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***NUTRITION* /**
NUTRICIONIZAM

MATERNAL DIETARY HABITS AND FOOD RESTRICTIONS DURING BREASTFEEDING

Nevena Ćorić[1,2*], Nevena Pandža[1], Ivona Bevanda[1], Andrea Karlović[2]

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ABSTRACT

Appropriate dietary intake during post-partum is of a particular importance since it strongly determines the health status of mother and infant. Many countries have developed national guidelines for a diet while breastfeeding. However, cultural tradition has a significant effect on lactation behavior. Popular myths about maternal diet during breastfeeding can become barriers to breastfeeding and lead to unnecessary dietary restrictions. So, the aim of this study was to examine the dietary habits of women during breastfeeding, self-food restrictions as well as increased consumption of some food. This study enrolled 148 voluntary breastfeeding mothers from Bosnia and Herzegovina. The data were obtained through a specially designed anonymous online questionnaire. Postpartum weight retention (PPWR) was calculated as difference between the postpartum weight at the time of completing the survey and the pre-pregnancy weight. The weight of breastfeeding women at postpartum was higher for 3,48 (95% CI, 2.70 to 4.26) kg compared to their pre-pregnancy weight [$t(147)=8.806$ $p<0.001$]. According to the BMI category, 12.2% of women were in pre-obese and 4.7% in the obese category before conception, compared to the postpartum time (24.3% and 6.1% respectively). Results from the study showed that 35.8% of women were taking supplements during breastfeeding, and 5.4% of all participating women were on some dietetic regimen. Only 28.4% did not avoid specific food or food groups, while others avoided one or more types of food during breastfeeding. Commonly restricted foods were citrus, vegetables from the Brassicaceae family, legumes, garlic, onion, and dairy products. Nursing mothers should be educated on proper diet practices while being warned about unscientific approaches to diet restriction, as well as dietetic regimens by non-professionals.

Keywords: lactating women, post-partum weight retention, dietary habits, food restrictions

INTRODUCTION

According to the guidelines of the World Health Organization (WHO) exclusive feeding with breast milk should be applied until the end of 6 months of age and should be continued during subsequent months with the simultaneous introduction of supplementary food (WHO, 2001). Breast milk is the best food for newborns and infants. Its nutrients come from the mother's diet or her own nutrient reserves (Segura et al., 2016). So appropriate dietary intake during post-partum is of a particular importance since it strongly determines the health status of mother and infant.

However, cultural tradition has a significant effect on lactation behavior where many cultures have some recommendations during lactation. Popular myths about maternal diet during breastfeeding can become barriers to breastfeeding and lead to unnecessary dietary restrictions (Karcz et al., 2021, Jeong et al. 2017). In Bosnia and Herzegovina, it is a common opinion that lactating women should not eat citrus, legumes and should increase fluid intake, especially sweet juices as they could increase breast milk production. If the mother has healthy eating habits, there is usually no reason to change them during breastfeeding. Although there are some nutritional guidelines for breastfeeding women, an evidence-based recommendation about food restriction during this period is still limited (Jeong et al., 2017).

Nutritional requirements increase during lactation mainly because of the loss of nutrients, first through colostrum and then through breastmilk, but also to support infant growth and development (Segura et al., 2016). Variations in mothers' diet may result in changes in the fatty acid profile and levels of some micronutrients, but they are not correlated with the volume or quality of the milk produced (Segura et al., 2016). Even from malnourished mothers, milk has an excellent nutritional and immunological quality. The mother's body always prioritizes the needs of the baby, and therefore most nutrients, such as iron, zinc, folate, calcium, and copper continue to be excreted in breast milk in adequate and constant amounts, at the expense of maternal stores. So, if a woman does not obtain sufficient nutrients from diet or supplements, she may be at risk of some nutritional deficiency (Segura et al. 2016).

Concern about growing obesity in many countries has directed increased attention to whether lactation can contribute to reducing the amount of body fat deposited during pregnancy (Kulakac et al., 2007). If more rapid weight loss is desired, a combination of proper diet and exercise should be combined (Bertz et al., 2012; Segura et al., 2016; Dewey and McCrory, 1994).

MATERIALS AND METHODS

This study enrolled 148 voluntary breastfeeding mothers from Bosnia and Herzegovina, aged 22 to 45 years (31.0 ± 4.1). The survey included mothers who had babies 1 week to 6 months old. The data were obtained through anonymous online questionnaire composed of 35 questions, designed by authors. Questionnaire included questions regarding general information such as maternal age, educational

attainment, number of children. Second section included questions about selfreported body height, body weight before gestation and in the time of completing the survey. Third section was questions about their food intake and presence of some conditions and complications during pregnancy (gestational diabetes mellitus (GDM), preeclampsia) as also question about commonly restricted foods and food groups during breastfeeding. A personalized message with a link to the online survey was sent to lactating women who delivered in University Hospital of Mostar but also to mothers who were members of Facebook groups for breastfeeding mothers in Bosnia and Herzegovina. The online survey format was easily accessible, time-saving for new mothers, and cost-effective. During this survey, the emails of respondents were not collected to keep the identities of the participants anonymous. We are aware that would be more relevant that Food frequency questionnaire (FFQ) or some other dietetic method was used in this survey and this is exactly what we consider a shortcoming for this research. A used questionnaire did not obtain information on the size of the consumed portion. However we consider that this paper presents a good insight into eating habits of breastfeeding mother, which has not been conducted in the mentioned area so far.

General questions were added to the questionnaire in order to gain a better insight into the eating habits of the examined population. These are issues such as the number of main meals and snacks, way of food preparation as well as the question of what type of fat they use most often.

The obtained data were compiled using the Google Forms platform and analyzed using IBM SPSS for Windows, version 25 (IBM Corp., Armonk, NY, USA). Absolute and relative frequencies were calculated for data description. Normality of distribution was tested using Shapiro-Wilk tests, and all variables were confirmed to be normally distributed. Within group weight and BMI, variations were analyzed by *t*-test for paired samples. Results were significant for p-value < 0.05. Since multiple response questions were present in the survey, frequencies and percentages for these questions were taken directly from the Google Forms survey.

RESULTS AND DISCUSSION

General characteristics and anthropometrics of breastfeeding mothers are shown in Table 1 and Table 2.

The weight of breastfeeding women at postpartum was higher for 3,48 (95% CI, 2.70 to 4.26) kg compared to their pre-pregnancy weight [$t(147)=8.806$ $p<0.001$]. Post-partum weight retention (PPWR) is important contributor to the risk for obesity one year postpartum, as also risk factor for gestational diabetes mellitus in second pregnancy (Liu et al., 2019; Endres et al., 2015). Postpartum weight retention (PPWR) was calculated as difference between the postpartum weight at the time of completing the survey and the pre-pregnancy weight. Mean of PPWR was 3.48 kg (Table 2). The average weight gain while pregnant for mothers in this study was 13.7±6.1, while mean of postpartum weight was 70.58 kg (BMI 24.08 kg/m^2) (Table 2). The recommended amount of pregnancy weight gain varies for everyone and strongly depends on pre-pregnancy weight and body mass index (BMI). Pregnant

women with overweight or obesity are generally recommended to gain less weight than women with underweight. The minimum recommended weight gain is between 5 and 8 kg, which accounts for the fetus, the placenta, amniotic fluid volume, and adaptations to maternal tissues (eg, uterus, breast, blood volume) (Rasmussen and Yaktine, 2009). A weight gain less than this amount implies that existing maternal adipose and protein stores would be mobilized to support the pregnancy. The maximum recommended weight gain during pregnancy is 18 kg (Kominiarek and Rajan, 2016; EUFIC, 2021). Gaining much more weight than the recommended amount can increase the risk of complications during pregnancy and delivery as well as increase the baby's risk of high birth weight (Moll et al., 2017). Being underweight or small weight gain during pregnancy may also increase some health risks in pregnancy, such as miscarriage, premature (preterm) birth, or delivering a baby with low birth weight (EUFIC, 2021).

Table 1. General characteristics of breastfeeding mother

Variable	Number of respondents n (%)
Age	
<20	0
20-29	59 (39.9)
30-39	84 (56.8)
≥40	5 (3.4)
Education Level	
Primary school	0
Secondary school	26 (24.3)
Non-university degree	13 (8.8)
University degree	92 (62.2)
PhD and other	7 (4.7)
Number of children	
1	81 (54.7)
2	43 (29.1)
≥3	24 (16.2)

Table 2. Basic anthropometrics of breastfeeding mothers

	N	Mean ± SD	Min	Max
Age (years)	148	31.0 ± 4,1	22.0	45.0
Height (cm)	148	171.16	158.0	187.0
Weight prior to pregnancy (kg)	148	67.10 ± 10.03	45.0	124.0
BMI prior to pregnancy (kg/m^2)	148	22.89 ± 3.16	16.26	40.49
Post-partum weight retention (PPWR) (kg)	148	3.48 ± 4.81	-9.0	25.0
Weight postpartum (kg)	148	70.58 ± 11.54	47.0	120.0
BMI postpartum (kg/m^2)	148	24.08 ± 3.72	16.26	39.18

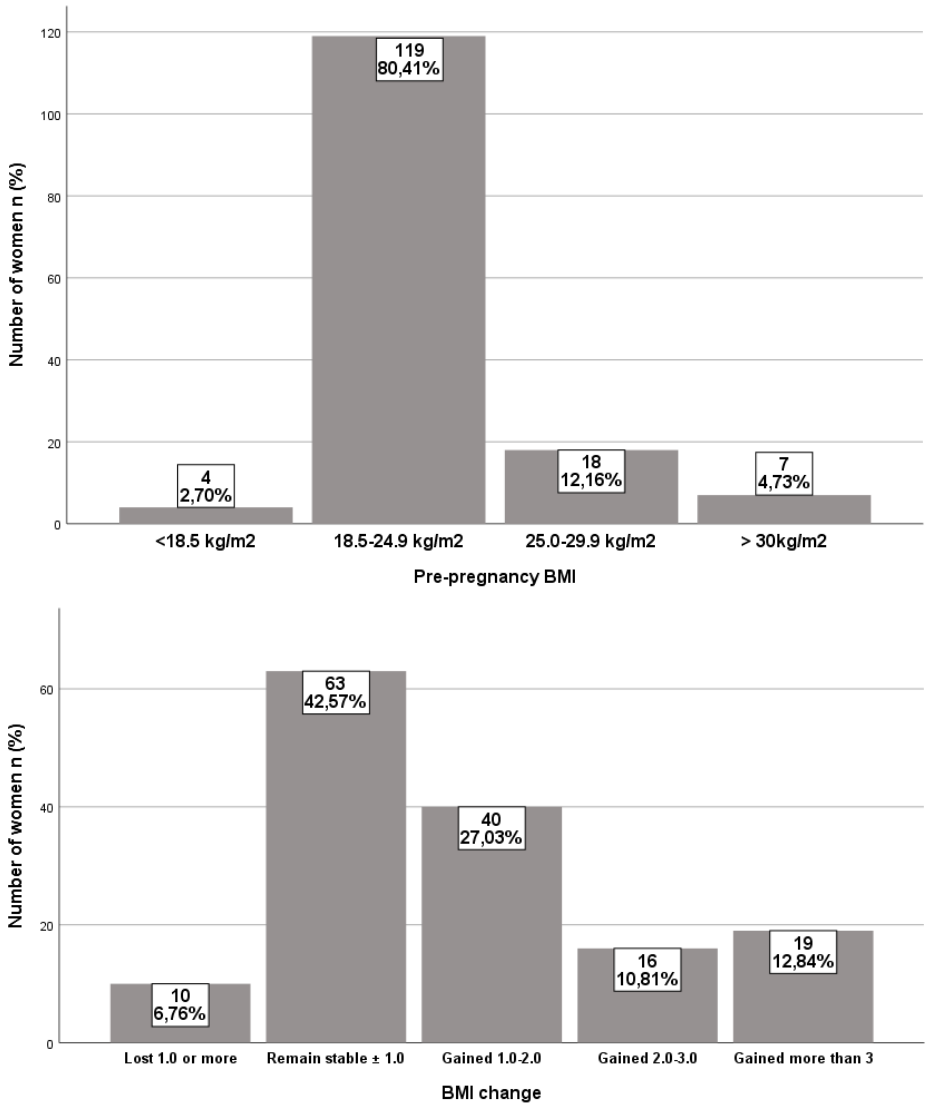

Figure 1. Pre-pregnancy BMI and BMI change calculated as as the difference between post-partum BMI and pre-pregnancy BMI

According to the BMI category, 12.2% of women were pre-obesity (BMI, 25–29.9) and 4.7% in the obesity category (BMI > 29.9) before conception (Figure 1). BMI change was calculated as the difference between post-partum BMI and pre-pregnancy BMI. We categoriezed BMI change as less than -1 (a lost in BMI more than 1 unit), -1 to less than 1, 1 to less than 2, 2 to less than 3, and 3 or more BMI units, based on system which has been used by Liu et al. (2019). A systematic review of Torloni et al. (2009) stated that pre-obese (BMI, 25–29.9), and obese I

women (BMI, 30–34.9) had an overall risk for preterm birth <37 weeks similar to women with normal BMI. Obese II women (BMI, 35–40) have an increased risk for preterm birth <37 weeks, moderate preterm (32–36 weeks) and very preterm (<32 weeks) birth, while the risk for preterm birth in women with grade III obesity almost doubled when compared to normal-weight women. According to the American Dietetic Association, pregnant women are considered to be at high risk if they have obesity, diabetes, or personal history of GDM or gestational hypertension (Kaiser and Allen, 2008).

Results from our study show 13.5% of women had some condition (GDM or preeclampsia) or other undefined complication during pregnancy.

During breastfeeding, 35.8% of surveyed mothers were taking one of the supplements: iron, folate, vitamin D. A systematic review of interventional studies (Keikha et al., 2021) suggest that maternal dietary vitamin and/or mineral supplementation, particularly fat-soluble vitamins, vitamin B1, B2, and C might be reflected in the breast milk composition. Continued use of a prenatal vitamin postpartum may exceed the iron and folic acid needs of a breastfeeding mother (CDC, 2021). However, some women such as vegans and vegetarians, and also others whose diet alone may not be sufficient to ensure adequate nutrition, may benefit from taking supplements (CDC, 2021). Vitamin D deficiency is fairly frequent in pregnant and lactating women (Segura et al., 2016). So, mothers that have a restricted diet, or limited exposure to UV radiation may have low plasma levels of this vitamin. Also, the transfer of this vitamin to mothers' milk is relatively poor, even if mothers take a supplement of Vitamin D in concentrations ranging from 400 to 2000 IU, their infants should be also supplemented with vitamin D until the first year of life (NIH, 2021).

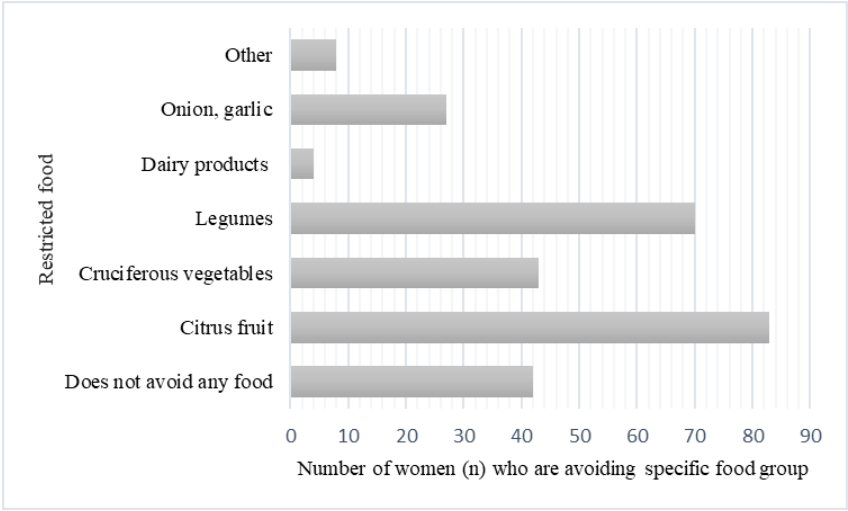

Figure 2. Commonly restricted food during breastfeeding

Of all participating women, 5.4% (n=8) were on some dietetic regimen, of which 6 were on a self-determined diet while for one, the diet was determined by a fitness trainer and for one by a specialist. Only 28.4% (n=42) did not avoid specific food or food group, while others (71,6%, n=106) avoided one or more types of food during breastfeeding. Commonly restricted food were citrus fruits, vegetables from the Brassicaceae family, legumes, garlic, onion, and dairy products (Figure 2). In many cultures, some foods are indicated as causing infant colic (Kulakac et al. 2007). One of them is legumes, so many breastfeeding mothers avoid this food group, which is common with these results also. Santos-Torres and Va´squez-Garibay (2003) found legumes to be the second most-avoided nutrient after vegetables among nursing mothers in Mexico. This could be very concerning since legumes and beans present an important source of plant protein as also dietary fibers. Karz et al. (2021) stated that there is no clear evidence regarding the association of dairy products, chocolate, vegetables from the Brassicaceae family, and legumes with colic symptoms, and therefore breastfeeding mothers should not be advised to exclude these products precautionary. As a result of advice to eliminate certain foods or entire food group, women can adapt to extremely restrictive diets, or stop breastfeeding entirely, out of concern that the food they eat would make their baby cry (Kidd et al. 2019).

Conover and Buehler (2004) determined that the smell of garlic passes on to mother's milk. So when mothers consume such food, the fetus and infant's early experience with smell and taste naturally determine the infant's reactions to the mother's milk. Believing that citrus fruit can make mothers' milk rancid and cause gastric illness in babies could be a reason for avoiding this food group (Kulakac et al., 2007).

Although dairy products were cited by mothers as restricted food, from Table 3 it is visible that 72,3% of mothers consume these products once a day. Table 3 summarizes the results of the frequency of consumption of different food groups. Vegetables and fruits are mostly consumed once a day. Red meat 3 to 4 times per week and poultry 1 to 2 times per week. Interesting but disappointing results are for fish and whole cereals, where 12,8% and 19,6% of women respectively, never consume these food groups. Fish is an important source of proteins and calcium and regular consumption of two servings of fish per week enables an adequate docosahexaenoic acid (DHA) content in breast milk to be reached and provides the infant with a proper amount of polyunsaturated fatty acids (Karcz et al., 2020) It is also a good source of iron and vitamin D which are frequently supplemented by mothers in this research. It is recommended to choose species that are less likely to accumulate mercury, such as the small and non-predatory varieties (Kominiarek and Rajan, 2016).

Table 3. Consumption frequency of different food groups by lactating mothers in % (n)

	2 and more times /day	1 time /day	3-4 times /week	1-2 times /week	1-2 times /month	Never
	Meal frequency % (n)					
Vegetables	12.2 (18)	48.6 (72)	27.7 (41)	10.8 (16)	0.7 (1)	-
Fruits	23 (34)	39.9 (59)	16.9 (25)	17.6 (26)	1.4 (2)	1.4 (2)
Legumes	-	-	-	43.2 (64)	38.5 (57)	18.2 (27)
Milk and dairy products	-	72.3 (107)	16.2 (24)	6.1 (9)	2.7 (4)	2.7 (4)
Red meat	-	13.5 (20)	48.0 (71)	35.1 (52)	2.0 (3)	1.4 (2)
Poultry	-	2.7 (4)	31.1 (46)	58.8 (87)	6.8 (10)	0.7 (1)
Fish	-	-	0.7 (1)	30.4 (45)	56.1 (83)	12.8 (19)
Whole cereals	-	23.0 (34)	17.6 (26)	21.6 (32)	18.2 (27)	19.6 (29)
Eggs	-	13.5 (20)	34.5 (51)	40.5 (60)	7.4 (11)	4.1 (6)

Also, we investigate the type of oil or fat that breastfeeding mothers use the most in their diet. Refined sunflower oil is the most consumed, followed by olive oil, butter, and in the last place animal fat such as pork and beef fat.

Statistically, a significant difference was not determined between higher consumption of some food groups and BMI, nor between the level of educational attainment and food restriction.

Water, cumin and fennel tea, fenugreek tea, chicken or baby beef soup, cow milk, chocolate, fruit compote, sweet juices, and non-alcoholic beer were most commonly used food of increased intake by 25,7% of lactating women. As it could be seen, a common opinion and method to increase milk supply are to increase fluid intake. Besides the above-mentioned fluids (tea, fruit compote), most women consume 1 to 2 L of clean water per day (Figure 3).

This is in accordance with research by Kulakac et al. (2007) on Turkish employed mothers. Dewey and McCrory (1994) pointed out that increasing fluid intake does not have any effect on milk volume. A pilot study of Morse et al. (1992) on 10 mothers concluded that although milk supply decreased with decreased fluids and increased with increased fluids, this change was not statistically significant, but as it could be seen, the mentioned research was on a very small number of participants. There is not enough evidence to support the theory of increased fluid intake of breastfeeding mothers beyond what they are likely to require to meet their physiological needs (Ndikom et al., 2010).

Fenugreek tea as well as cumin and fennel tea are considered to be herbal galactagogues. Those are medications used to promote breast milk production in lactating mothers. Fenugreek (lat. *Trigonella foenum-graecum*) is the most popular of the herbal galactagogues in the literature. Fenugreek and fennel have been used for centuries to improve breast milk supply. Fenugreek is often taken as a capsule, a

tea, or a sweetened paste, and fennel as a capsule or a tea (Jackson, 2010). Clinical evidence regarding the effectiveness of herbal galactagogues and there mechanism of action is still lacking (Jackson, 2010). Although it appears that fenugreek may have some benefit, randomized clinical trials are needed to determine its specific role as a galactagogue. Safety data are minimal since adverse events and tolerability were not reported. The Food and Drug Administration (FDA) lists fenugreek as generally regarded as safe (GRAS) but these data are not specifically aimed at nursing mothers and infants (Forinash et al., 2012).

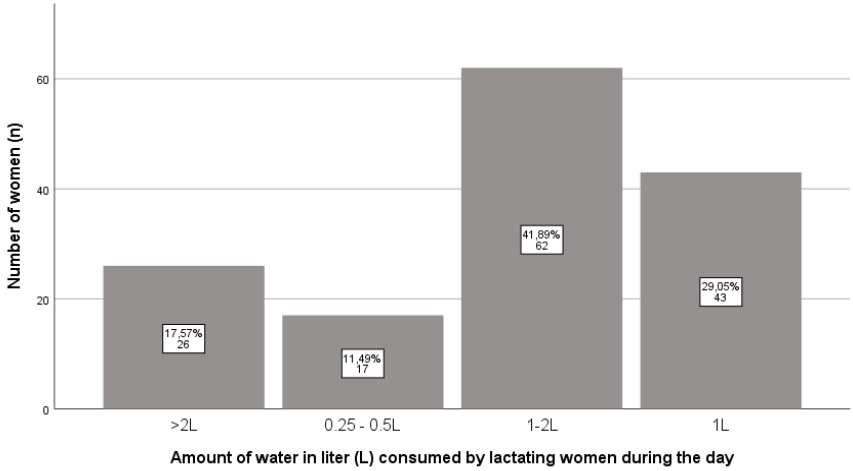

Figure 3. Daily water intake by lactating women

In Bosnia and Herzegovina, there is a popular opinion among new mothers about increased consumption of sweets during breastfeeding to stimulate milk production. That is supported by this research, where 17.6% of all mothers consume sweets more times in a day, 42.6% of them consume it once a day, while 36.5% state that they consume it "sometimes" and 3.4% "never". Similar results were also obtained in research by Kulakac et al. (2007). Since this is not scientifically proven, mothers should be encouraged to substitute sweets with healthier low caloric foods such as fruits, vegetables, root vegetables, cereals, dairy products. Also increased energy requirements during breastfeeding in a significant proportion could be met by fat stores that are increased in pregnancy (Kulakac et al. 2007).

In addition to general eating habits, in the questionnaire, some specific questions were included, such as "How often and what type of coffee do you drink during the day?", "Who usually prepares meals at home?", "Which is the most common way of preparing a meal?".

Mothers from this survey usually drink 1 to 2 coffees per day (n=81, 54.7%) and only 33 (22.3%) of all mothers, drink more than 2 coffees per day. The most commonly consumed type of coffee is the "Turkish" type of coffee (n=60, 40.5%) and instant (n=43, 29.1%).

Coffee is a common source of caffeine that is transferred to breast milk in less than 1% of the total amount consumed by the mother, and modest caffeine use does not negatively affect infants. With maternal consumption of up to three cups of coffee per day, no caffeine has been detected in an infant's urine so it is unlikely that they experience measurable exposure to caffeine (Jeong et al., 2017; Fisher et al., 2006, Berlin et al., 1984).

Breastfeeding mothers prepare food usually by themselves (n=120, 81.1%) and mostly with help of mother (n=24, 10.1%) and mothers in law (n=27, 18.2%). Cooking, baking, and simmering are the most common types of preparing the food, while only a small number of mothers prefer deep frying (n=19, 12.8%) and breading (n=9, 6.1%).

CONCLUSION

Nursing mothers should be educated on proper diet practices while being warned about unscientific approaches to diet restriction, as well as on dietetic regimens by non-professionals. An individualized approach to nutritional counseling that considers a woman's access to food, socioeconomic status, race-ethnicity, cultural food choices, and body mass index (BMI) is recommended. Generally, women do not need to limit or avoid specific foods while breastfeeding, except alcohol and raw animal products such as meat, eggs and sea products. Mothers should be encouraged to eat a healthy and diverse diet, which will help them return to a healthy weight, prevent problems in subsequent pregnancies, and reduce the risk of chronic diseases later in life.

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KURKUMA: ZAČIN ILI LIJEK ZA OSTEOARTRITIS?

CURCUMA: A SPICE OR MEDICINE FOR OSTEOARTHRITIS?

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SAŽETAK

Osteoartritis (OA) je multifaktorijalna degenerativna bolest s upalnom komponentom, čije su incidencija i prevalencija posljednjih desetljeća u značajnom porastu, te trenutno globalno pogađa oko 240 milijuna ljudi. Uzrok nije u potpunosti razjašnjen, lijek za sada ne postoji, a terapijske intervencije usmjerene su poglavito smanjenju simptoma te usporenju progresije destrukcije zgloba. Od terapijskih opcija koje su na raspolaganju najviše se propisuju nesteroidni antireumatici, koji su opterećeni su brojnim nuspojavama. Stoga se diferencira potreba za sigurnom, a djelotvornom tvari, koja će biti korištena u adjuvantnom liječenju, ali i prevenciji bolesti, a bit će bez ili sa što manje neželjenih učinaka. Jedna od takvih tvari je i kurkumin, hidrofobni polifenol koji čini aktivnu komponentu rizoma biljke Curcuma longa. Više studija pokazalo je njegovo jako antioksidativno i protuupalno djelovanje, uz netoksičnost i sigurnost primjene i pri visokim dnevnim dozama. Kurkumin osim što blokira apoptozu hondrocita, blokira i ekspresiju ciklooksigenaza, prostaglandina E-2 i proupalnih citokina u hondrocitima te tim mehanizmom potencijalno ublažava simptome upalne bolesti. Premda postoje velike varijacije kvalitete, metodologije i razultata do sada provedenih istraživanja, u ovom trenutku svoje mjesto u liječenju OA kurkumin prvenstveno nalazi kao sistemska kratkoročna i srednjeročna adjuvantna terapija u liječenju boli i smanjenju biokemijskih faktora upale. Navedeno u konačnici dovodi do bolje regulacije boli i poboljšanja funkcije zahvaćenog zgloba, što rezultira značajnom redukcijom standardno propisivanih doza lijekova koji sa sobom nose čitav niz nuspojava, a time i poboljšanjem kvalitete života pacijenta te stoga zaključujemo da postoji osnova za preporuku uzimanja kurkumina u terapijske svrhe. Potrebna su daljnja

istraživanja s ciljem utvrđivanja preventivne uloge kurkumina na razvoj OA, učinka dugoročne primjene kurkumina na prevenciju i/ili liječenje bolesti, te determiniranja optimalnih terapijskih doza.

Ključne riječi: kurkuma, kurkumin, ljekovite tvari, hrana kao lijek, osteoartritis

Keywords: curcuma, curcumin, medicinal substance, food as medicine, osteoarthritis

UVOD

Osteoartritis

Osteoartritis (OA) je kronična degenerativna bolest zglobova s upalnom komponentom čija su incidencija i prevalencija posljednjih desetljeća u značajnom porastu, što se pripisuje poglavito globalnom starenju populacije, kao i povećanju broja osoba s povećanom tjelesnom masom. Najzastupljeniji je tip artritisa koji može zahvatiti bilo koji zglob, a najčešće su aficirani mali zglobovi šaka, stopala i kralježnice, te zglobovi koljena i kuka. Procjenjuje se da bolest globalno pogađa oko 240 milijuna ljudi, što čini oko 12 % populacije, te je češći kod žena (Liu i sur., 2018). Premda je uzrok i dalje nepoznat, vremenom se diferencirao čitav niz rizičnih čimbenika za razvoj osteoartritisa. Zhang i sur. (2010) prihvaćaju podjelu patogenetskih rizičnih čimbenika OA koju u radu iz 2000. godine iznosi Felson, a koja ih dijeli na lokalne biomehaničke i sistemske. Kao sistemske čimbenike rizika navodi dob, spol, rasu, gustoću kosti, hormonalno nadomjesno liječenje te genetiku. Oni su ti koji sustav organa za kretanje čine podložnim za nastanak OA, ako na njega djeluju određeni lokalni biomehanički čimbenici, poglavito prekomjerna tjelesna masa, ozljeda ili deformitet zgloba, slabost mišića ili sindrom prenaprezanja. U konačnici, međuovisnost ovih čimbenika određuje lokalizaciju i težinu bolesti (Felson i sur., 2000; Zhang i sur., 2010; Martel-Palletier i sur., 2016).

Simptomi OA

Kao prvi i dominantan simptom javlja se bol, a osim nje, za ovaj tip zglobnih promjena karakteristična je ukočenost nakon spavanja ili mirovanja, koja tipično nestaje unutar pola sata od početka aktivnosti. Uz bol i ukočenost, u akutnoj fazi bolesti često je prisutna oteklina zahvaćenog zgloba, uz moguću prisutnost periartikularnog crvenila i topline kože. Napredovanjem patohistološkog supstrata, postupno dolazi do smanjenja pokretljivosti, uz često pucketanje ili škripanje („krepitacije") koje prati pokret, a rezultat je hipertrofije sinovije, širenja sinovijalnog zgloba i stvaranja koštanih izdanaka, poglavito u distalnim zglobovima šake. Posljedično istezanju i slabljenju aktivnih i pasivnih stabilizatora zgloba, primarno ligamenata i okolnih mišića, zglob postaje nestabilan, uz često 'klecanje' istog. Ako su zahvaćeni zglobovi kralježnice, osim boli, zbog kompresije korjenova moždinskih živaca i krvnih žila javljaju se simtomi u vidu mravinjanja, mišićne slabosti ekstremiteta, vrtoglavice, mučnine, povraćanja i smetnji vida. Simptomi se

nerijetko pogoršavaju prilikom izloženosti određenim vremenskim uvjetima, primjerice hladnoći ili povećanoj vlažnosti zraka.
Neliječene osteoartritične promjene zgloba rezultiraju poremećenom funkcijom istog. Bol koja ograničava aktivnosti svakodnevnog života, dovodi pacijenta liječniku. Pridodajući tome činjenicu da je osteoartritritis jedan od vodećih uzroka ugradnje endoproteze zahvaćenog zgloba (Zhang i sur., 2010), jasno je da ova bolest, osim što značajno utječe na kvalitetu života pojedinca, čini i jedan od vodećih javnozdravstvenih i ekonomskih problema zajednice.

Liječenje osteoartritisa

Premda trenutno nije moguće izliječiti OA, terapijske intervencije usmjerene su smanjenju simptoma te usporenju progresije destrukcije zgloba.
Prema važećim nacionalnim smjernicama (Grazio, 2015.) liječenje treba prilagoditi svakom bolesniku ponaosob uzimajući u obzir karakteristike pacijenta (dob, komorbiditeti, tjelesna aktivnost, očekivanja), ali i karakteristike bolesti (lokalizacija, stupanj i proširenost bolesti). Terapijski se postupci dijele na farmakološke, koji su višestrukim studijama znanstveno potkrijepljeni, te na nefarmakološke (gubitak tjelesne mase, edukacija o prehrani i tjelovježbi, fizikalno-terapijski modaliteti vježbanja), za koje postoji manje stručno-znanstvenih dokaza, a njihova je djelotvornost prvenstveno potvrđena iskustveno (Grazio i sur., 2015). Obzirom na multifaktorijalnu etiologiju bolesti, preporuča se objedinjeno farmakološko i nefarmakološko liječenje (Doherty i sur., 2012; Grazio, 2007; Grazio i sur., 2015). Sukladno preporukama EULAR-a (European League Against Rheumatism), AAOS smjernicama (engl. American Academy of Orthopaedic Surgeons) te ESCEO smjernicama (engl. European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis), u liječenju svih stadija OA temelj je edukacija pacijenta, kroz koje isti uči tehnike samopomoći, zaštitne pokrete i položaje, te ga se potiče na promjenu životnih navika s ciljem redukcije prekomjerne tjelesne težine (Grazio i sur., 2015; Fernandes i sur, 2013).
Prema hrvatskim smjernicama (Grazio i sur., 2015) u liječenju početnog, nekompliciranog oblika OA uz edukaciju bolesnika, primjenjuju se medicinske vježbe (aerobne vježbe, vježbe snaženja i vježbe opsega pokreta), preporučljivo kao hidrokineziterapija, a s ciljem redukcije boli i sprječavanja onesposobljenosti. Ukoliko to nije dovoljno, u ovom su stadiju prema Smjernicama Hrvatskog društva za fizikalnu i rehabilitacijsku medicinu Hrvatskog liječničkog zbora (Grazio i sur., 2015), indicirane i biomehaničke modifikacije, npr. taping, primjena klinastih uložaka u obući ili uporaba individualno izrađene obuće.
U farmakološkom pristupu, primarno se propisuje analgetska terapija (paracetamol), lokalna primjena nesteroidnih antireumatika (NSAR) ili kapsaicina. Propisivanje preparata hondroitina i glukozamina (SySADOAs, eng. symptomatic slow-acting drugs for osteoarthritis) dvojbeno je, a njihova se primjena u liječenju OA zasniva na osobnom stavu i iskustvu liječnika. Izuzevši formu kristaliziranog glukozaminsulfata, u Hrvatskoj ovi preparati uglavnom nisu registrirani kao lijekovi već kao dodaci prehrani, a sukladno nacionalnim smjernicama iz 2015. godine

njihova je primjena adjuvantna u liječenju OA kuka i koljena, kontinuirano tijekom najmanje 6 mjeseci. Ukoliko je riječ o srednje teškom stadiju bolesti, indicirana je sustavna (per os) primjena nesteroidnih antireumatika (NSAR) ili čak slabih opioda izolirano ili u kombinaciji s paracetamolom. Od nefarmakoloških mjera, prema istim nacionalnim i svjetskim smjernicama, primjenjuju se metode fizikalne terapije koje uključuju termoterapiju, laser i terapijski ultrazvuk, dok za primjenu elektroterapije nema dovoljno dokaza u literaturi (Cameron i sur., 2003; Grazio i sur., 2015). Bitno je naglasiti da se navedene metode fizikalne terapije primjenjuju isključivo komplementarno s medicinskim vježbama. U terapiji srednje teškog OA od drugih se terapijskih mogućnosti ističe intraartikularna primjena hijaluronske kiseline i njenih derivata (tzv. viskosuplementacija), danas sve zastupljenija metoda liječenja. U slučaju naglašene upalne komponente, intrartikularno se mogu primjeniti i glukokortikoidi, dok prilikom postojanja neuropatske sastavnice, u isti se mogu injicirati i analgetici. U ovoj fazi bolesti indicirana su pomagala za hod (štake, hodalice), a s ciljem rasterećenja bolesnog zgloba. Od ostalih terapijskih mogućnosti, citirani autori preporučuju radnu terapiju (osobito za OA šaka), te akupunkturu kao opciju tretiranja OA koljena. Kao posljednja terapijska mogućnost, u osoba s uznapredovalim stupnjem bolesti kod kojih nije postignut željeni učinak konzervativnog liječenja, pristupa se kirurškom liječenju i potom odgovarajućoj rehabilitaciji (Grazio, 2015). Najčešće je riječ o ugradnji parcijalne ili totalne endoproteze zahvaćenog zgloba, no bitno je naglasiti kako kirurško liječenje OA ima ograničenja u vidu kontraindikacija, a povezano je i s određenim operacijskim i postoperacijskim komplikacijama (Prince i sur., 2020).

Zašto potreba za novim lijekom?

Uzevši u obzir navedeno, a poglavito porast incidencije bolesti te ograničenje pacijenta u obavljanju aktivnosti svakodnevnog života, OA je bolest koja se i dalje intenzivno istražuje. Potrebno je naglasiti da, premda postoje nove terapijske mogućnosti, temelj farmakološkog liječenja i dalje ostaju NSAR lijekovi čija je učinkovitost ograničena, a opterećeni su širokim spektrom nuspojava (Ameye i sur., 2006; Liu X i sur., 2018; Grazio i sur., 2015). Iz toga se diferencira potreba za sigurnom a djelotvornom prirodnom tvari, koja će biti korištena u liječenju, ali i prevenciji bolesti, a bit će bez ili uz što manje neželjenih učinaka (Ameye i sur., 2006). Sukladno meta-analizi provedenoj od strane Liu X i sur. (2018), čak 69% ljudi s potvrđenom dijagnozom OA uzima neki od komercijalno dostupnih dodataka prehrani s ciljem ublažavanja simptoma bolesti, najčešće glukozamin i hondroitin, uprkos nedovoljno dokazanom učinku i neizvjesnoj sigurnosti primjene. Prema Ameye i sur. (2006), ljekovite hranjive tvari sporo su djelujuće i učinak postižu putem više različitih mehanizama, kumulativno, za što je potrebno vrijeme. Iz tog razloga, a i zbog činjenice da je OA progresivna multifaktorijalna bolest, upravo je ona jedna od kroničnih nezaraznih bolesti koja se nastoji prevenirati i/ili liječiti uporabom ljekovitih hranjivih tvari.
S obzirom na postojanje upalne komponente u OA, pozornost je usmjerena na terapiju onim ljekovitim tvarima koje pozitivno utječu na redukciju upalnih

parametara i simptoma bolesti, bez zabilježenih nuspojava i pri visokim dozama (Hewlings i sur., 2017). Jedna od takvih je i kurkumin.

Kurkuma i kurkumin

Kurkumin je hidrofobni polifenol, koji čini aktivnu komponentu rizoma biljke Curcuma longa. Više studija pokazalo je njegovo jako antioksidativno i protuupalno djelovanje, uz netoksičnost i sigurnost primjene i pri dozama od 8g dnevno (Mirzaei i sur., 2017). Ipak, zbog nestabilnosti molekule pri fiziološkim vrijednostima pH, kao i zbog slabe topljivosti u vodi, postavljena je sumnja u biodostupnost molekule kurkumina prilikom oralnog unosa. Slaba se biodostupnost, a time i učinkovitost, povezuje, osim s lošom apsorpcijom, i s brzim metabolizmom i eliminacijom same molekule kurkumina iz organizma (Hewlings i sur., 2017). Stoga se, a u cilju poboljšanja bioraspoloživosti molekule, ista spaja s različitim molekulama koje poboljšavaju njenu apsorpciju. Ona koju u svojoj studiji proučavaju Hewlings i sur. (2017) je alkaloid piperin crnog papra, čime se biodostupnost kurkumina poboljšava 2000 %. Mirazei i sur. (2017) kurkumin spajaju s fosfatidil-kolinom, čime su značajno poboljšana njena fizikalno-kemijska svojstva. Primjer je to primjene nove tehnologije koja donosi visokoiskoristive oblike kurkumina, poput miceliziranih ili liposomalnih oblika, što značajno pridonosi njenoj bioraspoloživosti.

Mehanizam djelovanje kurkumina

Mehanizam djelovanja kurkumina je blokiranje aktivacije NF-κB sustava u hondrocitima, čime se blokira apoptoza hondrocita, te suprimira otpuštanje proteoglikana i matriks metaloproteaza. Prema istim autorima, kurkumin blokira i ekspresiju ciklooksigenaza, prostaglandina E-2 te proupalnih citokina u hondrocitima (Goulard i sur., 2019; Hewlings i sur., 2017; Mathy-Hartert i sur., 2009; Henrotin i sur., 2014), modificirajući na taj način subjektivne smetnje i biokemijske markere upalne bolesti. Stoga je cilj ovog rada bio analizirati i kritički verificirati dosadašnje znanstvene spoznaje učinka kurkumina na prevenciju nastanka OA i njegovo liječenje.

