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Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno-tehnološki fakultet Osijek,
Franje Kuhača 20, 31000 Osijek, Hrvatska

Tehnička priprema i dizajn:
Damir Alihodžić (Tuzla, BiH), Ivana Lauš (Osijek, Hrvatska), Kenan Biberkić (Tuzla, BiH)

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Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia

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IRRITABLE BOWEL SYNDROME PREVALENCE AND THE ROLE OF DIET IN ADULT POPULATION

Josipa Dragičević[1], Jelena Balkić[2], Ines Banjari[1*]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, HR-31000 Osijek, Croatia
[2]University Hospital Osijek, Department for Dietetics and Nutrition, J. Huttlera 4, HR-31000 Osijek, Croatia

original scientific paper

Summary

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders with a very strong psychosomatic component in the etiology. The prevalence is higher in women and many IBS patients say that food triggers their symptoms. The aim of this study was to determine the prevalence of IBS symptoms (GSRS-IBS questionnaire) and the role of diet and dietary habits on IBS symptoms in adults. An observational online study with the study-specific questionnaire was completed by 109 adults (84.4% women, 15.6% men), average age 26 years (21-63 years). 12.8% of study participants had high IBS score (44-75 points). Participants with high IBS score tend to overeat (93.0% sometimes, 7.0% always) and skip meals (36.0%) which was proven to worsen IBS symptoms. Coffee consumption also worsens IBS symptoms, as well as the consumption of all drinks (without water) and sweets. IBS score was higher in participants who said that some food provokes symptoms. Beans (lentil, pea, bean) were proven to increase the risk for high IBS score, while strong spices (soup cube, chilli, curry) increase the risk of high IBS only in women (univariate logistic regression).

Keywords: irritable bowel syndrome, IBS score, diet, dietary habits, risk factors

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized with the recurrent abdominal pain followed by altered bowel movements (Bilić et al., 2015). IBS poses a significant healthcare burden (through direct or indirect expenses) because it is one of the most common reasons for sick leave and directly decreases person's quality of life (Lovell and Ford, 2012; Halland and Saito, 2015).

The pathophysiology of IBS is characterized by complete absence of histological, endoscopic or radiological manifestations of the digestive tract disorder (Ikechi et al., 2017). Mechanisms involved in the development of IBS are very complex, and intertwines biological and psychological factors (Saha, 2014; El-Salhy, 2015). Due to the fact that functional gastrointestinal disorders are classified only according to the symptoms manifested, Rome IV classification system was developed to enable clinical diagnosis of IBS (Drossman and Hasler, 2016). Rome IV criteria expands the concept of IBS by underlying alterations in the interaction between the enteric nervous system (gut and digestion in general) and brain. This interaction is related to disturbances in the gut motility, visceral hypersensitivity, leaky gut syndrome, and consequently has negative impact on the immune response, leads to dysbiosis, and alters processing of stimuli in the central nervous system (Schmulson and Drossman, 2017). Mentioned changes, along with visceral hyperalgesia (increased pain sensitivity) and post-infective state represented individually and variably are some of the possible etiological factors involved in IBS (El-Salhy, 2012; El-Salhy, 2015; Sikander et al., 2009).

Many IBS patients claim that their diet is linked to the symptoms they are experiencing, and that certain food worsens their symptoms (Krogsgaard et al., 2017). Today's standard for IBS diet therapy is the so called FODMAP diet characterized by elimination of foods rich in fermentable oligo-, di- and monosaccharides and polyols (Banjari et al., 2017). This diet seems efficient in improving the symptoms in IBS patients (fewer symptoms) (Sheperd et al., 2008).

IBS prevalence is higher among women, regardless of age and ethnic group, but almost half of those reporting IBS symptoms are up to 35 years old (Canavan et al., 2014). Various psychological disorders accompany 70 to 90 % of IBS patients (depression, anxiety and others), and they are positively correlated with worse IBS symptoms. Still, their interrelation in the pathophysiology of IBS is unclear (Longstreth et al., 2006; Bilić et al., 2015). Learned behaviour from parents is among possible etiological factors, while having a mother with IBS represents an independent risk factor for IBS (Levy et al., 2001).

Global prevalence of IBS is 11.2% (Lovell and Ford, 2012), and 11.5% for Europe (Hungin et al., 2003). Epidemiological studies conducted in continental part of Croatia showed that the prevalence of IBS was high; 28.00% for the area of Zagreb, 26.52% for Bjelovar-Bilogora County and 29.16% for Osijek-Baranja County (Grubić et al., 2014).

Subjects and methods

The aim of this study was to determine the prevalence of high IBS score in adult population, and analyse the role of dietary habits on IBS score.

*Corresponding author: ibanjari@ptfos.hr

Study Participants

An observational online study was carried out on general population from the area of Republic of Croatia. The research was conducted as a one-time anonymous study. Target group were women aged 20 to 40 years, but no restriction on gender was set. The questionnaire was created specifically for the purposes of this study by using a publicly-available program (Google Forms). All answers were automatically stored in tabular form (MS Office Excel). Social networks were used for the recruitment of potential participants. The questionnaire was completed by 109 adults, out of which only three had confirmed diagnosis of IBS.

