape of the double bond (cis and trans isomers). Regarding the possibility of synthesis in the body, they can be divided into essential (omega-6 and omega-3) and non-essential fatty acids (Whetsell et al., 2003). Saturated fatty acids (SFA) do not contain double (covalent) bonds or other functional groups in the molecular chain. SFA create straight chains of atoms and as a result can be stored in a compact form in the body, releasing a greater amount of energy per unit volume. Adipose tissue in humans and animals contains large amounts of long-chain saturated fatty acids. The most common SFA are: butyric acid (butyric acid): $CH_3(CH_2)2COOH$ or C4:0; caproic acid (hexanoic): $CH_3(CH_2)_4COOH$ or C6:0; caprylic acid (octanoic): $CH_3(CH_2)_6COOH$ or C8:0; capric acid (decadic): $CH_3(CH_2)_8COOH$ or C10:0; lauric acid (dodecadic): $CH_3(CH_2)_{10}COOH$ or C12:0; myristic acid (tetradecadic): $CH_3(CH_2)_{12}COOH$ or C14:0; palmitic acid (hexadecadic): $CH_3(CH_2)_{14}COOH$ or C16:0; stearic acid (octadecadic): $CH_3(CH_2)_{16}COOH$ or C18:0; arachidic acid (eicosanoic): $CH_3(CH_2)_{18}COOH$ or C20:0; behenic acid (docosanoid): $CH_3(CH_2)_{20}COOH$ or C22:0. (Gruevska, 2016).In the case of fatty acids that lack a pair of hydrogen atoms in the chain and in that case the fatty acid contains one double bond or monounsaturated fatty acid (*MUFA*). Polyunsaturated fatty acids (*PUFA*) contain more than one or double bonds in the carbon chain. Omega (ω) or n-number in the nomenclature of polyunsaturated fatty acids indicates the position of the first double bond in the carbon chain counted from the CH_3 group (Karolyi et al., 2007). The following Table 1 shows the most common unsaturated fatty acids in meat.

Table 1. Unsaturated fatty acids (Gruevska, 2016)

Myristoleic acid	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$	$C_{14:1}$	$C_{14}H_{26}O_2$	omega-5
Palmitoleic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	$C_{16:1}$	$C_{16}H_{30}O_2$	omega-7
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ or *cis*-Δ9	$C_{18:1}$	$C_{18}H_{34}O_2$	omega-9
Linoleic acid (LA)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	$C_{18:2}$	$C_{18}H_{32}O_2$	omega-6
Alpha-linolenic acid (ALA)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	$C_{18:3}$	$C_{18}H_{30}O_2$	omega-3
Stearidonic acid (SDA)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4COOH$	$C_{18:4}$	$C_{18}H_{28}O_2$	omega-3
Arachidonic acid (AA)	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$	$C_{20:4}$	$C_{20}H_{32}O_2$	omega 6
Eicosapentaenoic acid (EPA)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$	$C_{20:5}$	$C_{20}H_{30}O_2$	omega-3
Docosahexaenoic acid (DHA)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2COOH$	$C_{22:6}$	$C_{22}H_{32}O_2$	omega-3
Eruic acid	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	$C_{22:1}$	$C_{22}H_{42}O_2$	omega-9

In general, red meat contains about 40% SFA, 50% MUFA, 5% trans fatty acids and 4% polyunsaturated fatty acids (FRIDA, 2009). The amount of fat in the meat depends on the type and age of the animal as well as the part of the carcass (Irshad et al., 2012). The amount of fat and the composition of fatty acids also depends on the animal's diet. The representation of fatty acids in meat can be modified by the diet in monogastric animals such as pigs and poultry (Bolte et al. 2002; Smet et al., 2004). The total amount of fat can vary from 0.8 to 48 g/100 g. fats in meat mainly contain monounsaturated and saturated fatty acids. The most common are oleic (C18:1), palmitic (C16:0), stearic (C18:0) (Abbas et al., 2009). Poultry and pork contain more unsaturated fatty acids than beef and lamb, as well as a notable content of polyunsaturated fatty acids. Linolenic acid (C18:2) is the predominant PUFA, followed by α-linolenic acid. Trans fatty acids make up about 1-2% of the total fatty acids in all types of meat; and in ruminant meat they are represented by 2-4% (Belury, 2002). According to Krvavica et al. (2013), fatty acids in meat (consisting mainly of 12-22 C atoms) consist on average of 40% saturated, 40% monounsaturated and about 2-25% polyunsaturated fatty acids. The back bacon of fattening heads contains on average 44% MUFA, 36% SFA and 12% PUFA (Davenel et al., 1999). In lambs, SFA is 46.3%, MUFA 39.7% and 13.4% PUFA (Vnučec, 2011). Oleic fatty acid (C18:1 cis9) is the most important fatty acid of all types of meat, which is represented by more than 30% of total fatty acids and has a major biological role. In addition to it, palmitic (C16:0) and stearic (C18:0) are the most represented. These fatty acids in beef are represented by 80% of the total fatty acids, with a share of oleic acid of 33%, palmitic acid 27% and stearic acid 18% (Whetsell et al., 2003). The composition of fatty acids in the meat of ruminants (cattle, sheep and goats) is significantly more complex than the meat of non-ruminants because they contain more trans fatty acids (C18:1, elain trans fatty acid in beef is 2-5% of the total fat acids) (Krvavica et al., 2013). In ruminants, linoleic and α-linoleic fatty acids can be transformed into conjugated linoleic fatty acid (CLA) by rumen bacteria and have a potential positive effect on human health (Bergamo et al., 2003). CLA is the common name for a mixture of linoleic acid isomers that include double bonds at positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13. Each of these C18 isomers can occur in cis-trans, trans-cis, cis-cis and trans-trans form. (Whetsell et al., 2003). Pork meat has a high share of PUFA in the meat, and thus a favorable nutritional ratio P/S, which is usually within recommended limits ≥0.4 as a result of the high content of linoleic acid (C18:2n6). In pork, the omega 6 / omega 3 ratio is significantly higher than the nutritionally recommended values - lower than 4 (Enser, 1996). Kralik et al. (2001) analyzed the content of saturated, monounsaturated and polyunsaturated fatty acids in white and red meat (breast and thigh muscles). White meat contains significantly less fat than dark meat in poultry. In both types of meat, palmitic (C16:0) and stearic (C18:0) from the saturated fatty acids are the most abundant, and oleic (C18:1) from the monounsaturated and (C18:2n6) from the polyunsaturated fatty acids. The representation of individual groups of fatty acids in white and red chicken meat are: SFA 40.4%, ie 35.3%, MUFA 29.1%, ie 32.2%; PUFA omega- 6 17.4%-20.22% and PUFA omega-3 5.6% and 4.6%. The omega-6/omega-3 ratio is more favorable in white than in dark red meat. The ratio of omega-6/omega-3 in research by