MATERIJALI I METODE

Tijekom svibnja 2020. godine pretražene su PubMed/MEDLINE i SCOPUS baze podataka, prema ključnim riječima u naslovu ili sažetku: MeSH izrazi (curcumin OR curcuminoid OR Curcuma longa), AND (osteoarthritis). Pretraga je ograničena na istraživanja provedena na ljudima i na radove na engleskom i hrvatskom jeziku. U obzir smo uzeli metodološki relevantne radove unatrag 10 godina.

REZULTATI I RASPRAVA

Dosadašnja istraživanja

Hewlings i sur. (2017) svojom su kliničkom studijom dokazali učinkovitost i sigurnost primjene derivata kurkumina u liječenju prije svega upalnih bolesti, ali i psorijatične forme artritisa, gastrointestinalnih smetnji, anksioznosti, metaboličkog sindroma, te dijabetičke mikroangiopatije i retinopatije.

Dvostruko slijepu, randomiziranu studiju, koja je uključivala 40 ispitanika, Panahi i sur. (2014) proveli su s ciljem ispitivanja učinka kurkumina na simptome pacijenata s osteoartritisom koljena. Ispitanici su bivali podvrgnuti dnevnim dozama od 1500 mg kurkumina per os tijekom 6 tjedana. Učinci su praćeni trima skalama i nedvojbeno su dokazali značajno poboljšanje zglobne funkcije i redukciju boli u usporedbi s placebom, bez zabilježenih nuspojava.

Retrospektivna studija s ciljem proučavanja učinka kapsula koje sadržavaju ekstrakt Curcuma longa biljke provedena je od strane Appelbooma i sur. (2014). Obuhvaćeni su pacijenti s različitim lokalizacijama bolnog OA, a vrednovan je rezultat nakon 6 mjeseci primjene ljekovite tvari. Studija je dizajnirana kao upitnik kojim je obuhvaćeno 820 pacijenta, a rezultati istog su pokazali redukciju boli nakon 6 tjedana uzimanja lijeka, uz povećanje zglobne mobilnosti i poboljšanje kvalitete života. Uz to, studija je u više od 50 % ispitanika zabilježila smanjenje doze ili potpuni prekid uzimanja analgetske terapije. Ni ovim istraživanjem nisu zabilježene nuspojave primjene ekstrakta.

Meta-analiza Sahebhar i sur. (2016) obuhvatila je 8 radova, s uključenih 606 randomiziranih ispitanika. Cilj istraživanja je bio verificirati učinak kurkumina na redukciju boli, mjerene VAS skalom (vizualno-analognom skalom), bez obzira na podrijetlo boli, odnosno primarnu dijagnozu pacijenta, a uključujući i osteoartritis kao uzrok boli. Istraživanjem su obuhvaćene različite oralne doze kurkumina (500 do 6000 mg/dan), trajanja od 2 do 8 tjedana. Zaključno, ovom je studijom dokazana učinkovitost kurkumina u redukciji boli, neovisno o primjenjenoj dozi i vremenu uzimanja istog, uz dokaz sigurnosti primjene. Također, ovim istraživanjem nije detektiran niti jedan negativan učinak primjene ljekovite tvari.

Meta-analizom 11 randomiziranih studija provedenoj od strane Bannuru i sur. (2018) uspoređen je učinak kurkumina i ekstrakta biljke Boswellia serratae s placebom, te navedenih tvari i NSAR lijekova. Analiza je pokazala statistički značajnu razliku u smanjenju bolova i poboljšanju funkcije prilikom primjene ispitivanih tvari u odnosu na placebo. Usporedbom ovih tvari i NSAR lijekova nije bilo statistički značajne razlike u analiziranim parametrima, no bio je zabilježen značajno manji broj gastrointestinalnih nuspojava prilikom primjene ispitivanih ekstrakata. I ovim je radom dokazana sigurnost primjene kurkumina, ali i ekstrakta Boswellie.

Svojim radom Liu i sur. (2018) sistematski pregledavaju 69 randomiziranih, placebom kontroliranih kliničkih studija, u kojima je sudjelovalo ukupno 11586 ispitanika, dok je ispitivanjem obuhvaćeno 20 suplemenata uzimanih u različitim dozama, uključujući kurkumin. Analizirajući kratkoročni učinak, šest od ukupnog broja ispitivanih suplemenata, uključujući kurkumin, pokazalo je klinički značajnu

redukciju boli i poboljšanje funkcije zahvaćenog zgloba (pycnogenol, L-karnitin, ekstrakt Boswellia serrata, hidrolizat kolagena te ekstrakt marakuje). Srednjeročna i dugoročna primjena svih ispitivanih suplemenata, u usporedbi s placebom, nije pokazala statistički značajnu učinkovitost niti na redukciju boli, niti na poboljšanje funkcije zahvaćenog zgloba. Ovim je radom nedvojbeno dokazana sigurnost primjene svih ispitivanih suplemenata, osim diacereina, te je dokazana kratkoročna učinkovitost više različitih ljekovitih tvari na redukciju simptoma OA, među kojima i kurkumina.

Meta-analizom iz 2019. godine, sumirajući rezultate 9 kliničkih pokusa, heterogenih u duljini trajanja liječenja i dnevnoj dozi primijenjenog kurkumina, determinira se pozitivno djelovanje ispitivane tvari prvenstveno na redukciju boli i ukočenosti, ali i na poboljšanje funkcije zahvaćenog zgloba (Goulart i sur.). Kao adjuvantna terapija, kurkumin smanjuje potrebu za analgeticima, prvenstveno onima iz skupine NSAR-a. Posljedica primjene kurkumina je i redukcija upalnih markera u krvi i sinoviji, mehanizmom blokiranja aktivacije NF-κB sustava u hondrocitima.

Prospektivnim, randomiziranim, dvostruko slijepim istraživanjem kojim je praćeno 150 ispitanika tijekom 90 dana, Henroitin i sur. (2019) uspoređuju učinak dviju doza ekstrakta biljke Curcuma longa (90 mg i 140 mg) na simptome OA koljena. Osim PGADA skalom (Patient Global Assessment of Disease Activity), učinak je praćen mjerenjem koncentracije sColl2-1 molekule, serumskog markera razgradnje hrskavice. Istraživanjem je za obje ispitivane doze potvrđena sigurnost primjene ekstrakta, bez zabilježenih nuspojava primjene, uz biokemijski vidljivu redukciju koncentracije serumskog markera, te smanjenje boli i drugih subjektivnih smetnji povezanih s bolešću. Nije bilo statistički značajne razlike učinkovitosti između dvije primjenjene doze.

ZAKLJUČAK

Ovim preglednim radom obuhvaćene su studije provedene na više od 20 000 ispitanika koji su u različitim vremenskim intervalima i u različitim dnevnim dozama bili podvrgnuti djelovanju kurkumina u terapiji osteoartritisa, kao adjuvantnom terapijom ili monoterapijom.

Dosadašnjim je istraživanjima dokazana klinički značajna učinkovitost više različitih ljekovitih tvari na redukciju simptoma OA, među kojima prednjači kurkumin. Svoje mjesto u liječenju OA nalazi poglavito kao adjuvantna terapija u liječenju boli i smanjenju biokemijskih faktora upale, što rezultira značajnom redukcijom standardno propisivanih doza analgetika a time i njihovih potencijalnih nuspojava. Navedeno u konačnici dovodi do smanjenja boli i poboljšanja funkcije zahvaćenog zgloba, te time do poboljšanja kvalitete života pacijenta.

Ipak, obzirom da su pregledanim istraživanjima ispitani samo kratkoročni i srednjeročni učinci primjene kurkumina, ostaje za istražiti učinke dugotrajne primjene istog, te ga usporediti sa učincima dugotrajne primjene nekih drugih tvari korištenih u liječenju osteoartritisa.

Jednako tako, obzirom na manjkavost znanstvenih dokaza o preventivnom učinku primjene kurkumina na razvoj OA, uzimanje istog u svrhu prevencije ostaje za istražiti.

Zaključno, potrebna su daljnja istraživanja s ciljem utvrđivanja preventivne uloge kurkumina na razvoj OA, učinka dugoročne primjene kurkumina na prevenciju i/ili liječenje bolesti, te determiniranja optimalnih terapijskih doza.

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THE IMPORTANCE OF NUTRITION FOR COGNITIVE DEVELOPMENT OF CHILDREN WITH DOWN SYNDROME

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ABSTRACT

Down syndrome as the most common genetic disorder caused by excess chromosome on the 21st pair causes a number of phenotypic, psychomotor and cognitive specificities that are associated with excess genetic material. Depending on the intensity of gene expression, there are brain abnormalities of varying intensity such as differences in brain size and appearance, disruption of proteins crucial for neuromorphogenesis and optimal functioning of brain cells, which ultimately causes a number of conditions such as neurodegenerative diseases, oxidative stress, dendritic branching and intellectual disabilities. Excess genetic material in children with Down syndrome does not define special requirements regarding eating habits, however its overexpression very often causes conditions that require special dietary intervention. From an early age, feeding problems can be caused by hypotonia. Besides, sensory sensitivity to a certain consistency and texture of food is expressed, which leads to a deficient intake of nutrients through diet necessary for cognitive development. Numerous studies have focused on the importance of dietary intake of certain nutrients to improve the cognitive development of children with Down syndrome. Of exceptional importance for brain development is the intake of ω-3 fatty acids, iron, zinc, iodine, vitamin B12 and folic acid. The aim of this paper is to provide an overview of research on the importance of nutrient intake crucial for the development of cognitive abilities through diet in children with Down syndrome.

Keywords: Down syndrome, trisomy 21, nutrition, cognitive development

INTRODUCTION

Down syndrome is caused by trisomy of the whole or a part of 21st chromosome. It is the most common cause of mild to moderate intellectual disability and affects from one in 319 to one in 1000 liveborn infants, although this prevalence varies between countries because of differences in maternal age and prenatal screening (Park and Chung, 2013; Nordstrom, 2020).

Table 1. Possible physical features and congenital condition associated with Down syndrome (Eckdahl, 2018)

Physical features	
Head	Small, shortened skull that is flattened on the back, sloping forehead, missing or underdeveloped sinuses.
Eyes	Upward slanted and wide-set eyes, epicanthal folds, Brushfield spots.
Ears	Smaller ears with extra folds, ears set lower on the head.
Nose	Smaller nose, flattened nasal bridge.
Mouth	Smaller mouth, large tongue that tends to stick out more often, undersized teeth, crooked teeth, irregularly shaped teeth.
Hands	Broad hands, only one crease across the palm, short fingers, curved fifth finger.
Feet	Larger gap between the first and the second toe.
Limbs	Short and stocky arms and legs with hyperflexible joints.
Body	Short stature, shorter and wider neck, protruding stomach.
Congenital condition	
Heart	Septal defects (atrial septal defect, ventricular septal defect, atrioventricular septal defect), patent ductus arteriosus, tetralogy of Fallot.
Vision	Refractive errors, cataracts, amblyopia, blepharitis, glaucoma.
Hearing	Hearing loss (conductive and sensorineural), glue ear, otitis media.
Musculoskeletal	Hypotonia, ligamentous laxity, atlantoaxial instability, hip abnormalities, kneecap instability, flat feet.
Digestive	Hirschsprung disease, tracheoesophageal fistula, esophageal atresia, duodenal atresia, imperforate anus, gastroesophageal reflux disorder.
Immune	Hypothyroidism, celiac disease, respiratory infections.

Children with Down syndrome show large variability in physical features and congenital condition (Table 1.) because some have mild symptoms and complications, whereas others are more severely affected. This variability also relates to the risk of health and cognitive problems associated to poor nutrition in early age. Attention to nutritional intake and status is important for children with Down syndrome because some features and comorbidities have nutritional

implications and consequences (Freeman et al., 2008; Wong et al., 2014; Ergović Ravančić and Obradović, 2021).
Early life experiences have an impact on child health and development. Increasing evidence suggest that multidisciplinary aproach to children development needs to begin in early childhood because cognition related behaviors have early origins. Regardless of weight status, poor diet and activity levels may also have consequences for current and future health and development of children with Down syndrome. The early childhood years are a time for rapid and robust growth in cognitive development, but also a time of great vulnerability in this regard (Tandon et al., 2016).
Cognitive development is a complex, multidimensional set of abilities. In addition to the fundamental complexity of mental functions involved in any cognitive task, intellectual performance in specific test conditions depends on several factors including individual skills, motivation, general excitment, prior learning, fatigue and time of a day (Bhatnagar and Taneja, 2001). Given the number and complexity of factors than can affect cognitive performance at any given time, it is not surprising that very few nutritional effects on cognition have received scientific support. In this paper we consider the beneficial effects of nutrients from diet to improve the cognitive performance.

NUTRITION AND COGNITION

Cognitive development involve several of mental activities related to information storage and processing: attention, memory, language, learning, decision making, and problem solving. (Bhatnagar and Taneja, 2001; Lozoff and Georgieff, 2006).
Structural organization of the brain implies neurons who work on specific subsets of cognitive functions in a coordinated manner and within a region or structure. Myelin-coated neurons transmit electrical signals in single, double, or multiple directions, using neurotransmitters to communicate and interact (Figure 1). Between structures, nerve fibers tracts allow different regions to coordinate in the higher orders of cognitive function. The combination of the neural cell functions and the structural organization enables the higher order of cognitive functions.

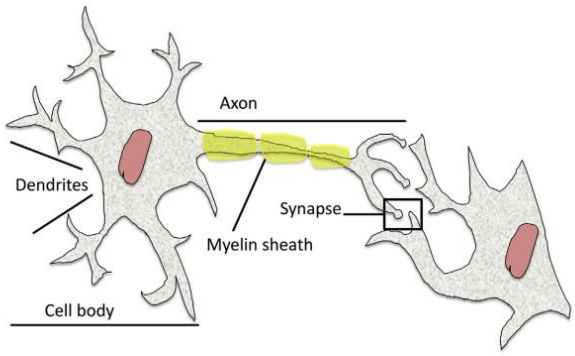

Figure 1. Structure of a neuron (Mehedint and Gulledge, 2014)

Nutrition as connection between nutrients and health, should provide the building blocks needed to build and maintain the structure of the central nervous system and function. There are five main classes of nutrients: proteins, fats, carbohydrates, minerals and vitamin. Insufficient amounts of any of these nutrients have been associated with various diseases (Bhatnagar and Taneja, 2001; Lozoff and Georgieff, 2006; Mehedint and Gulledge, 2014). Intellectual disability occurs when a child fails to fully develop the intellectual capacity to think, reason, learn, and understand. Children with an intellectual disability also have problems learning adaptive behavior, which encompasses the social and practical skills needed for everyday living. Intellectual impairment varies among children with Down syndrome. It ranges from severe intellectual impairment that makes people fully dependent on caregivers, to mild effects that enable people to think and learn at levels that enable them to pursue higher education, retain a job, and live independently (Stagni et al., 2015).

It's assumed that diet can play a key role in brain development and thus intellectual functioning. The brain, in a similar way to the rest of the body, need proteins, fats, carbohydrates, vitamins and minerals that are all ingested through food for growth and functioning. As the brain develops faster than the rest of the body, it is obvious to consider whether a lack of nutrition at a critical stage of development can lead to permanent changes in brain structure and functioning. In addition, the brain is the most metabolically active organ in the body, yet it has very limited stores of energy, so it relies on the diet for a continuous supply of glucose. Similarly, the minute-to-minute functioning of the brain requires an adequate supply of micronutrients that act as co-enzymes, or form structural parts of the enzymes required for optimal metabolic activity (Benton, 2005; Benton, 2010).

Vitamins, minerals, and antioxidants from the diet are important cofactors in many biochemical processes throughout the body. The presence of an extra copy of chromosome 21 in Down syndrome causes an overexpression of genes located on this chromosome and further metabolic changes, leading to increased levels of oxidative stress and several abnormalities in metabolism (Lima et al., 2010).

NUTRIENTS FOR IMPROVE THE COGNITIVE DEVELOPMENT

Studies have shown that the maturation of specific brain areas during childhood is associated with development of specific cognitive functions such as language, reading and memory (Nagy et al., 2004; Giedd et al., 2010). Since rapid brain growth occurs during the first 2 years of life (by the age of 2 the brain reaches 80 % of its adult weight), this period of life may be particularly sensitive to deficiencies in diet (Bryan et al., 2004).

ω-3 fatty acids

Dietary lipids are essential sources of metabolic energy, substrates for synthesis of active compounds, component of lipoprotein particles, and carriers for lipid soluble compounds. They participate in cell signaling and take active part in regulation of

gene expression. They are integral part of cellular membranes and precursors for bioactive compounds modulating a wide variety of biological functions.
Fatty acids are a part of lipids, which can be broken down into saturated and unsaturated acids. Eicosapentanoic acid (EPA) and decosahexanoic acid (DHA) are essential polyunsaturated lipids with an omega-3 desaturation that cannot be made in humans (Youdim et al., 2000).
In recent years, there has been an increasing interest in the effect of essential fatty acids, especially long chain polyunsaturated fatty acids, on cognitive brain development. 60 % of the dry weight of the human brain are lipids, of which 20 % are DHA and arachidonic acid (AA; an omega-6 fatt acid) the two core fatty acids found in gray matter (De Souza et al., 2011).
The supply of omega-3 fatty acids, including DHA and EPA, is frequently inadequate for children as well as for adults despite the knowledge that they play a central functional role in brain tissue. They are not only the basic components of neuronal membranes, but they modulate membrane fluidity and volume and thereby influence receptor and enzyme activities in addition to affecting ion channels. Essential fatty acids are also precursors for active mediators that play a key role in inflammation and immune reaction. They stimulate the growth of the neuronal and dendritic spine and synaptic membrane synthesis, so therefore influence signal processing and neural transmission. In addition, essential fatty acids regulate gene expression in the brain (Schuchardt et al., 2010; De Souza et al., 2011; Prado and Dewey, 2014). Zmijewski et al. (2015) presented results of research in which omega-3 containing fish oil with other healthy nutraceuticals can modestly suppress regulator of calcineurin 1 levels in mice with Down syndrome and supported the idea that fish oil could be an effective and cheap agent to treat genetically defined pathologies like cognitive decline.

Iron

Iron is a very important mineral for human health, primarily due to its structural and chemical roles in the heme rings of hemoglobin and cytochrome P450 and in various other structural and chemical roles in metabolic proteins (Carlson et al., 2007). Heme iron, in particular, is involved in the regulation of various cellular functions, such as respiration, proliferation, and differentiation. Iron also modulates specific brain functions by increasing the release and turnover of dopamine and other neurotransmitters. The brain is arguably the most metabolically active organ in the body and its internal concentration of iron is exceptionally high particularly during the phases of neurodevelopment due to iron's role in myelinogenesis (Barone et al., 2018).
Iron deficiency with or without anemia early in life has been associated with adverse long-term outcomes. Studies have shown that early iron deficiency affects neuronal function and myelination and is associated with behavioral abnormalities and, if left untreated, can lead to reduction cognitive functions and changes in behavior and mood. Therefore, it is crucial to recognize and treat iron deficiency for at an early stage of brain development (Hart et al., 2020).

Zinc

Zinc deficiency slows growth because it is involved in the activity of more than 200 enzymes, especially those associated with the synthesis of RNA and DNA.
In addition, zinc plays a role in neurogenesis, maturation, and migration of neurons and in synapse formation that are centrally involved in learning and memory (Black, 2003).
Experimental and clinical studies have found that zinc metabolism is altered in individuals with Down syndrome. Lima at al. (2010), reported that adequate zinc intake was observed in 40 % of children with DS and in 67 % of the control group and zinc concentrations were significantly lower in plasma and urine and higher in erythrocytes of children with Down syndrome. Many symptoms of children and adults with Down syndrome are conseqences by an excessive synthesis of multiple gene products, including an increase in the intracellular activity of copper-zinc superoxide dismutase due overexpression of genes present on chromosome 21. Zinc stabilizes the 3-D structure of superoxide dismutase and thus reduces the imbalance (Lima et al., 2010).

Iodine

Iodine deficiency is very often described as greatest single cause of preventable brain damage and intellectual disabilities, because iodine is necessary for the synthesis of thyroid hormones which in turn act by regulating the metabolic pattern of most cells of the organism. It also plays a crucial role in the process of early growth and development of most organs, especially the brain, which occurs in human subjects during the fetal and early postnatal life. Consequently, iodine deficiency, if severe enough to affect thyroid hormone synthesis during this critical period, will result in hypothyroidism and brain damage (Delange, 2000; Prado and Dewey, 2014).

Vitamin B12

Association between vitamin B12 and cognitive development has been mainly observed in infants born of vegetarian/vegan mothers or mothers on a macrobiotic diet. These diets can result in vitamin B12 deficiency, as vitamin B12 is largely found in animal products. Vitamin B12 deficiencies in infants included a variety of abnormal clinical and radiological signs such as: hypotonic muscles, involuntary muscle movements, apathy, cerebral atrophy, and demyelination of nerve cells (Louwman at al., 2000; Nyardi et al., 2013).
Vitamin B12 plays a key role in normal brain development and function and is required for enzyme methionine synthase, which is necessary for the synthesis of methionine who is the major methyl group donor used in human methylation reactions, including methylation of DNA and RNA. Deficient methylation reactions in the central nervous system can impair the methylation of myelin basic protein in the central as well as peripheral nervous system. The production of myelin is a key component of brain development from gestation, throughout childhood and well into

middle age. The myelination of the brain is of importance for multiple brain systems and is highly related to neurodevelopment and subsequent cognitive functioning.
Vitamin B12 also serves as a cofactor in numerous catalytic reactions in the human body, which are required for neurotransmitter synthesis and functioning. Vitamin B12 deficiency may cause pernicious anemia with similar effects on cognitive development and functioning as anemia caused by iron deficiency. Vitamin B12 deficiency can also result in neuropathy through degeneration of nerve fibers and irreversible brain damage (Winje et al., 2018).

Folic acid

Folates are water soluble vitamins that serve as coenzymes in a variety reactions, including de novo nucleotide biosynthesis and conversion of homocysteine to methionine which is required for various methylation reactions. Deficiency of folate results in a different clinical features like cytopenias (including megoblastic anemia), weakness, fatigue, headache, irritability, as well as gastrointestinal symptoms including nausea, vomiting, and mucosal aphthous ulcerations (Funk et al., 2020). Numerous enzymes involved in folate transport and metabolism are encoded by genes located on chromosome 21 and represent a potential mechanistic basis for folate dysregulation in children with Down syndrome.
Besade potential genetic causes of metabolic folate dysregulation in children with Down syndrome, non-genetic factors such diet, gender, and age must be considered because they must fully satisfy their folate needs through their diet since they lack the enzymatic machinery necessary to synthesize their own (Pfeiffer et al., 2012). Black (2008) explained two mechanisms for influence of folate and vitamin B12 deficiency on the brain: by disrupting myelination or influencing the inflammatory process.

CONCLUSION

Down syndrome is a condition characterized by an excess of genetic material on 21st pair of chromosomes. Many enzymes that are encoded on the extra 21st chromosome are known to be actively transcribed, which results in overexpression of many enzymes. Genetic overexpression of enzymes leads to overconsumption of their enzymatic substrates and overproduction of their metabolic end-products. Ultimately, children with Down syndrome have different nutrient needs and are often deficient which negatively affects on cognitive development. The majority of studies, which have investigated the association between nutrition and cognitive development, have focused on individual micronutrients but individuals consume combinations of food and poor overall diet can cause multiple macro-and micronutrient deficiencies and imbalances. If an overall healthy diet synergistically enhances cognitive development in children, then public health interventions should focus on the promotion of overall diet quality rather than isolated micronutrients or dietary components consumed by children.

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BELIEFS ABOUT WILD AND FARMED FISH AMONG CATERING CUSTOMERS

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ABSTRACT

The consumption of fish provides health benefits due to its nutritional value, and it is highly recommended to consume fish on as many occasions as possible. Despite capture fisheries not being able to meet the increasing demand, the consumption of farmed fish is still subject to prejudice. The objective of this paper is to better understand catering customers' consumption habits of, and beliefs about, wild and farmed fish. The nationally representative sample of the Croatian population was divided into two groups: frequent catering customers (FCC; n=264) and non-frequent catering customers (NFCC; n=654). Fish consumption habits and beliefs concerning wild and farmed fish were investigated using a tailored questionnaire. Results showed that the largest proportion of FCC consume white fish (56%) and fatty fish (52%) in catering facilities on a monthly basis, while one-third of the participants in the NFCC group eat white and fatty fish once a year or less (37% and 35%, respectively). The NFCC group believes that farmed fish contains more antibiotics ($p=0.003$), more fat ($p<0.001$), and is more artificial ($p<0.001$) than its wild counterpart. However, they have more positive beliefs regarding control ($p=0.001$), availability ($p=0.004$), and price ($p<0.001$). It can be concluded that one of the conditions for increasing fish consumption in catering facilities is to reduce prejudice against farmed fish.

Keywords: beliefs, catering customers, farmed fish, wild fish

INTRODUCTION

Eating out in catering facilities has become a common habit in modern society due to higher incomes, greater urbanization, a growing population that is also aging, and more hectic lifestyles. Furthermore, more women are working, thus they do not have as much time to spend on cooking, while foodservice outlets are becoming much more available and affordable. (Edwards, 2019). Households in the European Union (EU) spent over EUR 600 billion on catering services in 2018, representing 7% of their total consumption expenditure (EUROSTAT, 2020).

Eating out of the home has been associated with a sedentary lifestyle, increased energy intake (Orfanos et al., 2007), and, consequently, higher body weight (Bes-Rastrollo et al., 2010). It has been found that weight gain is higher when eating in fast food places than in restaurants (Nago et al., 2014). When comparing eating only at work and eating in restaurants, total energy intake was higher in people who ate in both places or only at restaurants, mainly due to increased alcohol consumption (Orfanos et al., 2017). In addition, sugar, desserts, savory baked goods, and beverages are consumed more frequently away from home than at home (Naska et al., 2015).

Nowadays, numerous recommendations advise the consumption of fish on as many occasions as possible, as it offers numerous health benefits in the prevention of modern non-communicable diseases such as cardiovascular disease, obesity, metabolic syndrome, cancer, and mental disorders (Bork et al., 2020; Jayedi, Shab-Bidar, 2020; Natto et al., 2019). A 2018 Eurobarometer survey found that nearly one-third of European citizens consume fishery products in restaurants and other food outlets at least once a month (European Union, 2018).

Following dietary recommendations, combined with population growth, global fish consumption has more than doubled since the 1960s. Also, capture fisheries have been relatively stable for the last few decades and, currently, more than one-third of the world's marine fish stocks are overfished. To ensure adequate fish supplies, more than half of all fish consumed now comes from aquaculture, and this trend is expected to continue to increase (FAO, 2020). Therefore, it is of interest to numerous stakeholders to understand the perception of farmed fish among different consumer groups. Although aquaculture is not necessarily perceived as negative *per se*, consumers generally tend to prefer wild fish (Carlucci et al., 2015, Krešić et al., 2020), and belief in the superiority of wild fish may be negatively related to the consumption of farmed fish (Hall, Amberg, 2013).

There is very little research data looking at farmed fish consumption in relation to eating away from home. The work that addresses this topic confirms that consumers who prefer wild fish are also more likely to eat fish in catering facilities (Cantillo et al., 2021). On the other hand, catering establishments often have farmed fish on offer due to its lower purchase price and constant availability.

With this in mind, the goal of this paper is to better understand catering customers' consumption habits and beliefs about wild and farmed fish. By grouping customers according to their usual frequency of visiting catering facilities, we aim to test the hypothesis that customers who visit catering facilities less frequently have less

experience with farmed fish and, consequently, have more prejudice and less positive beliefs than customers who visit them more often. The results are expected to provide valuable information for caterers to better understand their customers and tailor farmed fish offerings to them.

SUBJECTS AND METHODS

The study on catering customers is part of a large survey conducted in Croatia in December 2019 as part of the project AdriAquaNet – Enhancing Innovation and Sustainability in Adriatic Aquaculture. A quantitative approach was followed using online interviews (CAWI – Computer-Aided Web Interviewing), conducted by the market research agency Ipsos among its consumer panel members. The target population was a nationally representative sample of individuals responsible for household food purchases. Stratified random sampling and proportional stratification according to the national population distribution were used. Possible deviations of the sample structure from the population structure were eliminated by post-stratification (RIM weighting) by sex, age and region, according to the latest estimates of the Croatian Bureau of Statistics.

A total of 1002 participants, aged 18 to 65, were asked a filter question – whether they or their household members consumed fishery products at home or in a catering establishment in the last 12 months – resulting in 977 completed surveys. Respondents were additionally asked how often they visit catering facilities. The following frequencies were offered in response: almost every day, 4-5 times a week, 2-3 times per week, once per week, 2-3 times per month, once in 3 months, once in 6 months, once per year or less, and never. For the purposes of this study, those participants who reported that they never visit catering facilities were excluded, resulting in a final sample of 918 respondents.

Depending on the frequency of visiting catering facilities, respondents were classified as either frequent catering customers (FCC; n=264) or non-frequent catering customers (NFCC; n=654). Frequent catering customers were those whose frequency of eating in catering facilities was from “almost every day” to at least “2-3 times a month”, while non-frequent catering customers usually visited catering facilities “once in 3 months” to “once a year or less”. The habits of eating different types of fish in catering facilities were examined as the frequency of eating white and fatty fish, with responses provided on a 6-point scale (once a week or more, 2-3 times a month, once in 3 months, once in 6 months, once a year or less, never).

Customers' beliefs about wild and farmed fish were tested with 19 statements modified from Claret et al. (2014). Respondents were asked to indicate the extent to which they agree with the statements on a 5-point Likert scale (1=strongly disagree; 3=neither agree nor disagree; 5=strongly agree). The questionnaire also included 7 demographic questions on gender, age, income, household members, children, work status, and education level.

Statistical analyses were conducted using IBM SPSS Statistics version 26. Demographic differences between frequent and non-frequent catering customers were determined using Pearson's chi-square test. Descriptive statistics (means and

standard deviations) were used to describe beliefs about farmed and wild fish, and independent t-tests were conducted to determine differences between the two groups of catering customers. The statistical significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Since this study aims to improve the supply of farmed fish in catering facilities, the first step was to describe the customers who eat out of the home more frequently. Knowing customer characteristics could be a useful aid for stakeholders in creating specific marketing messages focused on the target audience. Demographic characteristics of the total study sample and the two groups of customers divided according to the frequency of visiting foodservice establishments are summarized in Table 1.

Almost one-third of the participants belonged to the FCC group (28.8%), while two-thirds (71.2%) were classified into the NFCC group. No statistically significant differences were found in terms of gender, age, and number of children and household members between the two groups of catering customers. However, there were differences in terms of income, work status and educational attainment.

Within the group of frequent catering customers, 88 (33%) had an income in the range of HRK 5001–10 000, while surprisingly, the majority of non-frequent catering customers (30.9%) were in the higher income bracket (HRK 10 001–15 000) ($p=0.042$). The majority of both groups of customers were employed full-time, but with a larger proportion in the frequent customer group (72.7% vs. 67.1%, $p=0.049$). Students were represented in a higher proportion in the non-frequent catering customer group, while the proportion of retired and unemployed participants was higher in the frequent catering customer group ($p=0.049$). More than half of the total study sample had the highest level of education, with a significantly higher proportion of highly educated among the non-frequent catering customers ($p=0.049$).

The results of this study are partially comparable to the results of the national survey on dietary habits of the adult population in Croatia. In this survey, also conducted on a representative national sample, the highest percentage of participants eat out of home 1-2 times per month (15.1%), while 11.2% eat out of home once in 2-3 months. The characteristics of the subgroup that eats out of home weekly are: more males (61.88%) than females (38.12%), 18–39 years old (51.38%), fully employed (65.77%), students (18.23%), and people with higher education levels (22.82%) (HAH, 2011–2012). The results of a large survey conducted on a general population in eleven European countries, which aimed to identify differences between eating habits at home and on eating occasions away from home, revealed somewhat different personal characteristics of customers than those in our study. Eating out of home was more common among men, younger and more educated participants. Those who ate out more reported similar food intake on those occasions as on home eating occasions. In contrast, for those who eat out less, these occasions are a type of special experience and they have different eating patterns than at home (Naska et al. 2015).

Table 1. Demographic characteristics of study sample (n= 918)

Parameter	Total study sample (n=918)	FCC* (n=264)	NFCC* (n=654)	p
	n (%)			
Gender				
Male	462 (50.3)	145 (54.9)	317 (48.5)	0.077
Female	456 (49.7)	119 (45.1)	337 (51.5)	
Age (yrs)				
18–30	192 (20.9)	66 (25.0)	126 (19.3)	
31–40	207 (22.5)	66 (25.0)	141 (21.5)	0.077
41–50	233 (25.4)	61 (23.1)	172 (26.3)	
51–65	286 (31.2)	71 (26.9)	215 (32.9)	
Income (HRK)**				
<5 000	73 (7.90)	21 (8.0)	52 (8.0)	
5001–10 000	275 (30.0)	88 (33.3)	187 (28.6)	
10 001–15 000	278 (30.3)	76 (28.8)	202 (30.9)	**0.042**
15 001–20 000	130 (14.2)	32 (12.1)	202 (15.0)	
>20 001	58 (6.3)	25 (9.5)	33 (5.0)	
DK/NA	104 (11.3)	22 (8.3)	82 (12.5)	
Household members				
1	54 (5.9)	17 (6.4)	3.7 (5.6)	
2	226 (24.6)	62 (23.5)	164 (25.1)	0.762
3	261 (28.4)	69 (26.1)	192 (29.4)	
4	228 (24.9)	71 (26.9)	157 (24.0)	
5+	149 (16.2)	45 (17.1)	104 (15.9)	
Children				
0	559 (60.9)	154 (58.3)	405 (61.9)	
1	201 (21.9)	64 (24.3)	137 (21.0)	0.257
2	114 (12.4)	29 (11.0)	85 (13.0)	
3+	44 (4.8)	17 (6.4)	27 (4.1)	
Work status				
Employed full time	631 (68.7)	192 (72.7)	439 (67.1)	
Employed part-time	22 (2.4)	8 (3.0)	14 (2.1)	**0.049**
Student	62 (6.8)	12 (4.5)	50 (7.7)	
Retiree	127 (13.8)	26 (9.9)	101 (5.4)	
Unemployed	76 (8.3)	26 (9.9)	50 (7.7)	
Education				
Primary school	12 (1.3)	7 (2.6)	5 (0.8)	
Secondary school	415 (45.2)	124 (47.0)	291 (44.5)	**0.049**
Bachelor, master or higher	491 (53.5)	133 (50.4)	358 (54.7)	

*FCC – Frequent catering customers; NFCC – Non-frequent catering customers

** HRK – croatian currency

Depending on the amount, type, and distribution of fat, there are two types of fish, commonly called white fish and fatty fish. White fish is very low in fat and calories, while fatty fish stores fat in its flesh and is, therefore, richer in omega-3 polyunsaturated fatty acids (PUFA) (Ariño et al., 2013). Most European Food-Based Dietary Guidelines recommend two servings of fish *per* week to obtain important nutrients, especially omega-3 PUFA, as well as vitamin D, iodine and selenium. When the type of fish to be consumed is specified, most of them recommend consuming half of the fish as fatty fish (EFSA, 2014). Since this study focused on fish consumption in catering facilities, participants were asked about their habits of eating white and fatty fish in catering facilities. The results, broken down by catering customer group, are summarized in Figure 1.

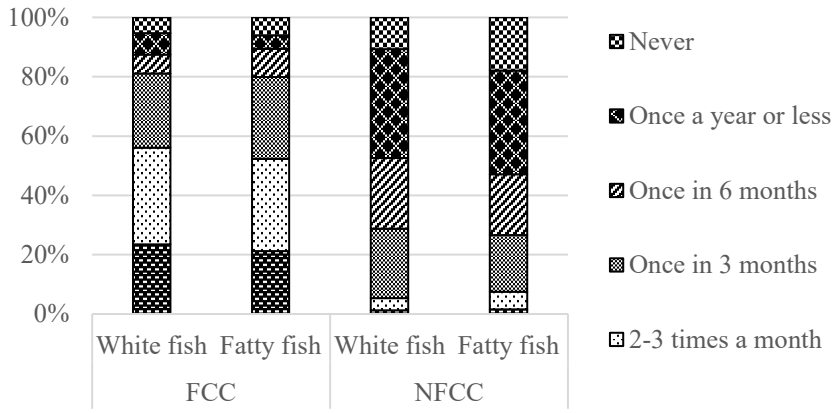

Figure 1. Frequency of consumption of fishery products in catering facilities (FCC – frequent catering customers, NFCC – Non-frequent catering customers)

The results show that the highest proportion of FCC consumed white fish (56%) and fatty fish (52%) in catering facilities on a monthly basis. More than one-third of the participants from the NFCC group consumed fish very infrequently in catering facilities. In this group, the frequency of white fish consumption "once a year or less" was reported by 37% of participants, while the same rare frequency for fatty fish was reported by 35% of participants from this group.

In the aforementioned study conducted in eleven European countries, male participants from Belgium, Germany, Italy, the Netherlands, and the United Kingdom reported higher consumption of fish when eating out compared to eating at home (Naska et al., 2015). In a recent analysis of the main determinants of eating fishery and aquaculture products away from home in the 28 countries of the European Union, the main reasons for eating these foods away from home were that they are cheaper than other foods, taste good, are healthy and are easy to digest. A higher frequency of eating fish away from home was reported by consumers who preferred wild fish (Cantillo et al., 2021).

Beliefs influence perceptions, attitudes, and intentions to purchase or consume certain foods. They represent information that a consumer possesses about a

particular object (in the case of this study, wild or farmed fish) and, therefore, link the attribute to the object (Fishbein, Azjen, 1975). With the aim of delving into the different aspects that influence beliefs about wild and farmed fish, belief statements were grouped according to Claret et al. (2014) into four main aspects: safety, quality, control, and purchase. The beliefs about wild and farmed fish for both groups of catering customers are presented in Table 2.

In general, farmed fish is considered a safe food, but there is still no consensus among consumers that it is safer than its wild counterparts (Krešić et al., 2020). However, a recent study by López-Mas et al. (2021) showed that European consumers, with the exception of the French, perceive farmed fish as safer, whereas the participants of our study showed a slight preference for wild fish. Regarding safety aspects, they believe wild fish is healthier, has a healthier diet, and contains less heavy metals and antibiotics than farmed fish. The NFCC group is significantly more convinced that farmed fish contains more antibiotics than wild fish ($p=0.003$). Other studies have also found that consumers generally believe that farmed fish contains higher concentrations of antibiotics (Claret et al., 2014; López-Mas et al., 2021; Solgaard, Yang, 2011; Verbeke et al., 2007). In a focus study among Belgian consumers (Verbeke, Brunsø, 2005), the perception of wild fish as "happier fish" contributed to its perception as more resistant to chemical and microbial contamination.

Our data confirm that customers are confident about the quality of wild fish. In five out of six statements, customers believe that wild fish is of superior quality. They believe that wild fish is of better overall quality, contains more nutrients, has firmer meat with less fat, and tastes better than farmed fish. Similar to previously mentioned results, customers who visit catering establishments frequently believe less strongly that farmed fish is fattier compared with wild fish ($p< 0.001$). Interestingly, neither group of customers considered either type of fish to be fresher. Similar results were obtained in a Spanish study where consumers preferred wild fish in all quality aspects (sensory characteristics, nutritional value, freshness) (Claret et al., 2014). In a study conducted in Belgium by Verbeke et al. (2007), wild fish performed better in the attributes of taste, health and nutritional value, although the majority of consumers did not perceive differences between farmed and wild fish. Taste preference for wild fish may also be influenced by the information conveyed to the consumer. In a blind experiment, consumers preferred farmed fish, but in the reverse case, they were in favour of wild fish (Claret et al., 2016; Rickertsen et al., 2017). Although quality is usually overestimated when comparing wild and farmed fish, such assumptions are not scientifically supported. The current scientific consensus is that farmed and wild fish are indistinguishable in terms of nutritional value and safety (EFSA, 2005). However, farmed fish has indeed higher fat content, but the amounts of omega-3 fatty acids contained in farmed fish *per serving* may be higher than in wild fish (Cahu et al., 2004).