Questionnaire

The questionnaire included general and socioeconomic characteristics, body mass and height, and questions regarding other medical conditions, regular use of medications, use of supplements. Participants were asked to assess their physical activity level, smoking habits, and their dietary habits (e.g. breakfast skipping, overeating, number of meals, etc.). Also, consumption of beverages (coffee, tea, alcohol, juices) and spicy foods, and use of spices (e.g. "Vegeta", salt, pepper, chilli) were assessed.
Gastrointestinal symptom rating scale for IBS (GSRS-IBS) was integrated in the questionnaire (Wiklund et al., 2017). On a 7 grade Likert scale participants had to assess their subjective experience of gastrointestinal symptoms they had felt for past 7 days prior completing the questionnaire. Participants' answers were scored and represent IBS score. Minimum score is 13 and maximum 91 points. Higher score indicates more IBS symptoms present in a person.
Finally, participants were asked to assess consumption of foods listed in the literature as possible propagators of the IBS symptoms. Monthly consumption of these foods, in a form of a semi-quantitative food frequency questionnaire (sFFQ) was assessed (2 or more times a day, once a day, 3 to 5 times a week, 1 to 2 times a week, 1 to 2 times in two weeks, 1 to 2 times in a month and rarely or never).

Statistical analysis

Statistical analysis was performed with Statistica (version 13.4, StatSoft Inc., SAD), at significance level 0.05 and 0.01. Graph plotting was performed with MS Office Excel tool (version 2013, Microsoft Corp., SAD).
Normality of data distribution was tested by the non-parametric Kolmogorov-Smirnov test for the comparison of medians and arithmetic mean, and histograms plotting. Spearman's correlation test and Mann-Whitney U test for the comparison of variables depending on the IBS score were used. Univariate logistic regression was performed and variables that were shown as significant were tested with multivariate logistic regression.

Results and discussion

Total of 109 participants, 92 women (84.4%) and 17 men (15.6%), average age 26 years (24 – 35, minimum 21 and maximum 63 years) completed the questionnaire.
Based on the GSRS-IBS scores, participants were divided into two groups. The first group achieved low IBS score (scores of 13 to 44) while the second group had high IBS score (from 44 to 75). The majority of study participants had low IBS score (87.1%), with almost equally represented men and women in both categories. However, more women than men had high IBS score (14.1% of women in comparison to 11.8% of men) (Fig. 1). The prevalence of high IBS score in study population is in line with previously mentioned epidemiologic data for IBS (Lovell and Ford, 2012).

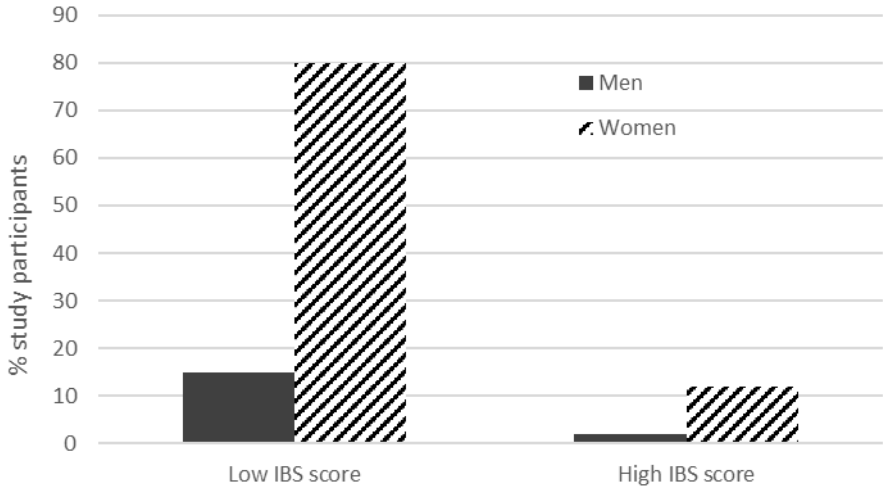

Fig. 1. Gender-based distribution of study participants according to their IBS score (N=109)

Median Body Mass Index (BMI) of study participants (BMI) was 22.1 (20.2 – 24.9) kg/m^2 with no statistical difference or correlation between BMI and the category of IBS score. The relationship between BMI and IBS is still unclear. Presence of gastrointestinal symptoms could lead to lower food intake consequently leading to malnutrition, therefore IBS patients would have lower BMI (El-Salhy, 2015). This was confirmed in a study by Kubo et al. (2011). However, Simrén et al. (2001) found that patients with IBS are mainly normal weighted or overweight. Results of a comprehensive review by Pickett-Blakely (2014) showed that obesity worsens IBS symptoms.

Among participants in the low IBS score, 27.4% said they have at least one more medical condition and 28.4% are regularly taking medications. In the high IBS score category, 35.7% said they have at least one more medical condition and 42.8% of them are regularly taking medications. A weak correlation was found between IBS score and presence of another medical condition (r=0.192) as well as for the use of medications (r=0.241). Studies have shown that a broad range of medical conditions accompany IBS, from gastroesophageal reflux, genitourinary symptoms, fibromyalgia, headache, back pain and other. Still, IBS is often misdiagnosed or not recognized timely and the question is whether other conditions are consequences or causes of IBS (Saha, 2014). Importantly, between 20 and 30% of IBS patients are prone to self-medication, mainly to antacids, which are ineffective for the symptoms experienced (Kua et al., 2012; Niknam et al., 2016).