Komprda et al. (1999) in white meat ranges from 3.1 to 13.6 and in dark meat from 4.5 to 19.2, depending on the content of the specified polyunsaturated fatty acids in the mixtures fed to chicks. In goat meat, the total representation of saturated fatty acids is 52.06% of the total amount of fatty acids, of which the most represented is palmitic with 28.25%, followed by stearic fatty acid with 13.24%, myristic fatty acid with 8.08 %, margarine fatty acid with 0.87%, etc. While from PUFA unsaturated fatty acids, the most represented is (oleic fatty acid) C18:1 n9c with 32.13%, followed by C18:2 n6c (conjugated linoleic fatty acid) with 4.2442%. The representation of other PUFAs in meat is in a much lower %, namely C20:3 n9 omega 9 (eicositrienoic fatty acid with 0.8228%, C18:3 n3 (alpha-linolenic fatty acid ALA) with 0.62714%. (Gruevska, 2016).

Unsaturated fatty acids and health

Meat and meat products are the most significant source of monounsaturated fatty acids (Woods and Fearon, 2009). In addition to the increased intake of saturated fatty acids in relation to unsaturated ones, concern also arises due to the increased ratio of omega-6/omega-3 polyunsaturated fatty acids, which in today's human diet is 10-30:1 (Lunn and Theobald, 2006), while the health recommendation is for that ratio to be 4:1 or less (HMSO 1994). According to Syvertsen et al. (2007) and Akahoshi et al. (2004) increased the intake of CLA in the human diet leading to a decrease in the percentage of body fat and an increase in protein in the human body. This unsaturated fatty acid works to reduce fat deposition and increase lipolysis in adipocytes (Azain, 2004). Metabolic disorders diabetes mellitus type-2 are associated with changes in fat metabolism at the intracellular level (Dobrzyn and Ntambi, 2005). Research shows that the 10-CLA isomer is the active isomer that affects the change in body mass observed in type-2 diabetics (Belury et al., 2003). Unsaturated fatty acids, most of which CLA has a role in reducing energy intake and reduced energy consumption, reducing proliferation, differentiation of preadipocytes and increasing lipolysis and fat oxidation (Salas-Salvado et al., 2006). CLA has been shown to inhibit tumor cells by blocking tumor cell growth and metastasis. CLA acts quickly, blocking malignant and benign tumors immediately after entering the body (Belury et al., 2002). It is assumed that isomer of 10-CLA this effect is achieved by influencing apoptosis and cell cycle control, while isomer-9 CLA affects through arachidonic acid (Ochoa et al., 2004). Atherosclerosis is a progressive disease of medium and large arteries that occurs with fat accumulation in inflammatory cells, cell proliferation, platelet adhesion and calcium deposits (Toomey et al, 2003; Desroche et al. 2005). CLA isomers have a strong antiarteriolosclerotic effect in animals (Weldon et al., 2004; Naumann et al., 2006). These effects are explained by the occurrence of a drop in LDL cholesterol and an increase in the desired HDL cholesterol, which is obtained by increased synthesis of A-I, A-II, increased expression of HDL receptors, i.e. increased behavior of cholesterol from cells and reduction of vascular inflammation through reduced synthesis in the nucleus of NFkB and A-I transcriptional activity resulting in a reduced risk of fibrinogen thrombosis (Salas-Salvado et al., 2006; Navarro et al., 2003; Smedman et al., 2004). Certain studies demonstrate that unsaturated fatty acids leading to the isomers of CLA (9-

CLA and 10-CLA) can enhance both acquired and innate immune responses (Albers et al., 2003; Oshea et al., 2004). These findings have been attributed to the ability of CLA to modify soluble mediators and factors of immunity such as eicosanoids (Chen et al., 2016), cytokines (Hur et al., 2007), and immunoglobulin production (Ringeis et al., 2006). MUFAs have shown a positive effect on cardiovascular diseases through several different mechanisms (Hammad et al., 2016). These mechanisms include factors that alter the lipid/lipoprotein profile, such as inactivation of the sterol regulatory protein element, a transcription factor that regulates cholesterol synthesis, and an increase in hepatic LDL receptor expression through stimulation of acyl-CoA, cholesterol acyltransferase (Kien et al., 2014). MUFA has been shown to induce greater diet- induced thermogenesis and a higher rate of fat oxidation compared to SFA (Krishnan and Cooper, 2014). In their analysis Mensink et al. (2016) showed that replacing SFA with MUFA reduced total cholesterol and HDL and LDL cholesterol as well as triglycerides. The importance of PUFA in human health and nutrition is clearly demonstrated by the individual concentration of n-6 and n-3 fatty acids, but mixing the two with the metabolism of the other may reduce the deposition of specific n-6 and n-3 PUFA in tissue lipids and thus alter the overall biological effects (Ruxton et al., 2004). According to Pantič (2014), the intake of n-3 polyunsaturated fatty acids has a beneficial effect on preventing the development and progression of atherosclerosis by changing the composition of plasmin lipids, reducing triglyceride levels and preventing the formation of coronary plaque that has a key role in the occurrence of ischemic changes. H-3 polyunsaturated fatty acids can stimulate endothelial relaxation that has an antiarrhythmic and cardioprotective effect on the heart.