Table 2. Beliefs about wild and farmed fish among catering customers (n=918)

Statement	FCC* (n=264)	NFCC* (n=654)	p
	Mean value ± SD		
Safety			
Wild fish is safer than farmed fish.	3.17 ± 1.12	3.10 ± 1.01	0.339
Wild fish is more affected by marine pollution than farmed fish.	3.03 ± 1.11	2.93 ± 1.08	0.251
Wild fish contains more heavy metals than farmed fish.	2.90 ± 1.06	2.79 ± 0.97	0.138
Wild fish contains more antibiotics than farmed fish.	2.39 ± 1.19	2.16 ± 1.03	**0.003**
Wild fish is more affected by parasites (anisakis) than farmed fish.	2.98 ± 0.98	2.86 ± 0.93	0.077
Wild fish has a healthier diet than farmed fish.	3.67 ± 1.04	3.66 ± 1.03	0.904
Wild fish is healthier than farmed fish.	3.52 ± 1.10	3.61 ± 1.05	0.243
Quality			
Wild fish is of better quality than farmed fish.	3.78 ± 1.04	3.75 ± 1.05	0.691
Wild fish is fresher than farmed fish.	3.16 ± 1.15	3.01 ± 1.12	0.081
Wild fish is more nutritious than farmed fish.	3.52 ± 1.04	3.39 ± 1.08	0.095
Wild fish is more fatty than farmed fish.	2.52 ± 1.30	2.21 ± 1.12	**<0.001**
Wild fish tastes better than farmed fish.	3.75 ± 1.04	3.80 ± 1.05	0.548
Wild fish is firmer than farmed fish.	3.57 ± 1.05	3.59 ± 1.02	0.757
Control			
Wild fish is more controlled than farmed fish.	2.73 ± 1.11	2.46 ± 1.01	**0.001**
Wild fish is more handled than farmed fish.	2.93 ± 1.09	2.86 ± 0.98	0.338
Wild fish is more artificial than farmed fish.	2.35 ± 1.23	1.86 ± 0.98	**<0.001**
Wild fish provides more guarantees than farmed fish.	3.34 ± 1.15	3.25 ± 1.06	0.229
Purchase			
Wild fish is easier to find than farmed fish.	2.56 ± 1.22	2.32 ± 1.05	**0.004**
Wild fish is cheaper than farmed fish.	2.70 ± 1.25	2.39 ± 1.17	**<0.001**

*FCC – Frequent catering customers; NFCC – Non-frequent catering customers

Farmed fish is preferred in terms of control, which is consistent with previous studies (Claret et al., 2014; López-Mas et al., 2021), whereas customers in the NFCC group are more convinced that farmed fish is better controlled ($p<0.001$). However, a significant difference between the groups of catering customers is evident in the statement "wild fish is more artificial than farmed fish", for which the NFCC and FCC groups had an agreement of 1.86 ± 0.98 and 2.35 ± 1.23, respectively ($p<0.001$). Consumers may not be familiar with aquaculture production systems and may perceive them as something new, industrial, and thus unnatural, while capture fisheries are idealised in their minds as a traditional method of fish production. As a result, wild fish is often referred to as natural, although there is no official definition of what natural means in relation to food. However, the term is already operative in the marketing of fish (Schlag, Ystgaard, 2013), and our findings support this fact.

The most positive aspects of the beliefs in favour of farmed fish refer to the possibility of purchasing it and to its price. Significant differences were found between the two groups of customers for both statements. Namely, the NFCC group was more convinced that farmed fish is easier to find ($p=0.004$) and that it is cheaper ($p<0.001$). The results are consistent with other European studies where farmed fish is perceived as more affordable and available, and these beliefs are often the most positive ones that consumers have towards farmed fish (Claret et al., 2014; López-Mas et al., 2021; Schlag, Ystgaard, 2013; Verbeke et al., 2007). However, price alone is not sufficient to determine overall preference for farmed *vs* wild fish (Hall, Amberg, 2013), and sometimes consumers are willing to pay more for wild fish (Davidson et al., 2012), as they may view a higher price as an indicator of higher quality (Claret et al., 2014).

CONCLUSION

The aim of this study was to investigate beliefs about wild and farmed fish in a group of Croatian customers who frequently visit catering facilities and in a group of those who do not. Overall, non-frequent catering customers are more likely to believe that farmed fish contains more antibiotics and more fat, and is more artificial. On the other hand, they are more positive about control, availability, and price. This may indicate that frequent catering customers may not be aware that farmed fish is often offered in catering facilities due to its constant availability. At the same time, they do not view it as much as a more affordable option, which may suggest that they generally view fish as an expensive product in catering facilities regardless of the production method. However, as there are already more positive beliefs of certain aspects in the group of frequent catering customers, these should be further emphasized in order to fully reduce prejudices against farmed fish and thus increase fish consumption in catering facilities. The results of this study provide valuable information for various stakeholders and can be used as a basis for effective promotional campaigns for farmed fish in catering. Future studies should aim to investigate the influence of beliefs about farmed fish on fish consumption in foodservice establishments serving mainly farmed or wild fish.

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THE IMPACT OF NUTRITION ON ENDOMETRIOSIS AND POLYCISTIC OVARY SYNDROME

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review paper

ABSTRACT

It is not clear to what extent altered concentrations of certain nutrients and food products may support disease onset, maintenance, and / or progression. Aim. The aim of this research was to describe the influence of different nutritional habits that are presumed to be associated with relief of symptoms of endometriosis and polycystic ovary syndrome (PCOS). Methodology. We included relevant studies that explored the influence of the intake of certain nutrients and food products on endometriosis and PCOS. Among the most frequently examined are polyunsaturated fatty acids, certain types of fruits and vegetables, and micronutrients such as vitamins C, B9, B12 and E, alpha and beta carotene, retinol, lycopene and trace elements such as selenium, copper, zinc, magnesium and calcium. Results. There is evidence that some PUFA, vitamins, antioxidants and green vegetables could reduce symptoms in endometriosis. Green vegetables, pulse-based diet, myo-inositol, certain bioflavonoids and quercetin showed positive effect in reducing hormonal imbalance, risk of cardiovascular diseases, hirsutism and amenorrhea among women with PCOS. Conclusion. More studies, especially those with precise quantification of intake are necessary to provide guidelines for reducing signs and symptoms of diseases such as endometriosis and PCOS.

Keywords: nutrients, food, endometriosis, polycystic ovary syndrome

INTRODUCTION

The modern age has brought numerous articles that are the result of research of the effects of certain food components on some diseases. It is mainly research that seeks to find a specific nutrient that would bring a disease under control or even eliminate it. Some researchers try to assess the degree of the disease or at least patients' own impression of the improvement of symptoms after the application of certain nutrients, while others try to measure indirect indicators of the degree of the disease. It is known that the type and composition of food can have a major impact on certain diseases such as diabetes, metabolic diseases such as phenylketonuria and diseases of the gastrointestinal system in which the body's response to changes in diet can be large and rapid. Aside from the commercial purpose of advertising certain food products in health promotion, without the cooperation of the health and food industries the progress of primary and secondary prevention of certain diseases may be slowed down. Some of the research has shown an impact on the improvement of certain female-specific diseases. There are studies that focus on the influence of micro / macronutrients on the development of cancer or their preventive effects. The aim of this paper is to present the results of some research on the impact of some micronutrients on the very common gynecological diseases of benign nature, but which have a great impact on the quality of life of the patient. By this we mean primarily endometriosis and polycystic ovary syndrome (PCOS).

Endometriosis

Endometriosis is one of the most common gynecological diseases. It is estimated that up to a total of 10% of women suffer from it, with large variations depending on the population (Shafrir et al., 2018). In women suffering from infertility, it is present in up to 50% of patients. The disease indicates the presence of endometrial tissue outside the uterus, which may be associated with a very complex and variable clinical picture. Some of the most common symptoms are dysmenorrhea, dyspareunia, dyschezia and infertility. However, the clinical picture includes pelvic pain (especially associated with the menstrual cycle), fatigue and back pain. Symptoms usually bring a woman to a doctor's office, and the diagnosis should primarily be made by direct visualization or laparoscopic, with biopsy or histological confirmation (Dunselman et al., 2014). The disease affects a woman's mental and sexual health and affects social well-being and productivity. The treatment of women is surgical and medical.

Polycystic Ovary Syndrome (PCOS)

The condition characterized by the ultrasound finding of numerous cysts in the ovary, associated with hormonal disbalance, chronic anovulation, and ovarian dysfunction is called polycystic ovary syndrome (PCOS). In addition to representing gynecological disease, PCOS has been associated with insulin resistance, type 2 diabetes mellitus, metabolic syndrome, endometrial cancer, and increased levels of androgenic hormones (Rosenfield et al., 2016). The disease is also common and is

thought to affect about 5-10% of women of reproductive age, and in some populations up to 20% (Lizneva et al., 2016).

METHODOLOGY

The PubMed database was explored on the topic of the association between food products, micro and macro nutrients on the one hand, and endometriosis and polycystic ovary syndrome on the other. The study included mostly interventional and randomized clinical trials, with the exception of individual case-control studies and observational studies, which quantified the intake of specific food or substances and measured the outcome related to improving clinical status or laboratory findings of certain parameters associated with endometriosis and PCOS. The time period in which articles included in the present study were published is 16 years, starting from 2003.

RESULTS AND DISCUSSION

Research on the impact of diet on endometriosis

Treatment of endometriosis involves a surgical approach, and in case the pregnancy is not planned in the near future, a very common therapeutic approach is the use of hormonal contraceptives (Dunselman et al., 2014). Due to results of some research focusing nutrition on relief of symptoms one part of the patients decides to change their life habits in order to improve their health. Some of the diets or instructions are based on the result of the experiences of other patients, and not on the results achieved by given biomedical research. On the other hand, avoiding certain food can create a deficiency of certain nutrients, and failure to adhere to a diet can create feelings of guilt. There is little scientific evidence of the impact of diet on endometriosis. Most studies are case-control, and the impact of diet in these studies was assessed by grading pain before and after the intervention or by the use of a particular product whose effectiveness is being examined.

De Leo et al. (De Leo et al., 2019) in their multicentric study compared the use of lipoic acid, palmitoiletanolamide and myrrh on the pelvic pain among 60 women suffering from endometriosis (endometriotic ovarian cyst diagnosed by ultrasound), aged 20-39. Patients used these nutrients for 6 months. Significantly less chronic pelvic pain associated with endometriosis has been reported. Dyspareunia and dysmenorrhea were also rated as mild, and interestingly, a statistically significant effect was achieved as early as three months. Nutrients had no effect on reducing the diameter of the endometriotic cyst. The limitation of the study was that there was no control group of subjects receiving placebo.

Cobellis et al. (Cobellis et al., 2011) observed the use of N-palmitoylethanolamine and transpolydatin in the treatment of endometriotic pain. The study included 61 subjects, with a previous laparoscopic diagnosis and who did not use any other drugs to relieve the symptoms of endometriosis. Subjects were divided into three groups - a control group receiving placebo, one using N-palmitoylethanolamine and trans polydatin, and one using celecoxib. All groups showed relief of symptoms, but there

was a statistically significant difference between the placebo group and the remaining two, with the group using celecoxib having the best results. But it should be borne in mind that celecoxib is a drug and take into account the side effects of long-term use of the drug.

There are results of a pilot study (Stochino Loi et al., 2019) in which ultramicronized-palmitoylethanolamide and co-micronized palmitoylethanolamide/ polydatin were used in the treatment of chronic pelvic pain related to endometriosis in 30 subjects. The research was also made without a control group. Using a visual-analog pain scale (VAS), improvement in dyspareunia, dysmenorrhea, and dyschezia has been shown. Moreover, patients reported a better quality of life and psychological relief.

Omega 3 and omega 6 have recently become one of the most researched fatty acids. Their influence was also examined in the case of endometriosis. Namely, foods rich in omega 6 fatty acids are associated with higher production of estradiol and estrone sulfate, which are associated with the development of endometriosis (Aris et al., 2010). A study conducted on 64 patients with endometriosis showed that the ratio of serum-measured eicosapentaenoic acid to arachidonic acid was related to disease severity (Khanaki et al., 2012).

Soy contains a small amount of phytoestrogens. Since endometriosis is related to the amount of estrogen, it seemed logical to avoid soy to reduce the symptoms of endometriosis. In a small description of two patients (Chandrareddy et al., 2008) it was shown that avoiding soy improved the clinical picture of endometriosis. However, since soy contains a very small amount of phytoestrogens, the usefulness of this approach is questionable.

Research has been conducted on the effect of certain vitamins and antioxidants in reducing the symptoms of endometriosis. One study did not show the benefits of vitamin D use over the placebo (Almassinokiani et al., 2016). This was a double-blind clinical trial in 39 subjects 8 weeks after surgery, and reevaluation of symptoms was performed 24 weeks after surgery, with a previous duration of therapy of 12 weeks (50,000 IU of vitamin D per week). Neither dysmenorrhea nor pelvic pain showed relief from vitamin D use.

Certain combinations of vitamins and minerals have also been examined in reducing the symptoms of endometriosis. In one study, 222 subjects underwent surgery for stage III and IV symptomatic endometriosis (Sesti et al., 2007). Subjects were divided into four groups - placebo, gonadotropin-releasing hormone agonist (GnRH), a combination of oral estrogens and progesterone, and diet therapy (vitamins, minerals, fish oil, and lactobacilli). The GnRH group showed the best results in the treatment of dysmenorrhea, but diet also was effective in reducing pain unrelated to menstruation. The combination of surgery and diet proved successful in increasing quality of life compared with placebo.

Studies involving curcumin have shown that the use of curcumin has an effect on reducing endometriosis. The research was conducted mainly on animal models, mostly mice. One such study showed that the use of curcumin can lead to a decrease in the proliferation of endometrial glands located ectopically in the peritoneum in a

group of mice in which curcumin was administered compared to mice in the control group (Jana et al., 2012).
Moreover, in a rat model, the use of curcumin has been shown to reduce endometrial proliferation by slowing cell growth and primarily by reducing estrogen secretion that stimulates ectopic endometrial growth (Zhang et al., 2013). Also, a similar study showed that the use of curcumin can reduce blood vessel proliferation in the ectopic endometrium, which is also one of the growth factors of endometriotic foci (Zhang et al., 2010).
Endometriosis is associated with an inflammatory process in the peritoneum. There are limited studies on the use of curcumin to reduce the inflammatory process, which would suggest an improvement in the clinical picture. In one study, the use of curcumin in vitro in ectopic human endometrial cells led to decreased secretion of certain inflammatory markers such as interleukin 6 and interleukin 8, and Monocyte Chemoattractant Protein-1, and activation of NF-κB (Kim et al., 2012). The effect of curcumin use on the reduction of adhesion and invasion of the ectopic endometrium, which included metalloproteinase matrix (MMP), was examined. In two studies in mice, the use of curcumin led to downregulation of MMP-2, MMP-9 (Jana et al., 2014) and MMP-3 (Jana et al., 2012).
One Italian case-control study (Parazzini et al., 2004) was conducted on 504 women with endometriosis (and 504 in the control group). It was observed that the risk of endometriosis was higher in the group of patients in the lowest tertile of intake of green vegetables, and the risk increased with higher consumption of red meat.
There are other studies on the influence of different dietary components on endometriosis, but mostly without a satisfactory level of evidence. In addition, some studies have such limitations that it is not possible to clearly distinguish whether it is the impact of diet on the intensity of the symptoms of the disease or some parallel procedure in which the patient found herself. However, an increasing number of studies emphasize the use of food as one of the components that could reduce the intensity of endometriosis symptoms.

Polycystic Ovary Syndrome and nutritional therapy

The etiopathogenesis of PCOS has not been elucidated, but the hormonal imbalance is certainly present. Given that hormonal imbalances in some conditions can be corrected by adhering to a specific diet, some studies have evaluated the effects of certain diets or certain nutrients on improving the clinical picture in patients with PCOS. Most of these studies focused on the impact on the reduction of insulin resistance and the reduction of cardiometabolic risk associated with PCOS.
Most research in which female patients have lost weight appears to show an improvement in the clinical picture in women with PCOS (Crosignani et al., 2003; Einarsson et al., 2017).
One study compared the impact of a pulse-based diet rich in low glycemic index food and a diet from Therapeutic Lifestyle Changes (TLC) (Kazemi et al., 2018). The study included 95 women aged 18-35 years, and the intervention lasted 16 weeks. A total of 61 respondents completed the study, of which 31 in the TLC

group. Interestingly, the subjects in the pulse diet, who had lentils, beans, chickpeas and split peas in their diet, had better insulin sensitivity, diastolic pressure, triglyceride concentration, low-density lipoprotein cholesterol and higher concentration of high-density lipoprotein cholesterol compared to TLC group. The authors concluded that such a diet may be more beneficial than the TLC diet in reducing cardiometabolic risk factors in women with PCOS.

Another study confirmed the impact of a diet rich in green vegetables on the reduction of cardiometabolic risk factors in women with PCOS. Respondents were divided into two groups according to whether they adhered to a DASH diet (Dietary Approaches to Stop Hypertension) rich in green vegetables or a control diet of the same amount of carbohydrates, fats and proteins, but different composition (Azadi-Yazdi et al., 2017). Final results after 12 weeks of diet showed that the group with the DASH diet had a significant decrease in body weight, body mass index, amount of adipose tissue and serum androstenedione, and a higher amount of sex hormone binding globulin (SHBG).

Research on the effect of polyunsaturated fatty acids in the diet on reducing hormonal imbalance has not shown the desired (or at least expected) effect. Namely, in a study by Phelan et al. (Phelan et al., 2011) in bovine cells found that long chain omega 3 fatty acids compared to omega 6 fatty acids had a lower atherogenic lipid profile and a lower concentration of free testosterone and vice versa - treatment with omega 6 fatty acids resulted in higher secretion of androgenic hormones. However, there was no difference in the reduction in triglyceride levels in patients with PCOS, nor in SHBG, total testosterone, and dehydroepiandrosterone sulfate.

A study of vitamin D administration showed an effect on hormonal status in women with PCOS. In a randomized, double-blind clinical trial, 90 subjects with insulin resistance and PCOS (Jamilian et al., 2017) participated. Subjects were divided into three groups according to the amount of vitamin D administered daily for 12 weeks (4000 IU, 1000 IU or placebo). The group with the highest amount of vitamin D supplementation had a statistically significant decrease in total testosterone, free androgen index (FAI), hirsutism, high-sensitivity C-reactive protein, and higher SHBG and total antioxidant capacity. Another study on the effect of vitamin D in combination with weight loss did not show the desired result of vitamin D (Jafari-Sfidvajani et al., 2018). In this study, 60 subjects with PCOS participated and were divided into two groups. In the first group, with weight loss, vitamin D was administered, in the amount of 50,000 IU orally per week, and in the second group with placebo weight loss. The intervention lasted 12 weeks. The results showed that there was no difference in the androgen profile (total testosterone, DHEAS, FAI and SHBG), although the frequency of the menstrual cycle was slightly better in the group with vitamin D.

Some studies on inositol have confirmed its effect on improving the metabolic profile in patients with PCOS, but the question of the direct effect of inositol on laboratory parameters remains. Namely, in studies examining the effect of some substance on the reduction of insulin resistance, improvement of lipid profile, weight loss or reduction of cardiometabolic risk, hormonal imbalance is often

corrected, so the question of the direct effect of certain dietary supplements on hormonal status remains. One such study was conducted on 22 obese women who received D-chiro-inositol (DCI) (500mg orally daily) over a 12-week period, without changes in other life habits. After the intervention, the results showed a better status of luteinizing hormone (LH), the ratio of luteinizing and follicle stimulating hormone, androstenedione as well as insulin (Genazzani et al., 2014). Likewise, patients showed a better response in LH secretion after administration of gonadotropin-releasing hormone (bolus of 10 μg). Another study compared the use of myo-inositol in one group of 25 PCOS patients (4g orally daily) and D-chiro-inositol in 25 PCOS patients (1 g orally daily) on improving ovarian function (Pizzo et al., 2014). Both groups received 400 mg of folic acid daily. The intervention lasted six months. The results showed that in both groups of subjects' ovarian function improved (measuring metabolic and hormonal status) and that better regularity of menstrual cycles was established. The difference was that the group with myo-inositol showed a better metabolic effect, and the group with D-chiro-inositol showed a greater reduction in hirsutism.

There are other, smaller studies that have focused on the use of everyday food products in improving the clinical picture of women with PCOS. One such is research on the use of green tea on the metabolic profile of patients with PCOS (Tehrani et al., 2017). In one group of subjects, green tea was administered for 12 weeks, and in the other a placebo. The study found that after the intervention, the green tea group had lower concentrations of free testosterone. Another study included quercetin as one of the most commonly represented bioflavonoids (Rezvan et al., 2018). In that study, a total of 84 patients with PCOS were divided into two groups, one who received 1 g daily of quercetin orally, while the other group received placebo. The intervention lasted 12 weeks. The quercetin group had significantly higher expression of the adiponectin receptor transcription ADIPOR1 and ADIPOR2 and the concentration of AMP-activated protein kinase. The authors conclude that it is likely that the result was achieved through the anti-inflammatory properties of quercetin and that the results of the study should be confirmed through in vitro studies on cell culture.

A very similar study was conducted in which patients with PCOS and overweight or obesity were divided into two groups, one with quercetin 1 g daily orally and the other with placebo, and the metabolic and hormonal parameters of quercetin were compared (Khorshidi et al., 2018). Administration of quercetin has been shown to lead to decreased serum concentrations of resistin and mRNA in peripheral blood mononuclear cells. Furthermore, testosterone and LH concentrations were lower in the quercetin group, but without a statistically significant effect on the reduction in insulin resistance compared to the placebo group.

CONCLUSION

There are numerous studies on the impact of diet on gynecological diseases. Some studies are observational and talk about the prevention of gynecological malignancies, while interventional research is aimed at secondary prevention and improvement of the clinical picture in women with benign gynecological diseases. These benign conditions often include those diseases in which the hormonal status has an impact on the development of the disease. In the absence of randomized interventional clinical trials, especially those in which individual food ingredients are given in a precisely determined amount, it is impossible to draw precise conclusions about the role of diet in individual gynecological diseases. Nevertheless, some studies, despite their limitations and lower level of evidence, show *in vivo* and *in vitro* conditions that certain food components have an effect on both reducing and increasing the intensity of endometriosis and PCOS symptoms. One of the main characteristics of such research is consistency in adherence to the diet of the respondents. Changing patients' life habits is one of the more difficult tasks, so it seems that gathering evidence based on well-structured research would be helpful in convincing patients of the possibilities of improving their quality of life through diet.

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ULOGA PREHRAMBENE INTERVENCIJE U RAZVOJU VJEŠTINA SAMOSTALNOG ŽIVLJENJA U OSOBA S DUŠEVNIM SMETNJAMA

THE ROLE OF DIETARY INTERVENTION IN THE DEVELOPMENT OF INDEPENDENT LIVING SKILLS IN PEOPLE WITH MENTAL DISORDERS

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SAŽETAK

Smatra se da prehrambene intervencije, osim u poboljšanju prehrambenih navika, mogu imati pozitivne učinke i na brojne aspekte tjelesnog i mentalnog zdravlja, među kojima je i razvoj određenih vještina samostalnog življenja. Unatoč tome, do danas se malo zna o učincima prehrambenih intervencija na razvoj spomenutih vještina u osoba s duševnim smetnjama. Stoga je cilj ovog preglednog rada sažeti trenutna saznanja o učincima različitih vrsta prehrambenih intervencija na vještine samostalnog življenja u ove populacijske skupine. Pregled literature putem bibliografske baze podataka PubMed uključio je izvorne znanstvene članke i pregledne radove. Prehrambene intervencije, različitog dizajna i duljine primjene, rezultirale su razvojem i unaprjeđenjem određenih vještina samostalnog življenja (veća samostalnost prilikom nabave namirnica i pripreme obroka, veća odgovornost kod raspolaganja prihodima, socijalna interakcija) u osoba s duševnim smetnjama. Daljnje studije na većem uzorku ispitanika su potrebne kako bi se potvrdili pozitivni učinci prehrambenih intervencija na vještine samostalnog življenja u ove populacijske skupine. Unatoč potrebi za daljnjim istraživanjima, rezultati publiciranih studija govore u prilog nužnosti uključivanja prehrambenih programa u cjelokupni tretman osoba s duševnim smetnjama. Pritom je od izrazite važnosti da u provedbu prehrambenih intervencija, uz nutricioniste, budu uključeni i radni terapeuti kako bi se postigao optimalan učinak na aspekte samostalnog življenja.

Ključne riječi: prehrambena intervencija, vještine samostalnog življenja, osobe s duševnim smetnjama, radni terapeut, nutricionist

Keywords: dietary intervention, independent living skills, people with mental disorders, occupational therapist, nutritionist

UVOD

Narušena sposobnost samostalnog življenja i funkcioniranja, koja je često prisutna u osoba s duševnim smetnjama, značajno povećava vjerojatnost hospitalizacije pripadnika ove populacijske skupine (Fortuna i sur., 2018). Osim u pogledu samostalnog življenja i funkcioniranja, osobe s duševnim smetnjama pokazuju i brojne poteškoće u socijalnom funkcioniranju, što u konačnici može rezultirati socijalnom izoliranošću te relapsom bolesti (Mihaljević-Peleš i sur., 2019).
Brojne su do danas publicirane znanstvene studije potvrdile i da osobe s duševnim smetnjama često imaju nezadovoljavajuće prehrambene navike (Osborn i sur., 2007; Casagrande i sur., 2011) koje se mogu značajno poboljšati uključivanjem u programe prehrambenih intervencija (Baker i sur., 2014). Općenito govoreći, prehrambene intervencije, osim u poboljšanju prehrambenih navika, mogu imati pozitivne učinke i na brojne aspekte tjelesnog (Ard i sur., 2016; Ozemek i sur., 2018) i mentalnog zdravlja (Firth i sur., 2019; Parletta i sur., 2019, Głąbska i sur., 2020), među kojima je i razvoj određenih vještina samostalnog življenja (Bogomolova i sur., 2018).
Unatoč tome, do danas se malo zna o učincima prehrambenih intervencija na razvoj vještina samostalnog življenja u osoba s duševnim smetnjama. Stoga je cilj ovog preglednog rada bio sažeti trenutna saznanja o učincima različitih vrsta prehrambenih intervencija (različitog dizajna i duljine trajanja) na vještine samostalnog življenja u ove populacijske skupine.

VJEŠTINE SAMOSTALNOG ŽIVLJENJA U OSOBA S DUŠEVNIM SMETNJAMA

Prema definiciji Svjetske zdravstvene organizacije, životne vještine su „sposobnosti za prilagodljivo i pozitivno ponašanje koje omogućavaju pojedincima da se efikasno nose sa zahtjevima i izazovima svakodnevnog života" (WHO, 1994). Kognitivna disfunkcija, često prisutna u osoba s duševnim smetnjama, može posljedično dovesti do pogoršanja životnih vještina (Samuel i sur., 2018). Brojne su do danas publicirane znanstvene studije utvrdile kako je u osoba s duševnim smetnjama nerijetko prisutan nedostatak samostalnosti u obavljanju svakodnevnih aktivnosti te kako pripadnici ove populacijske skupine često nisu spremni živjeti neovisno. Tako je prema rezultatima istraživanja provedenog na 100 ispitanika s dijagnozom shizofrenije u dobi 18-60 godina svega 2 % ispitanika bilo u potpunosti neovisno u obavljanju svih svakodnevnih aktivnosti, što je uključivalo sposobnost korištenja telefona ili mobilnih uređaja, izvršavanje jednostavnih zadataka kupovine, sposobnost pripreme hrane, samostalno pranje rublja, održavanje stambenog prostora čistim i urednim, razinu asistencije potrebne prilikom putovanja te potrebnu vrstu prijevoznog sredstva, samostalnu brigu o financijama te odgovornost prilikom uzimanja propisane farmakološke terapije (Samuel i sur., 2018). U pogledu samostalnog življenja, rezultati longitudinalnog istraživanja provedenog na 209 odraslih ispitanika (95 žena i 114 muškaraca) s prvom psihotičnom epizodom, koji

su praćeni kroz vremenski period od 10 godina, pokazali su kako je svega 58 % žena i 40 % muškaraca živjelo odvojeno od roditelja (Ayesa-Arriola i sur., 2020). Upravo poradi navedenog, za sveobuhvatnu rehabilitaciju osoba s duševnim smetnjama od izrazite su važnosti procjena postojećih životnih vještina te primjena odgovarajućih strategija i intervencija koje će za cilj imati kako razvoj novih životnih vještina tako i poboljšanje onih već postojećih (Samuel i sur., 2018).

U skladu s navodima Samuela i sur. (2018) te unatoč tome što se smatra da različite strategije i intervencije usmjerene na razvoj i poboljšanje životnih vještina mogu imati pozitivne učinke na ukupnu kvalitetu života osoba s duševnim smetnjama, veću samostalnost te posljedično i veće rasterećenje članova obitelji oboljelih, istraživanja na ovome području su, do danas, nedostatna, a navedene strategije i intervencije često u praksi nisu u dovoljnoj mjeri zastupljene kao integralni dio rehabilitacijskog tretmana.

ULOGA NUTRICIONISTA I RADNOG TERAPEUTA U PROVEDBI PREHRAMBENIH INTERVENCIJA U OSOBA S DUŠEVNIM SMETNJAMA

Kao što je u uvodnom dijelu rada već naglašeno, prehrambene intervencije kod osoba s duševnim smetnjama mogu rezultirati mnogobrojnim pozitivnim učincima na zdravlje (Gurusamy i sur., 2018) i kvalitetu života (Aucoin i sur., 2020). Nadalje, mogu voditi i k značajnim poboljšanjima prehrambenih navika i ukupne kakvoće prehrane (Sorić i sur., 2019), kao i rezultirati razvojem i poboljšanjem kulinarskih vještina (Duncombe, 2004; Clark i sur., 2015; Bogomolova i sur., 2018). Posljednje navedeno zauzima značajno mjesto u poticanju samostalnog življenja i povećanju sposobnosti osoba s duševnim smetnjama da samostalno brinu o sebi (Samuel i sur., 2018). Kulinarske vještine su u ovom kontekstu prvenstveno orijentirane na sposobnost samostalne pripreme jednostavnijih obroka, a posljedično mogu rezultirati i većom samostalnošću kod nabavke namirnica, planiranja obroka, raspolaganja financijskim sredstvima te poboljšanjem u socijalnoj interakciji (Bogomolova i sur., 2018).

Upravo je poradi toga, uz neizostavnu ulogu nutricionista, od izrazite važnosti naglasiti i značaj radnog terapeuta u provedbi prehrambenih intervencija. Dok je nutricionist prvenstveno orijentiran na edukaciju s ciljem poboljšanja prehrambenih navika, primarna uloga radnog terapeuta je razvijati vještine potrebne za samostalno obavljanje svakodnevnih zadataka (održavanje i poboljšanje postojećeg kognitivnog funkcioniranja, motoričkih i senzorskih komponenti), praćenje razine aktivnosti sudionika te pružanje psihosocijalne podrške tijekom provedbe samih intervencija (Malamud, 1986).

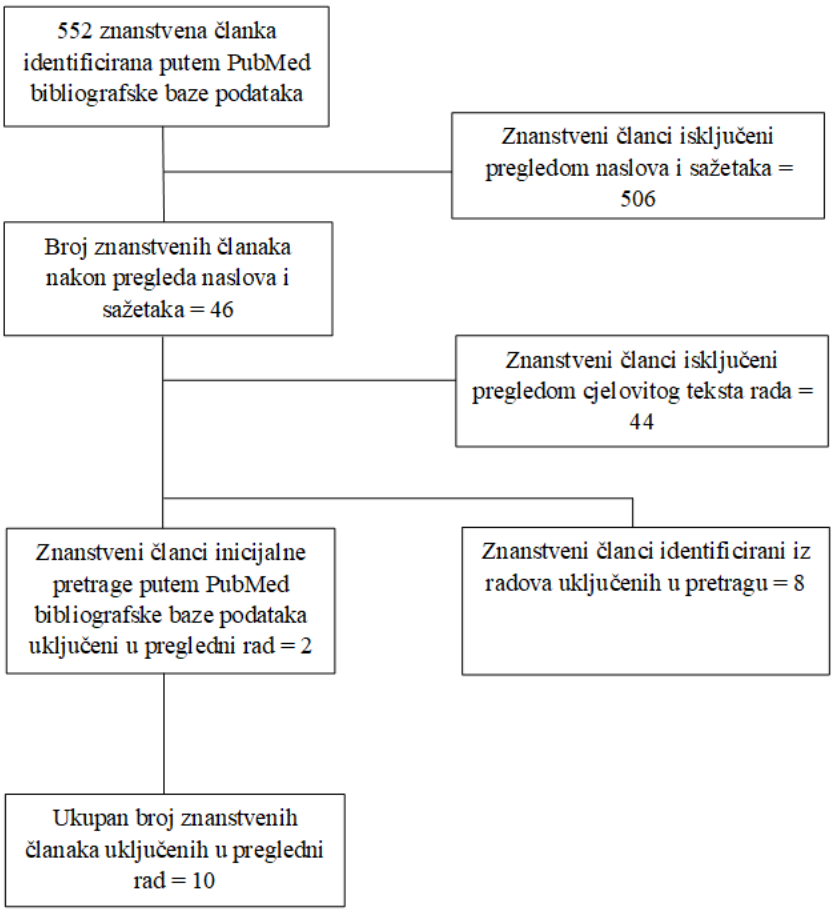
Također, važno je spomenuti i kako su prehrambene navike i ukupna kakvoća prehrane usko povezani s kulinarskim vještinama, čemu u prilog govore rezultati studije Hartmanna i sur. (2013) koji su potvrdili kako navedene vještine mogu

pridonijeti zadovoljavanju prehrambenih preporuka, što u konačnici može rezultirati odabirom nutritivno kvalitetnijih namirnica.

PREHRAMBENE INTERVENCIJE U RAZVOJU VJEŠTINA SAMOSTALNOG ŽIVLJENJA U OSOBA S DUŠEVNIM SMETNJAMA

Ovaj pregledni rad temeljen je na pretrazi literature putem bibliografske baze podataka PubMed te je uključio i izvorne znanstvene članke i pregledne radove. Za potrebe naprednog pretraživanja netom navedene bibliografske baze podataka korištene su sljedeće ključne riječi za identifikaciju ciljane populacijske skupine (mental disorders OR serious mental illness OR schizophrenia OR depression OR bipolar disorder OR dementia) u kombinaciji s jezičnim terminima za utvrđivanje vrste provedene intervencije (dietary intervention OR nutritional intervention) i ishoda od primarnog interesa ili usko povezanih s primarnim ishodima za provedeno istraživanje (independent living skills OR culinary skills OR social interaction OR food shopping OR budgeting OR meal planning OR occupational therapy). Tijekom pretrage u obzir su uzimane sve studije publicirane na engleskom jeziku, neovisno o godini njihove publikacije. Pretraga članaka za potrebe pripreme ovog preglednog rada provedena je dana 4. studenog 2021. godine. U strategiji pretraživanja bile su napravljene određene modifikacije u odnosu na pilot-verziju pretrage članaka provedenu dana 14. svibnja 2021. godine, a s ciljem što veće preciznosti same pretrage. Za potrebe identifikacije ciljane populacijske skupine, pretraga je s početnih pojmova „serious mental illness" i „schizophrenia" proširena i na pojmove „mental disorders", „depression", „dementia" i „bipolar disorder". Također, iz pretrage pojmova vezanih za ishode od primarnog interesa ili usko povezanih s primarnim ishodima za provedeno istraživanje isključeni su pojmovi „cognitive impairment" i „cognitive rehabilitation" budući da je ovom preglednom radu cilj bio utvrditi utjecaj prehrambenih intervencija isključivo na vještine samostalnog življenja, a uključeni su pojmovi „food shopping", „budgeting", „meal planning" i „social interaction". Kako bi se umanjila mogućnost pogreške odnosno mogućnost da se neki od relevantnih radova previdi, pretragu su, neovisno jedan o drugome, provela oba autora te su dobiveni rezultati potom uspoređeni, a razlike u rezultatima pretrage riješene su kroz raspravu. Pretraga je rezultirala pronalaskom ukupno 552 znanstvena članka. Pronađene studije su nadalje morale zadovoljiti sljedeće kriterije za uključivanje: (1) biti provedene na odraslim osobama oboljelima od shizofrenije, bipolarnog poremećaja, depresije i/ili demencije; (2) uključivati prehrambenu intervenciju, neovisno o dizajnu i duljini trajanja, koja je za jedan od ciljeva imala praćenje utjecaja iste na neke od vještina samostalnog življenja (kulinarske vještine, samostalnost u pogledu nabavke i pripreme obroka, samostalnost u raspolaganju financijama, socijalna interakcija). S druge strane, kriteriji za isključivanje bili su sljedeći: (1) studije provedene na osobama koje boluju od poremećaja u prehrani; (2)

studije provedene na osobama mlađima od 18 godina; (3) studije koje nisu uključivale prehrambenu intervenciju bilo zasebno ili kao dio tretmana; te (4) studije čiji cjeloviti tekst rada nije dostupan na engleskom jeziku. Slika 1 prikazuje faze selektiranja studija uključenih u ovaj pregledni rad. Pregledani su naslovi i sažetci svih identificiranih članaka te je izdvojeno ukupno 46 znanstvenih članaka koji su potom podvrgnuti detaljnijoj analizi kako bi se utvrdilo zadovoljavaju li kriterije za uključivanje odnosno neuključivanje. Konačno, pregledom cjelovitih tekstova izdvojenih znanstvenih članaka, u pregledni rad uključeno je njih ukupno 10.



Slika 1. Faze selektiranja znanstvenih članaka
Figure 1. Article selection process

Prikaz znanstvenih članaka uključenih u pregledni rad, zajedno s informacijama o populacijskoj skupini na kojoj je provedeno istraživanje, vrsti provedene intervencije te izdvojenim ishodima intervencije od interesa za ovaj pregledni rad dan je u Tablici 1.

Tablica 1. Karakteristike uključenih znanstvenih članaka (I.dio)
Table 1. *Characteristics of included scientific articles (I part)*

Literaturna referenca	Populacijska skupina	Provedena intervencija	Ishodi intervencije od interesa za pregledni rad
Bogomolova i sur., 2018.	Osobe s duševnim smetnjama oba spola, uključujući između ostaloga i osobe oboljele od shizofrenije i bipolarno afektivnog poremećaja	Tromjesečna intervencija tijekom koje su ispitanici bili uključeni u nutritivne edukacije i kulinarske radionice.	Navike samostalnog življenja (kulinarske vještine i sposobnost nabavke namirnica, raspolaganje financijskim sredstvima vezano uz nabavku namirnica, planiranje nutritivno kvalitetnih obroka, samopouzdanje vezano uz samostalnu pripremu obroka, socijalne vještine)
Clark i sur., 2015.	Osobe s duševnim smetnjama, oba spola (dijagnoze nisu specificirane)	Program nutritivno-kulinarskih edukacija u trajanju od šest tjedana.	Razina samostalnosti prilikom pripreme i nabavke namirnica.
Lloyd i Sullivan, 2003.	Osobe s duševnim smetnjama (u najvećem broju osobe muškog spola oboljele od shizofrenije)	Program edukativno-praktičnih radionica usmjerenih na prehranu, tjelesnu aktivnost i kontrolu tjelesne mase.	Raspolaganje financijskim sredstvima u području nabavke namirnica, planiranje nutritivno kvalitetnih i cjenovno prihvatljivih obroka, nabavka namirnica, priprema obroka.

Tablica 1. Karakteristike uključenih znanstvenih članaka (II. dio)
Table 1. *Characteristics of included scientific articles (II part)*

Literaturna referenca	Populacijska skupina	Provedena intervencija	Ishodi intervencije od interesa za pregledni rad
McDougall, 1992.	Osobe muškog spola oboljele od shizofrenije	Šestotjedni program koji je uključivao nutritivne edukacije (koje je provodio nutricionist) i radionice (koje je provodio radni terapeut).	Aktivnosti vezane uz nabavku namirnica.
Lloyd i Samra, 1996.	Osobe muškog spola oboljele od shizofrenije	HLP („Health Lifestyle Programme“) jednom tjedno kroz vremenski period od osam tjedana.	Vještine samostalnog življenja, uključujući raspolaganje financijskim sredstvima, prehranu i socijalnu interakciju.
Porter i sur., 2000.	Osobe s duševnim smetnjama oba spola	Program razvoja kulinarskih vještina.	Vještine samostalnog življenja, uključujući planiranje i pripremu obroka te raspolaganje financijskim sredstvima.
Bassett i sur., 2003.	Osobe ženskog spola s duševnim smetnjama koje su majke djece mlađe od pet godina	Food Cent$ program koji je uključivao praktični dio usmjeren na nabavku i pripremu namirnica.	Ponašanje i stavovi vezani uz odabir i pripremu obroka te visinu financijskih sredstava koja se izdvajaju za nabavku namirnica.