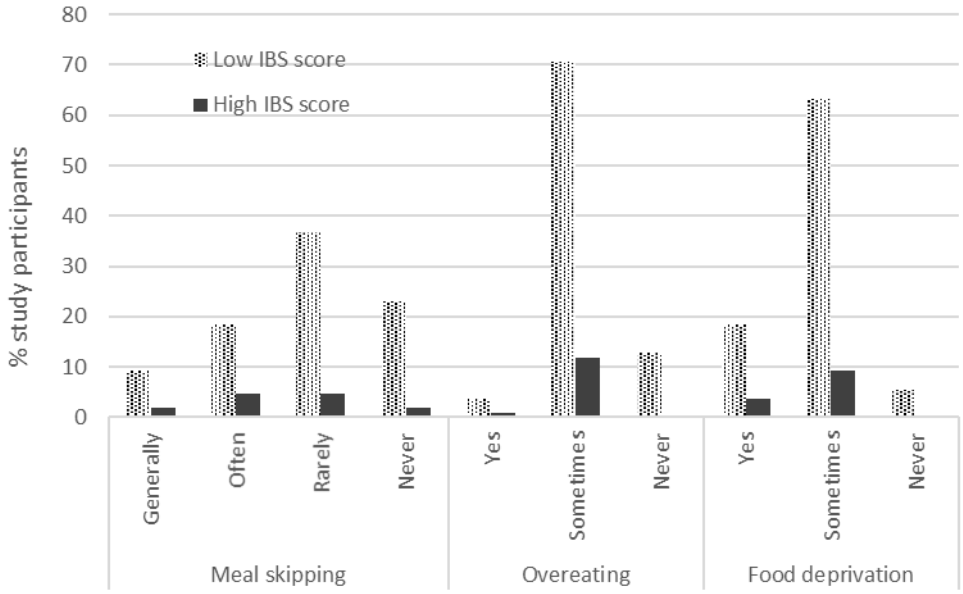

Fig. 2. Frequency of meal skipping, overeating and food deprivation in study participants according to their IBS score (N=109)

Participants do not differ in the average number of meals per day according to the IBS score category. However, 93.0% of participants with high IBS score sometimes overeat, even 71.0% tend to deprive of food, and 36.0% said they often skip meals (Fig. 2). Studies have shown that IBS patients tend to skip meals which can cause disturbances in gastrointestinal motility and contribute to the symptoms of IBS. It has also been confirmed that the relative risk for IBS is 2-4 times greater in persons who tend to overeat because large amounts of food in one meal can provoke symptoms of IBS (Cozma-Petruţ et al., 2017). The results of this research confirm these statements; week negative correlation between food deprivation and higher IBS score was found (Table 1). In other words, meal skipping is related to higher IBS score, i.e. indicates more symptoms of IBS present.

Higher consumption of all types of drinks (except water) shows strong positive correlation with IBS score. Also, participants who indicated that some food provokes gastrointestinal symptoms (is not well tolerated) have higher IBS scores (Table 1). Additionally, week correlation was found between the consumption of sweets (ice cream, chocolate, fruit yoghurt, milk deserts and creamy cakes) and higher IBS score (Table 1). This particular result can indicate the need for compensation for altered

function of serotonin, which has been found in IBS patients, and serotonin has a major role in gastrointestinal motility (Saha, 2014; Sikander et al., 2009).

Table 1. Correlations between some diet characteristics and IBS score of study participants

Diet characteristics	IBS score
Food deprivation	-0.193*
Coffee	0.284**
Beverages (except water)	0.328**
Sweets	0.199*
Some food is not well tolerated	0.300**

Spearman's correlation test, **correlation is significant at p<0.01
*correlation is significant at p<0.05

Univariate logistic regression showed that the consumption of beans (lentil, pea, bean) is the only positive predictor for high IBS score (OR = 1,880, 95% CI = 1,009 – 3,503; P=0,047). Interestingly, the use of strong spices represent positive predictor for high IBS score only in women (OR = 2,748, 95% CI = 1,015 – 7,443; P = 0,047).

These results on specific foods and IBS symptoms are in line with literature findings. Beans have high content of galactans that can induce gas production and consequently increase intraintestinal pressure, spices contain flavour enhancers which can also provoke gastrointestinal symptoms, and sweets can cause symptoms because of the high sugar content or lactose contained in milk-based products (El-Salhy, 2015; El-Salhy and Gundersen, 2015; Fedewa and Rao, 2014).

Conclusions

The prevalence of high IBS score determined is in accordance with previous findings. A number of diet characteristics have been identified as positive predictors of high IBS score. However, the results on the impact of specific foods are still unclear and more research on larger populations should be conducted to elucidate their role in IBS. Presented results favour the complexity of IBS pathophysiology and present valuable contribution to the field of IBS. Some of the findings could be used in practice for the education of IBS patients, especially regarding the foods that should be avoided to alleviate IBS symptoms.

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INFLUENCE OF STORAGE TIME ON QUALITY OF SPRAY-DRIED EXTRACTS OF BASIL (*OCIMUM BASILICUM* L.)

Aleksandra Gavarić, Senka Vidović, Zoran Zeković, Jelena Vladić*

University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia

original scientific paper

Summary

Sweet basil (*Ocimum basilicum* L.), a member of the *Lamiaceae* family, is the major essential oil crop which is cultivated commercially in many countries. The aromatic leaves of basil are used fresh or dried as a flavoring agent for foods, confectionery products and beverages. In the current study macerates of basil were spray dried with addition of 0%, 10%, 20% and 30% maltodextrin. In order to evaluate influence of storage time on quality of four basil powders the moisture content, rehydration, bulk density, total phenolic and total flavonoid contents were tested immediately after production and after 50 days long storage at room temperature in desiccator. This study showed that 50 days long storage time did not influence negatively on quality of basil powders regarding all investigated parameters except moisture content which rose for 30 - 40 %.