Conclusion

Quality and proper daily nutrition is of crucial importance for maintaining a good human health condition. Special attention is paid to the amount of fat entered in that diet, but also to the correct ratio of saturated versus unsaturated fatty acids that enter into the composition of fats. The increased intake of UFA (MUFA and PUFA) at the expense of SFA has a positive effect on the reduction of cardiovascular diseases as well as on cholesterol in adults and even in children. In addition to cardiovascular diseases, an increased intake of unsaturated fatty acids has a positive effect on cancer and immunological diseases, as well as diabetes mellitus- type 2.

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Proceedings

INTERMITTENT FASTING AND CARDIOVASCULAR RISK FACTORS: A REVIEW

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review paper

Summary

This article shows the impact of various dietary interventions on longevity and health span, focusing on intermittent fasting (IF), ketogenic diets (KD), low-carbohydrate diets, and vegan diets. IF, particularly time-restricted eating (TRE), is highlighted for its positive effects on weight loss, adiposity reduction, and cardiovascular health. The efficacy of alternate day fasting in improving cardiovascular indicators and body composition is also underscored. The KD, characterized by low carbohydrate and protein intake, is associated with extended lifespan, and improved metabolic, physical, and cognitive function in animal studies. The potential benefits of the KD on longevity are suggested to be linked to reduced protein/amino acid intake. Contrastingly, vegan diets, can have their benefits against aging and diseases, but there are also potential drawbacks, such as an increased risk of fractures due to specific amino acid deficits. Low-carbohydrate diets, including the ketogenic diet, are compared with low-calorie, low-fat/high-carb, and low-protein/high-carb diets, indicating similar effects on body mass index (BMI), cholesterol levels, and triglycerides in obese individuals. The conclusion advocates for a normocaloric longevity diet characterized by mid to high carbohydrate, low but sufficient protein intake, primarily plant-based, and including pesco-vegetarian-derived proteins. This diet is associated with extended lifespan and healthspan, as exemplified by populations like the Okinawans. The importance of a balanced diet, considering carbohydrate, protein, and fat intake, is emphasized for optimal health outcomes. Studies suggests that sustained dietary changes, such as transitioning to a plant-based, legume-rich diet, can significantly impact life expectancy. It underscores the need for further research to identify age-specific nutrition contributing to health span, emphasizing the integration of both animal and human studies. The findings highlight the dynamic nature of dietary effects on longevity and underscore the significance of considering various dietary components in promoting overall health and lifespan.

Keywords: intermittent fasting, ketogenic diet, longevity diet

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Introduction

IF typically involves 12-23 h of fasting per day. Although there are many different types of intermittent fasting regimens, ranging from restricting eating to a limited number of hours (time-restricted feeding [TRF] in model organisms and time-restricted eating [TRE] in humans) to alternate day fasting, to fasting for two days a week. There is mounting evidence that TRE has positive impacts on people as well. The majority of clinical trials conducted up to this point have involved participants who were obese, had the metabolic syndrome, or had type 2 diabetes, with the goals of losing weight or improving metabolic impairment (T2D).
There are various reversible risk factors that contribute to cardiovascular diseases such as heart attacks and strokes. These risk factors include being overweight, having high blood pressure, and diabetes. Fasting has been shown to help with all of these risk factors, as well as lower cholesterol, which is also a risk factor. Animal studies have shown that time-restricted eating, a form of short intermittent fasting, can reverse many of these risk factors, including cholesterol (Longo et al., 2021) However, most human studies have focused on healthy individuals, rather than those with diagnosed metabolic syndrome who are at a higher risk of cardiovascular disease. In recently published study, researchers specifically looked at people with metabolic syndrome who were already on medications and examined whether adding time-restricted eating could amplify some of the benefits (Chen et al., 2017; Li et al., 2019).

Discussion

Most TRE studies show weight loss and a decrease in adiposity (Wilkinson et al., 2020) or waist circumference (Schroder et al., 2021). According to several research circulating variables connected to cardiovascular disease have improved, but this is not always the case. Chronic calorie restriction (CR) improves cardiovascular function and several other physiological markers of health span (Martens et al., 2020). Improvements in glucoregulatory parameters are rarely reported. One prominent exception is research in which healthy weight adults' blood glucose levels were reduced yet cardiovascular risk indexes remained unaffected. Although harder to complete, longer TREs are useful in increasing insulin sensitivity (Sutton et al., 2018). Alternate day fasting, which involves people fasting every other day, was found to be equally as efficient for 4 weeks at improving cardiovascular indicators, lowering trunk fat, boosting β-hydroxybutyrate, and improving the fat-to-lean ratio, even on non-fasting days (Stekovic et al., 2020). In conclusion, TRE seems to have positive effects in both rodents and people, but side effects and compliance problems make an 11–12 h feeding window preferable, at least until more research identifies TRE lengths that are secure, practical, and efficient. Both in healthy subjects and in the context of treating disease, periodic fasting (PF) and fast mimicking diet (FMD) in humans have been examined. Reduced body weight, trunk, and total body fat were observed in a randomized crossover study of 100 patients, 71 of whom underwent three monthly 5-day FMD cycles. Blood pressure and IGF-1 levels were also dropped. Those with high baseline levels of these risk factors also showed a reduction in fasting glucose,