Tablica 1. Karakteristike uključenih znanstvenih članaka (III. dio)
Table 1. *Characteristics of included scientific articles (III part)*

Literaturna referenca	Populacijska skupina	Provedena intervencija	Ishodi intervencije od interesa za pregledni rad
Yarborough i sur., 2016.	Odrasle osobe, oba spola, s prekomjernom tjelesnom masom ili pretilošću, na terapiji antipsihoticima kroz vremenski period od najmanje mjesec dana	Dvije faze u trajanju od po šest mjeseci – intenzivna faza (tjedni grupni sastanci) i faza održavanja (mjesečni grupni sastanci i telefonska podrška po potrebi).	Znanja i vještine potrebne za održavanje, poboljšanje i brigu o vlastitom zdravlju, povećanje samopouzdanja i smanjenje stresa.
Duncombe, 2004.	Osobe oboljele od shizofrenije (neparanoidna shizofrenija ili shizoafektivni poremećaj), oba spola	Dvije skupine ispitanika (u bolničkom okruženju i u vlastitom kućanstvu) – ukupno četiri susreta, od čega tri praktične kulinarske radionice.	Kulinarske vještine.
Ohno i Inoue, 2014.	Osobe oboljele od shizofrenije ili shizoafektivnog poremećaja, oba spola	Dvije skupine ispitanika (program kulinarskih aktivnosti i socijalnih vještina) - jednom tjedno tijekom vremenskog perioda od šest mjeseci.	Radna memorija, socijalne vještine, motoričke sposobnosti.

Među uključenim studijama, četiri su provedene isključivo na osobama oboljelima od shizofrenije ili shizoafektivnog poremećaja (McDougall, 1992; Lloyd i Samra, 1996; Duncombe, 2004; Ohno i Inoue, 2014). Uključene studije uglavnom su bile provedene na relativno malom uzorku ispitanika. Konkretnije govoreći, studija Bogomolove i sur. (2018) uključila je ukupno 25 ispitanika s duševnim smetnjama (u najvećem broju ispitanika s dijagnozama shizofrenije i shizoafektivnog poremećaja), dok je svega 13 ispitanika završilo tromjesečni program. U

šestotjednom programu nutritivno-kulinarskih edukacija koje su proveli Clark i sur. (2015) sudjelovalo je ukupno 18 ispitanika s duševnim smetnjama, a na sličnom uzorku ispitanika provedena je i studija McDougalla (1992) koja je uključila ukupno 11 ispitanika, od kojih je njih sedam bilo raspoređeno u eksperimentalnu, a četvero ispitanika u kontrolnu skupinu.

Prehrambene intervencije, različitog dizajna i duljine primjene, uglavnom su rezultirale razvojem i unaprjeđenjem određenih vještina samostalnog življenja u osoba s duševnim smetnjama. Tromjesečna intervencija koja je uključivala nutritivne edukacije i kulinarske radionice rezultirala je razvojem vještina poput planiranja i pripreme nutritivno kvalitetnih obroka baziranih na jednostavnim recepturama, većom samostalnošću kod raspolaganja financijskim sredstvima prilikom nabavke namirnica te poboljšanjima u socijalnim interakcijama (Bogomolova i sur., 2018). Povećanje samostalnosti prilikom pripreme, planiranja i nabavke namirnica zabilježeno je, između ostaloga, i u studijama Clarka i sur. (2015) te Portera i sur. (2000). Tako je, prema navodima Clarka i sur. (2015), 90% ispitanika imalo osjećaj da se njihova samostalnost u pogledu izvršavanja kulinarskih zadataka povećala.

Pretragom bibliografske baze podataka PubMed nije pronađen nijedan pregledni rad koji se bavio isključivo tematikom utjecaja prehrambenih intervencija na vještine samostalnog življenja u osoba s duševnim smetnjama. Kod interpretacije rezultata ovog preglednog rada važno je naglasiti i određene nedostatke. Prije svega, rad je temeljen isključivo na pretrazi PubMed bibliografske baze podataka te stoga postoji rizik da određene studije koje bi tematikom odgovarale ovom preglednom radu nisu detektirane, pa samim time niti uzete u obzir. Također, u obzir su uzimani samo oni znanstveni članci za koje je cjeloviti tekst rada bio dostupan na engleskom jeziku.

ZAKLJUČAK

Nekolicina je do danas publiciranih znanstvenih studija utvrdila pozitivne učinke primjene prehrambenih intervencija na određene vještine samostalnog življenja u osoba s duševnim smetnjama. Daljnje studije provedene na većem uzorku ispitanika su potrebne kako bi se potvrdili ranije spomenuti pozitivni učinci u ove populacijske skupine. Unatoč potrebi za daljnjim istraživanjima, rezultati publiciranih studija govore u prilog nužnosti uključivanja prehrambenih intervencija u cjelokupni tretman osoba s duševnim smetnjama. Pritom je od izrazite važnosti da u provedbu prehrambenih intervencija, uz nutricioniste, budu uključeni i radni terapeuti kako bi se postigao optimalan učinak na aspekte samostalnog življenja.

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FUNCTIONAL FOOD AND DIETARY SUPPLEMENTS / FUNKCIONALNA HRANA I DODACI PREHRANI

DETERMINATION OF ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT IN AQUEOUS AND ETHANOL-AQUEOUS EXTRACTS (*Achillea millefolium*)

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ABSTRACT

Achillea millefolium is a medicinal plant that has wide application in the treatment of various diseases. It is also called yarrow and milfoil grass, and it is a source of various nutrients and biologically active substances. This plant has been used in traditional medicine as an antibacterial, against infections, in wound healing, as a sedative and diuretic. The essential oil of flowers and leaves contains achilles and azulene which have anti-inflammatory properties. The plant material was air-dried and then ground into a fine powder The homogenized sample was subjected to maceration extraction techniques at room temperature with distilled water and aqueous ethanol solutions (30%, 50% and 70% v/v ethanol). Over a period of 12 and 24 hours, 6 samples were macerated in light-free conditions, while the remaining 6 macerate samples were exposed to a daylight source.The sample macerated in the dark with 50% ethanol solution had the highest total phenol concentration of 120.27 mg/g dry matter. In this work, the and antioxidant activity of DPPH and FRAP method was determined. The results clearly showed that rabbit extract *(Achillea millefolium)* is a rich source of polyphenols and has high antioxidant properties. The plant material was air-dried and then ground into a fine powder.

Keywords: antioxidant activity, extract, rabbit, polyphenols

INTRODUCTION

Yarrow (*Achillea millefolium*) is a perennial plant that grows in meadows, pastures, fields and gardens, and its medicinal preparations have anti-inflammatory, antispasmodic, antimicrobial and antifungal effects. This plant is used to relieve problems such as dyspepsia, cramps in the gastrointestinal tract and skin diseases (Kuštrak, 2005). The plant is widespread and belongs to the *Asteraceae* family, and includes more than 100 species distributed in Europe, North America, South Australia and Northern Asia (Lakshmi et al., 2011). It is named after *Achilles*, a Trojan war hero who used this plant to stop bleeding and heal the wounds of Heracles' son Telephus, while millefolium was derived from the word mille, which means a thousand and a folium leaf (Benedek and Kopp, 2007).

It is believed that the high content of total phenols usually means a very good antioxidant activity of a certain plant species (Kahraman et al., 2013). The positive effect of medicinal herbs is explained by the presence of bioactive compounds, secondary metabolites in plants, and their role in the prevention of various degenerative diseases such as cancer, cardiovascular and neurodegenerative diseases. Of plants of the genus *Achillea, A. millefolium* s. l. is the most widely studied because it is the most widespread, and therefore the biological activities of its flavonoids are best studied, among which the most common are apigenin, luteolin and quercetin, and their glucosides, using various in vitro and in vivo tests. Among flavonoid heterosides were found monoglucosides (mainly O-glucosides, C-glucosides and O-glucuronides) and diglucosides (O-diglucosides, C-diglucosides, O-rutinosides) (Jokić et al., 2017). The phytochemical profile of *A. millefolium* shows the presence of organic acids (mainly oxalic, quinic and citric), fatty acids (with linolenic and palmitic as the main ones) and tocopherols (especially γ-tocopherol) (Gharibi et al., 2013; Dias et al., 2013; Benedek et al., 2007; Vitalini et al., 2011). Yarrow tea has a concentration of flavonoids high enough to exhibit a spasmolytic effect in the intestine, which is mainly caused by blockade of calcium input currents, but also by antagonistic effects of mediators (Lemmens-Gruber et al., 2006). Qualitative and quantitative research of bioactive components from plant materials largely depends on the selection of an adequate extraction method as well as its adequate application (Azmir et al., 2013).

MATERIALS AND METHODS

Prior to the extraction process, the plant material was air-dried and then ground into a fine powder using an IKA Tube Mill 100 control laboratory grinder. The homogenized sample was subjected to maceration extraction techniques at room temperature with distilled water and aqueous ethanol solutions (30%, 50% and 70% v/v ethanol). Over a period of 12 and 24 hours, 6 samples were macerated in light-free conditions, while the remaining 6 macerate samples were exposed to a daylight source.

In the prepared samples, total phenols were determined by the Folin - Ciocalteu method (Dewanto et al., 2002), and the results were calculated from the gallic acid calibration curve (Figure 1). The concentration of total phenols is calculated

according to the equation of direction obtained by Excel, with gallic acid concentrations (mg/L) plotted on the abscissa and absorbance values measured on the photoLlab 6600 UV-VIS WTW spectrophotometer at 765 nm. 0.2 ml of the sample, 1.8 mL of distilled water, 10 mL of Folin-Ciocalteu reagent and 8 mL of sodium carbonate solution were pipetted into a test tube. After the sample was left for 2 hours at room temperature, the absorbance was measured.

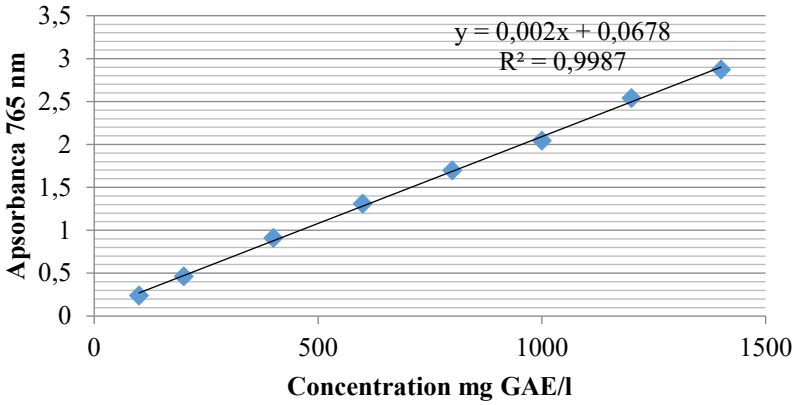

Figure 1. Standard diagram for determination of total phenols

The results are expressed as phenols equivalent to gallic acid mg GAE/g.

$y = 0.002x + 0.0678$
$R^2 = 0.9987$,
where is:
y - absorbance at 765 nm,
x - gallic acid concentration (mg/L),
R^2 - coefficient of determination.

One of the most commonly used methods in the determination of total flavonoids is the spectrophotometric method based on the formation of an aluminum complex by the addition of aluminum chloride solution to the sample. This method was first proposed for the analysis of plant material by Christ and Müller in 1960 and was later modified several times. The first commonly used method is based on the addition of a solution of aluminum chloride in a concentration of 2-10% to the test sample. After that, acid or acetate solution is added, and in some cases only water or methanol. The absorbance measurement is performed 2 to 60 minutes after incubation at a wavelength between 404-430 nm. A modified colorimetric method with $AlCl_3$ (Khlifi et al., 2011) was used to determine total flavonoids, and a standard quercetin solution was used to construct the calibration direction. Procedure: Pipette 0.2 mL of the sample into a test tube and add 1 ml of a 0.2% solution of $AlCl_3$ in methanol. The samples were kept in a dark place for 1 hour, after which their absorbance was measured at 415 nm. A test run containing

methanol instead of the sample for each sample was prepared. The absorbances obtained are proportional to the concentration.

$y = 0.0193x + 0.3734$
$R^2 = 0.9914$
where is:
y - absorbance at 415 nm,
x - quercetin concentration (mg/L),
R^2 - coefficient of determination.

FRAP is a colorimetric method for determining antioxidant activity based on the reduction reaction of a yellow colored iron-2,4,6-tripyridyl-s-triazine (TPTZ) complex. Fe^{3+} ions from the Fe (III) (TPTZ) 2 complex are reduced to Fe^{2+} ions in 2,4,6-tripyrid-s-triazine solution to give a blue colored product having an absorption maximum at 593 nm (Benzie and Strain, 1999). FRAP test: 0.1 ml of test extracts were added to 3 ml of FRAP reagent (0.3 M) acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 20 mM $FeCl_3 \times 6H_2O$ (10:1:1, v/v/v) and allowed to stand for 10 min at 37°C in the dark. The absorbance was measured at 593 nm. (Benzie and Strain, 1996).
DPPH test: (2,2-diphenyl-1-picrylhydrazyl) is one of the most stable organic nitrogen radicals with maximum absorption in UV-VIS at 517 nm. The DPPH radical is a stable nitrogen radical whose solution is dark purple, and with the addition of antioxidants the solution fades as the free radical is reduced to light yellow diphenylpicrylhydrazine, which is monitored spectrophotometrically via a drop in absorbance at 517 nm described (Jeong et al., 2010)., in 500 µl of extract of different concentration was added to 1000 µl of freshly prepared solution of DPPH (0.5 mM) in methanol. The sample was incubated for 30 minutes at 37 °C in the dark. Decreases in absorbance at 517 nm were measured using a WTW photoLab 6600 spectrophotometer, Germany.

RESULTS AND DISCUSSION

Mean values of total phenols depending on the applied solvent and extraction time of maceration at room temperature are shown in Table 1. From the table we can see that *Achillea millefolium* extract obtained by maceration at room temperature with 50% ethanol for 24 h shows the highest total phenol content of of the tested samples.

Table 1. Total phenol content in aqueous extracts and ethanol-aqueous extracts (*Achillea millefolium*) expressed in mg of gallic acid/g of dry matter.

Solvent	Maceration extraction 12h		Maceration extraction 24h	
	SD (n=6)			
	MT	MS	MT	MS
Water	87.54±1.74	76.34±1.43	94.55±1.25	85.22±0.67
30% ethanol	96.88±1.31	91.39±1.89	104.24±1.01	101.75±0.82
50% ethanol	116.94±2.18	107.26±1.18	120.27±0.92	109.99±1.04
70% ethanol	107.23±1.65	104.11±1.09	109.19±0.86	108.56±1.21

SD = mean ± standard deviation, n = number of samples, MT = maceration process carried out in the dark, MS = maceration process exposed to a daylight source.

Keser et al., (2013) who compared the polyphenol content of aqueous and ethanolic extracts, conducted separately on the leaves and flowers of the *Achillea millefolium* plant in Turkey, obtained almost twice the amount of polyphenols in ethanolic extracts. Total phenols in the 70-percent ethanolic flower extract of *Achillea millefolium* were previously investigated (Benedec et al., 2015), where they obtained values of 38.12 mg GE/g, which are much lower values compared to our results. Similar to the results obtained in this paper, other comparisons of the amount of total phenols show a slightly higher solubility of the same in organic solvents. Vitalini et al., (2011) reported a significantly higher content of total phenols of 281.7 mg/g in the methanolic extract of aboveground parts of *Achillea millefolium*. In contrast, Eghdami et al. (2010) observed a lower total phenol content in methanol extract of 123.9 ± 2.6 mg GAL/g, which is closer to the values determined in this paper in ethanol extracts. On the other hand, the content of total phenols was even lower in the aqueous extract of 48.4 ± 2.7 mg GAE/g, which are much lower values compared to our results. Also much lower values of total phenol content were recorded in the work of Wojdyło et al., (2007), 9.55 ± 0.11 mg GAE/100 g dry matter and Georgieva et al., (2015) in the range of 2.74 ± 0.01 and 7.92 ± 0.09 mg GAE/g dry matter in the four extraction techniques used.

Achillea millefolium macerates in which the maceration process was carried out in the dark show a higher concentration of total phenols compared to macerates that were exposed to daylight. In the research of Hanousek Čiča (2020) carob macerates in which the maceration process was carried out in the dark, show a higher concentration of total phenols during all 4 weeks of maceration compared to macerates that were exposed to daylight with the same alcohol content and solid: liquid ratio. Maceration in the dark prevents adverse reactions of phenolic

compounds degradation in order to maximize their concentration at the end of the process.

Table 2. Total content of flavonoid aqueous extracts and ethanol-aqueous extracts (*Achillea millefolium*) expressed in mg quercetin/g dry matter (maceration process carried out in the dark)

Solvent	**Maceration extraction 12h**		**Maceration extraction 24h**	
	SD (n=6)			
	MT	**MS**	**MT**	**MS**
Water	29.15±0.84	25.36±0.52	29.54±0.63	28.31±0.65
30% ethanol	32.29±0.80	30.36±0.74	34.86±0.54	32.71±0.60
50% ethanol	38.96±0.91	36.98±0.89	40.22±0.97	36.42±0.55
70% ethanol	34.59±0.63	33.58±0.96	36.39±0.50	35.90±0.84

SD = mean ± standard deviation, n = number of samples, MT = maceration process carried out in the dark, MS = maceration process exposed to a daylight source.

Moldovan et al., (2011) found, by testing ethanolic extracts of *Achillea millefolium*, a higher amount of flavonoids 46.59 mg QE/g. These values are slightly higher than our results. Also, from Table 2 it can be seen that, like the amount of total phenols, more flavonoids are contained in extracts with a higher ethanolic alcohol content.

Table 3. Antioxidant activity of aqueous extracts and ethanol-aqueous extracts (*Achillea millefolium*) measured by FRAP and DPPH test (maceration process exposed to a daylight source)

Sample	**FRAP sample test µmol Trolox/mg**	**DPPH test IC 50(µg/ml)**
Water	12.64±0.56	139.12±1.43
30% ethanol	13.45±0.89	10.25±0.79
50% ethanol	22.87±1.27	43.08±0.67
70% ethanol	18.61±0.83	41.63±0.58

SD = mean ± standard deviation, n = number of samples.

Table 4. Antioxidant activity of aqueous extracts and ethanol-aqueous extracts (*Achillea millefolium*) measured by FRAP and DPPH test (maceration process carried out in the dark)

Sample	FRAP sample test µmol Trolox/mg	DPPH test IC 50(µg/ml)
Water	10.27±0.84	126.04±2.09
30% ethanol	12.36±1.02	98.34±1.76
50% ethanol	21.28±1.13	40.26±0.54
70% ethanol	17.24±0.94	39.54±0.62

SD = mean ± standard deviation, n = number of samples.

Due to the number and chemical diversity of antioxidant molecules, different mechanisms of their action, as well as the target molecules on which their impact is to be investigated, a large number of antioxidant methods were developed. For in vitro testing of antioxidant activity of different foods, the best approach is considered to be a combination of several methods based on different principles of determining the antioxidant potential of the analyzed sample through different mechanisms of action (Mocan et al., 2016; Llorent-Martinez et al., 2016). The differences between the results of the methods for determining antioxidant activity can be explained by the different principles on which the methods are based. The DPPH method includes the ability of antioxidants to bind free radicals, while the FRAP method includes the ability of antioxidants to reduce iron ions (measure reduction capacity). Both reactions are based on the electron exchange mechanism (redox reaction). As for the reaction itself, the FRAP test takes place via the SET (single electron transfer) mechanism, while DPPH and ABTS are reactions in which these two radicals can be neutralized via the SET or HAT (hydrogen atom transfer) mechanism (Prior et al., 2005). Differences in affinities of DPPH and FRAP methods according to compounds occur due to the influence of pH value on the reduction capacity of antioxidants. Namely, at high pH, the proton dissociates from antioxidants and thus increases the reducing power of the sample. In contrast, at low pH, antioxidant protonation occurs and, consequently, a reduction in the reductive power of the sample (Huang et al., 2005). When performing, You should have in mind that reactions based on electron transfer depend on the pH of the reaction mixture, because the values of the standard potential decrease with increasing pH, so these reactions are sensitive to the presence of organic acids and metals (Mandić Vedrana, 2017). According to Prior , 2005, the DPPH method can be used in aqueous and nonpolar organic solvents and can be used to test hydrophilic and lipophilic antioxidants. In contrast to the DPPH method, which is suitable for the determination of antioxidants soluble in organic solvents (ethanol, methanol), the FRAP method can determine the antioxidant properties of water-soluble compounds (Dai and Mumper 2010).

Antioxidant activity can be determined by different tests with different principles that do not give the same results, and for that reason, it is difficult to make a concrete comparison of the results. The results depend on the applied test and the way the results are expressed. The results shown in Table 3 show that antioxidant activity was significant. In the study of antioxidant activity of plant parts of *Achillea millefolium* Wojdylo et al., (2007) give DPPH test values 200 ± 3.33 μM TE/100 g dry matter and FRAP test 191 ± 4.51 μM TE/100 g dry matter. The values of the DPPH test in Georgiev et al. (2015) ranged from 24.15 ± 0.15 to 116.74 ± 0.21 μM TE/g dry matter and the FRAP test values for the tested *Achillea millefolium* extracts ranged from 29.57 ± 0.40 to 132.71 ± 1.86 μM TE/g dry matter.
As none of the existing methods have been proven suitable to fully reflect the properties of the sample, it is common to use several methods based on different reaction mechanisms in research. Since individual tests cover different segments of antioxidant action, their combination has certain advantages. Integrating the values of individual tests into the Antioxidant Composite Index (ACI) provides a more comprehensive insight into the antioxidant activity of the analyzed samples.

CONCLUSION

Our results revealed that samples macerated in the dark contain a higher concentration of total phenols, and thus have stronger antioxidant activity because there is no degradation of biologically active molecules, which include phenolic compounds. The sample macerated in the dark with 50% ethanol solution had the highest total phenol concentration of 120.27 mg/g dry matter. The results also showed that the use of 50% aqueous ethanol solution gave the highest extraction content of total flavonoids.

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ZDRAVSTVENE POGODNOSTI JAPANSKE JABUKE

HEALTH BENEFITS OF JAPANESE APPLE

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pregledni rad */ review*

SAŽETAK

Japanska jabuka (*Diospyros kaki* L.) je bogat izvor biološki aktivnih spojeva koji su povezani s antikancerogenim i antimutagenim djelovanjem. Plod obilno raste na području Hercegovine, gdje se uzgaja bez upotrebe dodatnih sredstava za rast i razvoj. S obzirom da su plodovi meke konzistencije u zreloj fazi, dolazi do brzog truljenja svježeg voća. Japanske jabuke su prirodno bogate ugljikohidratima, a sadrže jako malo do gotovo nikako masti. Neke od značajnih komponenti utvrđenih kroz znanstvena istraživanja su: askorbinska kiselina, tanini, dijetalna vlakna, mineralne tvari, β-karoten, β-kriptoksantin, lutein, zeaksantin i likopen. Japanska jabuka ima potencijal u promicanju zdravlja jer smanjuje rizik nastanka i razvoja hiperkolesterolemije, dijabetesa, raka, kožnih oboljenja, hipertenzije i dr. Zbog takve fitokemije japanske jabuke, otvorila se mogućnost izoliranja korisnih sastojaka iz ploda za kozmetičku i farmaceutsku industriju, te kao funkcionalni dodatak s ciljem obogaćivanja drugih proizvoda. U ovom radu su obuhvaćeni rezultati znanstvenih istraživanja o zdravstvenim utjecajima japanske jabuke.

Ključne riječi: japanska jabuka, bioaktivni sastojci, zdravlje

Keywords: japanese apple, bioactive components, health

UVOD

Japanska jabuka (*Diospyros kaki L.*) prema botaničkoj podjeli spada u obitelj *Ebeneceae* (Matheus, 2020). Smatra se da je japanska jabuka pronađena u Kini (Luo i Wang, 2008) odakle se proširila na područje Koreje i Japana. Globalno se sadi preko 400 vrsta japanskih jabuka. Među njima su od većeg značaja *Diospyros kaki, Diospyros virginiana, Diospyros oleifera* i *Diospyros lotus* (Bibi i sur., 2007; Vieites, 2012). U Europi se japanska jabuka počinje uzgajati tek krajem 19. stoljeća, a kao najpogodnije područje za uzgoj jest područje Mediterana. Kao glavni uzgajivači japanske jabuke navode se Kina (2 533 899 t), Koreja (430 521 t), Japan (244 800 t), Brazil (169 000 t), Azerbajdžan (132 179 t), Španjolska (70 000 t), Italija (50 000 t), Izrael (30 089 t) i Uzbekistan (31 000 t) (Jung i sur., 2005; Luo, 2007; Bubba i sur., 2009; Primorac i sur., 2020).

Agroekološko područje južnog dijela Hercegovine pripada mediteranskoj klima pa osigurava dobre klimatološke uvjete za uzgoj japanke jabuke (*Diospyros kaki* L.), jer ona pripada voćnim vrstama koje ne traže posebne agrotehničke zahtjeve za uzgoj. Najčešće se uzgaja blizu okućnica, po nekoliko ukrasnih stabala, u rijetkim slučajevima kao nasad (Primorac i usr., 2020).

Plod japanske jabuke obično je svijetlo žute-narančaste do tamnocrvene-narančaste boje, te od 1,5 do 9 cm u promjeru, ovisno o vrsti. Može biti i sfernog oblika, nalik žitu ili bundevi. Čaška često ostaje vezana za plod nakon berbe, ali postaje lakša za uklanjanje kako sazrijeva (Merriam-Webster, 1983). Stablo japanske jabuke može dosegnuti i 18 m, sa širinom većom od 4,5 m. Stabla su listopadna, a listovi su smeđi i dlakavi, s peteljkama od 2 cm (Primorac i sur., 2020).

Japanska jabuka je suptropska listopadna voćka, koja relativno dobro podnosi hladnoću. Otpornija je prema niskim temperaturama od kultura kao što su masline, smokve i šipak, a može izdržati apsolutne minimalne temperature od -17 °C, pa čak i do-20 °C. Za rast i rodnost treba dosta vlage u tlu tijekom perioda vegetacije, a osobito u vrijeme intenzivnog rasta mladica i ploda, te za vrijeme bubrenja ploda. Smatra se da su za uzgoj japanskih jabuka prikladna područja gdje godišnje padne najmanje 600 mm kiše (Primorac i sur., 2020). Japanska jabuka je heliofit pa traži puno svjetla. Uz više svjetla plodovi ranije dozrijevaju. Za uzgoj treba birati položaje zaštićene od vjetra, a prikladna su duboka aluvijalna tla ilovaste do glinasto-ilovaste teksture, koja sadrže dosta silicija i koja su dobro vodopropusna (Jemrić, 2007).

Japanska jabuka je sezonsko voće: dostupan je samo u svježem obliku za kratko vrijeme tijekom cijele godine. U Europi se japanske jabuke mogu od rujna do početka prosinca (Giordani, 2001; Toplu i sur., 2009). Za vrijeme tehnološke zrelosti meso japanske jabuke je gorko i oporo zbog čega se ostavlja na dodatnu zriobu kojom meso postaje meko i slatko, bez tragova oporosti (Morton, 1987). Da bi se izgubila oporost japanske jabuke, neka istraživanja su pokazala da skladištenje japanske jabuke na temperaturi od - 20 °C i - 80 °C do 60 dana učinkovita metoda za uklanjanje trpkosti (Das i Eun, 2021).

KEMIJSKI I NUTRITIVNI SASTAV

Japanska jabuka je prepoznata kao izvanredan izvor biološki aktivnih spojeva. Uzgaja se uglavnom bez ikakve upotrebe zaštitnih sredstava i umjetnih gnojiva zbog čega su plodovi i proizvodi od plodova slobodni od štetnih ostataka pesticida i umjetnih gnojiva (Nazir i sur., 2013). Plod je bogat različitim hranjivim tvarima, kao što su ugljikohidrati, dijetalna vlakna, vitamini, mineralne tvari, karotenoidi i fenolni spojevi te druge bioaktivne komponente (Matheus, 2020).
Prema nekim autorima, plod sadrži oko 80,3 % vode, 0,58 % proteina, 0,19 % ukupnih lipida, 18,6 % ukupnih ugljikohidrata i neke minerale (kalcij, kalij, magnezij, natrij, željezo, cink, bakar, mangan, fosfor, selen), do 1,48 g ukupnih dijetalnih vlakana i 7,5 mg askorbinske kiseline (Ozen i sur., 2004; Ercisli i sur., 2008; Butt i sur., 2015) što je i prikazano u Tablici 1.

Tablica 1. Nutritivna vrijednost japanske jabuke izražena na 100 g (Butt i sur., 2015)
Table 1. *Nutritional value of Japanese apple per 100 g (Butt i sur., 2015)*

Nutrijent	Suha japanska jabuka	Sirova japanska jabuka
Voda (g)	23,01	80,32
Energija (Kcal)	274	70
Energija (kJ)	1146	293
Bjelančevine (g)	1,38	0,58
Ukupni lipitid (masti) (g)	0,59	0,19
Pepeo (g)	1,59	0,33
Ugljikohidrati (g)	73,43	18,59
Ukupna vlakna (g)	14,5	3,6
Kalcij (mg)	25	8
Željezo (mg)	0,74	0,15
Fospor (mg)	81	17
Kalij (mg)	802	161
Natrij (mg)	2	1
Vitamin C (mg)	0,0	7,5
Riboflavin (mg)	0,029	0,020
Niacin (mg)	0,180	0,100
Vitamin A (µg RAE)	38	81
β-karoten	374	253
β-kriptoksantin	156	1447
Vitamin A (IU)	767	1627

Ugljikohidrati i organske kiseline

Ugljikohidrati su glavni makronutrijenti prisutni u japanskoj jabuci, a plod sadrži više šećera u usporedbi s drugim voćkama koje se često konzumiraju. Većina ugljikohidrata sadrži mono- i disaharide, predstavljene glukozom, fruktozom i saharozom, dok su galaktoza i arabinoza sporedni sastojci. Slatki okus voća važan je zbog njegove kvalitete i senzorske vrijednosti, i oba primarna metabolita, šećeri i organske kiseline igraju važnu ulogu. Sadržaj ukupnih organskih kiselina iznosi oko 22,76 μg/mg (suha osnova), limunska, jabučna i jantarna kiselina prosječno 5,60, 9,07 i 8,09 μg/mg (suha osnova) (Novillo i sur., 2015). Šećeri su također povezani s ukupnim sadržajem topljivih krutih tvari (TKT) (Kuge i sur., 2018).

Bjelančevine

Sadržaj bjelančevina u japanskoj jabuci je nizak, a glavne slobodne aminokiseline su; 4-aminobutanojeva kiselina, aspartanska kiselina, glutaminska kiselina, glutamin, izoleucin, leucin, treonin, alanin i citrulin. Sadržaj citrulina smanjen je tijekom sazrijevanja, što je povezano s procesom sinteze i razgradnjom bjelančevina tijekom sazrijevanja i razvoja ploda (Santos i sur., 2018; Ryu i sur., 2016).

Vlakna

Prosječan ukupni sadržaj vlakana je relativno veći kada se usporedi s drugim voćem, koje je poznato po sadržaju netopljivih vlakana, poput argentinske jabuke (*Malus domestica*) (2,0 g/100 g) i marelice (*Prunus Armeniaca*) (2,0 g/100 g) (Dreher, 2018) što možemo vidjeti i iz rezltata istraživanja koje su radili Hernández-Carrión i sur. (2014). Naime, oni su pronašlivisok udio prehrambenih vlakana u japanskoj jabuci *Rojo Brillante*, oko 14,80 g/100 g (suha osnova), koja se sastojala uglavnom od netopljivih vlakana (9,40 g/100 g suha osnova) (Hernández-Carrión i sur., 2014).

Fenolni spojevi

Fenolni spojevi su biljni fiziološki metaboliti koji djeluju kao obrambeni mehanizam biljke (antioksidativno djelovanje) protiv stresa u okolišu, koji je povezan s velikom proizvodnjom slobodnih radikala i drugih oksidativnih vrsta (Lattanzio, 2013). Sentandreu i sur. (2015) otkrili su 32 fenolna spoja niske molekularne mase u plodu *Rojo Brilliante*. Galna kiselina i njeni derivati bili su vrlo zastupljeni u odnosu na flavonoide i glukozidnu kumarinsku, kvininsku i vaniličnu kiselinu (Sentandreu i sur., 2015). Sorta *Fuyu* se odlikuje većim sadržajem polifenola i većim antioksidativnim potencijalom (Altuntas i sur., 2011).

Karotenoidi

Karotenoidi su odgovorni za hvatanje svjetlosti i pružanje boje i foto-zaštite u fotosintetskom sustavu. Osim toga, karotenoidi imaju antioksidativnu aktivnost (Rodriguez-Concepcion i sur., 2018). Beta-karoten je glavni karotenoid u zrelom plodu, a kora ima veću količinu ovog spoja. Karotenoid u manjoj količini u kori

japanske jabuke je zeaksantin. Pulpa ima niže vrijednosti ukupnih karotenoida u usporedbi s korom. Najbrojniji karotenoidi u pulpi su β-karoten, β-kriptoksantin, α-karoten i zeaksantin (Veberic, 2010). Svi karotenoidi su lipofilni spojevi i zbog toga se kod njihove ekstrakcije iz voća, koje sadrži vodu, mora koristiti pogodno organsko otapalo koje se miješa s vodom u kojem su karotenoidi topljivi (Giordani, 2011).

Tanini

Tanini predstavljaju glavne fitokemikalije prisutne u ovom voću. Ulaze u interakcije s određenim makromolekulama, poput bjelančevina, škroba i drugih ugljikohidrata. Ovo je važan čimbenik tijekom konzumacije, kada tanini velike molekulske težine stupaju u interakciju s enzimima koji se nalaze u slini, uglavnom amilazom, što izaziva suh osjećaj u nepcu i karakterizira nepoželjnu trpkost. Stoga se koriste mnogi tretmani smanjenja tanina nakon žetve kako bi voće bilo pogodnije za konzumaciju (Matheus i sur., 2020). Važno je naglasiti da prisutnost topljivih tanina također utječe na sadržaj TKT. Oporo voće s većim sadržajem topljivih tanina ima više TKT nego neopore sorte (Primorac i sur., 2020).

Vitamin C

Vitamin C prisutan je u voću u dva oblika: L-askorbinska kiselina (AA) i njezin oksidacijski produkt, dehidro-L-askorbinska kiselina (DHAA). AA i DHAA imaju brojna važna biološka svojstva, od kojih su neka povezana s njihovim antioksidacijskim i antiradikalnim djelovanjem. AA i DHAA ne vrše iste antioksidacijske i antiradicke aktivnosti, jer je prva aktivnija, međutim, u ljudskom se tijelu oksidirani oblik lako i gotovo potpuno pretvara u reducirani (Gregory, 1996). Značajne promjene u AA i DHAA primijećene su tijekom razvoja i sazrijevanja ploda (Giordani, 2011).

ZDRAVSTVENE POGODNOSTI JAPANSKE JABUKE

U posljednjih nekoliko godina prehrambena i farmaceutska industrija provode istraživanja s fokusom na biljne bioaktivne komponente iz razloga što se smatra da upravo biljne bioaktivne komponente imaju povoljan učinak u liječenju i prevenciji ljudskih bolesti (Beecher, 2004).
Epidemiološka istraživanja na ljudima i životinjama pokazuju da je konzumacija voća i povrća povezana s niskim rizikom od kardiovaskularnih bolesti i raka. Zdravstvena svojstva ovih proizvoda ovise o komponentama bioaktivnih spojeva, uglavnom fenola, a djelomično i dijetalnih vlakana (Dauchet i sur., 2006).
Bogata fitokemija voća i povrća otvorila je mogućnosti za nova istraživanja hrane u liječenju različitih bolesti. Japanska jabuka se, osim zbog svoje nutritivne vrijednosti, koristi u tradicionalnoj kineskoj medicini zbog svojih blagotvornih učinaka na zdravlje, odnosno jer smanjuje rizik od nastajanja hipertenzije, krvarenja, u održavanju tjelesne temperature i općenito usporavanju oksidativnih procesa, kao diuretik, kod dijabetesa i ateroskleroze. Također se koristi za poboljšanje funkcije

pluća, želuca, slezene i crijeva te se koristi u prevenciji i liječenju bolesti kao što su upala grla i nesanica. Potencijal japanske jabuke u promicanju zdravlja je važan spomena i pažnje zbog činjenice da smanjuje rizik od nastanka kolesterolemije, dijabetesa, raka, kožnih bolesti, hipertenzije i slično (Butt i sur., 2015).
Utvrđeno je da su katehin i likopen učinkoviti u *in vitro* kao i u *in vivo* testovima u borbi protiv različitih vrsta raka (Giovannucci, 2002.). Loizzo i sur. (2009) su istraživali kemijski sastav,antioksidativna i antiproliferativna svojstva sorte *Diospyros L*., poznate i kao kavkavska japanska jabuka. Osam spojeva je izolirano iz *Diospyros L*. (galna kiselina, metilgalat, elaginska kiselina, kempferol, kvercetin, miricetin, miricetin 3-O-β-glukuronid i miricetin-3-O-α-ramnozid) te testirano u različitim *in vitro* sustavima (DPPH, ABTS, FRAP i Fe2+ kelacijska aktivnost). Istraživanje je pokazalo značajna antioksidativna djelovanja. Potencijalna antiproliferativna svojstva ekstrakta D. lotosa i izoliranih spojeva protiv devet humanih staničnih linija raka kao što su COR-L23, CaCo-2, C32, ACHN, A375, A549, Huh-7D12, MCF-7 i LNCaP ispitana su *in vitro* SRB testom. Najveća inhibitorna aktivnost se pokazala kod COR-L23, C32 i A375 (elaginska kiselina je pokazala najbolje djelovanje) te CaCo-2 (galna kiselina je pokazala najveću citotoksičnu aktivnost). Općenito, rezultati ove studije pokazuju da *Diospyros L*. ima dobro antioksidativno djelovanje i antiproliferativne učinke s tim da je važno naglasiti da je ovo djelovanje posljedica polifenolnih tvari koje posjeduje ova sorta japanske jabuke (Loizzo i sur., 2009). Ohguchi i sur. (2010) su otkrili da acetonski ekstrakt kore japanske jabuke (*Diospyros kaki 'Fuyu'*) inhibira biosintezu melanina u stanicama melanoma B16 kod miševa zahvaljujući flavonoidnim glikozidima: izokvercitrinu (kvercetin-3-O-glukozid) i hiperinu (kvercetin-3-O-galaktozid). Izokvercitrin i hiperin snažno su inhibirali proizvodnju melanina (IC50: 21,7 odnosno 18,2 μM) (Ohguchi i sur., 2010).Sljedeće godine, Itoh i sur. (2011) su radili istraživanje na istoj sorti ekstrahirajći iz kore *Diospyros kaki Fuyu* iste spojeve. Otkrili su da izokvercitrin i hiperin (Hyp) inhibiraju antigenom stimuliranu degranulaciju u stanicama bazofilne leukemije štakora RBL-2H3. Izokvercitrin i hiperin posjeduju aktivnost uklanjanja radikala DPPH sličnu onoj kod epigalokatehin galata, snažnog antioksidansa (Itoh i sur., 2011). Jang i sur. (2010) analizirali su zaštitni utjecaj neopora, ekstrahirani iz vrste Fuyu, na genotoksično oksidativno oštećenje DNA H_20_2 u ljudskim leukocitima. U ovom slučaju fenoli β-karoten i askorbinska kiselina imali su zaštitni učinak (Mori i sur., 2004.). S obzirom da je rak štitnjače najzastupljeniji oblik raka od kojega obolijevaju žene u Korei, Jung i sur. (2013) su istraživali kako prehrana utječe na prevenciju boleesti. Pokazali su da velika konzumacija sirovog povrća, japanske jabuke i mandarina može smanjiti rizik od raka štitnjače i pomoći u prevenciji raka štitnjače u ranoj fazi (Jung i sur., 2013). Park i Shin (2020) su pokazali da ekstrakt lista japanske jabuke također ima antikancerogena svojstva. Direito i sur. (2017) su istraživali utjceaj fenolnog ekstrakta japanske jabuke (80 % acetona u vodi) na protuupalne i antiproliferativne učinke, koristeći *in vivo* model eksperimentalnog kolitisa i model invazije stanica raka. Dokazano je da fenolni ekstrakt japanske jabuke ublažava kolitis i potencijalni antiproliferativni učinak na kultivirane stanice raka debelog crijeva. Primjena

fenolnog ekstrakta japanske jabuke na miševima s kolitisom izazvanim TNBS-om dovela je do smanjenja nekoliko funkcionalnih i histoloških biljega upale debelog crijeva, i to: slabljenje smanjenja duljine debelog crijeva, smanjenje opsega vidljive ozljede (formiranje ulkusa), smanjenje težine proljeva, smanjenje stope mortaliteta, smanjenje krvarenja sluznice i smanjenje općih histoloških obilježja upale debelog crijeva (Direito i sur. (2017).