Keywords: Ocimum basilicum, spray drying, dry powder characterization, storage time

Introduction

Sweet basil (*Ocimum basilicum* L.), a member of the *Lamiaceae* family, is native to Asia, Africa, South America, and the Mediterranean but widely cultivated commercially in many countries (Grayer et al., 1996) in natural and green house conditions in order to improve its yield and obtain a regular supply of the material (Johnson et al., 1999). Among 150 species of the genus *Ocimum*, basil is the major essential oil crop (Sajjadi, 2006) which has been extensively utilized in food as a flavoring agent (Telci et al., 2006), due to its foliage adding a distinctive flavor to many foods. Basil is also a rich source of aromatic compounds and essential oils containing biologically active compounds which possess insecticidal (Deshpande and Tipnis, 1997), nematicidal (Chatterjee et al., 1982), fungistatic (Reuveni et al., 1984) and antimicrobial properties (Wannissorn et al., 2005). According to its chemical composition, sweet basil belongs to aromatic herbs whose quality is determined by the content of essential oil. The essential oil content varies between 0.5 - 0.8 % (Tucakov, 1990). Essential oil comprises around 30 characteristic compounds, mostly terpenes (monoterpenes and sesquiterpenes, and their oxygenated derivates) and phenolic compounds. Dominant components of essential oil are primarily phenolic compounds: methylchavicol, linalool, eugenol, methyleugenol and methylcinnamate (Filip, 2014).

This aromatic herb has been used traditionally as a medicinal herb in the treatment of headaches, diarrhea, constipation, warts, worms and kidney malfunctions (Simon et al., 1999). The leaves and flowering tops of the plant are recognized as carminative, galactogogue, stomachic and antispasmodic in folk medicine (Sajjadi, 2006). It was reported previously that the leafy parts of basil had tonic and antiseptic activity (Kosekia et al., 2002). It is also known that leaves of basil are suitable for the treatment of pain and cough (Basilico and Basilico, 1999). In addition, basil is used for inflammations and dyspepsia (McClatchey, 1996). Recently, the potential uses of basil essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (Lee et al., 2005, Politeo et al., 2007, Sartoratotto et al., 2004, Suppakul et al., 2003, Wannissorn et al., 2005). The basil essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Da-Silva et al., 2003, Sajjadi, 2006). Since sweet basil is scarce during off-seasons and highly perishable, it has to be preserved against deterioration and spoilage, which makes its drying a primary issue (Parmar et al., 2017). Spray drying is perceived as the most economic technique maintaining high quality of powder by rapid dehydration. It ensures a large surface area in the form of fine liquid droplets obtained through atomization in the drying chamber, which leads toward production of regularly and spherically shaped powder particles (Fazaeli et al., 2012; Turchiuli et al., 2011).

The main aim of this study was to assess the efficiency of spray drying to microencapsulate phenolic compounds from basil extracts obtained by maceration. In order to estimate influence of storage time on quality of spray dried extracts of basil, obtained powders were tested for moisture content, rehydration and bulk density immediately after their production and after 50 days long storage. In addition, the content of polyphenols in basil powders was determined promptly after their

*Corresponding author: vladicjelena@gmail.com

production and after 50 days of storage at room temperature.

Materials and methods

Plant material

Sweet basil (*O. basilicum* L.) was cultivated at the Department for Organic Production and Biodiversity, Bački Petrovac, Serbia. The aerial parts of basil were stored in a paper bags, at a room temperature. The dried basil was grounded in a domestic blender prior extraction, and the particle size of grounded material was determined using sieve sets (Erweka, Germany). Mean particle size of basil used in investigation was 0.2138 mm.

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Folin-Ciocalteu reagent and (±)-catechin were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Gallic acid was purchased from Sigma (St. Luis, MO, USA). All other chemicals and reagents were of analytical reagent grade.

Liquid extract and liquid feed preparations

Basil liquid extracts were generated by classical extraction technique-maceration with 50% ethanol. Plant material and 50% ethanol (ratio 1 g/10 mL) were mixed twice a day in a glass flask for 5 days. Extraction was performed at room temperature. After extraction, obtained extracts were immediately filtrated through filter paper under vacuum. Extracts were collected into glass flask and stored at 4 °C until further experiments. Maltodextrin (MD) of dextrose equivalent (DE) 16 was used as a carrier material and its solution was added to liquid feed in concentration of 10%, 20% and 30%, and both were mixed with a magnetic stirrer at temperature of approx. 40 °C prior to the spray drying (SD) process. Therefore, four basil powder samples were obtained (0% MD, 10% MD, 20% MD and 30% MD) and further investigated.

Spray drying process and its efficiency

The prepared liquid feed was spray dried using an Anhydro lab scale spray dryer (APV Anhydro AS, Denmark). A peristaltic pump was used to pump the feed into the dryer. Liquid feeds were dried at inlet temperature, T_i =120 °C, while outlet temperature, T_o was kept constant at 80 °C. During the production of the dry powder extract, atomizer's speed ranged from 20,000 to 21,000 rpm. The obtained powder was separated from air by a cyclone. Obtained dry extracts were collected in glass bottles, sealed and kept protected from air and humidity. The production yield of the SD process was determined according to the mass of total solids measured in the feed and the mass of dry powder obtained at the end of the process in powder receptacle.

Moisture content

Powder (dry extract) moisture content was carried out according to standard procedure described in European Pharmacopeia (Ph. Eur. 8). All experiments were performed in three replicates. Moisture content was determined in dry extracts of basil immediately after their production and after 50 days of storage in desiccator.

Rehydration

Rehydration of dry extracts was determined by adding 2 g of powder into 50 mL distilled water, at room temperature. Mixture of powder and water is mixed via magnetic stirrer in glass flask. Time needed for powder to completely rehydrates, expressed in seconds, represents rehydration time (Goula and Adamopoulos, 2005; Goula and Adamopoulos, 2008). Rehydration was determined in dry extracts of basil immediately after their production and after 50 days of storage in desiccator.