triglycerides, total and low-density lipoprotein cholesterol, and C-reactive protein, according to a post hoc study (Wei et al., 2017). This dietary intervention has the potential to be effective and should be tested in further clinical trials for the prevention and treatment of many diseases when applied for only 3–4 times per year without necessitating but preferring improvements in daily eating habits. This is because the beneficial changes caused by FMD cycles can last for months. Most low-carbohydrate diets for people restrict daily carbohydrate intake to 50–60 g, with the remaining calories coming from high fat and moderate to high protein sources. The factor that most affects the ketogenic diet (KD) is the carbohydrate content in the diet and less protein. More than 100 years ago, the ketogenic diet was used to treat epilepsy in children (Wilder, 1921). Robert Atkins developed and popularized the ketogenic diet in the 1970s to help people lose weight and have more compliance by allowing considerably higher protein intake while keeping carbohydrate intake low to very low (Weber et al., 2020). Yet, the KD's widespread use led to the establishment of a low-carbohydrate diet that permits more than 15 g of carbohydrates per day while simultaneously encouraging the consumption of foods common to Western diets. In adult mice, a KD application extends lifespan and enhances markers of metabolic, physical, and cognitive function (Roberts et al., 2017). The diet has positive effects on metabolism and cognition when followed in cycles (Newman et al., 2017). KD enhances cerebrovascular function in mice (Ma et al., 2018), and it enhances cognitive performance in rats along with modifications to the prefrontal cortex's metabolite transport networks (Hernandez et al., 2018). According to Pawlosky et al. (2020), treatment with ketone bodies in Alzheimer's disease mice fed a standard diet improves cognition and lowers plaque burden in a way that was connected to hippocampal neuronal mitochondrial function. Within hours, the KD causes the liver to undergo autophagy, which is necessary to produce ketone bodies. The autophagy-dependent clearance of an inhibitor complex and the important lipid metabolism regulator peroxisome proliferator activated receptor alpha (PPAR-α) are related to how the ketogenic program is activated. Since that both lipid metabolism and autophagy are connected to the regulation of longevity in shorter-lived animals, they are likely to contribute to the health advantages of the KD. The KD involves a low protein diet in many studies so it's probable that some of the benefits of the KD on longevity and disease may be related, at least in part, to decreased protein/amino acid intake. KD triggers autophagy in the liver within hours, which is essential to produce ketone bodies. Given that both are associated with the regulation of longevity in shorter-lived animals, the relationship between lipid metabolism and autophagy is expected to contribute to the health advantages of the KD. The KD is notable for involving low protein intake in studies, therefore it's probable that some of the reported benefits of the KD on longevity and disease may be connected, at least in part, to decreased protein/amino acid intake. Several human studies have been conducted on low-carbohydrate diets, including the ketogenic diet. According to a recent meta-analysis, eating a ketogenic/low carbohydrate diet had similar effects on body mass index (BMI), circulating levels of total cholesterol, lipoprotein profiles, and triglycerides in obese humans as low-calorie, low-fat/high-carb, or low-protein/high-carb diets (López-Espinoza et al., 2021). Several epidemiological studies have explicitly examined the relationship between dietary carbohydrates and mortality. One of

these studies lasted for 26 years with 85,168 women (baseline age: 34–59) and 44,548 men (baseline age: 40–75) who were free of diabetes, cancer, or heart disease. This study demonstrated that a low-carbohydrate diet high in plant-based foods was related with lower all-cause and cardiovascular disease mortality rates, but a low-carbohydrate diet low in plant-based foods was associated with greater all-cause mortality in both men and women. Males who followed a low-carb, animal-products-based diet showed a 66% increase in the risk of developing cancer, compared to women, who showed a 26% increase (Fung et al., 2010). In a meta-analysis of many cohorts encompassing 432,179 participants, it was discovered that, in comparison to moderate carbohydrate intake, both low carbohydrate intake (40% of energy) and high carbohydrate intake (>70% of energy) increased mortality risk. Compared to the group receiving 50-55% of their energy from carbohydrates, the risk of overall mortality increased by more than 50% in the group consuming less than 20% of their energy from carbohydrates (Seidelmann et al., 2018). Here we can criticize the performance of the study itself, since the intake of 40% of carbohydrates in the diet can hardly be called a low intake. Interestingly, consuming less carbohydrates forces people to consume more protein and fat, which raises the prospect that eating more protein and/or fat may have a greater impact on mortality than consuming fewer carbohydrates. The source of the macronutrients was also discovered to be important, in addition to the macronutrient balance. When animal-derived proteins or fats were substituted for carbs, the mortality risk was roughly 18% greater, but it was 18% lower when plant-based proteins or fats were used. These epidemiological studies considered those whose carbohydrate intake was low but not as low as the very low levels (50 g) permitted in the rigorous ketogenic diets. Although it is widely known that the vast majority of people cannot sustain a very restrictive KD over the long term, these researches are crucial to determining whether specific plant-based diets with relatively low carbohydrate levels might offer a more practical choice for the general population. They also stress the significance of looking at the relative macronutrient composition rather than concentrating on a single one and highlight the very different impacts of consuming fats and proteins from animal vs plant sources on health, mortality, and longevity. These findings also highlight the significance of integrating fundamental and human research to start identifying the age-specific nutrition that can increase health span. Although fat consumption has declined in the United States, obesity rates have climbed despite this, which suggests that factors more than just dietary fat intake are to blame, including higher total calorie intake and the makeup of modern diets. In fact, when 7,447 participants at high risk for cardiovascular disease were randomized to either a control diet with the advice to reduce dietary fat or to a Mediterranean diet supplemented with extra-virgin olive oil or mixed nuts, the risk of major cardiovascular events was about 30% lower in the Mediterranean diet groups supplemented with healthy fats from olive oil or nuts than in the group advised a low fat diet (Estruch et al., 2018). However, a vegan dietary pattern is also linked to a lower risk of cancer, hypertension, and diabetes compared to that for regular meat eaters (Segovia-Siapco and Sabate, 2019). Several studies show that pesco-vegetarians, but not vegans, display reduced risk for overall mortality when compared to meat eaters. Importantly, compared to non-vegan diets, the vegan diet has been linked to a 43% higher risk of total