Ekstrakt japanske jabuke od davnina se koristi za crijevnu mikrofloru odnosno za probleme s probavom. Yang i sur. (2021) istražili su učinak polisaharidnih ekstrakata iz japanske jabuke na proliferaciju *Lactobacillusa* i crijevnu mikrobiotu miševa. Rezultati su pokazali je da su korisne bakterije u crijevima proliferirale i da je smanjeno obilje štetnih bakterija odnosno da ekstrakt japanske jabuke ima značajan pozitivan učinak i na proliferaciju *Lactobacillus*-a i na crijevnu mikrobiotu miševa.

Fenolni spojevi su bioaktivni kemijski spojevi koji se nalaze u voću i povrću, a posjeduju mnoštvo zdravih svojstava. Posljednjih godina raste interes za japanskom jabukom zbog prisutnosti mnogih različitih grupa fenolnih spojeva. Esteban-Munoz i sur. (2021) su radili procjenu pojedinih fenolnih spojeva i antioksidativnog kapaciteta pulpe dviju sorti japanske jabuke (*Rojo Brillante* i *Triumph*) poboljšanim postupkom ekstrakcije uz UPLC-Q-TOF-MS platformu. Istraživanja su pokazala da je sastav fenolnih spojeva japanske jabuke karakteriziran prisutnošću hidroksibenzojeve i hidroksicimetne kiseline, hidroksibenzaldehida, dihidrohalkona, tirozola, flavanola, flavanona i flavonola. Identificiran je ukupno 31 spoj i kvantificirano je 17 spojeva. Galna kiselina je bila dominantni fenolni spoj pronađen u sorti *Rojo Brillante* (0,953 mg/100 g), dok je koncentracija p-hidroksibenzojeve kiseline bila viša kod sorte *Triumph* (0,119 mg/100 g) (Esteban-Munoz i sur., 2021). Fenoli u cijelom plodu japanske jabuke ukazuju na izrazito antioksidativno djelovanje te su 20 puta jači *in vitro* od klasičnog antioksidansa vitamina E (Uchida i sur., 1990.). Već 1998., Gorinstein i sur. su pokazali da plod japanske jabuke ima hipokolesterolemijski i antioksidativni učinak te se stoga smatra antiaterosklerotskom dijetom. Isto istraživanje je pokazalo da je prehrana obogaćena suhom korom japanske jabuke učinkovitija od iste prehrane obogaćene suhom pulpom (Gorinstein i sur., 1998).

Japanska jabuka se preporuča i kod osoba oboljelih od dijabetesa. Istraživanja su pokazala da kora japanske jabuke ima visok udio dijetalnih vlakana, ukupnih karotenoida, vitamina C i ukupnih fenola. Dodatak prehrani kore japanske jabuke kroz 2 tjedna u 5 % i 10 % (w/w) značajno je smanjio unos hrane, razinu glukoze u krvi, triglicerida u plazmi i ukupni kolesterol u štakora s dijabetesom. Kora japanske jabuke u prehrani je utjecala i na djelomičnu ili potpunu obnovu razine aspartat amino transferaze (AST) u plazmi, odnosno kreatinina, koji služe kao pokazatelji poremećaja funkcije jetre i bubrega (Lee i sur., 2006). Slično istraživanje proveli su i Izuchi i sur. (2011), koji su ekstrahirali spojeve iz kore japanske jabuke topiv u mastima. Ovakva prehrana kroz 12 tjedana, značajno je smanjila aktivnost glutamin-piruvat transaminaze u plazmi, uz nakupljanje β-kriptoksantina u jetri kod štakora. Ekspresija gena povezanih s inzulinskim signalnim putem značajno je obogaćena u

različito eksprimiranim genskim skupovima. Štoviše, Western blotting analiza je pokazala povećanje fosforilacije inzulinskih receptora beta tirozina kod štakora. Lee i sur. (2007) primijetili su da zečji polimeri i oligomeri iz proantocijanina kore japanske jabuke mogu imati ulogu antidijabetičkih spojeva inhibicijom α-glukozidaze ili α-amilaze, kao i enzima koji povećava apsorpciju glukoze u crijevima.

Poznato je da glavni faktor nastanka ateroskleroze jesu povišena razina lipoproteina niske gustoće (LDL) i snižena razina lipoproteina visoke gustoće (HDL). Upravo je istraživanje Parka i sur. (2008) doprinijelo otkriću da prehrana obogaćena cijelim plodovima japanske jabuke pozitivno utječe na određene pokazatelje ateroskleroze. Naime, štakori su bili hranjeni prehranom bogatom kolesterolom uz dodatak voća, odnsono japanske jabuke. Tri sorte japanske jabuke su pokazale smanjenje lipidnog seruma kod štakora (Park i sur., 2008). Također, povišena razina LDL uz smanjenje HDL uzorkuje displidemiju. Skupina suradnika na čelu s Matsumato (2006) istražila je kako japanska jabuka utječe na sprečavanje displidemije. Naime, istraživanje je provedeno na pretilim miševima kojima je uvedena različita prehrana: jedna s visokim udijelom masti te druga s dodatkom dvije sorte japasnke jabuke *Fuyu-kaki* i *Hachiya-kaki*. Prehrana obogaćena objema vrstama voća značajno je smanjila porast lipida u plazmi, uključujući ukupni kolesterol ($p<0,005$), trigliceride ($p<0,05$) i LDL kolesterol ($p<0,05$) (Matsumato i sur., 2006).

Artiritis je autoimuna kronična bolest koja zahvaća zglobove, a podaci govore da od nje 1,5 % pučanstva. Direito i sur. (2019) proučavali su kako ekstrakt japanske jabuke utječe na upalne procese artritisa. Istraživanje je rađeno na štakorima. Ekstrakt japanske jabuke smanjuje stupanj kronične upale i oštećenja tkiva karakterističnih za kolagenom induciranim artritis kod štakora, najvjerojatnije zahvaljujući snažnim antioksidativnim svojstvima ekstrakta.

Kashif i sur. (2017) iznijeli su podatke o potencijalnoj upotrebi japanske jabuke u dermatološkoj i kozmetičkoj industriji. Aktivni sastojci iz različitih dijelova biljke pokazuju protuupalno, antialergijsko, foto-zaštitno djelovanje, imaju antiage svojstva, s blagim djelovanjem na enzim tirozinazu, elastazu i kolagenazu. Obećavajući antioksidativni učinak, njegov potencijal posvjetljivanja kože i povećano smanjenje sadržaja sebuma i pora kože, čine ga prikladnim izborom za kozmetički sastojak. Dosadašnja istraživanja su pokazala da japanska jabuka ima povoljan učinak na alergijski dermatitis zbog čega su Kim i sur (2013) istražili zaštitne učinke vodenog ekstrakta japanske jabuke na alergijsku upalu posredovanu mastocitima te utvrditi moguće mehanizme djelovanja pomoću *in vitro* i *in vivo* modela baziranih na mastocitima. Utvrđeno je da su inhibitorni učinci vodenog ekstrakta japanske jabuke na alergijsku reakciju i oslobađanje histamina slični onima dinatrijevog kromoglikata, poznatog antialergijskog lijeka (Kim i sur., 2013). Itoh i sur. (2011) pokazali su da su izokvercitrin i hiperin, izolirani iz kore japanske jabuke, korisni za ublažavanje simptoma alergije tipa I.(Itoh i sur., 2011). Sun i sur. (2021) su istraživali utjecaj tanina ekstrahiranoj iz japanske jabuke na pigmentaciju kože. Rezultati su pokazali da su u usporedbi s modelnom grupom, superoksid dismutaza, katalaza, glutation peroksidaza, sadržaj DKK1 u razinama inhibitora

signalnog puta Wnt/-katenina i inhibitorna aktivnost tirozinaze povećani za 24,3 %, 33,3 %, 59,3 %, 36,81 % i 17,16 %, respektivno. U međuvremenu, ekspresija interleukina-6 i interleukina-8 smanjena je za otprilike 22,2 % i 54 %. Rezultati su također pokazali da ekstrakt tanina japanske jabuke može značajno smanjiti gustoću melanina (Sun i sur, 2021).

Do razvoja sarkopenije nastaje uslijed gubitka mišićne mase uslijed oksidativnih oštećenjanastalih djelovanjem stresa. U pokušaju smanjenja potrošnje tjelesnih mišića najčešće se rabe određeni enteralni pripravci koji sadržavaju povećan udio proteina i vitamina D te hidrolizate kolagena. Nažalost, komponente hrane ne dolaze izravno u dodir s mišićnim stanicama stoga se pristupa pronalaženju novih oblika liječenja. Jedno od istraživanja je rađeno na smanjenju oksidativnog stresa u stanicama mioblasta pomoću ekstrakta japanske jabuke. Istraživanja su pokazala da bi ekstrakt japanske jabuke mogao stimulirati crijevne epitelne stanice da proizvode izlučevine koje smanjuju oksidativni stres u mioblastima (Alfarafisa i sur., 2021).

Japanska jabuka se pokazala i kao dobro antibakterijsko sredstvo. Naime, ekstrakt japanske jabuke se pokazao dobrim u suzbijanju *Salmonella typhimurium, Klebsiella pneumonia*, *Bacillus subtilis*, *Aeromonas hydrophila* i *Pseudomonas aeriginosa*. Ovo istraživanje pokazalo je da etanolni ekstrakt voća ima značajnu količinu fitokemikalija i antibakterijsko djelovanje u odnosu na njegov vodeni ekstrakt (Pandey i sur., 2021). Ekstrakti japanske jabuke imaju detoksikacijski učinak na ugrize zmija, kao i na druge toksine koje proizvode mikroorganizmi (Okonogi i sur., 1979).

Spomenute zdravstvene pogodnosti u liječenju raznih oboljenja (Tablica 2) povezane su s bogatim antioksidativnim spojevima uključujući vitamine, fenolne spojeve i karotenoide (Chen i sur., 2008). Naime, na temelju podataka o preporučenim dnevnim unosima (RDA) za vitamin C (40-90 mg/dan), evidentno je da u većini slučajeva 100-150 g japanske jabuke (otprilike polovina cijelog voća) zadovoljava dnevne potrebe (Giordani, 2011).

Osušeni plod japanske jabuke djelotvoran je u smanjenju razine alkohola u krvi. Konzumacija sušenog voća prije konzumiranja alkohola smanjila je sadržaj alkohola u krvi za 40 %, a acetaldehida za 30 % (George i sur., 2008). Također, katehini u sirovim taninima japanske jabuke spriječili su simptome epilepsije u mozgu štakora (Mori i sur., 2004.). Važno je napomenuti da nakon prerade ploda, zaostaju nusproizvodi kao što su kora, peteljka i sjemenka. Spomenuti nusproizvodi uglavnom se odbacuju zbog čega su u posljednjih par godina provedena mnoga tehnološka i biotehnološka strateška istraživanja za recikliranje otpada japanske jabuke nakon prve upotrebe ploda (Matheus, 2020). U nekoliko studija opisano je da je koncentracija hranjivih i drugih funkcionalnih komponenti japanske jabuke veća u koži nego u pulpi (Gorinstein i sur., 2001) zbog čega treba posebnu pažnj posvetiti ponovnoj upotrebi spomenutih nusproizvoda.

Tablica 2. Bioaktivne komponente japanske jabuke i njihov pozitivan učinak na zdravlje ljudi

Table 2. *Bioactive components of Japanese apples and their positive effect on human health*

Namirnica	Utjecaj na čovjekovo zdravlje
Karotenoidi	Antioksidativni učinak Sprječavanje kardiovaskularnih oboljenja
Tanini	Hipertenzija Antialergijski učinak Smanjenje slobodnih radikala Antikancerogeno djelovanje Antimutageno djelovanje
Flavanoli	Antikancerogeno djelovanje Antimutageno djelovanje Sprječavanje kardiovaskularnih oboljenja Antioksidativni učinak
Anti-cijanid	Antioksidativni potencijal Hiperkolesterolemija Smanjenje slobodnih radikala

ZAKLJUČAK

Japanska jabuka je dobar izvor biološki aktivnih spojeva poput askorbinske kiseline i tanina koji imaju razne fiziološke funkcije, uključujući zaštitnu uloga protiv oksidativnih bolesti povezanih sa stresom, antimutageno i antikarcinogeno djelovanje (Suzuki i sur., 2005). Plod japanske jabuke ima veliki značaj za zdravlje zbog antiateroskleroznih i antioksidativnih učinaka, ali i korisnih sastojaka za kozmetičku i farmaceutsku industriju te kao dodatak u druge proizvode.

S obzirom na spomenute blagodati japanske jabuke, te na njen jednostavan uzgoj koji ne zahtjeva primjenu pesticida i umjetnih gnojiva, potrebno je iznaći načine novih tehnologija prerade i očuvanja roka trajnosti ovog voća. Također, veliku je pažnju potrebno posvetiti nusproizvodima koji nastaju, te njihovoj ponovnoj upotrebi. Široka je paleta produkata koji se mogu proizvesti iz ovog ploda, no potrebna je provedba daljnjih ispitivanja s ciljem otkrivanja načina razvoja novih proizvoda, ali i terapijskih mehanizama bioaktivnih komponenti japanske jabuke.

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FOOD SAFETY /
ZDRAVSTVENA SIGURNOST HRANE

VODOM DO ZDRAVLJA

WITH WATER TO HEALTH

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SAŽETAK

Voda je jedina namirnica koju svakodnevno koriste svi stanovnici Zemlje, neovisno o vjeri, rasi i socioekonomskom statusu. Pravo na dostupnost zdravstveno ispravne vode za piće temeljno je ljudsko pravo dano svakom ljudskom biću rezolucijom Opće skupštine UN-a naslovljenom „Ljudsko pravo na vodu i sanitarne uvjete" od 28. srpnja 2010. Globalizacija i nezaustavljivi industrijski razvoj, urbanizacija te prekomjerno korištenje prirodnih resursa značajno je narušilo ravnotežu svih segmenata ekosustava te ubrzalo i osnažilo učinke klimatskih promjena koje se naročito očituje u sve češćoj pojavi problema dostupnosti vode kao i pogoršanju kvalitete vodnih resursa. Stoga je prerada i isporuka zdravstveno ispravne vode danas zahtjevna i jedna od najodgovornijih zadaća naše civilizacije.

Redoviti unos vode u organizam osnovni je uvjet za život, a udio vode u organizmu ovisi o starosnoj dobi pojedinca te drugim stanjima organizma. No, redovitim unosom vode, čovjek svakodnevno u organizam unosi i niz kemijskih tvari koje prirodno ili kao rezultat ljudskih aktivnosti kruže u okolišu. S obzirom na potrebe ljudskog organizma, pojedine kemijske tvari prisutne u vodi su nužne za metaboličke procese i zdravlje ljudskog organizma, no često se u vodi mogu naći i one kemijske tvari koje pri dugotrajnom unosu u organizam mogu uzrokovati pojavu niza nepoželjnih i štetnih učinaka na organizam. U ovom radu prikazani su učinci kvalitete vode za piće na zdravlje ljudi.

Ključne riječi: voda, zdravlje, mikrobiološki sastav, kemijski sastav

Keywords: water, health, microbiological composition, chemical composition

UVOD

Hidrološki ciklus je proces neprestanog kruženja vode na Zemlji uzrokovan djelovanjem sunčeve energije, pri čemu voda mijenja agregatna stanja. Ovisno o dijelu ciklusa u kojem se trenutno nalazi, voda može biti (i) oborinska, (ii) površinska ili (iii) podzemna. U površinske vode ubrajaju se vode rijeka i potoka, oceana i mora te vode prirodnih i umjetnih jezera i bara. Sve navedene skupine imaju različita fizikalno-kemijska, kemijska i mikrobiološka svojstva, prije svega uvjetovana dužinom kontakta vode i okoliša u kojem se voda nalazi. Tako oborinske vode najčešće karakterizira visok udio otopljenih plinova i potpuna odsutnost otopljenih mineralnih tvari, dok podzemne vode najčešće karakterizira visoka tvrdoća te značajni udjeli otopljenih minerala i metala (USGS, 2019).
S aspekta mikrobiološke kvalitete, površinske vode u značajnijoj mjeri mogu biti opterećene mikroorganizmima, a pojavnost pojedinih vrsta mikroorganizama u vodi ovisi o vrsti vodnog tijela. Vode manjih potoka najčešće su dobre mikrobiološke kvalitete, dok rijeke i jezera najčešće imaju povećani broj mikroorganizama uslijed svakodnevnog prihvata manjih ili većih količina otpadnih voda. Podzemne vode se nalaze u vodonosnicima koji se mogu nalaziti na različitim dubinama. Ukoliko su blizu površine (plići vodonosnici) oborinske vode se lako procjeđuju u njih te utječu na njihov kemijski i mikrobiološki sastav i čine ga promjenjivim. S druge strane podzemne vode smještene u dubljim vodonosnicima (od par desetaka metara do nekoliko stotina metara dubine) karakteriziraju ujednačene fizikalno-kemijske karakteristike, odnosno vrlo mali udjeli plinova ili njihova odsutnost, najčešće neutralna pH vrijednost, konstantna temperatura te konstantan udio mineralnih tvari, prije svega kalcijevih i magnezijevih soli, a često sadrže i povećane koncentracije huminskih kiselina, željeza i mangana te dušikovih spojeva. Navedene ujednačene karakteristike podzemnih voda osnovni su razlog što su podzemne vode najčešća sirovina u procesima prerade vode za piće (Gulić, 2003; Puntarić i sur., 2012; WHO, 2019).
Vodu za piće svakodnevno upotrebljavaju i konzumiraju svi stanovnici Zemlje, a dostupnost zdravstveno-ispravne vode za piće putem vodoopskrbe jedan je od najznačajnijih pokazatelja socioekonomskog stanja svake države.
Nažalost, globalizacija i urbanizacija te prekomjerna upotreba vode u svim ljudskim djelatnostima značajno su narušili kvalitetu vodnih resursa na globalnoj razini, stoga je danas jedna od zahtjevnijih i najodgovornijih zadaća modernog društva snabdijevanje stanovništva dovoljnim količinama zdravstveno ispravne vode za piće (IFPRI, 2015).

Voda za piće

Mikrobiološki i kemijski sastav vode uvjetovan je klimatskim, reljefnim, hidrološkim i hidrogeološkim značajkama, dok se zdravstvena ispravnost vode, njena kategorija kvalitete i pogodnost za piće određuje prema preporukama, smjernicama i pravilnicima mjerodavnih ustanova i institucija na međunarodnoj, međudržavnoj i državnoj razini.

Voda za piće, s obzirom na porijeklo vode u prirodi i kvalitetu vode, dijeli se u sljedeće kategorije:

- voda za ljudsku potrošnju
- prirodna mineralna voda
- prirodna izvorska voda
- stolna voda.

Globalno tržište pakirane vode na tržištu klasificira vode kao:

- negazirane vode
- gazirane vode
- aromatizirane pakirane vode
- funkcionalne pakirane vode.

Navedene kategorije voda i njihova zdravstvena ispravnost ocjenjuje se prema vrijednostima mikrobioloških, fizikalnih i kemijskih pokazatelja, a parametri kojima se iskazuju podijeljeni su prema utjecaju na ljudsko zdravlje u tri kategorije:

- mikrobiološki parametri značajni za zdravlje ljudi
- kemijski parametri značajni za zdravlje ljudi, odnosno zdravstvene ispravnosti vode za ljudsku potrošnju (pojedini metali i organski spojevi te pesticidi i nusproizvodi dezinfekcije)
- indikatorski parametri, odnosno parametri čija vrijednost pruža podatke o postupcima pročišćavanja te o organoleptičkoj (boja i miris) i estetskoj kakvoći vode. Indikatorski parametri mogu biti mikrobiološki, kemijski i radiološki.

Odstupanje vrijednosti indikatorskih parametara ukazuje na potencijalno postojanje problema pri preradi i distribuciji vode te indicira nužnost daljnjeg ispitivanja kakvoće vode i utvrđivanja mogućeg rizika za zdravlje ljudi (WHO, 1997; Ministarstvo zdravstva, NN 125/2017, NN 39/2020).

Određivanje prisutnosti i količine mikroorganizama u vodi ograničeno je činjenicom da se mikroorganizmi u vodi pojavljuju povremeno, u nepravilnim vremenskim razdobljima i u raznim koncentracijama. No, usprkos navedenom, nadzor nad mikrobiološkom ispravnosti vode osnovni je dio sustava monitoringa kvalitete svih navedenih kategorija vode jer mikrobiološka kontaminacija vode, naročito patogenim mikroorganizmima, može u vrlo kratkom vremenu uzrokovati značajne posljedice po zdravlje ljudi, a naročito kod osjetljivih dobnih skupina kod kojih posljedice mogu biti i smrtonosne (WHO, 1997).

Važnost vode za zdravlje ljudi

Voda je čovjeku nezamjenjiva i neophodna za život (Slika 1), a udio vode u organizmu ovisi o nizu čimbenika kao što je starosna dob, zdravstveno stanje te fizička aktivnost pojedinca. Udio vode u ukupnoj tjelesnoj masi kod novorođenčadi može iznositi i do 83 %, a starenjem organizmai, udio vode se smanjuje i do 50 % (Habuda-Stanić i sur., 2016).

Voda je osnovni sastojak svih tjelesnih tekućina, uključujući krv, limfu, slinu, izlučevine iz žlijezda i tekućinu leđne moždine, a ima i važnu ulogu u svim procesima ljudskog organizma. Voda otapa minerale, vitamine i druge tvari čime

omogućuje njihov transport u organizmu, podmazuje zglobove, štiti organe i tkiva, regulira tjelesnu temperaturu, olakšava probavu te potpomaže eliminaciju otpadnih tvari i toksina iz organizma. Kontinuirano održavanje neophodnog udjela vode u organizmu tijekom 24 sata postiže se izravnim unosom tekućine (1500-2000 mL), unosom putem krute hrane (300-450 mL), dok dio vode nastaje oksidacijom organskih tvari tijekom metaboličkih procesa (300-450 mL). Redoviti unos vode u organizam pridonosi i redovitom izlučivanju štetnih tvari iz organizma (Habuda-Stanić i sur., 2016).

Slika 1. Funkcije vode u ljudskom organizmu
Figure 1. *Functions of water in the human body*

Nutricionisti preporučuju da minimalni dnevni unos vode u organizam kod odraslog čovjeka, s prosječnom fizičkom aktivnošću, treba biti između 20 i 25 g vode po kilogramu tjelesne težine, što znači da čovjek s težinom od 75 kg treba dnevno popiti oko 1500 g ili 1,5 litre vode. Kod veće fizičke aktivnosti te kod povećanja temperature zraka, potreba za vodom se povećava (UNESCO, 2003).

Mikrobiološka i kemijska kvaliteta vode

Voda je važan put unosa minerala i oligoelemenata u tijelo nužnih za normalno funkcioniranje organizma, a kemijski elementi i njihovi spojevi prisutni u vodi mogu na ljudski organizam imati pozitivni, neutralni ili negativni učinak. Učinak na organizam određene tvari ovisi o vrsti, dozi i dužini izloženosti određenoj tvari, njenoj reaktivnosti u organizmu, fiziološkom stanju i imunitetu organizma, kao i genetskim predispozicijama razvoja pojedinih bolesti te spolu i starosti organizma (UNESCO, 2003; WHO, 2019).

Na zdravlje ljudi značajan učinak, osim kemijskog ima i mikrobiološki sastav vode. Temeljni mikrobiološki kriteriji kvalitete vode za piće definirani su Pravilnikom o parametrima sukladnosti, metodama analize, monitoringu i planovima sigurnosti vode za ljudsku potrošnju te načinu vođenja registra pravnih osoba koje obavljaju

djelatnost javne vodoopskrbe (NN 125/2017, NN 39/2020) te Pravilnikom o prirodnim mineralnim, prirodnim izvorskim i stolnim vodama (NN 85/2019) prema kojima u vodi za piće ne smije biti utvrđena prisutnost parazita i patogenih mikroorganizama, bakterije *Escherichia coli* i drugih koliformnih bakterija, fekalnih streptokoka, odnosno enterokoka te sporogenih sulfitoreducirajućih anaerobnih bakterija. Također, nije dozvoljena prisutnost bakterije *Pseudomonas aeruginosa*, dok je ukupni broj mikroorganizama sposobnih za razmnožavanje u vodi definiran za svaku vrstu vode za piće, odnosno za vodu za ljudsku potrošnju te vrste pakiranih voda. Kako je mikrobiološka kvaliteta vode od iznimnog značaja za ljudsko zdravlje i u kratkom vremenu može uzrokovati negativne posljedice po zdravlje ljudi, dezinfekcija vode je obvezan tretman vode za ljudsku potrošnju, dok je dezinfekcija prirodnih mineralnih i prirodnih izvorskih voda nedozvoljena. Naime, mikrobiološka kvaliteta vode i odsutnost bakterija temeljni su kriterij klasifikacije navedenih kategorija pakiranih voda (NN 85/2019).
Pojedine vrste virusa također mogu biti prisutne u prirodnim vodama, a u vode najčešće dospijevaju putem otpadnih voda. Analizama je utvrđeno da se u vodama najčešće može detektirati prisutnost virusa hepatitisa A, „Norwalk-like“ virusa, rotavirusa i polio virusa te adenovirusa (tipovi 40 i 41). No, analitičke metode za utvrđivanje pristunosti virusa u vodi podrazumijevaju uporabu najmodernijih i visokosofisticiranih analitičkih uređaja, stoga postupak njihovog određivanja u vodi nije dio standardnih analiza kvalitete vode.
Niz je kemijskih elemenata i njihovih spojeva koji su esencijalni za metaboličke procese i zdravlje ljudskog organizma, a među njima najznačajniji su kalcij, magnezij, kalij, natrij, mangan, željezo te bakar, cink, krom i nikal.

Kalcij je najzastupljeniji metal u ljudskom organizmu i sudjeluje u nizu fizioloških procesa. Procjena je da se u tijelu odrasle osobe nalazi približno 1,2 kg kalcija, od čega se oko 99 % nalazi u kostima, dok je ostali udio kalcija u mekim tkivima i tjelesnim tekućinama. Stoga, kalcij ima dvojaku funkciju u organizmu, funkcionalnu i strukturnu jer izgrađuje koštani sustav i zube te sudjeluje u nizu funkcija mišićnog, živčanog, endokrinološkog, krvožilnog i probavnog sustava. Niz je čimbenika koji utječu na apsorpciju kalcija u organizmu, a znanstvenici bioraspoloživost kalcija u vodi uspoređuju s bioraspoloživosti kalcija u mlijeku za koje je utvrđeno da se u organizmu apsorbira u gotovoj najvišim količinama. Unos kalcija preporuča se kao način smanjenja rizika pojave osteoporoze, karcinoma dojki i debelog crijeva, za ublažavanje tegoba menopauze i bolova u mišićima i zglobovima te kao pomoć pri smanjenju kolesterola (Heaney, 2001; Cano i sur., 2018).

Magnezij je rasprostranjen u prirodi u obliku oksida, a u vodama magnezij dolazi u ioniziranom obliku i predstavlja značajan izvor bioraspoloživog magnezija važnog za ljudski organizam. Magnezij sudjeluje u gotovo svim metaboličkim procesima, a iznimno je važan za pravilan rad živčanog i mišićnog sustava te doprinosi jačanju imunološkog sustava, održava stabilan rad srca i doprinosi čvrstoći kostiju. Magnezij također doprinosi procesima održavanja normalne razine glukoze u krvi te

sudjeluje u proizvodnji energije i bjelančevina. Nedostatak magnezija u organizmu može uzrokovati poremećaje u radu živčanog, kardiovaskularnog i mišićnog sustava, no izloženost visokim koncentracijama magnezija može uzrokovati dijareju, a kod osoba s bubrežnim oboljenjima može uzrokovati hipertenziju, slabost mišića te komatozno stanje (Sengupta, 2013).

Kalij je esencijalni element, prisutan u svim biljnim i životinjskim tkivima. Primarni izvor kalija za opću populaciju je hrana. Izloženost organizma visokim koncentracijama kalija može uzrokovati povraćanje te dodatne tegobe kod osoba s oštećenom bubrežnom funkcijom, kardiovaskularnih bolesnika te djece s ograničenim bubrežnim funkcijama (WHO, 2009).

Natrijev ion je sveprisutan u vodi, a vode u prirodi najčešće sadrži manje od 20 mg/L natrija. Izloženost visokim koncentracijama natrija putem vode za piće može uzrokovati mučninu, povraćanje, konvulzije, grčenje i ukočenost mišića te moždani i plućni edem. Dojenačka populacija je dodatno ugrožena zbog nezrelosti bubrega. Unos visokih koncentracija natrija može uzrokovati trajna neurološka oštećenja, a epidemiološke studije ističu i povezanost povišenog unosa natrija i pojave hipertenzije, odnosno negativan utjecaj natrija na zdravstveno stanje kardiovaskularnih bolesnika (WHO, 1996a).

Mangan je čest sastojak prirodnih voda, a njegove povećane koncentracije u vodi mogu biti posljedica i ljudskih aktivnosti. Mangan je također esencijalni element jer pojedini enzimi u svom sastavu sadrže mangan, a pojedini se aktiviraju manganom. Istraživanja ukazuju da izloženost prevelikim koncentracijama mangana može imati štetan utjecaj na funkcioniranje neurološkog sustava (WHO, 2011).
Uz mangan, i željezo se često pojavljuje u prirodnim vodama. Količina željeza koje će tijelo iskoristiti ovisi o zdravstvenom stanju pojedinca, a najznačajnije količine željeza se apsorbiraju u dvanaesniku i gornjem jejunumu. Nedostatak željeza u organizmu se manifestira pojavom anemije (WHO, 2003).

Deficit bakra u organizmu također može uzrokovati pojavu anemije te koštanih deformacija, reproduktivnih anomalija i oštećenja živčanog sustava. Nasuprot navedenom, izloženost velikim količinama bakra može uzrokovati akutne posljedice kao što su želučane smetnje, anemije, oštećenje jetre i bubrega (WHO, 2004).
Krom u vodi može biti prisutan u trovalentnom i šesterovalentnom obliku, a sudjeluje u mnogim reakcijama u organizmu poput metabolizma glukoze. Dugotrajna izloženost kromu putem vode za piće djeluje genotoksično i kancerogeno na ljudski organizam, a zabilježena je i pojava gastrointestinalnih poremećaja i konvulzije kod akutne izloženosti (WHO, 1996b).

Na području istočne Hrvatske, uslijed geološkog sastava tla, u podzemnim vodama prisutne su povišene koncentracije arsena. Epidemiološke studije povezuju povišenu prisutnost arsena u vodi za ljudsku potrošnju s pojavom karcinoma mokraćnog

mjehura, kože i pluća, dijabetesa, kao i pigmentacijskim promjenama te pojavom perifernih vaskularnih bolesti. Utvrđeno je da na pojavnost navedenih oboljenja utječe više čimbenika kao što su dobna starost, spol, stanje imunološkog sustava, obiteljska anamneza te dodatna izloženost rizičnim agensima poput alkohola i dima cigareta (Habuda-Stanić, 2011).

Nitrati i nitriti također dolaze kao čest sastojak podzemnih i površinskih voda. Njihova pojava u vodama može biti posljedica geološkog sastava tla ili posljedica ispiranja poljoprivrednih površina tretiranih umjetnim gnojivima. Konzumacija vode s povišenom koncentracijom nitrata može imati dvojako djelovanje. Akutna izloženost nitratima se najčešće manifestira kao methemoglobinemija pri čemu se nitrati u probavnom sustavu reduciraju na nitrite koji se vežu za željezo u hemoglobinu crvenih krvnih zrnaca tvoreći methemoglobin. U navedenom obliku željezo ne može više vezati kisik što rezultira pojavom plavičaste kože, a pri velikim koncentracijama i oštećenje neurološkog sustava i smrt. Kod kronične izloženosti nitratima, moguća je pojava karcinoma kao rezultat izlaganja organizma nitrozaminima koji nastaju tijekom reakcije nitrata s u tijelu prisutnim aminima (Nujić i Habuda-Stanić, 2017).

ZAKLJUČAK

Voda je neophodna za život, a količina vode koju treba unijeti u organizam ovisi o starosnoj dobi, zdrastvenom stanju te fizičkoj aktivnosti pojedinca. Redoviti i dovoljan unos vode u organizam nužan je za normalno funkcioniranje organizma. No, voda može sadržavati kemijske tvari koje mogu imati i negativan utjecaj na zdravlje ljudi.

Kruženjem u prirodi, voda otapa kemijske tvari, a čovjek svojim aktivnostima dodatno utječe na kemijski i mikrobiološki sastav voda. Kvaliteta podzemnih voda općenito je ujednačenija i bolja u odnosu na kvalitetu površinskih voda, stoga su podzemne vode česta sirovina za preradu vode za piće.

Cilj i zadatak svake države je svojim stanovnicima osigurati kontinuiranu opskrbu zdravstveno-ispravnom vodom, odnosno primjenom odgovarajuće tehnologije prerade vode osigurati njenu kemijsku i mikrobiološku kvalitetu kako bi unos vode zadovoljio potrebe organizma za esencijalnim tvarima, ali i kako bi se spriječila pojava pojedinih vrsta oboljenja te osiguralo zdravlje populacije uslijed unosa zdrastveno-ispravne vode za piće.

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MICROBIOLOGICAL AND PARASITOLOGICAL QUALITY OF DIFFERENT FRESH-CUT SALADS

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ABSTRACT

Fresh-cut salads are "ready-to eat" food products intended for immediate consumption after minimal processing including washing, (cutting) and packaging. Although raw salads washing procedure reduces microbial load up to 90%, working surfaces contamination spreads through chopped salad while injured cell juices provide a favourable substrate for microbial growth. If present, higher loads or pathogenic microorganisms and parasites pose a serious health threat for consumers since these products are eaten fresh, without thermal treatment. The aim of this work was to examine the microbiological and parasitical quality of ready-to-eat salads from retail locations. In total 80 samples were collected from retail locations during February, May, June and July 2020. The presence of microorganisms in analysed samples was determined by standard microbiological methods while the presence of parasites was tested using sedimentation, differential staining and microscopy. Yeasts, moulds and *Enterobacteriaceae* as well as *Staphylococcus aureus* were detected in higher counts in expired salads, making them unsafe for consumption. *Salmonella*, coliforms, sulphite-reducing bacteria and parasites or their life forms (cysts/oocysts) were not detected. The presence of fungi *Alternaria* spp., possible mycotoxin producers, was higher in whole leaf salads, compared to chopped salads. Fresh-cut salads are perishable minimally processed products and it's microbiological and parasitical contamination should be closely monitored.

Keywords: fresh-cut salads, minimally processed, microorganisms, parasites, food safety

INTRODUCTION

Ready-to-eat salads are minimally processed products since their production includes mainly washing, cutting, peeling, etc. At the end of the production process they are packed in packaging that provides them longer storage time on the refrigerated shelves of shopping centres. Refrigeration ensures appropriate nutritional composition and the freshness of the product itself is maintained (Caradonna et al, 2017). Due quick and easy preparation and high nutritional value, demand, and production of ready-to-eat salads, has increased in recent years (Garreth, 2002).

Since these products are consumed raw, foodborne illnesses in consumers are more frequent. Salads can be contaminated by parasites and bacterial pathogens during primary production, processing, storage or transport. Additionally, pathogens can be found in irrigation water or manure as well as contamination occurs during transportation of raw materials or inappropriate manual harvesting (Garreth, 2002). The most common bacterial pathogens that can be found in ready-to-eat salads are enterobacteria (*Salmonella*), *Clostridia*, *Staphylococcus aureus*, and sometimes yeasts and moulds.

The aim of the experimental part of this paper was to determine the microbiological contamination and the parasite`s presence of in samples of ready-made salads ("fresh-cut" salads) from retail chains in the city of Osijek from February to July 2020. Furthermore, the diversity of the microbial population and the presence of pathogenic organisms was also determined in analysed samples.

MATERIALS AND METHODS

Random samples of ready-to-eat salads were collected from retail locations from February to July of 2020. A total of 80 samples were analysed. 60 g of the sample was washed in 300 mL of 0.85% NaCl solution and homogenized. Microbiological methods have been used to determine: bacteria of the *Salmonella* genus, *Staphylococcus aureus*, enterobacteria, sulphite-reducing clostridia, and yeasts and moulds. Microscopic examination of parasites was performed in the sediment by staining with 0.5% lugol solution (El Said Said, 2012) and the Kinyoun carbol fuchsin method. The presence of cysts or eggs was checked at magnifications of 200-10000×.

All samples were analysed for Salmonella spp., *Enterobacteriaceae*, Coagulase-positive staphylococci, sulphite-reducing *Clostridia*, yeasts and moulds, according to criteria from Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and Croatian National Guidelines on microbiological criteria for foodstuffs.

Detection of Salmonella spp.

Detection and enumeration were performed according to the International Standard method ISO 6579 for detection of *Salmonella* spp. After homogenization in stomacher for 2 minutes, 25 g of sample was analyzed in: Buffered Peptone Water, Rappaport-Vassiliadis Soy (RVS) Broth medium, and XLD agar (Liofilchem, Italy).

Enumeration of Enterobacteriaceae, Coagulase-positive staphylococci, Sulphite-reducing Clostridia and Yeasts/Moulds

Tested microorganisms were analysed by the ISO 21528-2 for enumeration of Enterobacteriaceae, ISO 6888-1 for enumeration of Coagulase-positive staphylococci, ISO 15213 for enumeration of sulphite-reducing *Clostridia* and ISO 21527-1 for enumeration of yeasts and moulds. After homogenization in stomacher for 2 minutes, a 10 g of sample 10-fold serial dilutions were prepared and processed for enumeration of specified microorganisms using VRBG agar, Baird-Parker Agar, TSC agar and DRBC agar (all Liofilchem), respectively. Temperature and incubation period as well as identifications were performed according to specific ISO standard method.

Data analysis

Parallels of microbial counts were analysed in log scale ($\log_{10}$ CFU g^{-1}), and Box plots were used for visualisation of results (MS Excel 2019, Microsoft Corporation, 2019).

RESULTS AND DISCUSSION

Fresh produce, like ready-to-eat salads have been associated with 4.2% of total foodborne outbreaks in the European Union (EFSA, 2017) and 14.8% of illness outbreaks that accounted for 22.8% of all foodborne illnesses in the US (Amin et al, 2012). This experimental work was conducted to evaluate microbiological quality and the presence of parasites in ready-to-eat salads obtained from retail centres in Osijek, Croatia. Microscopical examination of ready-to-use salads (Table 1 and 2) showed numerous artefacts (pollen, insect parts…) in soft leafed uncut salads (rocket, lamb`s lettuce and spinach) representing 96.52% of total samples analysed. Lamb`s lettuce was responsible detection of 94.12% fungal spores of *Alternaria* spp. Mentioned lettuce types have soft and fragile leaves so this could be the reason of increased *Alternaria* sp. growth. Every manipulation during processing causes the leaf tissue damage suggesting that washing procedures were not performed as they should. In cut salads (Table 2) although some artefacts and *Alternaria* spp. spores were detected, due to improved washing or the less fragile structure of plant parts (stronger leaves of cabbage or roots like carrot and beet) artefacts and spores were distributed somehow evenly. Still, inclusion of softer and more fragile lettuce in mixtures (rocket, spinach and lamb`s lettuce) increased artefact counts.