Bulk density

Bulk density was determined by measuring the volume of the powder mass. 20 g of basil powder was placed to a 100 mL graduated glass cylinder. The glass cylinder was held on the vibration plate for 2 min. After that, bulk density was calculated from the difference of the empty glass cylinder and the mass of the glass cylinder with powder (Vidović et al., 2014). Bulk density was expressed as mg of powder per mL. Bulk density was determined in basil powders immediately after their production and after 50 days of storage in desiccator.

Total phenols content

The contents of total phenolic compounds (TP) in herbal powders were determined by the Folin–Ciocalteu procedure (Kähkönen et al., 1999). TP was expressed as mg of gallic acid equivalent per g of dry extract (mg GAE/g DE). All experiments were performed in three replicates.

Total flavonoids content

The total flavonoids content (TF) was determined using aluminum chloride colorimetric assay (Markham, 1989), using catechin as a standard compound. The content of total flavonoids was expressed as mg of catechin equivalent per g of dry extract (mg CE/g DE). All experiments were performed in three replicates.

Results and discussion

Process efficiency

Several drying techniques (spray drying, vacuum drying, freeze drying and spouted bed drying) have been proposed for the production of dry extracts on the industrial scale. According to Hammami and Rene (1997), an industrial scale comparison showed that the spray drying process is around 4-5 fold more economic than that of freeze drying due to its less electricity consumption and short drying time. Santivarangkna et al. (2007) reported that spray drying is 8-fold more economic than freeze drying and 4-fold more economic than vacuum drying. Since inlet temperature of the drying air affects the process of the liquid removal from the dispersion, it is necessary to adjust this temperature to allow the best possible thermal efficiency of the process, without the risk of destroying the product. The drying air temperature and air humidity simultaneously affect the final solvent content in the product, however the temperature is the only variable that can be changed at any time. Also, the humidity of the inlet air has a significant effect on the performance and efficacy of the drying process (Baker, 1997).

In our study 50% ethanolic extract, obtained by maceration of basil, was used as liquid feed. One powder was obtained with no added maltodextrin (0% MD), while other three powders were prepared with addition of 10%, 20% and 30% maltodextrin (10% MD, 20% MD and 30 % MD). Maltodextrin of dextrose equivalent (DE) 16 was used as a carrier material. As stated in the literature, maltodextrins with a DE in the range from 10 to 20 proved to be the best for carriers in spray dried powders due to less turbidity at high concentrations (Raja et al., 1989). Peng et al. (2013) observed that maltodextrin was superior to β-cyclodextrin for protecting the antioxidant components. The carrier material also needs to be inexpensive, food grade, readily available, and legally allowed (Mahdavi et al., 2014). Partanen et al. (2002) observed that maltodextrin was more heat stable than β–cyclodextrin under dry conditions. According to Bhandari et al. (1997), recovery higher than 50% in the cyclone is regarded as the criteria of efficient drying in lab-scale dryers.

The efficiency of four investigated spray drying processes can be considered high since in all cases it was above 50% (0% MD: 53.80%; 10% MD: 64.15%; 20% MD: 67.18%; 30% MD: 69.66%) (Table 1). In addition, efficiency of microencapsulation was increased by adding maltodextrin which can be related to the effect of carrier's concentration on the formation of surface core prior to the formation of crust around the drying droplets (Young et al., 1993).

Table 1. Process efficiency for basil powder samples

Basil powder	Process efficiency [%]
0% MD	53.80
10% MD	64.15
20% MD	67.18
30% MD	69.66

Basil powder properties

Moisture content

Moisture content is essential factor which affects stability, particle size, morphology and rheological properties of powders (Bhandari and Hartel, 2005). The spray dried product is highly stable, due to its low moisture content and water activity. Common ranges of moisture content and water activity of spray dried fruit and vegetable powders are 2 – 5 % and 0.2 - 0.6, respectively (Shishir et al., 2016; Patil et al., 2014; Tze et al., 2012). Under these conditions, the powdered products are rather resistant to microbiological and oxidative degradation, i.e. browning and hydrolitical reactions, lipid oxidation, auto-oxidation and other enzymatic activities (Marques et al., 2007; Tan et al., 2011b). Moisture contents of basil powders are presented in Table 2.

Table 2. Moisture content in basil powders determined immediately after production and after 50 days of storage

Basil powder	Moisture content [%]	Moisture content after 50 days [%]
0% MD	7.97	11.66
10% MD	5.96	10.57
20% MD	7.83	11.11
30% MD	8.48	10.20

Moisture content of obtained basil powders ranged between 5.96 and 8.48 %, which is similar to moisture content of *A. millefollium* powders (6.10 - 7.68 %) (Vladic et al., 2016). The lowest moisture content was determined in powder with 10% MD, whereas the highest maltodextrin concentration provided powders with highest moisture content. However, after 50 days of storage, moisture content deteriorated and rose for 30 - 40 % except in the case of 30% MD sample where it increased for 16.86%. It can be concluded that the highest carrier concentration exhibited the highest efficacy with regard to content of moisture in powders during storage time.

Rehydration time

Rehydration is a significant step in the utilization of dried fruits and vegetables. Since consumers have shown an increased interest in healthy and ready-to-use foods (De Belie, Laustsen et al., 2002), convenience, freshness, high quality, flavor (Hollingsworth, 2002) and adequate reconstitution are essential in meeting their expectations. Optimal reconstitution conditions are of outmost importance since pre-drying treatments, drying and rehydration processes induce many changes in the structure and composition of plant tissue which result in impaired reconstitution properties (Lewicki, 1998). Optimal reconstitution can be achieved by controlling the drying process and adjustment of the rehydration conditions (Marabi et al., 2004, Marabi et al., 2003, Marabi and Saguy, 2004). Rehydration time in basil powders is presented in Table 3.