fractures and a 2.3-fold higher risk of hip fractures (Tong et al., 2020). Deficits in specific amino acids may help to explain this fragility.
In reality, the EPIC-Oxford study found that 8.1% of vegan women and 16.5% of vegan men consumed less protein than they needed, which may have been made worse by the vegans' exclusive reliance on legumes, which have very low quantities of the amino acid methionine and other essential amino acids (Mariotti and Gardner, 2019). In conclusion, the data are consistent with the remarkable benefits of a vegan diet against aging and diseases but also with an association of vegan diets with fewer benefits compared to vegetarian or pesco-vegetarian diets, possibly because these diets stop the general population's tendency toward frailty that is associated with vegan diets.

Conclusion

We can start to identify a common factor for healthy longevity based on all of the studies presented in this review and representing all of the pillars of longevity listed above. These pillars suggest that the normocaloric longevity diet for daily use is characterized by a mid to high carbohydrate and low but sufficient protein intake that is primarily plant-based and regularly includes pesco-vegetarian-derived proteins. This diet is associated with low or very low side effects and an extended lifespan and healthspan. For instance, animal products made up about 1% of the traditional diet of the Okinawans, who have the longest life expectancy records (Willcox et al., 2007), and populations in the Sardinian and Loma Linda regions with high rates of centenarians and long life expectancies also tended to consume meat or animal products on occasion (Levine et al., 2014). Evidence from studies on calorie and protein restriction in short-lived animals, epidemiological statistics presented in earlier sections, and evidence from significant clinical trials all support the benefits of such a diet. Hence, a diet with a moderate protein intake and a high legume intake, which results in a relatively low content of methionine and other amino acids, leads to a decrease in the levels and activity of the pro-aging GHR, IGF-1, insulin, and TOR-S6K signaling. Nevertheless, the low-protein diet may instead lead to lean body mass loss and frailty in people over 65 because it does not appear to further lower the circulating IGF-1 levels that have already been decreased by the aging process. The relatively high complex carbohydrate intake may also help prevent frailty, especially in the elderly, in the absence of obesity and insulin resistance by supplying energy without raising insulin levels and activating glucose signaling pathways. Although the traditional Okinawan diet provided a much lower level of fats, it is confirmed that there are variations of the ideal longevity diet that could be equally effective. The longevity diet also includes a fat consumption providing about 30% of energy, mostly from plant-based and pro-longevity sources. This is consistent with basic research, epidemiological and clinical data. Because fat catabolism, fatty acids, and ketone bodies lie at the core of fasting responses, the high circulating fat level may not have the pro-aging effects of the protein- and sugar endocrine axes. The longevity diet is supported by a recent study that used meta-analyses and data from the Global Burden of Disease 2019 project, which included studies from China, Europe, and

the United States. When starting at age 20, a sustained switch from the conventional Western diet to one high in legumes, whole grains, and nuts and low in red and processed meats is linked to increases in life expectancy of 10.7 years for women and 13 years for men, and more than 8 years when starting at age 60 (Fadnes et al., 2022).

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FRONT-OF-PACK NUTRITION LABELLING

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Summary

The vast majority of prepackaged foods have a nutritional declaration, which is often found on the back of the packaging and is not easy accessible for the average consumer. According labelling regulations, basic nutritional information can be repeated and additionally supplemented in the main visual field (front-of-pack, FOP). Several different types of front-of-pack nutrition labeling have been proposed, however the different approaches and formats used can further confuse consumers and obstruct the purpose of such schemes. By the end of 2022, the European Commission was supposed to come up with harmonized nutrition labels for food products that would be mandatory in all member states to make it easier for consumers to choose healthy and sustainable food based on reliable information. Because there are big differences among Member States in relation of on front-of-pack labeling systems, the European Commission needs more time for additional regulation of nutrition labelling than it planned. The aim of this work is to provide an overview of the regulations the and current situation to the topic of front-of-pack nutrition food labeling within the member states of European union and plans for next period.

Keywords: food labelling, nutrional lebelling, front-of-pack labelling, food regulation

Introduction

The nutritional information on the back of the packaging of food products is not easy for everyone to understand. For this reason, product labeling was developed on the front of the packaging, where consumers can see essential information about nutritional values at a glance. There is a lot of scientific evidence that shows that FOP systems can increase consumer understanding and encourage them to eat healthier. In Chile, the government requires nutrient warnings. After the introduction of FOP, the share of food and beverages requiring at least one label that the product has a high proportion of unwanted nutrients decreased from 51% to 44% (CSPI, 2023).

According to the current rules of the EU- a, it is not mandatory to provide information on the nutritional value on the front of the package, but subjects in the food business can provide it voluntarily under certain conditions. The European Commission has announced a mandatory harmonized system of nutritional labeling on the front of the packaging at the EU level as part of the Farm to Fork action plan (EFSA, 2022).

There is evidence that food companies selectively label healthier foods while omitting them from less healthy foods, using them solely for marketing purposes rather than promoting healthier eatin (CSPI, 2023; Ganderats-Fuemtes et al., 2023).