Table 1. Artefacts and spores of *Alternaria* sp. in uncut salads

Uncut salads			
Salad type	N	N (*Alternaria* sp.)	N artefacts
Rocket	15	1	24
Lamb's lettuce	14	16	48
Baby spinach	1	0	3
Spinach	2	0	39
Lamb's lettuce + radicchio	1	0	0
Misancija salad (spinach + radicchio)	1	0	1
Total	34	17	115
Artefacts (%)		29.57	
Alternaria sp. (%)		50	

Table 2. Artefacts and spores of *Alternaria* sp. in cut salads

Cut salads			
Salad type	N	N (*Alternaria* sp.)	N artefacts
Diet salad (carrot, red and white cabbage)	4	0	7
Red cabbage	4	0	12
Carrot and cabbage	1	0	2
Celery root	2	0	1
Carrot and apple	2	0	1
Iceberg, radicchio, carrot, spinach	2	0	0
Mix (endive, radicchio lamb`s lettuce, rocket spinach)	5	2	11
Romana (iceberg, endive, radicchio, carrot)	1	0	0
Rica salad (radicchio, lamb`s lettuce)	4	0	7
Carrot	1	0	3
Baby mix (spinach, swiss chard, kale)	1	0	7
Lamb`s lettuce, radish	1	0	2
Green and red lettuce	5	1	13
Cabbage (white)	2	0	8
Endive and radicchio	4	0	4
Iceberg mix (radicchio, carrot, endive, spinach)	3	0	12
Rocket, lamb`s lettuce, red and green lettuce, spinach, beet	4	0	13
Total	46	3	103
Artefacts (%)		44.66	
Alternaria sp. (%)		6.52	

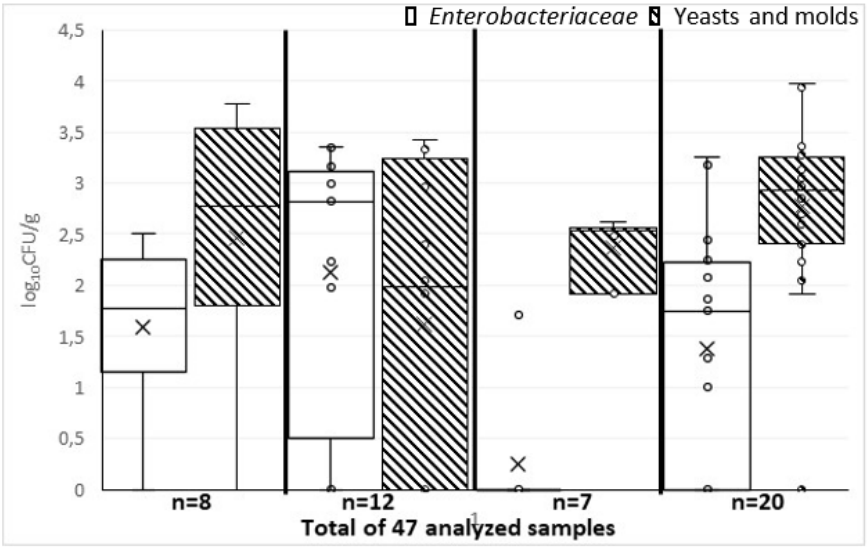

Figure 1. Contamination of ready-to-eat salads before expiration date

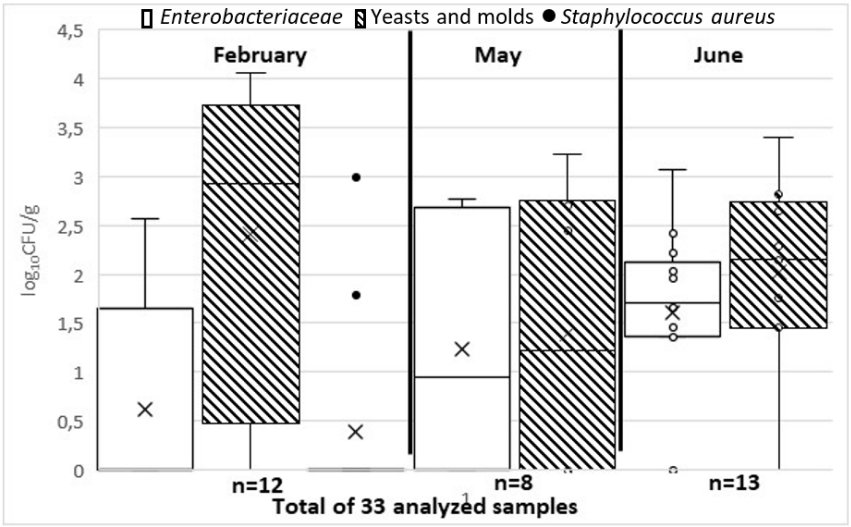

Figure 2. Contamination of ready-to-eat salads after expiration date

In total of 80 analysed samples, 43 was obtained from retail centres before expiration date (Figure 1), while 33 samples were on shelves of the same centres after expiration date (except in July). Conversely to expectation, counts of most tested microbes were lower in samples after expiration date. Ready-to-eat salads, specially cut, have very large surface area and probably samples with expired usage date were washed more thoroughly reducing thus the level of contamination. Additionally, washing water should be closely supervised for effective chlorine

concentration since it is unstable and reduces it`s microbicidal activity in the presence of organic matter. In February the number of yeasts and moulds was higher which could be connected with their ability to grow at lower temperature. On the other hand, warmer months had beneficial effect on *Enterobacteriaceae* multiplication. *Staphylococcus aureus* was detected only in one ready-to-eat salad from February, which passed expiration date. In total, 28 of 80 (35%) of analysed ready-to-eat salads did not meet the microbiological criteria (labelled as unsatisfactory samples), due to high levels of yeasts/moulds and *Enterobacteriaceae*.

Similar results were reported in several papers regarding microbiological quality of ready-to-eat salads (Calonico et al, 2019; Koushki et al, 2021; Ljevaković-Musladin et al, 2019) where *Salmonella* or *Listeria monocytogenes* were rarely detected although higher than legislation of coliforms, yeasts and moulds was the main reason of unsatisfactory microbiological quality of tested samples.

Salmonella and sulphite-reducing *Clostridia* or parasites or parasitic elements (oocysts) were not detected in any of analysed samples.

Ready-to-eat salads are raw and minimally processed products and washing is crucial step in production of safe products. Nevertheless, every washing procedure affects the quality of the vegetables, making them susceptible to contamination. Although chlorine products are usually used for washing, active chlorine is unstable and can be ineffective while residual of washing water offers more opportunity for microbial growth (Gill et all, 2009).

CONCLUSION

Our study showed that ready-to-eat salads can harbour spoilage microorganisms as well as unwanted and potentially hazardous ones. Pathogens like *Salmonella* spp. and *Staphylococcus aureus* as well as sulphite-reducing *Clostridia* were not isolated nor were parasite elements found, but there is high contamination of spoilage indicators, especially in cut salads and salads passed expiration date. Higher counts of spoilage indicators (yeasts and moulds) as well as *Enterobacteriaceae* suggests improper washing procedure and storage. Specially in ready-to-eat food products washing and hygiene are crucial in providing safe product for consumers. Additional research is needed as well as control since expired ready-to-eat products pose considerable threat for consumer`s health.

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TEŠKI METALI U HRANI I NJIHOV UTJECAJ NA ZDRAVLJE LJUDI

HEAVY METALS IN FOOD AND THEIR IMPACT ON HUMAN HEALTH

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SAŽETAK

Zdravstvena sigurnost hrane je prioritetno pitanje za zdravlje ljudi. Metali, kao što su arsen, kadmij, olovo, nikal, živa, krom i uran, su jedni od onečišćivača hrane koji utječu na zdravlje ljudi, uzrokujući promjenu metabolizma, utječući na morbiditet i na mortalilitet, a neki od njih su klasificirani kao kancerogenici. Teški metali predstavljaju prirodne sastojke okoliša, ali izvor kontaminacije potječe i od antropogenih izvora (sagorijevanje fosilnih goriva, industrijska postrojenja, ekstrakcija rude, motorna vozila, deponije industrijskog i komunalnog otpada, gnojiva i atmosferski talozi). Kontaminacija hranidbenog lanca s ovim elementima potječe iz zraka, vode i tla. Antropogeni izvori zagađenja posebno dolaze do izražaja u regijama koje imaju velike industrijske zagađivače. Nepostojanje sustavnog monitoringa kontaminacije hranidbenog lanca, ili neadekvatnog sustavnog monitoringa, uzrokuje da konzumiramo hranu upitnog kvaliteta, koja s vremenom može postati uzrok bolesti kod ljudi. Teški metali predstavljaju jednu od najhitnijih briga u raspravi o sigurnosti i kvalitetu hrane kao i eventualne mjere saniranja kontaminiranih područja. Unatoč važnosti za pojačanim praćenjem i poduzimanjem mjera za saniranjem kontaminiranih regija, veoma mali broj istraživanja je usmjeren ka tom javnozdravstvenom problemu.

Ključne riječi: teški metali, hrana, zdravlje ljudi

Keywords: heavy metals, food, human health

UVOD

Zagađenje okoliša je ozbiljan ekološki problem svijeta koji je sve više u fokusu javnosti zbog zabrinutosti za sigurnost i zdravlje, kako ljudi tako i okoliša. Od industrijske revolucije do danas, broj, vrsta i količina zagađivača u okolišu se eksponencijalno povećala. Različiti anorganski i organski zagađivači u okolišu mogu uzrokovati ozbiljne zdravstvene probleme živim bićima (Rai i sur., 2019). Jedni od tih zagađivača su teški metali. Teški metali se definiraju kao metali s gustoćom većom od 5 g/cm^3. Dijele se na esencijalne (bakar, cink, krom, mangan, željezo, molibden, selen, nikal) i neesencijalne (olovo, živa, kadmij, arsen, aluminij, kositar, kobalt, paladij, platina). Mnoge svjetske agencije za zaštitu okoliša smatraju neesencijalne teške metale kao prioritetne zagađivače. Budući da nisu biorazgradivi, akumuliraju se u velikim količinama u okolišu i uzrokuju ozbiljna zagađenja vode i tla, a na živim bićima ispoljavaju toksične, genotoksične, kancerogene, mutagene i teratogene učinke, čak i nakon izlaganja niskim koncentracijama. (Dixit i sur., 2015; Sarwar i sur., 2017) Stoga je sigurnost hrane postala globalni problem, zbog njene neraskidive veze sa zdravljem. (Tóth i sur., 2016).

Teški metali se prenose iz abiotičkog okoliša (vode, tla, zraka, sedimenta) u žive organizme, što dovodi do akumulacije ovih elemenata u bioti i posljedične kontaminacije prehrambenog lanca sa ovim elementima. Duž prehrambenog lanca dolazi do biomagnifikacije, jer u svakoj narednoj trofičkoj razini dolazi do sve veće akumulacije ovih elemenata. Trofički prijenos, bioakumulacija i biomagnifikacija teških metala u prehrambenom lancu ima važne implikacije na sva živa bića. Akumulacija teških metala kod beskralježnjaka, vodozemaca i gmazova (zbog apsorpcije kroz visokopropusnu kožu), te ptica (zbog konzumacije kontaminirane hrane i vode) nepovoljno utječe na njihov razvoj, rast, zdravlje, fiziološke procese i reprodukciju (Ali i Khan, 2019). Onečišćenje vode teškim metalima utječe na različite fiziološke procese u ribama, uključujući rast i razvoj (Jezierska i sur., 2009). Prehrambene namirnice, životinjskog i biljnog porijekla, kontaminirane teškim metalima predstavljaju potencijalni rizik za ljudsko zdravlje.

Opsežna studija ispitivanja uzoraka tla na prisustvo teških metala u EU pokazala da je velika većina poljoprivrednih zemljišta sigurna za proizvodnju hrane (Tóth i sur., 2016), ali hrana koju konzumiramo ne dolazi samo sa ovih područja. Globalna trgovina omogućava nam konzumaciju prehrambenih proizvoda koji su proizvedeni širom svijeta. Nedovoljna sustavna kontrola prehrambenih namirnica dovodi u pitanje sigurnost hrane koju konzumiramo. Nedavna istraga u SAD-u je pokazala da hrana za bebe koja se prodaje na tržištu SAD-a sadrži opasne razine arsena, olova, žive i kadmija. (U.S. House of Representatives, 2021). Brojna istraživanja su pokazala prisustvo teških metala u mnogim namirnicama biljnog i životinjskog porijekla koje koristimo u svakodnevnoj prehrani. Analiza začinskih biljaka kupljenih u lokalnim trgovačkim centrima je pokazala da je sadržaj olova u većini uzoraka bio iznad maksimalno dopuštenih koncentracija koje preporučuje WHO (Huremović i sur., 2014). Prisustvo teških metala, a posebno sadržaj olova koji je bio veći od dopuštenog, utvrđen je u uzorcima proizvoda sa propolisom, koji se smatraju dodacima prehrani (González-Martín i sur., 2018).

S obzirom na veliku raznolikost izvora teških metala i prirode tih metala, nemoguće je izbjeći prisutnost teških metala u okolišu, a time i u prehrambenom lancu.

PRIRODNI IZVORI ZAGAĐENJA TEŠKIM METALIMA

Teški metali su sastavni dio zemljine kore i prisutni su na Zemlji od njenog nastanka. Pod utjecajem različitih prirodnih pojava metali se oslobađaju iz svojih depozita i ulaze u prirodne biogeokemijske cikluse i na taj način u sve dijelove biosfere, gdje se akumuliraju (Callender, 2014). Najveći prirodni izvor teških metala u okolišu su erozija stijena i ispiranje tla, djelovanjem atmosferilija, i vulkanska aktivnost. Ovi izvori predstavljaju 80% prirodnih izvora teških metala u okolišu, a preostalih 20% potječe od šumskih požara i biogenih izvora. (Nriagu, 1990) Ispiranjem i usitnjavanjem stijena metali ulaze u rijeke i oceane, gdje procesima taloženja postaju dio sedimenta iz kojeg se u određenim uvjetima mogu mobilizirati. Isparavanjem vode, metali ulaze u atmosferu, a nastale čestice se zračnim strujanjima raznose na velike udaljenosti. Vulkanske erupcije izbacuju u atmosferu velike količine čestica stijena i pepela, ali i emisije iz vulkana u stanju mirovanja su također značajne. Prema procjenama godišnje emisije kadmija, žive, selena, bakra i cinka iz vulkana Etna su u razini emisija iz antropogenih izvora na području Mediterana (Garrett, 2000). Biogeni izvori, kao npr. aerosoli iz biljaka, organski otpad, pelud i sl., predstavljaju više od trećine prirodnih emisija nekih metala (Nriagu, 1990).

ANTROPOGENI IZVORI ZAGAĐENJA TEŠKIM METALIMA

Globalni rast ljudske populacije, kao i industrijski i ekonomski razvoj država uvjetuju kontinuirano povećavanje proizvodnje i uporabu metala u svim segmentima života ljudi: energetici, građevinarstvu, automobilskoj i elektroničkoj industriji, agronomiji, medicini itd. Rudnici, talionice, termoelektrane, metaloprerađivačka, kemijska, tekstilna industrija kao i poljoprivredne prakse su najveći antropogeni izvor zagađenja okoliša sa teškim metalima. Nekontroliranim ispuštanjem (industrijskih, poljoprivrednih, komunalnih) otpadnih voda i plinskih emisija teški metali ulaze u vodotokove i atmosferu, i cirkuliraju u okolišu procesima suhog (vjetar) ili mokrog (oborine) taloženja. Ovisno o meteorološkim uvjetima, čestice se prenose na velike udaljenosti, tako da ne postoje područja na Zemlji koja su pošteđena zagađenja, bez obzira koliko su udaljeni od izvora zagađenja.
Antropogena djelatnost pridonosi zagađenju okoliša, pri čemu su emisije nekih metala karakteristične za pojedinu industrijsku ili komunalnu djelatnost. Izgaranje fosilnih goriva, posebice ugljena, je glavni izvor emisija Cr, Hg, Mn, Sb, Se, Sn i Tl, a izgaranje nafte je glavni uzrok povećanim emisijama Ni i V. Promet, odnosno izgaranje olovnog, nisko-olovnog i bezolovnog benzina, najviše doprinosi emisijama Pb, dok je metaloprerađivačka industrija najznačajniji izvor emisija As, Cd, Cu, In i Zn (Pacyna i Pacyna, 2001).

TEŠKI METALI U OKOLIŠU

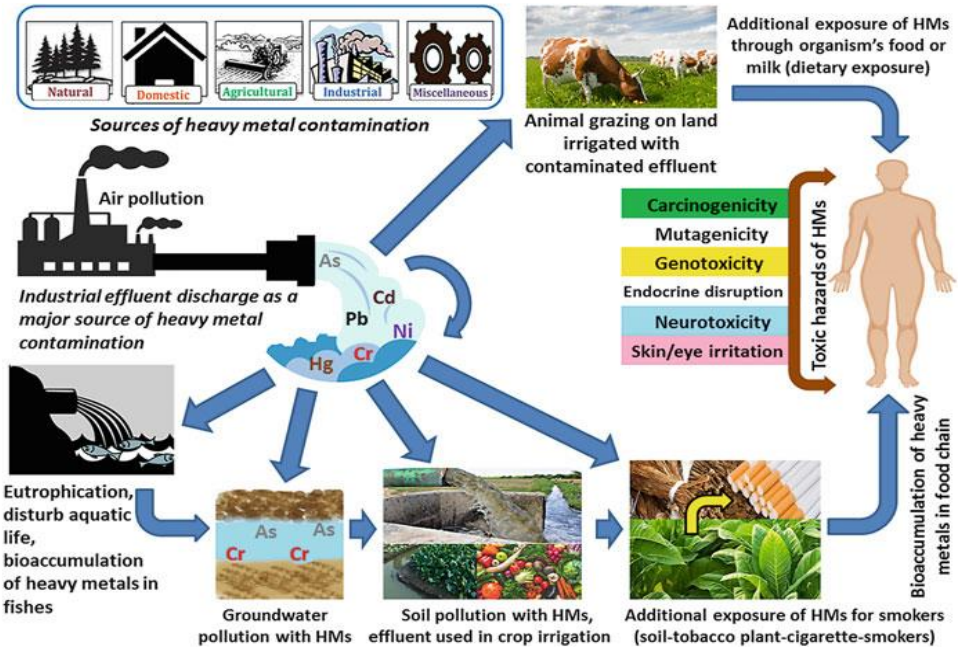
Teški metali nisu biorazgradivi, sastavni su i trajni dio našeg planeta i našeg okoliša. Antropogena djelovanja, uz prirodne geološke aktivnosti, u značajnoj mjeri doprinose kontaminaciji površinskih slojeva Zemlje sa ovim polutantima. Ovi elementi kada jednom uđu u prehrambeni lanac u njemu ostaju trajno. Rezultati brojnih studija potvrđuju kontinuirano obogaćivanje sedimenta i tla s metalima kao posljedica povećanih emisija metala iz antropogenih izvora. (Callender, 2014) Emisije teških metala u svijetu variraju ovisno o razvijenosti država. U većini razvijenih zemalja, trendovi emisije teških metala su u opadanju zbog strožih zakonskih propisa, tehnoloških poboljšanja u proizvodnji, kontroliranog zbrinjavanja otpada itd. Najveće emisije skoro svih metala zabilježene su na području Azije što je posljedica sagorijevanja fosilnih goriva, uglavnom ugljena koji se koristi i u industrijskim pogonima i za proizvodnju električne energije, kao i brzog industrijskog razvoja. Na Azijske države odnosi se gotovo 40 – 60% ukupnih svjetskih emisija svih metala, a samo Kina emitira 1/3 ukupnih svjetskih emisija žive. Na području Azije smještena je i snažna metaloprerađivačka industrija iz koje se u atmosferu ispuštaju velike količine arsena i kadmija, te čak 45% svjetskih emisija olova. (Pacyna i Pacyna, 2001)

Metali ne ostaju lokalizirani na izvoru zagađenja. Nošeni zračnim strujanjima i vodom mogu biti prenešeni na velike udaljenosti. Tijekom procesa precipitacije i taloženja akumuliraju se u tla i sediment, odakle bioakumulacijom u biljkama i životinjama ulaze u prehrambeni lanac. Povišene koncentracije toksičnih metala u okolišu predstavljaju potencijalnu prijetnju svim živim bićima (Pacyna i Pacyna, 2001).

UTJECAJ ONEČIŠĆENJA NA BILJKE

Tlo je osnovni izvor opskrbe biljaka svim esencijalnim tvarima potrebnih za rast i razvoj. Kontaminacija tla sa teškim metalima ne utječe samo na biljke, nego i na druge organizme koje žive u tlu, bilo da žive u simbiozi sa biljkama ili pridonose plodnosti tla, tako i onih kojima je tlo habitat. Pogođeni su kako korisni insekti (posebno u poljoprivredi), beskralježnjaci, sisavci, tako i mikrobiološka biota (Gall i sur., 2015). Primarni izvori kontaminacije biljaka i tla sa teškim metalima su atmosferska depozicija, stajsko gnojivo, navodnjavanje otpadnom ili zagađenom vodom, metalo-pesticidi ili herbicidi, gnojiva na bazi fosfata i gnojiva na bazi kanalizacijskog mulja (Gall i sur., 2015). Apsorpcijom ovih metala, iz tla i zraka, biljke uvode ove kontaminante u prehrambeni lanac ljudi i životinja. Kada uđu u biljke, teški metali utječu na različite biljne enzime, i na taj način remete mnoge fiziološke procese, kao što su: stanični metabolizam, klijavost sjemena, metabolizam i fiksacija dušika, fotosinteza, transpiracija, asimilacija CO_2, regulacija vode i stanične ionske homeostaze, apsorpcija minerala, fotofosforilacija, antioksidatvni zaštitni sustav, što u konačnici dovodi do smrti biljke (Yadav, 2010; Asgari Lajayer i sur., 2017). Osim ovih procesa, također se remete i citološki procesi u biljkama, kao što su rast i produljenje korijena, permeabilnost stanične membrane, mitoza,

stabilnost genetskog materijala i dolazi do pojave kromosomskih abnormalnosti (Nagajyoti i sur., 2010; Yadav, 2010).



Slika 1. Načini prijenosa teških metala putem hrane do čovjeka i njihova toksičnost (Saxena i sur., 2020)

Figure 1. *Methods of transmission of heavy metals via food to humans and their toxicity (Saxena i sur., 2020)*

Povrće, voće i žitarice su važne namirnice u svakodnevnoj prehrani ljudi jer sadrže vitamine i minerale, i imaju važnu ulogu u održavanju zdravlja i prevenciji različitih bolesti. Ove namirnice sadrže različite koncentracije kako esencijalnih tako i toksičnih elemenata. Teški metali su glavni k koji utječu na nutritivnu vrijednost povrća, voća i žitarica (Singh i sur., 2010). Navodnjavanje prehrambenih usjeva sa otpadnim vodama, često bogatim teškim metalima, uobičajena je poljoprivredna praksa u mnogim zemljama u razvoju, koja dovodi do nakupljanja teških metala u tlu i njihovog kasnijeg prijenosa u prehrambene usjeve kao što su žitarice i povrće, a indirektno i u mlijeko i mliječne proizvode (Singh i sur., 2010). Osim apsorpcijom putem korijena, teški metali iz atmosfere mogu se taložiti na listovima biljaka i ući u biljke putem folijarnog prijenosa, kroz kutikulu i puči lista. Akumulacija teških metala u biljkama ovisi o biljnim vrstama i svojstvima tla (Rattan i sur., 2005). Nedavno istraživanje pokazuje da pšenica općenito ima veću sklonost nakupljanju teških metala u usporedbi s kukuruzom (Wang i sur., 2017), a uzgoj riže je vrlo zabrinjavajući jer ova kultura zahtijeva puno više vode (Arunakumara i sur., 2013). Lisnato povrće (špinat, kupus, korijander) apsorbira metale u većoj mjeri u usporedbi s lukovičastim i gomoljastim povrćem (Mahmood i Malik, 2014). Mjesto

akumulacije teških metala u biljkama varira ovisno o biljnim vrstama. Povrće kao što su salata, rotkvice, grah i rajčice akumuliraju teške metale u različitim koncentracijama u korijenu, lišću i plodovima (Cobb i sur., 2000). Literatura o teškim metalima u prehrambenim biljkama pokazuje je povrće, lisnato i nelisnato, dobar „akumulator" teških metala (Khan i sur., 2015).
Budući da se u tlu akumuliraju teški metali, tlo je i izvor onečišćenja podzemnih i površinskih voda, sedimenta i oceana sa ovim elementima (Facchinelli i sur., 2001).

UTJECAJ ONEČIŠĆENJA NA ŽIVOTINJE

Životinje su izložene teškim metalima iz svog okoliša. Teški metali utječu na sve životinjske vrste, vodene i kopnene, koje akumuliraju ove elemente iz abiotskog okoliša i putem hrane. Potrebno je razlikovati nekoliko pojmova koji se tiču načina prijenosa onečišćenja u organizme: biokoncentracija, bioakumulacija, akumulacija hranom, trofički prijenos i biomagnifikacija. Biokoncentracija se odnosi na nakupljanje onečišćenja u organizmu kao rezultat njegovog preuzimanja iz abiotičkog okoliša. Bioakumulacija se odnosi na nakupljanje onečišćenja u organizmu kao rezultat njegovog unosa iz abiotičkog okoliša i iz hrane. Akumulacija hranom se odnosi na nakupljanje onečišćenja u organizmu kao rezultat njegovog unosa iz hrane. Trofički prijenos odnosi se na prijelaz onečišćivača u prehrambenom lancu, s jedne trofičke razine na drugu. Biomagnifikacija se odnosi na povećanje koncentracije onečišćenja duž prehrambenog lanca, odnosno duž uzastopnih trofičkih razina prehrambenog lanca. (Ali i Khan, 2019)
Onečišćenje vode teškim metalima je ekološki problem koji negativno utječe na biljke, životinje i ljudsko zdravlje (Rezania i sur., 2016). Teški metali ulaze u vodene sustave suhim i mokrim taloženjem iz atmosfere i putem otpadnih voda - industrijskih, komunalnih i poljoprivrednih. Kada dospiju u vodu teški metali se nakupljaju u sedimentima i bioti, a dio ostaje otopljen u vodi. Zadržavanje teških metala u sedimentima ovisi o različitim fizikalno-kemijskim procesima kao što su adsorpcija/desorpcija, kompleksiranje i taloženje, koji ovise o različitim fizikalno-kemijskim parametrima, kako vode tako i metala. Topljivi oblici ovih elemenata imaju veću bioraspoloživost i time veću toksičnost (Ali i Khan, 2018). Ribe, i drugi vodeni organizmi, su u prirodnom okruženju izložene mješavinama više metala, čiji učinci mogu biti aditivni, sinergistički ili ponekad antagonistički. Ribe unose metale u organizam na tri načina: iz vode preko škrga, iz prehrane putem crijeva i putem kože (Sauliute i Svecevičius, 2015). Akumulacijom ovih elemanata u organizmima riba dolazi do poremećaja struktura i funkcija različitih tkiva i organa. Teški metali utječu na različite fiziološke procese u ribama, uključujući razmnožavanje i razvoj. Negativno utječu na različite metaboličke procese u razvoju embrija, što rezultira zaostajanjem u razvoju, morfološkim i funkcionalnim anomalijama ili smrću najosjetljivijih pojedinaca. Osim toga, teški metali aktiviraju procese detoksikacije koji troše energiju potrebnu za rast. Rani razvojni stadiji riba posebno su osjetljivi na intoksikaciju (Jezierska i sur., 2009). Riba je važan dio ljudske prehrane jer osigurava bjelančevine i nezasićene masne kiseline koje su vrlo važne za zdravlje ljudi, međutim konzumacija ribe kontaminirane teškim metalima predstavlja

potencijalni rizik za ljudsko zdravlje. I druge vodene životinje koje koristimo u prehrani, kao što su školjke, rakovi, hobotnice, lignje, mogu biti izvor teških metala, zbog bioakumulacije ovih elemenata u organizmima (Ali i Khan, 2019).
Prehrambene namirnice kopnenog porijekla (meso i mlijeko) predstavljaju važan izvor lipofilnih kontaminanata za ljude. Različite studije su pokazale kako se teški metali mogu akumulirati, u značajnoj količini, u hidrofobne odjeljke u životinjskom organizmu udisanjem i ingestijom kontaminirane vode, stočne hrane i tla tijekom ispaše (Di Bella i sur., 2020). Prijenos ovih kontaminanata na ljude putem hrane ne uključuje samo konzumaciju mesa kontaminiranih životinja, nego i konzumaciju namirnica porijeklom od životinja, kao što su jaja, mlijeko i mliječni proizvodi (Bilandžić i sur., 2011; Kabeer i sur., 2021).

TEŠKI METALI – UTJECAJ NA ZDRAVLJE LJUDI

S toksikološkog gledišta najopasniji metali su živa, olovo, kadmij, krom i arsen, i izloženost tim metalima je glavna prijetnja zdravlju ljudi.

Živa

Živa je metala koji se na sobnoj temperaturi nalazi u tekućem stanju. U okoliš dospijeva iz prirodnih i antropogenih izvora te je široko rasprostranjena. U okolišu se nalazi u tri kemijska oblika, a to su elementarna (Hg^0), anorganska (Hg_2^{2+}i Hg^{2+}) i organska živa. Sagorijevanje krutih goriva kao što su ugljen, lignit i drvo, u industriji i kućanstvima, predstavljaju jedan od najvećih izvora onečišćenja živom u svijetu. Zbog dugotrajne uporabe, stotine tisuća tona žive su ispuštene u okoliš. Atmosfera je glavni put prijenosa žive širom svijeta, i taloženja na kopnu i u vodi. Živa koja se nalazi u atmosferi i tlu ne predstavlja značajan izravan rizik za zdravlje ljudi ili životinja. Vodeni okoliš je važniji jer djeluje kao dugotrajno skladište žive i, što je još važnije, živa u vodenom okolišu se pretvara u metil-živu (EEA, 2018). Životni vijek žive u površinskim vodama se procjenjuje se na 30 godina, dok u dubokim vodama ostaje stoljećima. Živa prisutna u površinskim vodama se oslobađa isparavanjem natrag u atmosferu, odakle će ponovno doći u vodu ili će se taložiti na kopnu (UNEP, 2013).
U prehrambenom lancu metil-živa je najčešći oblik organske žive, i najvažniji je oblik žive u okolišu koji se akumulira u životinjama i čovjeku zbog činjenice da je 50 puta otrovnija od elementarne (HAH, 2014). Temeljni problem onečišćenja okoliša živom je u tome što se njezini organometalni spojevi mogu nakupljati i metabolizirati u biosferi. Metil-živa nastaje kao rezultat metilacije anorganskih oblika žive od strane mikroorganizmima koji se nalaze u tlu i vodi (Dopp i sur., 2004). Riba i proizvodi od ribe su glavni izvor izloženosti metil-žive u prehrani, za sve dobne kategorije ljudi. Posebno ribe koje su na vrhu hranidbenog lanca, kao što su tuna, sabljarka, bakalar, štuka, oslić, najviše doprinose izloženosti metil-živi u prehrani, kako odraslih tako i djece. Najvažniji izvori izloženosti anorganskom živom u prehrani su riba i ostali morski plodovi, bezalkoholna pića i obrađena/kompozitna hrana (EFSA, 2012).

Živa je jedan od najopasnijih metala koji je prisutan u ljudskoj prehrani, a pritome nema nikakvu biokemijsku funkciju u organizmu (Akpor i sur., 2014). Toksično djeluje na kardiovaskularni sustav, a ispoljava i neurotoksično djelovanje kako za odrasle tako i za djecu. Anorganska živa toksično djeluje na bubrege, ali može djelovati i na druge organe i sustave poput jetre, pluća, živčanog, imunološkog i reproduktivnog sustava (HAH, 2014).

Ljudi su izloženi svim kemijskim oblicima žive kroz onečišćenje okoliša, kontaminaciju hrane, njegu zuba, industrijske i poljoprivredne aktivnosti, i putem profesionalne izloženosti (Bhan i Sarkar, 2005). Kemijski oblici žive koje se najviše apsorbiraju su elementarna živa (Hg^0) i metil živa (MeHg). Pare elementarne žive su vrlo lipofilne i dobro se apsorbiraju kroz pluća i sluznice. Nakon što Hg^0 uđe u krv brzo prolazi kroz stanične membrane, kao i krvno-moždanu i placentnu barijeru (Guzzi i La Porta, 2008). Nakon što uđe u stanicu, Hg^0 se oksidira u visoko reaktivnu Hg^{2+}. Metil-živa iz hrane lako se apsorbira u gastrointestinalnom traktu i zbog svoje topljivosti u lipidima može lako prijeći i placentnu i krvno-moždanu barijeru. Nakon što se apsorbira, živa ima vrlo nisku brzinu izlučivanja iz organizma i veliki dio apsorbirane žive nakuplja se u bubrezima, jetri i neurološkom tkivu (Tchounwou, Ayensu, i sur., 2003).

Pokazalo se da, i organska i anorganska, živa u organizmu mijenja homeostazu kalcija kroz različite mehanizme. Smatra se da organski živini spojevi (MeHg) povećavaju koncentraciju unutarstaničnog kalcija, ubrzavajući priljev kalcija iz međustaničnog prostora u stanicu i mobilizirajući unutarstanične zalihe, dok anorganski živini (Hg^{2+}) spojevi povećavaju unutarstanične zalihe kalcija samo kroz dotok kalcija iz međustaničnog prostora (Tchounwou i sur., 2012). Živa inducira stvaranje reaktivnih spojeva kisika (ROS) za koje je poznato da uzrokuju oštećenja DNK u stanicama, a takvi procesi mogu dovesti do pokretanja kancerogenih procesa u stanicama (Valko i sur., 2006). Izravno djelovanje ovih slobodnih radikala na nukleinske kiseline može izazvati genetske mutacije (Inoue i sur., 2005). Ovi slobodni radikali također mogu izazvati konformacijske promjene u proteinima koji su odgovorni za popravak DNA, formiranje diobenog vretena i odvajanje kromosoma (Valko i sur., 2006).

Olovo

Olovo je plavkasto-sivi metal prisutan u malim količinama u zemljinoj kori. Antropogene aktivnosti, poput izgaranja fosilnih goriva, uporaba olova u bojama, proizvodnja vodovodnih cijevi, rudarstvo, proizvodnja baterija, streljiva, doprinose onečišćenju okoliša s ovim polutantom u visokim koncentracijama. Od 1970-ih se poduzimaju kontrolne mjere za reguliranje olova u bojama, limenkama za hranu, cijevima za vodu i benzinu, što je dovelo da smanjenja izloženosti olovu (ATSDR, 2020). Unatoč napretku sa ovim programima, izloženost ljudi olovu ostaje ozbiljan zdravstveni problem, a trovanje olovom i dalje je jedan od najčešćih pedijatrijskih zdravstvenih problema u Sjedinjenim Američkim Državama (Pirkle i sur., 1998).

U općoj populaciji izvori izloženosti olovu su zrak, hrana, voda, tlo i prašina, ali i niz potrošačkih proizvoda kao što su baterije, kozmetika, boje za kosu, nakit, glazure

za keramiku, stakleno posuđe od olovnog kristala, oružje i streljivo, dječje igračke, tradicionalni ili narodni lijekovi, ambalaža za hranu/slatkiše (ATSDR, 2020). Olovo u okolišu može lako kontaminirati hranu putem vode i preko atmosferskog taloženja na poljoprivredne usjeve (EFSA, 2010). Odrasle osobe apsorbiraju 35-50% olova putem vode za piće, a apsorpcija kod djece može biti veća od 50%. Na apsorpciju olova utječu čimbenici kao što su dob i fiziološki status. U ljudskom tijelu olovo ulazi u bubrege, jetru i ostala meka tkiva poput srca i mozga, a glavno mjesto akumulacije su kosti. Vrijeme polueliminacije za anorgansko olovo u krvi je otprilike 30 dana, dok je za olovo deponirano u kostima ta vrijednost između 10 i 30 godina (EFSA, 2010). Toksičnost olova se ispoljava na nekoliko organa u tijelu uključujući bubrege, jetru, središnji živčani sustav, hematopoetski sustav, endokrini sustav i reproduktivni sustav. Nervni sustav je najranjiviji kod trovanja olovom. Glavobolja, nedostatak pažnje razdražljivost, gubitak pamćenja i tupost su rani simptomi toksičnog djelovanja olova na središnji nervni sustav (ATSDR, 2020).

Toksični učinci olova na neurološki sustav su najopasniji, jer se ti učinci opažaju kod dojenčadi i djece, i mogu rezultirati doživotnim smanjenjem neuroloških funkcija. Dojenčad se rađa sa opterećenjem olovom koje je posljedica prijenosa iz majke tijekom trudnoće, a nakon rođenja se može nastaviti putem majčinog mlijeka. Djeca su također izloženija i zato što je gastrointestinalna apsorpcija olova viša u usporedbi s odraslim osobama, vjerojatno zbog kombinacije fizioloških razlika i razlika u prehrani. Brojne studije utjecaja izloženosti djece olovu pružaju dosljedne dokaze o smanjenju neuroloških funkcija, uključujući smanjenje kognitivne funkcije (učenje i pamćenje), promjene ponašanja i raspoloženja (pažnje, hiperaktivnost, impulzivnost, razdražljivost, delinkvencija) i promijeni neuromotoričkih i neurosenzornih funkcija (spretnost, promjene sluha i vida) (ATSDR, 2020).

Danas ne postoji procjena podnošljivog tjednog unosa (PTWI) olova, budući da se ne može utvrditi granična vrijednost za toksično djelovanje olova (EFSA, 2010; ATSDR, 2020). SZO je procijenila da je pri vrijednostima prethodno utvrđenog PTWI, od 25 μg/kg tjelesne težine, dolazilo do toksičnog djelovanja olova, što je kod djece povezano sa smanjenjem kvocijenta inteligencije od najmanje 3 IQ boda, a kod odraslih je dovelo do povećanja sistoličkog krvnog tlaka (ATSDR, 2020).

Jedan od glavnih mehanizama toksičnog djelovanja olova je kroz biokemijske procese, zbog sposobnost olova da inhibira ili oponaša ulogu kalcija u organizmu, i na taj način se veže za biološke molekule i ometa njihovu funkciju putem brojnih mehanizama (ATSDR, 2020). Unutar kostura, olovo se ugrađuje u koštano tkivo umjesto kalcija. Zbog dugog vremena polueliminacije, kronična toksičnost olova je razlog najveće zabrinutosti, kada se uzme u obzir potencijalni rizik za ljudsko zdravlje (EFSA, 2010). Različita fiziološka stanja (npr. trudnoća, menopauza, napredna dob) ili stanja povezana s bolešću (npr. osteoporoza, produljena imobilizacija) kod kojih dolazi do povećane resorpcije kosti, mogu uzrokovati otpuštanje Pb iz kostiju, što može doprinijeti povećanju koncentracije Pb u krvi (ATSDR, 2020). Međunarodna agencija za istraživanje raka (IARC) 2006. godine klasificirala je anorgansko olovo kao vjerojatno kancerogeno za ljude (Skupina 2A) (EFSA, 2010).

Arsen

Arsen je element koji se u niskim koncentracijama nalazi u gotovo svim segmentima životne sredine. U prirodi se najčešće pojavljuje u anorganskom obliku, kao trovalentni arsenit i pentovalentni arsenat. Organski oblici arsena, monometilarsenska kiselina (MMAA), dimetilarsenska kiselina i trimetil-arsin oksid, nastaju metilacijom djelovanjem mikroorganizama. Zbog sposobnosti obavljanja ovih procesa, mikroorganizmi imaju važnu ulogu u ciklusu kruženju arsena u prirodi. Osim prirodnih izvora zagađenju okoliša s ovim metalom pridonose i antropogeni izvori – industrija, upotreba herbicida, insekticida, pesticida, algicida, drvnih konzervansa i boja (Tchounwou i sur., 2012). Opća populacija je izložena anorganskom arsenu konzumacijom onečišćenje vode, korištenjem onečišćene vode u pripremi hrane i pića kao i za navodnjavanje poljoprivrednih usjeva, industrijskim procesima, konzumacijom kontaminirane hrane i pušenju duhana. (WHO, 2017)
Arsen se u organizam unosi oralnim putem (ingestijom), inhalacijom, dermalnim kontaktom i parenteralnim putem u određenoj mjeri. Koncentracija As u raznim prehrambenim namirnicama kreće se od 20 do 140 μg/kg (ATSDR, 2007). Riba, školjke, meso, perad, mliječni proizvodi i žitarice također mogu biti izvori arsena, iako je izloženost sa ovim namirnicama općenito mnogo niža u usporedbi s izloženošću kroz kontaminirane podzemne vode. U morskim plodovima, arsen se uglavnom nalazi u svom manje toksičnom organskom obliku (WHO, 2017). Prehrana je za većinu pojedinaca najveći izvor izloženosti, s prosječnim unosom od oko 50 mg dnevno, ali kod pojedinaca koji su profesionalno izloženi spojevima arsena, unos arsena može biti značajno viša (ATSDR, 2007).
Izloženost arsenu utječe na gotovo sve organske sustave uključujući kardiovaskularni, respiratorni, živčani, hepatobilijarni, bubrežni, gastrointestinalni i dermatološki sustav (Tchounwou, Patlolla, i sur., 2003). Kod velikog broja ljudi koji su konzumacijom vode bili izloženi visokim koncentracijama arsena, u Bangladešu, Zapadnom Bengalu, Tajlandu, Tajvanu, Kini, Mongoliji, Meksiku, Argentini, Čileu, Finskoj i Mađarskoj, došlo je do pojave različitih kliničko-patoloških stanja, kao što su kardiovaskularne i periferne vaskularne bolesti, razvojne anomalije, neurološke i neurobihevioralne poremećaje, dijabetes, gubitak sluha, portalna fibroza, hematološki poremećaji (anemija, leukopenija i eozinofilija) i karcinomi (ATSDR, 2007). Istraživanja su također pokazala da su u mnogim područjima koja su onečišćenja arsenom značajno veći mortalitet od karcinoma mokraćnog mjehura, bubrega, jetre i kože. Isto tako povećana je incidenca kožnih oboljenja, kao što je hiperkeratoza i pigmentacijske promjene. Štetni učinci arsena na zdravlje vezani su s kemijskim oblikom, dozi i vremenu izloženosti (WHO, 2017). Toksičnost arsena za ljude povezana je s izlaganjem anorganskom arsenu. Arsenit - As(III) je 2-10 puta toksičniji od arsenata - As(V). Vezanjem na tiolne ili sulfhidrilne skupine na proteinima, As(III) može inaktivirati preko 200 enzima. Ovo je vjerojatni mehanizam kojim arsen utječe na različite organske sustave. As(V) može zamijeniti fosfat koji je uključen u mnoge biokemijske putove (Hughes, 2002). IARC je klasificirao anorganske spojeve arsena u skupinu 1 (kancerogeni za ljude) na temelju dovoljnih dokaza o karcinogenosti kod ljudi (WHO, 2017).