Table 3. Rehydration time in basil powders determined immediately after production and after 50 days of storage

Basil powder	**Rehydratation time** [s]	**Rehydratation time after 50 days** [s]
0% MD	6	11.2
10% MD	8	12.5
20% MD	8.2	13.2
30% MD	9.1	18.3

Powders are intended for rehydration with water or, respectively, an aqueous liquid. An ideal powder should be wetted quickly and thoroughly, sink into the liquid rather than float on the surface and disperse/dissolve within a short period of time without lump formation. This ideal behaviour is difficult to achieve, since the manufacturing processes usually yield particles of rather small size and/or unfavourable structure (Hogekamp and Schubert, 2003). In our study, rehydration tends to rise with addition of carrier. Rehydration ranged between 6-9.1 s, which is rather satisfying and deteriorated after 50 days of storage from 11.2 to 18.3 s.

Bulk density

Bulk density is a very important parameter to characterize powders which have to meet bulk density targets to provide consistent weight during packaging (Legako & Dunford, 2010). Measurement of bulk density of herbal extracts is particularly significant due to its further use in the formulation of final pharmaceutical product which is restricted in volume (Goula and Adamopulos, 2010). The preferable powder properties for packaging and storage are higher bulk density and low moisture content (Shishir and Chen, 2017). Tze et al. (2012) reported that the bulk density is associated with the particle size. Smaller particles reduced the void spaces among them and arranged the particles in a close form, therefore the lower particle size led to higher bulk density (Tze et al., 2012). The bulk densities in investigated basil powders were 48 mg/mL in carrier-free powder, 40 mg/mL in both 10% MD and 20% MD powders and 51 mg/mL in 30% MD powder (Table 4). These values are lower than the one obtained in *S. montana* powder by adding 10% MD (82.4 mg/mL) (Vidovic et al., 2014), but in agreement with bulk density measured in *A. millefollium* powder with 10% MD addition (41.31 mg/mL) (Vladic et al., 2016). After 50 days of storage, bulk density of powders increased. The most significant rise was determined in the powder with 30% MD (app. 2-fold), while in the case of powder with 20% MD bulk density increased slightly.

Table 4. Bulk density in basil powders determined immediately after production and after 50 days of storage

Basil powder	Bulk density [mg/ml]	Bulk density after 50 days [mg/ml]
0% MD	48	65
10% MD	40	51
20% MD	40	42
30% MD	51	101

Polyphenol content in basil powders

Polyphenols are abundant micronutrients in our diet, and proof for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The health effects of polyphenols depend on the amount consumed and on their bioavailability. Knowledge of the bioavailability and metabolism of the various polyphenols is necessary to evaluate their biological activity within target tissues (Manach et al., 2004). A large pool of preclinical research and epidemiological data suggests that plant polyphenols can slow the progression of certain cancers, reduce the risks of cardiovascular disease, neurodegenerative diseases, diabetes, or osteoporosis, suggesting that plant polyphenols might act as potential chemopreventive and anti-cancer agents in humans (Arts and Hollman, 2005; Scalbert et al., 2005; Scalbert et al., 2005; Surh, 2003). Since high bioactive potential has been assigned to polyphenolic compounds, it is necessary to determine their content in basil dry extracts which could be further implemented in pharmaceutical formulations and dietary supplements. Polyphenol contents in obtained basil powders are presented in Table 5.

Table 5. Total phenols (TP) and total flavonoids (TF) contents of basil powders determined immediately after production and after 50 days of storage

Basil powder	TP [mg GAE/g DE]	TP after 50 days [mg GAE/g DE]	TF [mg CE/g DE]	TF after 50 days [mg CE/g DE]
0% MD	148.55	139.59	109.83	93.03
10% MD	135.32	127.68	106.48	90.60
20% MD	133.45	125.36	84.98	78.36
30% MD	113.63	111.21	81.24	80.04

The highest value of total phenols in basil powders (148.55 mg GAE/g DE) was obtained in carrier-free powder, while the lowest value of TP was gained in 30% MD sample. This outcome is expected since sample is diluted with addition of carrier. TP value obtained in 0% MD is comparable with TP values obtained in *S. montana* powder with 10% MD (153.61 mg GAE/g) and *A. millefolium* powder with 10% MD (151.86 mg GAE/g). After 50 days of storage at room temperature in desiccator TP in all samples deteriorated and decreased for 2 – 6 %.

Total content of flavonoids was in the range from 111.21 to 139.59 mg GAE/g. As in the case of TP, TF content also decreased with the addition of maltodextrin due to dilution of dry extracts' bioactive compounds with carrier. Determined values were lower than the one obtained in *S. montana* powder with 10% MD (118.69 mg CE/g). Total flavonoids in spray dried rosemary extracts obtained by maceration with aqueous ethanol ranged between 46 and 76.4 mg/g (Couto et al., 2012). Pavlic et al. (2017) investigated polyphenolic contents in two powders (carrier-free and powder with 20% MD) of *Salvia officinalis* obtained by spray drying of subcritical water extracts. They reported lower values for total phenols (106.26 mg GAE/g for carrier-free powder and 91.35 mg GAE/g for 20% MD added powder) and total flavonoids (58.97 mg CE/g for carrier free powder and 56.98 mg g CE/g for 20% MD added powder). After 50 days of storage at room temperature in desiccator TF in all samples deteriorated and decreased by 1.5 – 15 % and 30% was the most efficient concentration of carrier for preservation of polyphenols.