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Regulations regulating

Nutritional labeling in European union is defined by Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers (Regulation (EU). In addition to defining mandatory food nutrition information, the regulation also provides for the option to re-list the basic elements in the main visual field (front-of-pack, FOP) to help consumers easily spot key nutritional information when purchasing food. The European Commission (EC) requested EFSA's Sscientific adevice about possibilities to develop a future EU-wide system for front-of-pack nutrition labelling and conditions for restricting nutrition and health claims on foods. EFSA's scientific opinion was published in the EFSA Journal on April 19, 2022 (EFSA, 2022). A Joint Research Centre (JRC) study on front-of-pack nutrition labeling showed that consumers view front-of-pack nutrition labels as a quick and easy way to obtain nutritional information when making purchasing decisions, but also prefer simple, colorful, and evaluative nutrition information with a summary on the front of the package, which is easier to understand than more complex, single-color labels without evaluation (Thogersen et al., 2022). It is believed that labeling nutritional values on the front of the package encourages food companies to improve the nutritional quality of their products, for example by reducing added salt or sugar, and to obtain a more favorable rating from consumers (EC, 2020; Thogersen et al., 2022). 71% of respondents in a dutch consumer survey and 78% in a german consumer survey consider front-of-pack labeling useful (EP, 2023). Although FOP labeling plays an important role in making healthy food choices for consumers and encourages food manufacturers to reformulate their products and move towards healthier products, the different approaches and formats used in front-of-pack labels can further confuse consumers and defeat the purpose of such schemes Figure 1 (EUFIC, 2022).

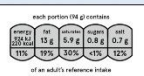
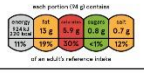
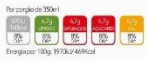
Examples of FOP schemes		The main characteristics of the system	EU Member State
	Reference Intakes label - numerical	The label provides numerical information on how much energy and nutrients are present in a portion of a food and how much this represents as a percentage of the daily reference intake	Across the EU
	Colour codes - Traffic light	The system provides information on the content of fat, saturated fat, sugars and salt, and the energy value by serving or portion of the food. Colours are used to classify those nutrients as 'low' (green), 'medium' (amber) or 'high' (red); colour thresholds are based on 100 g/ml of food/drinks . For products sold in large portions, portion thresholds apply for the red colour.	UK
	Colour codes	FOP nutrition label - based on a traffic light format that adds colors to the Reference Intakes label.	PT; ES
	Nutri-Score-graded indicators	The label is represented by a scale of five colours, from dark green indicating food products with the highest nutritional quality to dark orange for products with lower nutritional quality, associated with letters from A to E. The algorithm to calculate the nutritional score considers both negative (sugars, saturated fats, salt and calories) and positive elements (protein, fibre, fruits, vegetables, legumes and nuts).	FR, BE, ES, DE, NL, LU
	Keyhole logo – positive logos	The Keyhole is a voluntary free-of-charge that identifies the healthier choice within 33 defined food groups based on nutritional criteria such as the level of fat, sugars, salt, wholegrain or fibre. The logo cannot be used on products that have a low nutritional value, such as salted snacks or soft drinks.	SE, DK, LT

Figure 1. Different FOP nutritional schemes used in different European countries

Around 270 scientists signed a petition in March 2021 proposing that Nutri-Score become a single form of FOP labeling for the entire European Community, but this was not accepted because several EU countries were against it such as Italy, Cyprus, Greece, the Czech Republic, Romania, and Hungary (DJ, 2023). It was pointed out that the system should "take into account the specificities of the food culture of each member state, typical diets and national dietary guidelines" (EP, 2023). The European Public Health Association (EUPHA) expressed in March 2023 its concerns regarding the delays of revisions of Regulation 1169/2011 on food information to consumers. The deadline that the Commission initially announced has passed and there was no official indication of when its proposal will be published (Figure 2) (EUPHA, 2023).

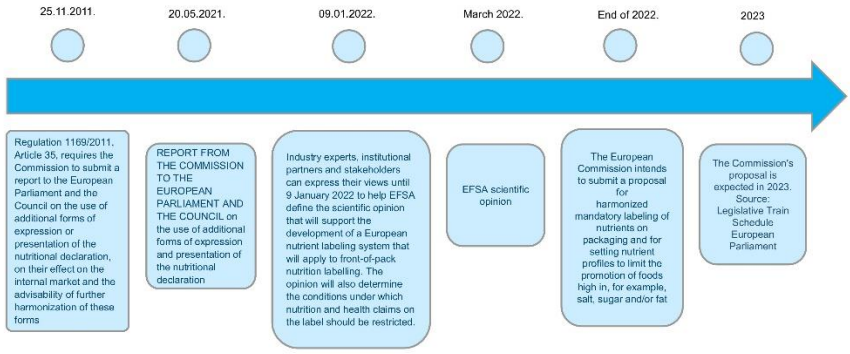


Figure 2. Additional forms of nutritional labelling EU -EFSA

More than half of adults in the EU are overweight or obese, and unhealthy diets lead to an increase in chronic diseases such as cancer, cardiovascular disease, type 2 diabetes, hypertension, and coronary heart disease (EC, 2020). Although the Commission announced that the proposed mandatory harmonized front-of-pack nutrition labeling system would be adopted by the end of 2022, this has not happened, so the new FOP labeling system is expected to be adopted by the end of 2023 (EC; EP, 2023).

However, some scientists believe that it is necessary to reopen the debate on the appearance of the label on the front of the package, and to adopt a multidisciplinary approach that would include the choice of food depending on the environmental impact (Muzzioli et al., 2023).