Kadmij

Kadmij je metal koji se koristi u industriji čelika, proizvodnji baterija, pigmenata, legura, stabilizatora, a nalazi se i u plastici. Kadmij u okoliš dospijeva u otpadnim vodama, upotrebom gnojiva i lokalnim onečišćenjem zraka (ATSDR, 2012a;WHO, 2017). Zbog kontinuirane upotrebe kadmija u industriji tijekom prošlosti, onečišćenje okoliša i izloženost ljudi kadmiju dramatično su se povećali.
Glavni putevi izlaganja organizma kadmiju su inhalacija i ingestija, a hrana je glavni izvor svakodnevnog unosa. Procjenjuje se da je dnevni oralni unos kadmija 10-35 µg. Pušenje je značajan dodatni izvor unosa kadmija. Kadmij je prisutan u određenim namirnicama kao što su lisnato povrće, krumpir, žitarice i sjemenke, iznutrice, rakovi i mekušci (Satarug i sur., 2003). Osim navedenih, namirnice bogate kadmijem mogu uvelike povećati koncentraciju kadmija u organizmu, npr. jetra, gljive, školjke, dagnje, kakao prah i sušene morske alge. Apsorpcija kadmijevih spojeva ovisi o njihovoj topljivosti. Kadmij se prvenstveno nakuplja u bubrezima i u ljudima ima dugo poluvrijeme eliminacije od 10-35 godina (WHO, 2017). Kadmij je jak iritans pluća i gastrointestinalnog trakta i može biti smrtonosan ako se udahne ili proguta. Simptomi akutne toksičnosti nakon ingestije obično se pojavljuju unutar 15-30 minuta, a manifestiraju se kao bol u trbuhu, osjećaj peckanja, mučnina, povraćanje, salivacija, grčevi u mišićima, vrtoglavica, šok, gubitak svijesti i konvulzije (Baselt i Cravey, 1995). Akutni unos kadmija, ovisno o putu unosa, također može uzrokovati eroziju gastrointestinalnog trakta, oštećenja pluća, jetre i bubrega, i komu (Baselt i Cravey, 1995). Kronična inhalacijska izloženost česticama kadmija je povezana s poremećajima funkcije pluća i smanjenjem olfaktorne funkcije (ATSDR, 2012a). Nekoliko epidemioloških studija dokumentiralo je povezanost između kronične izloženosti niskim koncentracijama kadmija sa smanjenjem mineralne gustoće kostiju i osteoporozom (Schutte et al., 2008). Postoje dokazi da je kadmij kancerogen nakon unosa inhalacijskim putem, a IARC je kadmij, i spojeve kadmija, svrstao u grupu 2A (vjerojatno kancerogen za ljude). Međutim, nema dokaza o karcinogenosti nakon oralnog unosa niti jasnih dokaza o genotoksičnosti kadmija (WHO, 2017).

Krom

Krom (Cr) se u prirodi nalazi u oksidacijskim stanjima u rasponu od +2 do +6 (WHO, 2017). Spojevi kroma su stabilni u trovalentnom obliku Cr(III) i u prirodi se javljaju u tom obliku, u rudama kao što je ferokromit, a heksavalentni oblik Cr(VI) je drugo najstabilnije stanje (Patlolla et al., 2009). Krom koji se u okoliš oslobađa antropogenim djelovanjem je, uglavnom, u heksavalentnom obliku Cr(VI). Heksavalentni krom Cr(VI) je industrijski polutant kojeg je nekoliko regulatornih i neregulatornih agencija klasificirao kao karcinogen za ljude (ATSDR, 2012b). Toksičnost kroma ovisi o njegovom oksidacijskom obliku i topljivosti spojeva, u rasponu od niske toksičnosti Cr(III), do visoke toksičnosti Cr(VI). Kod ljudi i životinja, Cr(III) je esencijalni element koji sudjeluje u metabolizmu glukoze, masti i proteina, tako što pojačava djelovanje inzulina (Goyer RA, 2001).

U općoj populaciji, glavni izvori izloženosti kromu su hrana i voda. Voda značajno doprinosi izloženosti kada je koncentracija kroma >25 µg/L. Inhalacija iz zraka također pridonosi izloženosti, što je vrlo značajno kod profesionalne izloženosti. Koncentracije kroma u većini namirnica obično se kreću od <10 do 1300 µg/kg, a najveće koncentracije nalaze se u mesu, ribi, voću i povrću. Sadržaj kroma u hrani uvelike varira i ovisi o postupcima prerade i pripreme hrane (ATSDR, 2012b).

Osim ingestije i inhalacije, može doći i do izloženosti dermalnim putem, nakon kontakta kože s proizvodima koji sadrže krom. Neki od potrošačkih proizvoda za koje se zna da sadrže krom su određeni konzervansi za drvo, cement, materijali za čišćenje, tekstil i koža štavljena kromom (WHO/IPCS, 1998). Poznato je da i Cr(III) i Cr(VI) prodiru u kožu, a Cr(VI) prodire u većem stupnju (ATSDR, 2012b). Poznato je da izloženost spojevima koji sadrže Cr(VI) uzrokuje multiorgansku toksičnost, kao što su oštećenje bubrega, alergija i astma, te karcinomi dišnog sustava kod ljudi (Goyer RA, 2001;WHO/IPCS, 1998). Udisanje visokih koncentracija Cr(VI) može uzrokovati iritaciju sluznice nosa. Glavni zdravstveni problemi uočeni kod životinja nakon ingestije Cr(VI) spojeva su iritacija, pojava čireva u želucu i tankom crijevu, anemija, oštećenje muškog reproduktivnog sustava i oštećenje spermija. Kod ljudi i životinja koji su konzumirali vodu kontaminiranu sa Cr(VI) uočena je povećana incidence tumora želuca. Ingestija iznimno visokih doza Cr(VI) spojeva je rezultirala teškim posljedicama na respiratorni, kardiovaskularni, gastrointestinalni, hematološki, jetreni, bubrežni i neurološki sustav, što može dovesti i do smrti (ATSDR, 2012b).

Apsorbirani krom se distribuira u gotovo sva tkiva, a najveće koncentracije su nađene u bubrezima i jetri. Kosti su također glavni depo i doprinose dugotrajnom zadržavanju kroma u organizmu. U organizmu Cr(VI) se reducira u Cr(III), preko međuprodukata Cr(V) i Cr(IV). Redukcija Cr(VI) u Cr(III) može dovesti do nastanka reaktivnih međuprodukata, adukata kroma s proteinima i deoksiribonukleinskom kiselinom (DNK), i sekundarnih slobodnih radikala. U krvi, Cr(VI) ulazi u eritrocite, gdje se reducira i stvara stabilne komplekse s hemoglobinom i drugim intracelularnim proteinima, i tako se zadržava u eritrocitima većim dijelom njihovog životnog vijeka. Apsorbirani krom može se prenijeti na fetus kroz placentu i na dojenčad putem majčinog mlijeka. Iz organizma krom se uglavnom izlučuje urinom, i u manjoj mjeri preko kose i noktiju (ATSDR, 2012b).

IARC je klasificirao krom (VI) u skupinu 1 (kancerogen za ljude) i krom (III) u skupinu 3 (ne može se klasificirati prema njegovoj kancerogenosti za ljude) (WHO, 2017).

ZAKLJUČAK

Za potpunu procjenu stanja okoliša i emisija teških metala potrebno je obuhvatiti ukupne podatke o emisijama u sve segmente okoliša, iz prirodnih i antropogenih izvora. Kontrola koncentracije tih elemenata u hrani nije samo važan pokazatelj kvalitete hrane, već se na taj način neizravno prati utjecaj tih elemenata na tlo, zrak i vodu. Oni su također rani pokazatelji pojave zagađenja, u kontroli kvalitete tla i kakvoće zraka. Hrana i voda su glavni izvor izloženosti ljudi teškim metalima, dok se preostalih unos ostvaruje putem udisanja onečišćenog zraka i dermalnih kontakata. Posljednjih decenija, sigurnost hrane je goruć problem za zdravlje ljudi, zbog rasta populacije i sve veće potražnje za hranom. Teški metali su povezani sa različitim utjecajima na zdravlje, ali potrebno je istražiti i kumulativne učinke istovremene izloženosti različitim metalima na ljudski organizam, i na okoliš. Iako stupanj toksičnosti teških metala za ljude ovisi o dnevnom unosu, rizik od teških metala je očit, jer čak i vrlo male količine mogu dovesti do štetnih učinaka. Više pozornosti u budućim istraživanjima treba posvetiti djeci i trudnicama zbog veće osjetljivosti te populacije na teške metale.

Nepostojanje sustavnog monitoringa kontaminacije prehrambenih namirnica sa različitim kontaminantima, ili neadekvatnog sustavnog monitoringa, uzrokuje da konzumiramo hranu upitnog kvaliteta, koja s vremenom može postati uzrok bolesti kod ljudi. Teški metali predstavljaju jedno od važnih pitanja u raspravi o sigurnosti i kvalitetu hrane, kao i eventualne mjere sanacije kontaminiranih područja.

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FOOD ANALYSIS /
ANALIZA HRANE

DIFFERENCES IN GLUTEN PROTEINS CONTENT BETWEEN SOME HISTORICAL AND MODERN WHEAT CULTIVARS (*Triticum aestivum* L.)

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ABSTRACT

It is well established that bread-making quality of wheat is mostly related to the gluten proteins composition and quantity and at the same time, some of these proteins are responsible for human health problems. The wheat gluten proteins are classified according to electrophoretic mobility into monomeric gliadins (GLI) (α-, γ- and ω-) and polymeric glutenins (GLU) (HMW-GS and LMW-GS). Six common wheat cultivars grown at the Agricultural Institute Osijek were subdivided into two groups (historical and modern ones, released before and after the late 60s) and evaluated during two consecutive years. On average, the similar protein and wet gluten content were found between historical and modern cultivars (14.3% vs. 14.2% and 27.0 % and 26.5%, respectively), while significant differences were found for gluten index (84 vs. 92) and sedimentation values (27 cm^3 vs. 36 cm^3) as good indicators of gluten strength. Considering gluten proteins a decrease in expression of α-GLI (27.1% vs. 28.8%) and γ-GLI (15.7% vs. 18.7%) as major trigger of coeliac disease was observed in modern cultivars, while ω-GLI with highly immunogenic potential (6.7% vs. 5.6%) was increased. The obtained better technological properties of modern cultivars were correlated with an increase of glutenins sub-fractions HMW-GS (10.6% vs 9.4%) and LMW-GS (22.9% vs. 19.1%) and consequently reducing the GLI/GLU ratio (1.48 vs 1.90).

Keywords: wheat, cultivars, gluten proteins, RP-HPLC

INTRODUCTION

Wheat is one of the most important cereals in the world. It is a major source of energy, protein and dietary fibre in human nutrition and animal feeding. It provides approximately one-fifth of the total calorific input of the world's population (FAO, 2009). Wheat is unique among the edible grains because wheat flour has the protein (P) complex called „gluten" that can be formed into a dough with the rheological properties required for the production of leavened bread (Uthayakumaran et al., 2002). Traditionally, proteins are classified into four different groups according to their solubility: albumins and globulins (AG) as non-gluten proteins, gliadins (GLI) (ω-, α- and γ-GLI) and glutenins (GLU) (HMW-GS and LMW-GS) as gluten proteins (Wieser and Kieffer, 2001). Although starch is the major component of wheat grains (60–75%), the proteins of the grain (9–18%) are essential for bread-making quality. AG have metabolic and structural functions with a minor role in quality, they are responsible for about 20% of total wheat protein (Shewry et al., 2002). In contrast, gluten proteins represent about 80% of the total grain proteins and they are mainly responsible for the rheological properties of the dough. The storage proteins of wheat contain about 10% HMW-GS, about 40% LMW-GS and about 50% prolamin fraction (GLI). Prolamin fraction of wheat includes ω-, α- and γ-GLI. Although GLI and GLU differ in molecular weights, they have a very similar amino acid composition. They contain a higher proportion of glutamic acid and proline, and lower proportions of the essential amino acids lysine and arginine. Most of the GLI are monomeric proteins and they form intramolecular disulphide bonds; however, GLU are polymeric complexes linked by inter-and intramolecular disulphide bonds to glutenins and gliadins (Shewry et al., 2003). More precisely as we stated earlier, gluten and related proteins from other cereals are classified as "prolamins". This name was coined by T.B. Osborne, the father of plant protein chemistry who worked at the Connecticut Agricultural experiment station from 1886 till 1928. During this period he published about 250 papers, including studies of seed proteins from 32 species. This allowed him to develop a broad classification of proteins based on their extraction in a series of solvents (Osborne, 1924). As we said, GLI and GLU are particularly rich in proline and glutamine amino acids; the high proline content renders these proteins fairly resistant to proteolytic processing by gastric and pancreatic enzymes as well as mammalian small intestinal brush-border membrane enzymes. Prolamin fraction of gluten, which is the main storage protein of wheat, is harmful for patient with coeliac disease. As a major structural component of cereals, gluten is also essential for dough formation owing to its unique viscoelastic properties (Kasarda, 1996). Wheat gluten has an immense impact on human nutrition as it largely determines the processing properties of wheat flour, and in particular the ability to make leavened breads, other baked products, pasta and noodles. However, there has been increasing interest in wheat gluten over the past two decades because of its well-established role in triggering coeliac disease, and its perceived role in other adverse reactions to wheat. Coeliac disease is generally defined as a chronic immune-mediated enteropathy driven by dietary gluten, which is present in grains including wheat, rye and barley and is one

of the most common lifelong food-related disorders worldwide (Abadie et al., 2011). Several studies have shown that peptides derived from incomplete digestion of all gluten proteins, but in particular those of the α- and γ-type of GLI are major fractions that trigger coeliac disease in susceptible individuals (Camarlengo et al., 2017; De Santis et al., 2017).

MATERIALS AND METHODS

Six common wheat cultivars grown at the Agricultural Institute Osijek during two consecutive years were subdivided into two groups depending on the year of released. The historical group included cultivars released until the late 60s (San Pastore, U-1 and Libellula), while the modern group consisted of cultivars released in the last 20 or 30 years (Kraljica, OS Olimpija and Srpanjka). Grain samples in replicate, for about 0.5 kg were collected after the harvest, polled, homogenized and used for protein analysis. In whole grain, the crude protein content on dry matter basis (P) (N x 5.7, DM) was measured using an Infratec 1241 Grain Analyzer (Infratec 1241, Foss Tecator, Hillerød, Denmark). Wet gluten content (WG) and gluten indeks (GI) of approximately 70% extraction flour (Quadrumat Senior mill, Duisburg, Brabender, Germany) were analysed using a Glutomatic 2200 Gluten System (Perten Instruments, Hagersten, Sweden) and Glutomatic Centrifuge (Perten, 2015), according to ICC standard No. 155. WG in wheat flour is a plastic-elastic substance consisting of the proteins gliadin and glutenin, obtained after washing out the starch from wheat flour dough. Gluten separated from wheat flour is centrifuged to force wet gluten through a specially constructed sieve under standardized conditions. The percentage of WG remaining on the sieve after centrifugation is defined as the Gluten Index (GI). Zeleny sedimentation (SED) test was carried out in accordance with the requirements of the ICC Standard Method 116/1, and SD is determined. Prior to the reversed phase-high-pressure liquid chromatography (RP-HPLC) analysis, the single protein fractions were sequentially extracted according to Wieser et al. (1998) with some modifications. Briefly, 50 mg of wholemeal flour (Retsch Centrifugal Mill ZM1, Haan, Germany, equipped with 1 mm sieve) were extracted by 1 mL of 0.4 M NaCl for 30 min at RT AG extract. From the remaining pellet, the GLI were extracted with 1 mL of 50% 1-PrOH for 60 min at RT and finally, the GLU were extracted with 1 mL of a solution containing 50% (v/v) 1-PrOH, 2 M urea, 1% (w/v) dithioerythritol and 0.05 M Tris-HCl (pH 7.5) for 60 min at 60 °C. During all extractions, the suspensions were vortexed for 1 min every 10 min and then centrifuged for 15 min at 14,000 rpm and RT. The obtained supernatants were stored at -20 °C and filtered through a 0.45 µm PVDF syringe filter prior to HPLC analysis. Two extractions were performed for each sample. Gluten proteins were separated according to the method of Wieser et al. (1998) using RP-HPLC (Perkin Elmer Instruments, Walthman, MA, USA) coupled with C18 column, photodiode array detector and Total-Chrom software. Elution was performed with linear gradient of acetonitrile (ACN/0.1%TFA) in water (H_2O/0.1%TFA) from 24-54% in 30 min at flow rate of 1mL min^{-1} and column temperature of 50 °C. Proteins were separated on a C18 reverse phase column (5 µm

4.6 x 150 mm; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Quantification of protein fractions was based on measuring their peak area at 210 nm. The peak areas under AG, GLI and GLU chromatograms were used for further calculations of their relative proportion (%) in total extracted proteins.

RESULTS AND DISCUSSION

Rheological properties are important parameters in determining the functional properties of wheat flour. Values of P, WG, GI and SED value, as indirect quality parameters, in our research are in accordance with Horvat et al. (2012). GI is a good indicator of gluten strength and it can give more information about the quality of wheat flour than just determining wet gluten (Magdić et al., 2006). On average, the very similar P and WG content were found between historical and modern cultivars (14.3% vs. 14.2% and 27.0% vs. 26.5%, respectively). Moreover, GI as good indicators of gluten strength was not statistically significant, but tends to increase in modern cultivars in comparison to traditional (92 vs. 84, respectively). SED values, also as good indicators of gluten quality, were significantly higher in modern cultivars in contrast to traditional (27 cm^3 vs. 36 cm^3, respectively) (Table 1). Gluten forming proteins are primary responsible for the functional properties of wheat flour (Jurković et al., 1996). The unique viscoelastic and cohesive properties of the dough are most influenced by gluten, and it is accepted that the GLIs define the viscosity and extensibility of the dough, while the GLUs are responsible for the dough firmness and elasticity (Autran et al., 2001). The elasticity of the dough depends on the unique ability of GLU to form a large number of intramolecular hydrogen bonds stabilized by interchain disulphide (S-S) bonds (Kolster et al., 1993). A significant increase of total GLU and its sub-fractions HMW-GS (10.6% vs. 9.4%, respectively) and LMW-GS (22.9% vs. 19.1%, respectively) was noticed in modern cultivars compared to historical (Fig 1.), as well as a reduced GLI/GLU ratio (1.48 vs. 1.90, respectively) (Fig 2.). Considering GLI proteins, a decrease in expression of α-GLI (27.1% vs. 28.8%, respectively) and γ-GLI (15.7% vs. 18.7%, respectively) as major trigger of coeliac disease was observed in modern cultivars, while ω-GLI with highly immunogenic potential (6.7% vs. 5.6%, respectively) was increased. Our results of α-GLI are in part correlated with results of De Santis et al. (2017) who considering old and modern Italian durum wheat cultivars showed similar α+γ-GLI content (61% vs. 58%, respectively), while in the modern ones a marked decrease in ω-GLI was observed.

Table 1. Average values of proteins, wet gluten, gluten index and sedimentation volume

CULTIVARS	P	WG	GI	SED
	%	%		cm^3
HISTORICAL	14.3	27	84	27
MODERN	14.2^{ns}	26.5^{ns}	92^{ns}	36^{**}

*P = grain protein content; WG = wet gluten content; GI = gluten index; SED = Zeleny sedimentation volume

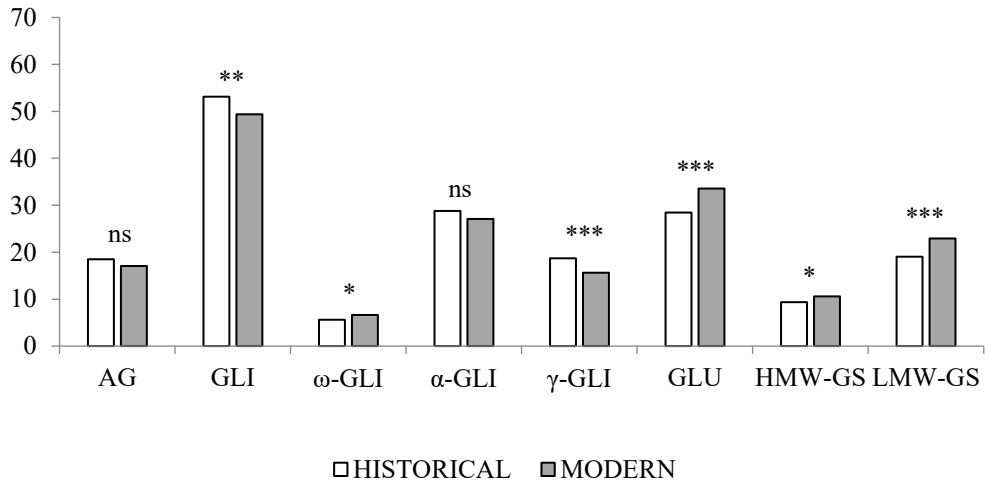

Figure 1. Average values of AG, GLI, ω-GLI, α-GLI, γ-GLI, GLU, HMW-GS, LMW-GS in historical and modern wheat varieties; *, **, *** represent significant differences between historical and modern wheat varieties at 0.05, 0.01 and 0.001 respectively; according to t-test

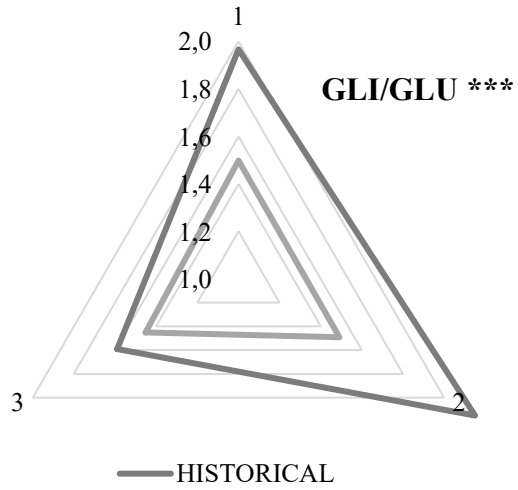

Figure 2. GLI/GLU ratio in historical and modern wheat

CONCLUSION

The better technological properties of modern cultivars were correlated with an increase of total GLU, HMW-GS and LMW-GS, while observing modern ones from a health aspect, trend of decrease of α-GLI and γ- GLI content as the main triggers of coeliac diseases was noticed.

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UTJECAJ RAZLIČITIH OMJERA I VRSTA (*Coffea arabica* i *Coffea robusta*) NA SENZORSKU OCJENU I PRIHVATLJIVOST NAPITKA OD KAVE

EFFECTS OF DIFFERENT TYPE AND RATIO (*Coffea arabica* and *Coffea robusta*) TO SENSORY ASSESSMENT AND ACCEPTANCE OF COFFEE BEVERAGE

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SAŽETAK

Kava spada u najpopularnije napitke širom svijeta. Na prostoru Bosne i Hercegovine postoji duga tradicija konzumiranja napitka crne kave. Kava, kao i svaka namirnica, vrjednuje se osnovnim senzorskim svojstvima (boja, miris, okus, konzistencija). Precizan opis arome, okusa i boje napitka crne kave, kao najvažnijih senzorskih svojstava, moguće je dati primjenom deskriptivne senzorske analize. Cilj ovog rada bio je ispitati senzorsku kvalitetu i prihvatljivost mješavina napitaka crne kave pripremljenih na tradicionalan način. Za pripremanje napitka crne kave koristile su se dvije vrste kave, Arabika (*Coffea arabica*, sorte *Rio Minas* i *Santos*) i Robusta (*Coffea robusta*, sinonim *Coffea canephora*, sorte *Sheery* i *Camerun*) u različitom omjeru. Sudionici u senzorskom ocjenjivanju uzoraka kave bili su upućeni ocjenjivači i potrošači. Rezultati senzorskih svojstava ispitivanih uzoraka napitaka kave uglavnom su bili pod utjecajem vrste i omjera korištene kave. Miješanje kave Arabike i Robuste značajno je poboljšalo senzorska svojstva proizvoda, naročito mirisa i okusa. Rezultati instrumentalnih mjerenja boje pokazuju da su ispitivani uzorci kave tamno i srednje prženi. Test prihvatljivosti je pokazao dobru prihvatljivost uzoraka različitih napitaka crne kave od strane potencijalnih potrošača.

Ključne riječi: kava, *Coffea arabica*, *Coffea robusta*, senzorska analiza, boja

Keywords: coffee, *Coffea arabica*, *Coffea robusta*, sensory analysis, color

UVOD

Kava kao jedan od najpopularnijih napitaka odrasle populacije pruža iznimno zadovoljstvo potrošačima zbog specifične arome, mirisa i okusa (Džinić i sur., 2013; Odžaković i sur., 2015). Razlog zbog kojeg je ovaj napitak popularan je svakako i njegovo pozitivno djelovanje na centralni živčani sustav. Utvrđeno je da kava pored stimulativnog djelovanja pokazuje i antioksidativna svojstva koja inaktiviraju štetna djelovanja virusnih infekcija (Dias i sur., 2015). Među različitim senzorskim modalitetima, aroma (miris) je najznačajnija za kvalitetu kave. Kako bi osjetili aromu kave, potrebno je da oslobođeni hlapljivi organski spojevi iz kave dospiju do olfaktornog epitela smještenog u gornjem dijelu nosne šupljine, gdje se nalaze živčani završetci koji omogućavaju osjet mirisa. Do sad je otkriveno oko 1000 hlapljivih spojeva u sirovoj i prženoj kavi, međutim samo 5 % njih je odgovorno za aromu kave. Sredinom devedesetih godina ustanovljeno je da je manje od 30 hlapljivih organskih spojeva važno za aromu pržene kave. Naknadnim eksperimentima utvrđeno je da bi stvarni broj nezamjenjivih spojeva arome kave mogao biti najmanje devet. Među spojevima od značaja za aromu kave zastupljene su organske kiseline, spojevi koji sadrže sumpor, terpeni, polifenoli, pirazini, piridini i dr. (Yeretzian i sur., 2019). Na senzorsku kvalitetu napitka kave značajan utjecaj imaju i nehlapljive komponente zrna kave koje uključuju kiseline, alkaloide, ugljikohidrate, lipide, proteine, minerale, a u prženom zrnu i melanoidine (Buffo i Cardelli-Freire, 2004). Napitak od kave je bogat izvor bioaktivnih komponenata posebno polifenola, odnosno fenolnih kiselina, od kojih je najzastupljenija klorogenska kiselina (u sirovom zrnu) i kava kiselina (nastaje procesom prženja). Fenolne kiseline doprinose unosu ukupnih polifenola hranom i povoljno djeluju na zdravlje ljudi. U prehrani ljudi kava je glavni izvor klorogenske kiseline za koju je poznato da pokazuje antioksidacijska svojstva (Krol i sur., 2020). Klorogenska kiselina značajno doprinosi konačnoj kiselosti i gorčini kave. Također, sudjeluje u nastanku boje, okusa i arome kave tijekom prženja (Faraz i sur., 2010). *Coffea arabica* (Brazil) i *Coffea robusta* (Indija) su kave koje se u svijetu najviše koriste, a ima ih više od 25 vrsta. Kava koja se svakodnevno konzumira ne sastoji se samo od jedne vrste kave, nego se miješaju najčešće dvije vrste kave (Martin i sur.,1999; Parras i sur., 2007). Miješanje različitih vrsta kave se vrši kako bi se postigao bolji okus, aroma i miris napitka kave. Razliku u kvaliteti kave čini i sam način pripreme napitka, postupak prerade te stupanj prženja samog zrna kave, što diktira i cijenu proizvoda (Oliveira i sur., 2005). Tradicija, kultura društva, ali i navike potrošača u različitim dijelovima svijeta rezultat su postojanja velikog broja različitih vrsta napitaka kave, kao i sam način njihove pripreme i konzumiranja (Odžaković, 2015). Senzorska analiza napitka crne kave pripremljenog od prženog, mljevenog zrna kave jedan je od najvažnijih kriterija za ocjenu kvalitete kave (Sunarharum i sur., 2014; Ribeiro i sur., 2010). Za identifikaciju i objektivnu ocjenu odabranih senzorskih svojstva napitka crne kave, koja utječu na kvalitetu proizvoda, primjenjuje se deskriptivna senzorska analiza. Senzorska svojstva kave se najbolje mogu odrediti uz pomoć senzorskih analitičara (Odžaković, 2015). U ovom radu su skupine ocjenjivača za senzorsku analizu podijeljeni na upućene ocjenjivače i potrošače.

Upućeni ocjenjivači su prošli određenu obuku i trening kako bi prepoznali različite nijanse okusa, dok potrošači na osnovu dopadljivosti napitka daju ocjene za uzorak. Uzorci pripremljenih napitaka kave trebali bi da imati specifičnu, konstantnu i ugodnu aromu te odgovarajući odnos kiselosti i gorčine koji su karakteristični za ovu vrstu napitka. Cilj ovog rada bio je ispitivanje utjecaja različitih omjera dvije vrste kave, Arabika (sorte *Rio Minas* i *Santos*) i Robusta (sorte *Sheery* i *Camerun*) na senzorsku kvalitetu i prihvatljivost pripremljenih napitaka crne kave.

MATERIJALI I METODE

U istraživanju rada korištene su dvije vrste kave: *Coffea arabica* (sorte *Rio Minas* i *Santos*) i *Coffea robusta* (sorte *Sheery* i *Camerun*), a koje su dobivene od lokalnog prerađivača kave. Detaljniji pregled korištenih vrsta kave prikazan je u Tablici 1.

Tablica 1. Uzorci pojedinačnih vrsta sirove kave, šifre uzoraka, trgovački naziv, porijeklo
Table 1. *Samples of individual types of raw coffee, sample codes, trade name, origin*

Naziv i vrsta sirove kave	Oznaka uzorka	Trgovački naziv	Proizvođač
ARABIKA	CA1	Rio Minas	Brazil
	CA2	Santos	
ROBUSTA	CR3	Sheery	Indija
	CR4	Camerun	

*CA - *Coffea Arabica*; CR - *Coffea Robusta*

U Tablici 2. prikazane su korištene mješavine različitih omjera dvije vrste kave (Arabika i Robusta) za pripremu napitaka crne kave.

Tablica 2. Uzorci različitih mješavina prženih uzoraka mljevene kave (Arabica i Robusta)
Table 2. *Samples of different mixtures of roasted samples of ground coffee (Arabica and Robusta)*

Oznaka uzorka	Mješavine mljevenih uzoraka kave	Oznaka uzorka	Mješavine mljevenih uzoraka kave
C1	CA1:CA2:CR3 = 40%:40%:20%	**C4**	CR4:CR3:CA2 = 40%:40%:20%
C2	CA1:CA2:CR3 = 35%:50%:15%	**C5**	CR4:CR3:CA2 = 35%:50%:15%
C3	CA1:CA2:CR3 = 30%:45%:25%	**C6**	CR4:CR3:CA2 = 30%:45%:25%

Priprema napitka kave

Napitak kave za senzornu analizu je pripremljen prema ISO 6668:2008 (E) standardu. Prema ovom standardu, napitak od kave se dobiva otapanjem 7 g mljevene kave u 100 mL kipuće vode. Kava je pripremljena klasičnim načinom kuhanja kave. Dakle, uliveno je 0,5 L hladne vode u džezvu i grijano do vrenja. Nakon što je voda prokuhala, džezva se sklonila s grijaće ploče i u nju je dodano 35 g mješavine mljevene kave. Napitak se dobro promiješa kako bi se stvorila pjena i lagano dogrijava na grijaćoj ploči do ponovnog razvitka pjene. Prije posluživanja, preporuka je da napitak stoji od 3 do 5 minuta kako bi talog potonuo na dno džezve. Napitci kave su servirani u bijelim čašama kako bi se što relevantnije odredila boja i miris uzorka. Uzorci mješavine pržene mljevene kave su posluženi u bijelim tanjurima kako bi se odredila tekstura i miris kave. Kako se ne bi miješali okusi uzoraka kave te kako bi se dobili što objektivniji rezultati, potrošači i upućeni ocjenjivači su između svakog uzorka konzumirali vodu, kifle i slane štapiće. Ocjenjivanje kave je provođeno u dvije sekcije gdje je servirano po tri uzorka kave koja se kuhala neposredno prije konzumacije. Kvalitetu i prihvatljivost kave u različitim omjerima su ocjenjivali upućeni ocjenjivači (n=7) i potrošači (n=12) u prostorijama laboratorija Biotehničkog fakulteta u Bihaću. Ispitivanja su obuhvaćala deskriptivnu senzorsku analizu, metodu rangiranja i metodu bodovanja pripremljenih napitaka kave po standardnom postupku. Instrumentalno određivanje boje sirove i pržene kave određeno je kolorimetrijskom metodom.

Parametri za senzorsko ocjenjivanje kave

Senzorska svojstva pripremljenih napitaka crne kave (izgled, miris, okus i aroma) ocijenili su upućeni ocjenjivači i potrošači. Svako svojstvo je ocijenjeno ocjenom od 1 do 5, koja je zatim pomnožena s faktorom značajnosti da bi se dobili ponderirani bodovi. Na osnovu ukupnog broja ponderiranih bodova (maksimalno 20 bodova) uzorci napitaka crne kave su razvrstani u razrede. Rezultati ocjena senzorskih svojstava uzoraka su navedeni kao prosječna vrijednost svojstava svih ocjenjivača.

Deskriptivna senzorska analiza napitka crne kave

Primjenom deskriptivne senzorske analize provedeno je ispitivanje boje, arome, okusa, punoće okusa i općeg dojma u ustima uzoraka napitka crne kave (ISO 13299:2003 (E)).

Metoda rangiranja – ocjena prihvatljivosti

Sveukupan dojam o prihvatljivosti kvalitete napitaka različitih uzoraka crne kave provedena je diskriminatornom senzorskom analizom, metodom rangiranja (ISO 8587:2006).

Instrumentalno određivanje boje

Boja kave mljevenih uzorka sirove kave i mješavina uzoraka pržene mljevene kave određena je upotrebom kromametra. Kromametar je opremljen standardnim izvorom svjetlosti D65 (2º kut standardnog promatrača). Karakteristike boje se iskazuju u CIE L*a*b* sistemu prema kojem se boja definira preko psihometrijske svjetlosti (L*) ili svjetloće boje, psihometrijskog tona (a*) (udjela crvene i zelene boje) i psihometrijske krome (b*) (udjela žute i plave boje).

Statistička obrada podataka

Rezultati analiziranih uzoraka dobivenih mjerenjem senzorskih karakteristika različitih mješavina napitaka crne kave kao i instrumentalnim mjerenjem boje kave prikazani su kao srednje vrijednosti ponavljanja ± standardna devijacija. Analizom varijance (ANOVA) te Duncanovim testom utvrđene su statistički značajne razlike u boji kave pripremljenih napitaka crne kave, različitih omjera.

REZULTATI I RASPRAVA

Rezultati deskriptivne senzorske analize napitka kave

Deskriptivna senzorska analiza se koristi za ispitivanje jednog ili više uzoraka prehrambenih proizvoda, za kvalitativno i kvantitativno određivanje njihovih svojstava. Za vrijeme izvođenja deskriptivne analize, korištenjem odgovarajućih atributa izrađuje se detaljan opis senzorskih svojstava proizvoda (Murray i sur., 2001; Grujić i Spaho, 2010). Dobiveni rezultati su podijeljeni na pokazatelje kvalitete i pokazatelje intenziteta. Kombinacijom tablice s uputama za obavljanje deskriptivne senzorske analize i ocjenjivačkog listića dobiveni su rezultati (pokazatelji kvalitete i intenziteta) napitka od kave od strane potrošača i upućenih ocjenjivača.
Primjenom deskriptivne senzorske analize moguće je dati precizan opis boje, arome, okusa, (punoće okusa i općeg dojma u ustima) napitka kave, kao najvažnijih senzorskih svojstava, sa značajnim utjecajem na kvalitetu i na prihvatljivost proizvoda. Ispitivanjem i ocjenjivanjem odabranih senzorskih svojstava proizvoda dobiva se precizna slika o njegovoj realnoj kvaliteti koji se može usporediti s očekivanom ili standardnom kvalitetom i na taj način donijeti ocjena o prihvatljivosti ukupne kvalitete prehrambenih proizvoda (Grujić, 2015).

Tablica 3. Rezultati deskriptivne senzorske analize napitaka kave treniranih ocjenjivača
Table 3. *Results of descriptive sensory analysis of coffee beverages by trained evaluators*

Pokazatelji kvaliteta Oznake i odabrana senzorska svojstva		**Oznaka uzoraka i ocjena kvalitete senzornog svojstva za n=7 (senzoričari)**					
		C1	**C2**	**C3**	**C4**	**C5**	**C6**
A1	Aroma kave	4,6±0,55	4,33±0,58	5,00±0,00	4,00±1,00	5,00±0,00	5,00±0,00
A2	Aroma na prženo	3,5±0,71	3,25±0,50	-	4,00±0,00	-	5,00±0,00
A3	Aroma čokolade	-	-	-	-	5,00±0,00	-
A4	Aroma karamela	-	-	-	-	-	-
A5	Cvjetna aroma	-	-	-	-	-	-
A6	Sladunjava voćna aroma	-	-	-	-	-	3,00±0,00
A7	Aroma žitarica i slada	-	-	3,00±0,00	-	-	2,00±0,00
A8	Aroma oraha, (gorkog) badema	-	-	-	-	-	-
A9	Aroma trave	-	-	-	-	-	-
A10	Aroma zemlje	-	-	-	-	-	-
A11	Aroma prašine	-	-	-	-	-	-
A12	Aroma na zagoreno	-	-	-	-	2,52±0,71	-
A13	Aroma dima	-	-	-	-	-	-
A14	Aroma lijekova	-	-	-	-	-	-
A15	Aroma na plijesni	-	-	-	-	-	-
GORČINA		3,71±0,75	3,71±0,76	4,43±0,79	3,43±0,97	4,14±1,21	4,14±1,07
KISELOST		3,71±0,75	4,00±0,82	4,57±0,79	3,43±0,79	4,57±0,79	4,28±0,75
PUNOĆA OKUSA		3,57±0,79	3,71±1,11	4,43±0,97	3,71±0,49	4,43±0,97	4,14±0,90
OPĆI DOJAM U USTIMA		3,57±0,78	3,57±0,97	4,42±0,97	3,85±0,69	4,28±0,95	4,02±0,92

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja).

U Tablici 3. je dat prikaz rezultata deskriptivne senzorske analize upućenih ocjenjivača. Na osnovu dobivenih rezultata svi uzorci napitka kave se mogu opisati kao uzorci s izraženom aromom kave te arome na prženo. Značajnijih odstupanja prilikom ocjenjivanja arome kave je bilo kod C5 uzorka kave, gdje su upućeni ocjenjivači naveli prisustvo arome na zagoreno. Prisustvo ove arome u uzorku C5 nije narušilo krajnju ocjenu dodijeljenu na osnovu postotka od maksimalne moguće kvalitete gdje ovaj uzorak napitka kave ima visoku ocjenu. Gorčina i kiselost kave su ocijenjene približno istom ocjenom kod svih uzoraka napitka kave, nedovoljno izražene, ali prisutne kod svih uzoraka. Punoća okusa je najmanje izražena kod uzorka kave C1, a najviše kod uzoraka C3 i C5 što opravdava to da su ovi uzorci ocijenjeni kao najprihvatljiviji. Opći dojam u ustima je najbolje ocijenjen kod uzoraka C3 i C5 i to kao blag, skladan i harmoničan dojam.