Conclusions

Spray drying is a well-established technique for obtaining powders from fruit juices but not so widespread when liquid feed is prepared as water/hydroalcoholic extract of herbal material. The major challenge in spray drying is to produce a standardized herbal dried extract that has the desired content of bioavailable active compounds. This is

particularly difficult since herbal extracts contain number of chemical constituents and are inconsistent in composition. This study has shown that 50 days long storage time did not influence negatively on quality of basil powders regarding all investigated parameters except moisture content. After 50 days of storage, moisture content significantly deteriorated and rose for 30 – 40 %. On the contrary, TP and TF contents in all samples slightly deteriorated and decreased for less than 15% after 50 days of storage at ambient temperature.

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DISRUPTION OF *ASPERGILLUS FLAVUS* CELLS: A BEAD MILL HOMOGENIZATION METHOD

Tihomir Kovač[1*], Matea Stuburić[1], Biljana Crevar[1], Marija Kovač[2], Ante Nevistić[2], Ante Lončarić[1], Bojan Šarkanj[3]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia
[2]Inspecto Ltd., Industrijska zona Nemetin, Vukovarska cesta 239b, 31000 Osijek, Croatia
[3]University North, University centre Koprivnica, Department of Food Technology, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia

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Summary

The most important mycotoxigenic fungus involved in pre- and post-contamination of crops is *Aspergillus flavus* which causes great health and economic loses worldwide due to production of the most potent natural hepatocarcinogen – aflatoxin B_1. Contamination with this secondary metabolite is getting even worst by global climate changes and other abiotic stressors present in environment. Accordingly, researches with the aim of synthesis or identifying the antiaflatoxigenic and antifungal compounds are of interest. For such efforts realization, use and manipulation with intracellular content of *A. flavus* cells is necessary. The aim of this study was to apply Omni® Bead Ruptor 12 Homogenizer on disintegration of *A. flavus* cells, to find optimal parameters of homogenization and prepare biologically active extracts which can be used for determination of possible strategies for control of contamination with aflatoxins. Results of study showed that bead mill homogenizer Omni® Bead Ruptor 12 Homogenizer can be applied for disintegration of *A. flavus* mycelia and preparation of enzymatically active cell-free extracts. The homogenization mixture in 2 mL homogenization tubes should contain 100 mg of fresh wet mycelia, 1 g of precooled acid washed glass beads of 0.5 mm in diameter and 1 mL of ice-cold buffer. Such mixture should be homogenized at speed of 6 m/s during 120 s, in six cycles of 20 s with cooling of samples in ice-bath between cycles.

Keywords: bead mill, disintegration, *Aspergillus flavus*, catalase, proteins, aflatoxins

Introduction

Saprotrophic mycotoxigenic fungus *Aspergillus flavus* is producer of extremely toxic secondary metabolite - aflatoxin B_1 (AFT B_1), one of the dangerous known natural hepatocarcinogen (IARC, 1993; 2002; 2012), and opportunistic pathogen of crops, animals and humans (Klich, 2007; Šarkanj et al., 2018a). This fungus is continually the subject of scientific research, since its discovery, especially due to health and economic challenges. Moreover, it is affected by global climate changes factors (Battilani, 2016; Helfer, 2014; Magan et al., 2007; Trnka et al., 2014), as temperature, drought stress and CO_2 concentration or new environmental pollutant, abiotic stressors, which causes oxidative status perturbations (Kovač et al., 2017; 2018a; 2018b; 2018c); *A. flavus* is sensitive on such stimuli (Jayashree and Subramanyam 2000; Narasaiah et al. 2006; Reverberi et al. 2010; Miskei et al., 2010; Reverberi et al., 2012; Hong et al. 2013) which results with changes in AFTs production. Moreover, besides aflatoxins (AFTs) production, impact of climate change on the emerging toxins is receiving increasing attention (Battilani, 2016; Abdallah et al., 2017; Kovač et al., 2019).

Above mentioned studies aimed on decrease of pre- and post-harvest contamination with AFTs, imply use and manipulation with intracellular content (Fountain et al., 2018; Šarkanj et al., 2018b; Kovač et al., 2018c), whichrequire application of certain disruption processes. Here stands out disruption of cells with bead mill homogenization, mechanical method of disruption effective for disintegration of filamentous fungi, such as *Aspergillus* spp. fungi. Method is based on intracellular components release due to circulating beads dispersed in homogenization mixture of mycelia and buffer (Doucha and Livansky, 2008). Despite to that, the most of literature data on this topic are about disruption of Gram-negative, Gram-positive bacteria and yeast (Klimek-Ochab et al., 2011). Furthermore, the same group of authors reported that bead milling disruption is not the best option for disruption of *A. fumigatus*. Therefore, the aim of this study is to found optimal parameters for disintegration of *A. flavus* cells by Omni® Bead Ruptor 12 Homogenizer and prepare biologically active extracts which can be used for research of possible AFTs contamination control strategies.

Materials and methods

A. flavus cultivation in yeast extract sucrose (YES) medium

Before *A. flavus* NRRL3251 growth in YES medium with 2% sucrose, stimulation of conidia production was performed by growth on potato dextrose agar during 168 hr, at 29 °C in dark.

*Corresponding author: tihomir.kovac@ptfos.hr

Fungal conidia suspension preparation ($2.5 \cdot 10^4$), inoculation of aflatoxin-inducing YES broth in 250 mL flasks, as well as incubation, were conducted at 29 °C (which favours aflatoxin production) according to Kovač et al. (2017).

For the incubation of the inoculated YES media flasks during 144 hr, the rotary shaker (KS 260 basic, IKA, Germany) settled at 200 rpm was used. After ending of the incubation period, samples of YES broth and *A. flavus* mycelia were collected from the flasks. Separation of mycelia from media was performed by filtration. For comparison of mycelia growth rate and aflatoxins production ability, six random samples were collected. All collected samples of mycelia and media were stored at in 2 mL tubes at −80 °C, for at least 24 hr before experiment. Additionally, mycelia portion was taken prior freezing and lyophilisation, and dried until constant mass (24 hr at 105 °C) in order to determine the dry mycelial weight.