It is known that the Mediterranean diet is part of a healthy diet and it has a small impact on the main indicators of environmental impact (Serra-Majem et al., 2020)

A recent study showed the importance of including frequency and/or serving size in nutrient profiling algorithms that showed better correlation with the main indicators of environmental impact than algorithms that were developed on the basis of 100 g as a reference standard (Muzzioli et al., 2023)

Conclusion

The mandatory nutrition declaration on prepackaged foods is often found on the back of the package and is not easily accessible to the average consumer. Front-of-pack (FOP) labeling can help consumers make health-conscious decisions and help prevent diet-related illnesses. Also, they can encourage food manufacturers to reformulate their products and get better quality of their products. However, the different approaches and formats used in front-of-pack labels can further confuse consumers and defeat the purpose of such schemes. The European Commission will come up with harmonized nutrition FOP labels during 2023 that would be mandatory in all member states, and which would help consumers make healthier food choices. As food consumption contributes a significant share to the overall impact of greenhouse gases, it would be desirable when making decisions about FOP labels for healthy eating to think about communicating sustainable nutrition, which would achieve two goals with one label. But additional discussion would likely extend the already overdue time for adoption of a harmonized nutrition FON labels.

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ENERGY AND NUTRITIONAL VALUE OF DRIED MEAT PRODUCTS AT SANSKI MOST

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professional paper

Summary

Meat is a highly valued food product for human consumption because it is a good source of energy, fat, protein, essential amino acids, vitamins and minerals. Cured meat products mean products obtained by processes that include salting or brining, and drying or heat treatment with or without smoking beef, sheep, goats, pigs and ungulates. The research was carried out in the laboratory for testing chemical and biological residues and food quality at the Veterinary Faculty of the University of Sarajevo. The established energy values of samples of beef prosciutto (4.19 kJ/100 g), dried sausage (sudžuk) was 4.16 kJ/100 g) and dry lamb meat "stelja" 4.14 kJ/100 g). It can be said that the difference in energy value was not significant between the samples. Looking at the aggregate results, the chemical composition varied among the samples, so the highest moisture values were the sample of dry meat (52.05%), dry lamb meat "stelja" (35%) and dried sausage (38.95%). The same happened with dry matter, the highest value was the sample of dry lamb meat "stelja" (65%), followed by the sample of dried sausage (61%) and finally the sample of dry meat (47.95%). The total protein values ranged from: dry meat 32.25%, dried sausage 39.88% and finally dry lamb meat "stelja" 21.85%. The percentage of lipids (fats) in the analyzed samples was 61% in dried sausage, 38.11% in dry lamb meat "stelja", and 10% in the dry meat sample. The results obtained in our work on the nutritional values of the analyzed dried meat products match or coincide with the data used in the literature for this researched area. The aim of this research was to determine the energy-nutritional value of Bosnian dried sausage, dry lamb meat "stelja", and prosciutto (dried meat) in the area of Sanski Most municipality.

Keywords: dried meat, nutritional value, fermented and dried sausages, dry lamb meat "stelja"

Introduction

Natural processed meats have been a very significant part of the market growth that is occurring in natural and organic foods. Producers and processors have responded to consumer demand for foods perceived by many to be healthier and more wholesome than conventionally

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produced food products (Sebranek and Bacus, 2007). The production of dried meat products in Bosnia and Herzegovina has a long tradition, especially when it comes to Bosnian beef prosciutto, one of the indigenous products. The production of beef prosciutto was most often related to the individual needs of rural households, and only small quantities were brought to the markets. The industrial development of Bosnia and Herzegovina leads to the formation of a market, and thus to a greater demand for traditional products (Toroman et al., 2013; Ganić et al., 2013). However, due to the properties of fresh meat such as relatively high-water activity (aw), slightly acidic pH and the availability of carbohydrates (glycogen) and proteins, it becomes a good substrate for the growth of microorganisms and is considered a highly perishable product (Operta et al., 2008).

Long-life dried meat products are thermally unprocessed, dried with or without smoke and are marketed as prosciutto, dry lamb and goat meat "stelja", Dalmatian prosciutto, Bosnian prosciutto, Bosnian sudžuk and others. In traffic, they must meet the following conditions: that they have a light or dark brown color of smoked meat, that their surface is free of cuts and properly processed, that they have a pleasant taste and smell of smoked or smoked meat. During the production of dry lamb meat "stelja", the shoulders are first separated from the carcasses by cutting the natural muscle (synsarcoses) connection with the carcass. Then the carcass is completely deboned. The carcasses were deboned by first cutting all the ribs on one and the other side in the region of the spinal column, after which the ribs were separated, and the intercostal musculature was left as part of the dry lamb meat "stelja". The sternum (sternum) was also separated from the carcasses with the ribs (Petrović et al., 1977).

According to the type and category of meat from which they are produced, and according to the method of technological processing and sustainability, the products are marketed as permanent and semi-permanent. The most important criterion for the aforementioned classification is thermal treatment in the technological production process (Operta et al., 2008).

The aim of this research was to determine the energy-nutritional value of Bosnian sudžuk, dry lamb meat "stelja", and prosciutto in the area of the municipality of Sanski Most.

Material and methods

The practical part of the research was carried out in the laboratory for testing chemical and biological residues and food quality at the Veterinary Faculty of the University of Sarajevo.

During the examination of the nutritional and energy values of cured meat products from the municipality of Sanski Most, 400 grams of beef prosciutto, 400 grams of Bosnian sudžuk and 400 grams of dry lamb meat "stelja", were used.

Sample analysis included: physical-chemical analysis, protein content, fat content, carbohydrate content, energy value of samples.

Determination of fat content was done in accordance with BAS ISO 1443 Soxhlet extraction method.