Tablica 4. Postotak od maksimalne moguće kvalitete deskriptivne senzorske analize napitka kave za upućene ocjenjivače
Table 4*. Percentage of maximum possible quality of descriptive sensory analysis of coffee beverage for referred evaluators*

Odabrana senzorska svojstva (pokazatelji kvalitete)	**Oznaka uzoraka i ukupna ocjena kvalitete senzorskog svojstva korigirane koeficijentom važnosti (n=7)**					
	C1	C2	C3	C4	C5	C6
Aroma	32,4	30,32	32	32	33,3	30
Okus	22,6	23,13	27	20,58	26,13	25,26
Punoća okusa i opći dojam u ustima	21,42	21,42	26,5	22,68	26,13	24,42
% od maksimalne moguće kvalitete	76,42	74,87	85,5	75,26	85,56	79,68

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja).

U Tablici 4. prikazan je postotak od maksimalne moguće kvalitete za odabrana senzorna svojstva u uzorcima napitaka kave. Rezultati deskriptivne senzorske analize napitka kave kod upućenih ocjenjivača, pokazuju da su najbolje ocijenjeni uzorci C3 i C5. Uzorak C6 je rangiran na trećem mjestu, a uzorci C1, C4 i C2 na četvrtom, petom i šestom mjestu.

Tablica 5. Rezultati deskriptivne senzorske analize napitaka kave ocijenjeni od strane potrošača
Table 5. *Results of descriptive sensory analysis of coffee beverages evaluated by consumers*

Pokazatelji kvalitete Oznake i odabrana senzorska svojstva		**Šifre uzoraka i ocjena kvalitete senzornog svojstva za n=12 (potrošači)**					
		C1	**C2**	**C3**	**C4**	**C5**	**C6**
A1	Aroma kave	4,66±0,52	3,88±1,05	3,75±1,28	3,6±0,55	4,46±0,55	3,75±0,5
A2	Aroma na prženo	3,60±0,55	4,00±0,00	3,33±0,58	3,25±0,50	4,00±0,00	3,55±0,84
A3	Aroma čokolade	-	-	-	-	-	-
A4	Aroma karamela	-	-	-	-	3,00±0,00	-
A5	Cvjetna aroma	3,00±0,00	-	3,00±0,00	-	-	-
A6	Sladunjava voćna	-	2,52±0,71	-	2,52±0,71	2,00±0,00	2,00±1,41
A7	Aroma žitarica i slada	-	-	-	-	-	-
A8	Aroma oraha, (gorkog) badema	-	-	-	-	-	-
A9	Aroma trave	-	-	-	-	-	-
A10	Aroma zemlje	-	4,00±0,00	-	4,00±0,00	-	-
A11	Aroma prašine	-	-	-	-	-	-
A12	Aroma na zagoreno	5,00±0,00	-	4,00±0,00	-	-	-
A13	Aroma dima	-	-	-	-	-	-
A14	Aroma lijekova	-	-	-	-	-	-
A15	Aroma na plijesni	-	-	-	-	-	-
GORČINA		3,50±1,00	3,75±0,96	3,33±1,07	3,51±0,90	3,00±1,15	3,25±1,29
KISELOST		3,25±1,05	3,08±0,79	3,25±0,75	3,42±0,99	3,33±1,07	3,17±0,83
PUNOĆA OKUSA		3,66±0,89	3,51±1,09	3,09±0,94	3,42±0,99	3,33±0,98	3,33±1,07
OPĆI DOJAM U USTIMA		3,58±0,99	3,50±1,0	3,25±0,96	3,51±0,90	3,25±1,14	3,33±1,07

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja).

Na osnovu rezultata potrošača ustanovljeno je prisustvo arome na kavu i arome na prženo kod svih uzoraka napitaka crne kave (Tablica 5). Cvjetna aroma je prisutna kod uzoraka C1 i C3, dok je sladunjava voćna aroma prisutna u C2, C4, C5, C6 uzorcima kave. Promatrajući rezultate koji su dobiveni za gorčinu i kiselost uočava se da su potrošači za ova dva svojstva utvrdili nešto manju razliku između uzoraka. Uzorak C5 ima umjerenu, ugodnu punoću okusa, dok uzorak C3 ima tešku i neugodnu punoću okusa. Opći dojam u ustima je ocijenjen kao blag, skladan i harmoničan u gotovo svim uzorcima napitaka kave.

Tablica 6. Postotak od maksimalne moguće kvalitete deskriptivne senzorske analize napitka kave za potrošače
Table 6. *Percentage of the maximum possible quality of descriptive sensory analysis of coffee beverage for consumers*

Odabrana senzorska svojstva (pokazatelji kvalitete)	**Šifre uzoraka i ukupna ocjena kvalitete senzorskog svojstva korigirana koeficijentom važnosti (n=12)**					
	C1	**C2**	**C3**	**C3**	**C5**	**C6**
Aroma	32,52	28,76	28,16	26,7	26,8	24,67
Okus	20,25	20,49	19,74	20,76	18,99	19,26
Punoća okusa i opći dojam u ustima	21,72	21	19,02	20,76	19,74	19,98
% od maksimalne moguće kvalitete	74,49	70,25	66,92	68,22	65,53	63,91

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja).

U Tablici 6. prikazani su % od maksimalne moguće kvalitete deskriptivne senzorske analize napitka kave od strane potrošača. Prema konačnim rezultatima deskriptivne senzorske analize uočava se da je najbolje ocijenjen C1 uzorak (74,49 % od maksimalne moguće kvalitete). Uzorak C1 je dobio najveće ocjene od strane potrošača za sve pokazatelje kvalitete, dok su ostali uzorci ocijenjeni sa nižim ocjenama približnih vrijednosti. Prema % od maksimalne moguće kvalitete potrošači su napitke kave rangirali prema sljedećem redoslijedu: prvorangirani je uzorak C1 koji se sastoji od Rio Minas, Santos i Sheery vrste kave. Drugoplasirani je uzorak C2 u kojem je postotno najviše zastupljena Arabika vrsta kave. Na trećem i četvrtom mjestu je C4 i C3 uzorak, a na petom i šestom mjestu su uzorci C5 i C6.

Rezultati senzorske ocjene napitka kave –metoda bodovanja

Prilikom provođenja senzorske analize, prvo se ocjenjuje vanjski izgled proizvoda, zatim se pristupa ocjenjivanju mirisa i arome odnosno prepoznatljive mirisne note, a potom tekstura i konzistencija. Na kraju provođenja senzorske analize ocjenjuje se okus proizvoda preko kemijskih receptora smještenih u ustima (Radovanović i

Popov – Raljić, 2001). Vrijednosti senzorskih svojstava su date na osnovu srednje vrijednosti ocjena svih ocjenjivača. Ponderirani bodovi se dobiju na način da se srednja dobivena vrijednost pomnoži s faktorom zančajnosti, a služe kako bi odredili kategoriju kvalitete proizvoda. U Tablici 7. i 8. prikazane su ocjene kao srednje vrijednosti date od strane upućenih ocjenjivača i potrošača za mješavine mljevene pržene kave.

Tablica 7. Ocjena senzorskih svojstava mješavine kava od strane upućenih ocjenjivača
Table 7. *Evaluation of the sensory properties of the coffee mixture by referred assessors*

SENZORSKO SVOJSTVO	**A- srednja vrijednost ocjena (n=7) B- ponderirana vrijednost ocjena (n=7)**											
	C1		C2		C3		C4		C5		C6	
	A	B	A	B	A	B	A	B	A	B	A	B
Izgled mljevene kave	4,85	1,94	4,78	1,91	4,85	1,94	4,64	1,86	4,78	1,91	4,64	1,86
Izgled napitka	4,61	1,84	4,71	1,86	4,83	1,93	4,32	1,73	4,38	1,74	4,71	1,88
Miris napitka	4,7	5,64	4,15	4,99	4,3	5,16	4,33	5,19	4,56	5,45	4,14	4,97
Okus i aroma napitka	4,22	8,46	4,07	8,14	4,66	9,31	4,74	9,48	4,4	8,8	4,17	8,34
Ukupna ponderirana ocjena	17,88		16,93		18,34		18,25		17,93		17,04	
Opisna ocjena	ODLIČAN		VRLO DOBAR		ODLIČAN		ODLIČAN		ODLIČAN		VRLO DOBAR	

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja).

Izgled mljevene kave treba biti prikladan, svojstvene boje s potpuno ujednačenim mljevenjem. Promatrajući rezultate date od strane upućenih ocjenjivača primjećuje se da svaka mješavina kave zadovoljava kriterije svojstvene ovom proizvodu. Nadalje, izgled napitka kave zavisi od boje, zatim količine, strukture i gustoće pjene. Boja kave nam daje dojam o izgledu i prihvatljivosti napitka. Tekućina napitka kave treba biti tamno smeđe boje, a pjena crvenkasto smeđe do smeđe boje, obilna i dobre gustoće, strukture i trajnosti. Upućeni ocjenjivači su izgled napitaka kave ocijenili sa ocjenama svojstvenim navedenim odlikama, jedino su se uzorci kave C4 i C5 razlikovali od ostalih, jer su sadržavali manje pjene i time ocijenjeni nešto nižom ocjenom.
Miris, odnosno aroma napitka kave je jedna od najcjenjenijih karakteristika kave. Miris kave se doživljava kao olfaktorni dojam izazvan nadražajem receptora slobodnim hlapljivim mirisnim supstancama koje se oslobađaju iz pripremljenog toplog napitka kave (Ribeiro i sur., 2010). Uzorci C2, C3, C4, C5 i C6 su ocijenjeni

kao napitci kave s mirisom slabe jakosti, dok je C1 dobio ocjenu sa značajkama dobre kave. Okus i aroma napitka zajedno stvaraju osjet u ustima, odnosno punoću okusa. Punoća okusa može imati dobre značajke, bez primjedbi, zatim može biti slabe punoće okusa pa gotovo bez arome, bezukusan napitak kave. Ocjene koje su upućeni ocjenjivači dodijelili na osnovi okusa i arome napitka ukazuju da su napitci kave osrednje punoće okusa, sa slabo izraženom aromom. Uzorci mješavina kava C3 i C4 imaju dobre značajke napitka kave sa dobrom punoćom okusa i arome.
Izgled mješavine mljevene kave za potrošače je svojstvene boje s potpuno ujednačenim mljevenjem. Izgled napitka kod uzoraka C1, C2, C3, C4, C5 potrošači su ocijenili kao napitak kave s malom količinom nestabilne pjene tamno smeđe boje. Uzorak C6 je dobio veću ocjenu izgleda napitka tamno smeđe boje sa obilnom pjenom dobre strukture i smeđe boje. Potrošači su miris napitka ocijenili sukladno s ocjenama upućenih ocjenjivača, dakle svi uzorci imaju miris slabe jakosti kave. Uzorci C1, C2 i C5 su bolje kvalitete s osrednjom punoćom okusa i slabo izraženom aromom. Okus i aroma napitka u uzorcima C3, C4 i C6 je ocijenjen sa ocjenom niže kvalitete sa značajkama slabe punoće okusa.

Tablica 8. Ocjena senzorskih svojstava mješavine kava od strane potrošača
Table 8*. Evaluation of sensory properties of coffee mixture by consumers*

SENZORSKO SVOJSTVO	**A- srednja vrijednost ocjena (n=12)** **B- ponderirana vrijednost ocjena (n=12)**											
	C1		C2		C3		C4		C5		C6	
	A	B	A	B	A	B	A	B	A	B	A	B
Izgled mljevene kave	4,34	1,74	4,33	1,73	3,79	1,52	4,39	1,76	4,66	1,86	4,57	1,83
Izgled napitka	3,20	1,28	3,28	1,31	3,76	1,51	3,99	1,59	3,97	1,59	4,85	1,94
Miris napitka	3,52	4,23	4,06	4,88	4,03	4,84	3,93	4,72	3,86	4,64	4,45	5,34
Okus i aroma napitka	3,7	7,4	3,73	7,46	3,46	6,93	3,36	6,73	3,70	7,41	3,44	6,88
Ukupna ponderirana ocjena	14,65		15,39		14,80		14,80		15,51		15,99	
Opisna ocjena	DOBAR		VRLO DOBAR		DOBAR		DOBAR		VRLO DOBAR		VRLO DOBAR	

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja).

Rezultati rangiranja na osnovu ocjene prihvatljivosti ukupnog kvaliteta

Metodom rangiranja su obrađene dobivene ocjene o prihvatljivosti ukupne kvalitete napitka kave. Sume rangova imaju vrijednosti za svaki analizirani uzorak. Ukoliko su vrijednosti sume rangova veće to znači da je taj uzorak napitka kave ocijenjen nižom ocjenom, odnosno, najmanje je ugodan. Uzorci napitaka kave koji imaju

manju vrijednost sume rangova imaju višu razinu kvalitete i ocijenjeni su kao najugodniji.

Tablica 9. Rangovi dodijeljeni na osnovu ocjene prihvatljivosti ukupne kvalitete ispitivanih uzoraka napitaka kave od strane upućenih ocjenjivača
Table 9. *Ranks assigned on the basis of the assessment of the acceptability of the overall quality of the examined samples of coffee beverages by the referred assessors*

Uzorci kave	Broj ponavljanja			Suma rangova
	1. mjesto	2. mjesto	3. mjesto	
C1	-	1	6	20
C2	1	5	1	14
C3	6	1	-	8
C4	3	-	4	15
C5	3	4	-	11
C6	1	3	3	16

Promatrajući rezultate za rangiranje uzoraka napitka kave ukupne kvalitete i postotak od maksimalne moguće kvalitete (Tablica 9.) dolazi se do zaključka da su upućenim ocjenjivačima najprihvatljiviji C3 i C5 uzorak. Najmanje prihvatljiv uzorak ukupne kvalitete napitka kave od strane upućenih ocjenjivača je uzorak C1.

Tablica 10. Rangovi dodijeljeni na osnovu ocjene prihvatljivosti ukupne kvalitete ispitivanih uzoraka napitaka kave od strane potrošača
Table 10*. Ranks assigned based on the assessment of the acceptability of the overall quality of the tested coffee beverage samples by consumers*

Uzorci kave	Broj ponavljanja			Suma rangova
	1. mjesto	2. mjesto	3. mjesto	
C1	6	1	5	23
C2	3	6	3	24
C3	3	5	4	25
C4	-	6	6	30
C5	6	1	5	23
C6	6	5	1	19

Potrošači su kao najprihvatljiviji uzorak ukupne kvalitete napitka kave ocijenili uzorak C6 (Tablica 10.). Deskriptivnom senzorskom analizom utvrđeno je da najveći postotak od maksimalne moguće kvalitete ima uzorak C1, tako da pored uzorka C6 ubrajamo i uzorak C1 kao najprihvatljiviji napitak. Kao najmanje prihvatljiv uzorak ukupne kvalitete napitka kave od strane potrošača je ocijenjen C4 uzorak. Usporedbom suma rangova u uzorcima C2, C4 i C6 može se uočiti da su ovi uzorci ocijenjeni kao manje prihvatljivi i to s po jednim bodom razlike. Promatrajući sume rangova za uzorke C2, C3 i C5 koji su dodijeljeni od strane potrošača uočava

se razlika u rangiranju s jednim bodom razlike, pa su tako ovi uzorci ocijenjeni kao manje prihvatljivi uzorci napitaka kave.

Rezultati analize boje sirove i pržene mljevene kave

Tablica 11. Pokazatelji boje ispitivanih uzoraka različitih mješavina pržene mljevene kave
Table 11. *Color indicators of the tested samples of different blends of roasted ground coffee*

Parametri	C1	C2	C3	C4	C5	C6
L*	(30,06±1,89)^{c}	(30,39±1,83)^{b}	(30,81±2,13)^{a}	(29,04±1,25)^{e}	(28,15±0,50)^{f}	(29,93±1,38)^{d}
a*	(6,23±1,68)^{c}	(6,62±1,93)^{b}	(8,21±1,91)^{a}	(4,9±0,86)^{f}	(4,29±0,77)^{e}	(5,51±1,16)^{d}
b*	(4,14±5,47)^{b}	(4,69±3,09)^{b}	(5,13±3,89)^{a}	(2,48±1,73)^{d}	(1,25±0,62)^{e}	(3,71±1,21)^{c}

Legenda: C1 (40% Rio Minas, 40% Santos, 20% Sheery). C2 (35% Rio Minas, 50% Santo, 15% Sheery). C3 (30% Rio Minas, 45% Santos, 25% Sheery). C4 (40% Camerun, 40% Sheery, 20% Santos). C5 (35% Camerun, 50% Sheery, 15% Santos). C6 (30% Camerun, 45% Sheery, 25% Santos). Podaci su predstavljeni kao srednje vrijednosti ± SD (s tri ponavljanja). ^{abc} Duncanovim testom potvrđena je statistička značajna razlika između srednjih vrijednosti (±SD).

Tablica 12. Parametri boje sirovih uzoraka kave
Table 12. *Color parameters of raw coffee samples*

Parametri	CA1	CA2	CR3	CR4
L*	55,37^{c}±1,13	56,68^{b}±3,4	56,6^{b}±2,18	57,24^{a}±0,95
a*	2,52^{c}±0,70	5,04^{a}±0,39	2,32^{c}±0,45	3,9^{b}±0,27
b*	26,65^{c}±1,26	31,05^{a}±0,68	28,67^{b}±0,94	25,81^{c}±3,26

Legenda: CA1- Rio Minas; CA2 - Santos; CR3 - Sherry; CR4 - Camerun; A- Arabica; R - Robusta; Podaci predstavljaju srednje vrijednosti ± SD (s tri ponavljanja). ^{abc} Duncanovim testom potvrđena je statistička značajna razlika između srednjih vrijednosti (±SD).

U Tablicama 11. i 12. prikazani su rezultati instrumentalnog određivanja boje ispitivanih uzoraka sirove mljevene kave i uzoraka različitih mješavina pržene mljevene kave. Ispitivani uzorci sirove mljevene kave (CA1, CA2, CR3 i CR4) imaju statistički značajno veću ($P<0,05$) L* vrijednost u odnosu na uzorke različitih mješavina pržene mljevene kave (C1, C2, C3, C4, C5 i C6). Uzorci različitih mješavina pržene mljevene kave C1, C2 i C3 imaju statistički značajno veću ($P<0,05$) L* vrijednost u odnosu na uzorke pržene mljevene kave C3, C4 i C5. Vrijednost L* se kreće od 0 za crnu boju do 100 za bijelu boju (Gokmen i Senyuva, 2006) što znači da manje L* vrijednosti imaju tamniji uzorci. Prema literaturnim podacima (Mendes i sur., 2001; Sacchetti i sur., 2009) može se reći da se L* vrijednost linearno smanjuje povećanjem temperature prženja. Prema rezultatima boje sirovi uzorci kave imaju statistički značajno manju ($P<0,05$) a* vrijednost (udio

crvene i zelene boje) i statistički značajno veću ($P<0,05$) b* vrijednost (udio plave i žute boje) u odnosu na različito prženje uzoraka kave.
Prema navodima Džinić i sur. (2013) za srednje prženo zrno kave L* vrijednost je 26,0, a za tamno prženo zrno kave 24,3. Sacchetti i suradnici (2009) su stupanj prženja odredili na osnovu izmjerenih L* vrijednosti i to: $L^*>35$ za svijetlo prženje, $25<L^*<35$ za srednje prženje i $L^*<25$ za tamno prženje. Uspoređujući ove vrijednosti sa dobivenim rezultatima za različite uzorke mješavina mljevene pržene kave, može se zaključiti da svi uzorci spadaju u srednje pržene. Vrijednosti a* i b*, kao pokazatelji kromatičnosti opadaju s povećanjem stupnja prženja (Džinić i sur., 2013). Izmjerene CIE L* i b* vrijednosti opadaju eksponencijalno s vremenom prženja, dok se vrijednost a* povećava i dostiže svoj maksimum, a zatim opada s vremenom prženja. Prema rezultatima Pittia i suradnika (2007) vrijednost a* se mijenja od 10,8 za slabo prženu, preko 8,8 za srednje, do 6,5 za tamno prženu kavu.

ZAKLJUČAK

Kava spada u najpopularnije napitke širom svijeta. Razlog zbog kojeg je ovaj napitak popularan je svakako i njeno pozitivno djelovanje na centralni živčani sustav. Napitak od kave se na prostorima Bosne i Hercegovine priprema i konzumira na tradicionalan način. Koristeći deskriptivnu senzorsku metodu za ispitivanje senzorskih svojstava napitka crne kave upućeni ocjenjivači su kao najprihvatljivije uzorke ocijenili C3 i C5 uzorak, dok su potrošači najbolje ocijenili uzorak kave C1. Postotno je u uzorku C1 zastupljeno više Arabika vrste kave, a glavna odlika ove vrste kave je što je aromatično-slatkog okusa, a što je potrošačima bilo najprihvatljivije za konzumiranje ovog napitka. Na osnovu rezultata koji su upućeni ocjenjivači dodijelili za senzorska svojstva napitka kave, može se zaključiti da su svi uzorci ocijenjeni sa relativno dobrim značajkama kvalitete. Opisnom ocjenom odličan ocijenjeni su uzorci C1, C3, C4, C5, dok su uzorci C2 i C6 ocijenjeni opisnom ocjenom vrlo dobar. Potrošači su, promatrajući opisnu ocjenu, senzorska svojstva napitka kave ocijenili s nešto nižim ocjenama u odnosu na upućene ocjenjivače. Uzorci C1, C3, C4 su ocijenjeni opisnom ocjenom dobar, dok su C2, C5, C6 uzorci ocijenjeni kao vrlo dobri. Postotak od maksimalno moguće kvalitete u uzorcima napitka kave za upućene ocjenjivače kretao se od 74,87 do 85,6%, dok se kod potrošača kretao od 63,91 do 74,49 %. Upućeni ocjenjivači su kao najprihvatljiviji ocijenili uzorak C3, a potrošači C6. Rezultati boje kave pokazuju da su dobivene vrijednosti u skladu s propisanim odlikama srednje pržene kave.
Na osnovu rezultata dobivenih ispitivanjem kvalitete komercijalnih uzoraka kave može se zaključiti da se željena kvaliteta napitka crne kave specifičnih i prihvatljivih senzorskih svojstava i sastava, može dobiti pravilnim izborom vrste i sorte kave, njihovog odnosa u mješavini i kontinuiranom kontrolom procesa prženja i kvalitete proizvoda.

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CHROMATOGRAFIC DETERMINATION OF BISPHENOL A IN BOTTLED WATER

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ABSTRACT

Bottled water can contain numerous toxins, such as bisphenol A. These toxins can pass from the container into the water. Bisphenol A (BPA) is often used in the synthesis of epoxy resins. Its unreacted particles migrate into water and can cause numerous diseases and abnormalities in the human body when consumed. Due to the BPA negative effects on human health, many plastics manufacturing companies have decided to eliminate bisphenol A from their synthesis, but it is still generally used. Therefore, we have developed useful methods for the extraction of bisphenol A by solid phase extraction on Strata X and its quantitative analysis by liquid chromatography with UV detection. 15 samples of bottled water were analysed. 10 of them were purchased on the Serbian and Bosnian markets while 5 samples were of Slovenian origin. Isocratic reverse phase elution was performed, and bisphenol A was detected at a wavelength of 210 nm. Bisphenol A is present in the bottled water in extremely low concentrations and the obtained results were below the detection limits of the UV detector.

Keywords: bisphenol A, bottled water, liquid chromatography, ultraviolet light, drinking water

INTRODUCTION

Bisphenol A (BPA) is an environmentally harmful chemical that is increasingly produced and worldwide used (Oluranti et al., 2021). It improves the properties of plastics but is not harmless. Its unreacted particles migrate into water and, if consumed, can cause numerous diseases and abnormalities in the human body. Due to the BPA-related negative effects on human health, many plastics manufacturing companies have decided to eliminate bisphenol A from their synthesis, but it is still generally used. For this reason, we decided to find out if bottled water still contains bisphenol A, which can migrate from the packaging.

Bisphenol A (2,2-bis[4-hydroxyphenyl] propane) is an industrially important chemical that is widely used as a raw material for the production of polycarbonate plastics (Jiang et al., 2018) and epoxy resins (Dreolin et al., 2019), which have a variety of applications, such as plastic food containers and epoxy coatings for food-cans. Applications of BPA include printed circuit boards, composites, adhesives, etc. (Gallart-Avala et al., 2009). Bisphenols exhibit estrogenic activity and are suspected as potential carcinogenic and mutagenic compounds, producing adverse effects also in humans (Cacho et al., 2012).

Various analytical methods such as gas chromatography-mass spectrometry, gas chromatography-tandem mass spectrometry, enzyme-linked immune sorbent assay (ELISA) and electrochemical methods have been used for the determination of BPA. A highly sensitive and selective photoelectrochemical sensor was developed for measurement of the concentration of BPA in solution based on AuNPs/g-C3N4 nanocomposite film (Deiminiat and Rounaghi, 2021). The good performance of the sensor can be attributed to the high binding affinity of the aptamer toward the BPA molecules on the one hand and, the surface plasmon resonance effect of the gold nanoparticles on the other hand. The main objective of the work reported here, was to determine the BPA concentrations in the selected bottled water samples using HPLC-UV analysis at wavelength 210 nm. The solubility of BPA was evaluated, and validation of the method was performed.

MATERIALS AND METHODS

Samples

A total of 15 bottled water samples were analysed for BPA. 5 bottled water samples were obtained from Slovenian market (Maribor, Slovenia), 5 from Bosnia and Herzegovina and 5 Serbia. The packaging material of most bottles was specified as polyethylene terephthalate (PET). Bottle packaging of one water sample was made of 30 % plant material. Bottle packaging of another water sample was produced from PET and high-density polypropylene (HDPE). The water samples names are confidential and are not listed here. BPA was extracted and quantified in triplicates, and recovery data were calculated.

Calibration curve preparation

Prior to the HPLC analysis the appropriate solvent was selected among methanol, acetonitrile, and water based on the solubility of BPA (Gallart-Avala et al., 2009). Commercially available BPA, 2,2-(4,4-dihidroxydiphenyl) propane was provided by Sigma (St. Louis, MO; USA). 10 mg of BPA was dissolved in 2 mL of solvent.
A stock solution of 1 g/ L was prepared in methanol and stored in the darkness at −10 °C. Working standard solutions were prepared daily by diluting stock solution with Milli-Q water in range from 0.5 mg/L to 100 mg/L in 10 mL glass flasks (Figure 1). BPA absorption maximum was determined at 210 nm. For separation the reverse phase column ZORBAX Eclipse XDB-C18 (4.5 x150 mm, 5 µM) was used. Isocratic elution of the compounds was performed where mixture of the methanol and water (methanol:water =70:30, V/V) was used as mobile phase at the 0.5 mL/min flow. The analysis time was 12 min with 10 µL sample injection. Analyses were performed on the Varian ProStar 210 HPLC-UV system, equipped with an autosampler, automatic injector and Varian ProStar 310 UV-VIS detector.

Figure 1. The calibration curve samples

Water sample analysis

The recoveries of three samples were tested using different columns. 1 mL of standard solution, 0.25 mL of standard solution, and no standard solution were added to the sample. The VAC-ELUT, Varian vacuum control valve, was used for sample extraction. SPE was performed as follows: 2x3 mL of methanol was used for conditioning, the same amount for loading, then 25 mL for washing and 3x3 mL mixture methanol/acetonitrile= 1/1 for elution of BPA. Samples were concentrated using rotavapor Buchi Rotavapor R-100 at 40 °C and p = 153 mbar. Dry samples were re-dissolved in 0.1 mL of the solvent. The sample was analysed on HPLC-UV. Strata X cartridge was selected and used for all further analyses, as recoveries in the ranges from 94 % to 106 % were confirmed for this column.

RESULTS AND DISCUSSION

Calibration curve

Calibration curve is presented in Figure 2. The coefficient of determination R2 values were greater than 0.999, Quality coefficient was 2.14 %. The linearity was confirmed. Reproducibility was confirmed with RSD lower than 5 % as can be seen from the Table 1. Limit of detection and limit of quantification were determined at 1.7 mg/L and 5.6 mg/L, respectively. The lowest concentration of 0.5 mg/L was below the LOD.

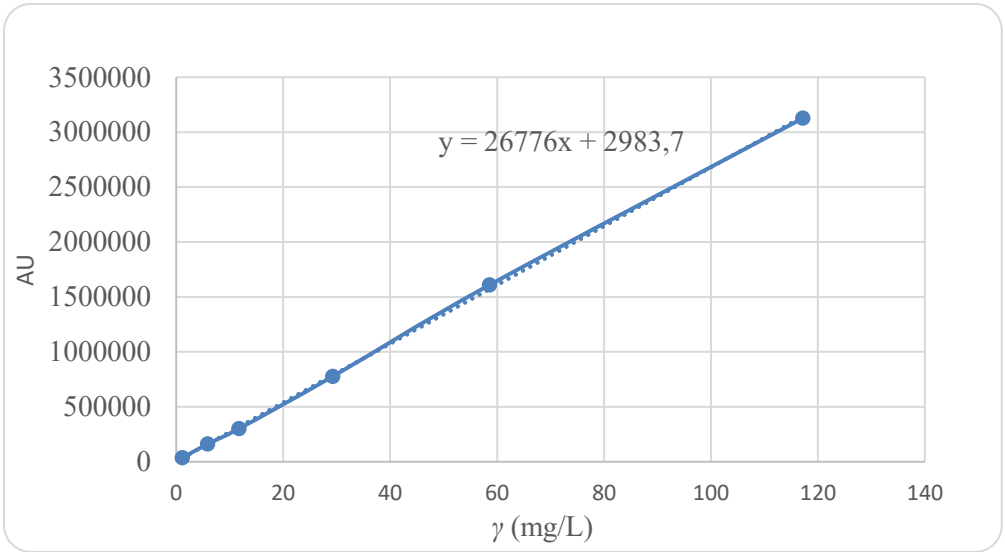

Figure 2. The calibration curve for BPA in the concentration range from 0.5 mg/L to 100 mg/L

Table 1. Reproducibility

γ (mg/L)	*RSD* (%)
0.5	-
1.17	3.64
11.72	1.64
29.30	1.88
58.60	0.27
117.20	0.27

To evaluate reproducibility, 3 replicates were prepared for each concentration, and these were used to measure the recovery of BPA in solution.

Standard solution chromatogram

Figure 3. represents the chromatogram of the standard solution containing 100 mg/L BPA. The repeatability of areas obtained for the blank chromatograms was good. Complete, satisfactory separation of BPA was achieved on the used RP column (Figure 3).

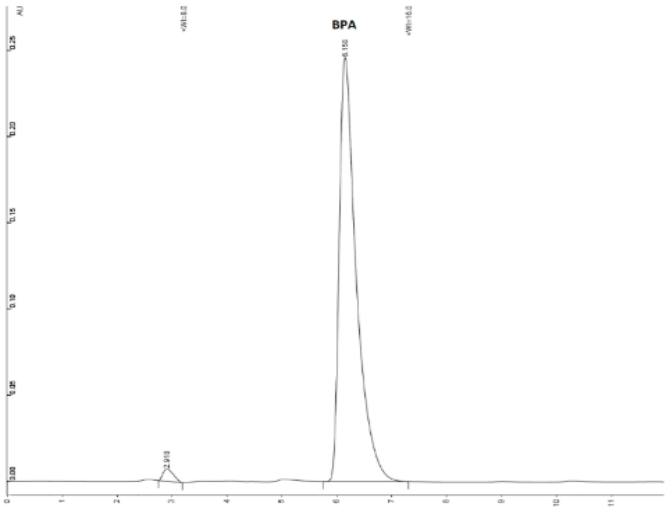

Figure 3. Standard BPA solution chromatogram

Real sample analyses

Three replicates were made for each sample. Typical chromatogram of the selected Slovenian bottled water sample can be seen on the Figure 4. and the chromatogram of the selected Bosnian bottled water sample can be seen on the Figure 5.

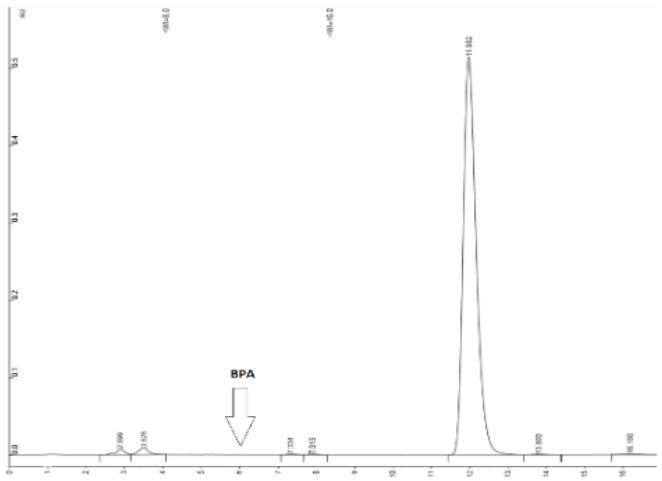

Figure 4. Typical chromatogram of the selected Slovenian bottled water sample

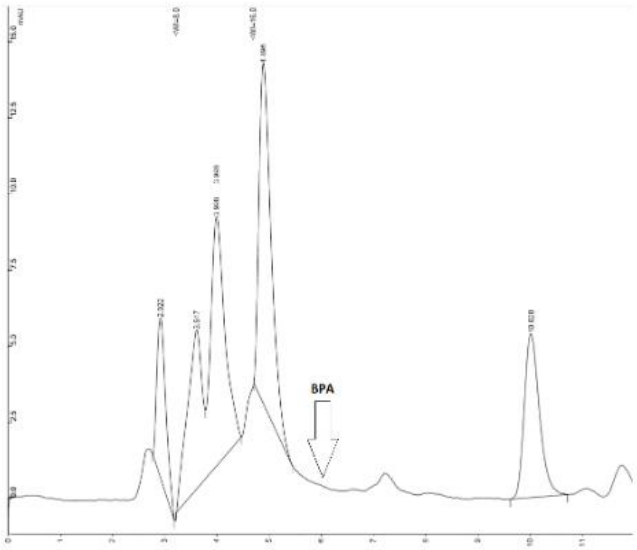

Figure 5. Typical chromatogram of the selected Bosnian bottled water sample

Under the described experimental conditions, the retention time of BPA was 6.15 min. As can be seen from Figures 4 and 5, BPA was not detected in the real bottled water samples or was far below the determined LOD. Similar results were obtained for all 15 samples analysed.
From Figure 5 it is seen that some other compounds were present in the sample, but they were not identified or quantified. In general, the BPA values in bottled waters obtained in this study are in good agreement with other published studies (Santhi et al., 2012).

CONCLUSION

The aim of this study was to determine BPA in 15 different bottled water samples obtained from Slovenian, Bosnian and Serbian markets. BPA was successfully isolated by solid phase extraction using Strata X cartridges and then separated and determined using HPLC-UV method. The linearity of the method was confirmed with the coefficient of determination greater than 0.999. The recoveries ranged from 94 to 104%, which was very satisfactory. The low RSD ($\leq$ 4.64%) clearly indicates the reproducibility and the successful application of the developed method for the quantitative measurement of BPA in various real water samples. BPA was not detected in any of the 15 water samples analysed. In the analysed samples some other compounds were detected but they were not identified or quantified. It could be concluded that samples were all without the presence of BPA.

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Korisnik bespovratnih sredstava: **Podravka d.d.**

Vrijeme provedbe: **1.9.2020.- 1.9.2022.**

Opis projekta: istražiti inovativni tehnološko učinkoviti proces odvajanja nutritivno vrijednog biootpada te mogućnost njegovog recikliranja u svrhu razvoja novih i inovativnih prehrambenih proizvoda više dodane vrijednosti; istražiti potencijal proizvodnje bioplina iz svih otpadnih tokova proizvodnje Tvornice Kalnik, Varaždin, Hrvatska.

Total project value: ***6.899.956.33 Croatian kuna***

Amount co-financed by the EU: ***3.055.545.56 Croatian kuna***

Grant beneficiary: ***Podravka Ltd., Croatia***

Implementation time: ***1.9.2020.- 1.9.2022.***

Project description: *investigate the innovative technologically efficient process of separation of nutritionally valuable by-products and the possibility of its recycling for the purpose of developing new and innovative food products of higher added value; investigate the potential of biogas production from all waste production streams of the Kalnik Factory, Varaždin, Croatia.*

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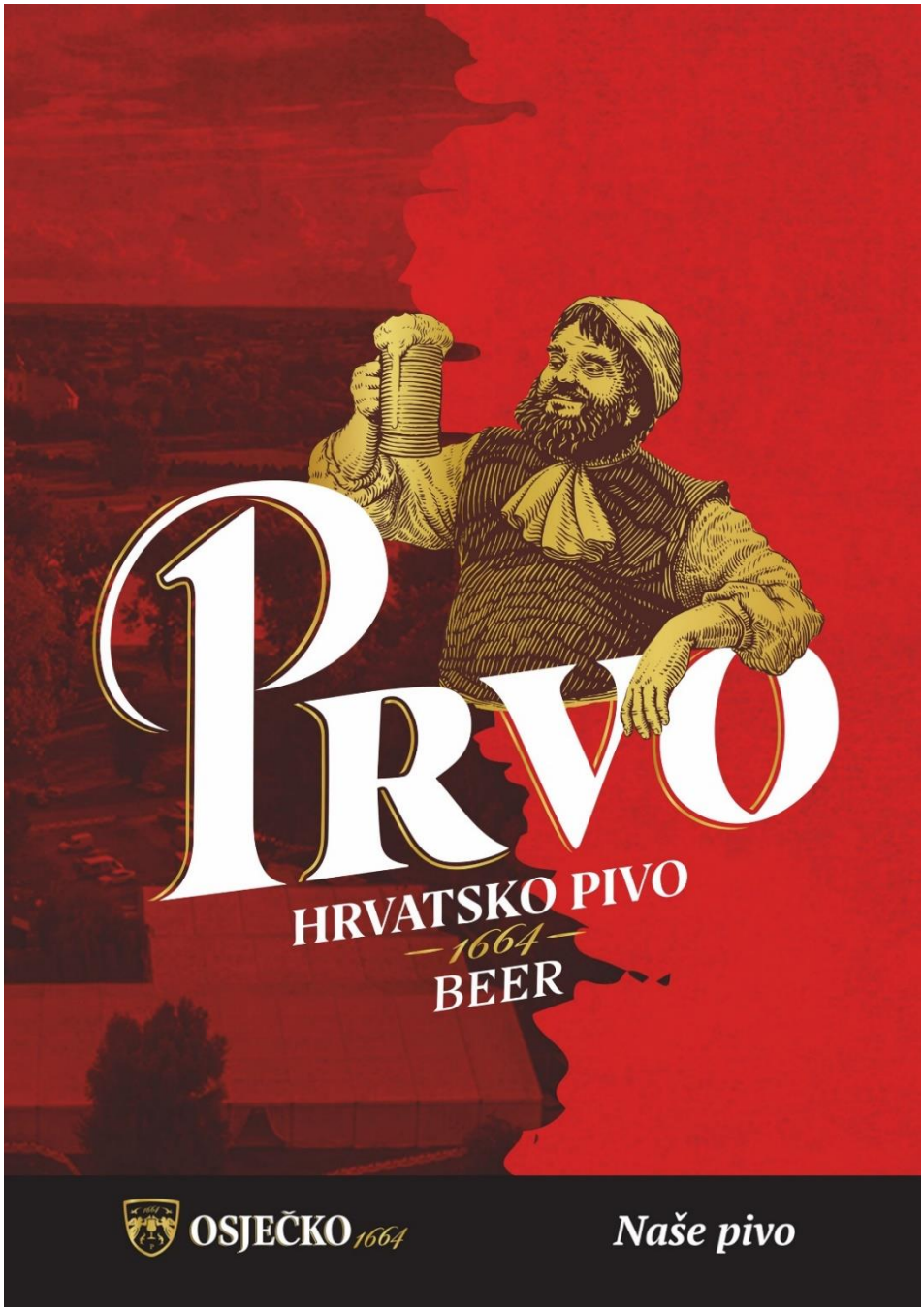

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