Determination of protein content was done in accordance with BAS ISO 936:2007 Kjeldahl extraction method.
Determination of moisture content (%) was done in accordance with BAS ISO 1442.
Sample preparation – The thin metalic dish is dried for 30 minutes in an oven set at 103 °C, then cooled in a desiccator to room temperature and weighed with an accuracy of 0.001 g. Part of the test sample 5 g to 8 g of the prepared test sample is transferred into the prepared container and measured with the content with an accuracy of 0.001. Determination – The container with the content is heated for 2 hours in an oven set at 103 °C, and then take it out and put it in a desiccator. The container and its contents are left to cool to room temperature and then weighed to the nearest 0.001 g. The heating, cooling and measurement operations are repeated until the result of 2 consecutive measurements separated by heating for 1 hour differ by more than 0.1% of the mass of the test sample part.
The values of carbohydrate (%) were obtained by subtracting the total percentage water, mineral, protein, and fat from hundred. This is known as carbohydrate by difference and is used because no satisfactory method exists for determining carbohydrate by direct analysis (Helen, 1979).
After homogenization of the samples, the mass fraction of salt (%) was determined in the samples using the Mohr method. Based on the amount of meat product sample taken in the analytical procedure and the volume of AgNO3 solution used for titration, the mass fraction of sodium chloride NaCl (%) in the analyzed sample is calculated.
After obtaining the results on fat and protein content, the energy value of the analyzed samples was calculated in kcal or kJ according to the following formula: Energy value = fat x fat coefficient (9.3) + protein x protein coefficient (4.1).

Results and discussion

As shown in Table 1, the total dry matter value in the sample was 47.95%. The percentage of water in the sample was 52.05%. Of the nutrients in the examined sample, proteins had the highest value in the amount of 32.25%. A slightly lower value was recorded in the fat content and amounted to 10.00%. The percentage of carbohydrates was 0.20%. The energy value of 100 g of beef prosciutto was 4.19 kJ.

Table 1. Analysis of the nutritional value of dried meat

Parameter	Obtained value (%)	Reference value	Methods
Water	52,05	Max.60	VI-HBR-SOP-12
Dry matter	47,95	NP*	Calculated
Fat	10,00	NP*	VI-HBR-SOP-08*
Total protein	32,25	NP*	VI-HBR-SOP-10*
Carbohydrates	0,20	NP*	Calculated
Salt content (NaCl)	4,95	NP*	BAS ISO 1841-1

*No Parameters

As can be seen from Table 2, the percentage share of dry matter in the sample of Bosnian sudžuk was 61.05%. The percentage of water in the analyzed sample of Bosnian sudžuk was 38.95%. Of the nutrients, fats had the highest value in the sample, the estimated share of which was 61.05%. In this sample, the proportion of protein was 29.88%. The relative share of carbohydrates in the sample of Bosnian sudžuk was 3.63%. The analyzed sample of 100 grams of Bosnian sudžuk had an energy value of 4.16 kJ.

Table 2. Analysis of the nutritional value of Bosnian sudžuk

Parameter	Obtained value (%)	Reference value	Methods
Water	38,95	Max.40	VI-HBR-SOP-12
Dry matter	61,05	NP*	Calculated
Fat	61,05	NP*	VI-HBR-SOP-08*
Total protein	29,88	Min.16	VI-HBR-SOP-10*
Carbohydrates	3,63	NP*	Calculated
Salt content (NaCl)	3,98	NP*	BAS ISO 1841-1

*No Parameters

As it shown in the Table 3, the percentage of dry matter in the analyzed sample of dry lamb meat "stelja" was 65%, and the proportion of water was 35%. Of the nutrients, fats had the highest value in the sample with an amount of 38.11%, while the percentage of proteins was much lower and amounted to 21.85%. The proportion of carbohydrates in the analyzed sample was not registered.

Table 3. Analysis of the nutritional value of dry lamb meat "stelja"

Parameter	Obtained value (%)	Reference value	Methods
Water	35,00	Max.40	VI-HBR-SOP-12
Dry matter	65,00	NP*	Calculated
Fat	38,11	NP*	VI-HBR-SOP-08*
Total protein	21,85	Min.16	VI-HBR-SOP-10*
Carbohydrates	0,00	NP*	Calculated
Salt content (NaCl)	4,40	NP*	BAS ISO 1841-1

*No Parameters

The results obtained in our work on the nutritional values of the analyzed dried meat products match or fit with the data known from the literature for this scientific research area: Jašić et al. (2018), Operta et al. (2008), Čaušević et al. (1989), Soyer et al. (2005), Papadima et al. (1999), Siriken et al. (2009), Comi et al. (2005), Babiker and Abuelfatah (2018).

According to literature Soyer et al. (2005) the amount of moisture in the research for dried meat products obtained from beef, goat meat or lamb in the wet base was 69.29%,

74.02% and 66.71% while the amount of moisture in the dry base was 2.26%, 2.86% and 2.00% for cow meat, goat meat and sheep meat respectively.

Conclusions

After the physico-chemical analysis, it was shown that Bosnian sudžuk contains a significantly higher percentage of fat compared to dry lamb meat "stelja" and prosciutto, and thus has a higher caloric and energy value compared to other analyzed products. The energy value of the analyzed samples confirmed that the energy value of dry lamb meat "stelja" was 4.14 kJ, beef prosciutto 4.19 kJ, and Bosnian sudžuk 4.16 kJ. After summarizing the physico-chemical characteristics of sudžuk, dry lamb meat "stelja" and beef prosciutto, we obtained results that confirm that the tested samples meet all the norms set by the rules and guidelines on the quality and hygiene of foodstuffs, and that the analyzed samples from the area of the municipality of Sanski Most have no significant deviations from literature data and that the mentioned dried meat products are safe for human consumption.

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