After weighting and freezing of mycelia samples intended for comparison, lyophilisation was performed (Christ, Alpha 1-4 LD, Germany). Drying conditions were as described by Kovač et al. (2018) and as follows: freezing temperature −55 °C; temperature of sublimation −35 to 0 °C; vacuum level 0.22 mbar. The temperature of isothermal desorption varied from 0 to 22 °C under the vacuum of 0.06 mbar. Freeze-drying lasted until the constant mass of mycelia was obtained, which was approximately 5 hr. However, before freezing collected mycelia samples were homogenized by pestle and mortar, separated on portions and transferred into 2 mL tubes. Mycelia stored as this, as well as lyophilised mycelia was used for determination of catalase activity, proteins and aflatoxins content. Also, YES media separated from six samples intended for comparison was subjected to an analysis of aflatoxin content.

A. flavus extract preparation

The extracts of *A. flavus* mycelia were prepared by disintegration of mycelia by homogenization using Omni® Bead Ruptor 12 Homogenizer (Omni International, Kennesaw GA, USA). Disintegration was performed at 2.1, 4 and 6 m/s during 20, 40, 60, 120, 180 and 240 s with sample cooling on ice after every 20 s of disruption. In 2 mL disintegration tubes, 0.1 g of mycelia with 1 g of glass beads (acid washed and precooled; diameter 0.5 mm; Sigma-Aldrich, Germany) and ice-cold extraction buffer (50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA-2Na) was added. Such content of disintegration mixture was previously experimentally determined (Kovač et al., 2017) and is selected according to tubes and device manufacturer technical recommendation. After disintegration process, extracts were clarified by centrifugation (15000 x *g* /4 °C /20 min) at centrifuge Thermo Scientific SL 8R (Thermo ScientificTM, Finland). The prepared extracts were immediately used for analysis with the aim of optimal parameters of described disintegration process determination.

Analysis of catalase activities and protein concentration in extracts

For determination of catalase (CAT; EC 1.11.1.6) activity, spectrophotometric measurements of the decrease in absorbance at 240 nm, due to H_2O_2 decomposition in the presence of CAT, according to Reverberi et al. (2005) were performed.

Concentration of proteins in extracts was determined by Bradford assay according to Bradford (1976). As a standard, bovine serum albumin was used.

Analysis of aflatoxins content in mycelia extracts and YES growth media

Content of aflatoxins (AFTs) in prepared mycelia extracts and separated YES media samples were estimated by *dilute and shoot* LC-MS/MS method described by Kovač et al. (2017). Separation was performed using an Acquity UPLC H-Class system (Waters, MA, USA) on Acquity BEH C18 column (2.1 x 100 mm, 1.7 μm) (Waters, USA), while detection and quantification were performed using a Xevo TQD mass spectrometer (Waters, USA). MassLynx and TargetLynx software (v. 4.1., Waters, USA) was used for data acquiring and processing. Recovery was 92% for all aflatoxins and was assessed by spiking blank YES medium with aflatoxin standard mix (Biopure, Austria) solution at a concentration of 10 ng/mL. Instrumental limits of detection were 0.15 ng/mL, while limits of quantification were 0.5 ng/mL, for all aflatoxins.

Mycelial extracts used for aflatoxin content determination were prepared as described above, but instead of extraction 20% acetonitrile (HPLC grade) solution was used.

Statistical analysis

All data presented here are expressed as the mean value ± SEM. Shapiro-Wilk test was used for normality distribution checking of pooled datasets which were compared by nonparametric statistics methods (Friedman ANOVA and Kendall coefficient of concordance; Kruskal-Wallis ANOVA). The Statistica 13.3 programme package (TIBCO Software Inc, Palo Alto, CA, USA) was used and differences were considered significant when the p value was < 0.05. For the drawing

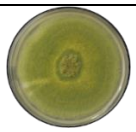
of the Sankey diagrams, Flourish studio was used (Flourish Studio, Kiln Enterprises Ltd, London, UK).

Results and discussion

A. flavus growth and aflatoxin production

At first step of the experiment, *A. flavus* growth rate and aflatoxins production ability was determined. From the all inoculated flasks, six of them were excluded after 144 hr, at the end of the growth period. All actions regarding conidia suspension preparation, inoculation of aflatoxin-inducing YES broth, as well as growth during incubation period, resulted by the pattern typical for tested fungi (Table 1), as previously showed (Kovač et al., 2017; 2018a; 2018b). The results for aflatoxin content are expressed as the sum of mycelial and medial concentrations, as well as the sum of AFT B_1 and B_2, which were detected, where AFT B_1 contributed with more than 99% in the total sum.

Table 1. *Aspergillus flavus* NRRL 3251 growth rate and aflatoxin production ability

 ***A. flavus* NRRL 3251**	**Aflatoxin sum (B_1 and B_2) [ng/mL]**	Mycelia	YES media	Σ
		4.04 ± 0.37	4.39 ± 0.27	8.42 ± 0.64
	Mycelia growth rate [g $_{d.m.w.}$ /50 mL]	0.167 ± 0.04		

Impact of bead mill homogenization speed and time on catalase activity, protein content and aflatoxins concentrations in A. flavus mycelia extracts

The *A. flavus* mycelia extracts prepared under different speed and time of homogenization at bead mill Omni® Bead Ruptor 12 Homogenizer were subjected to determination of the catalase activity, protein and aflatoxin concentrations (Fig. 1-3).

The results of catalase activity in prepared extracts showed proportional dependence to the speed and time of the homogenization (Fig. 1). There was observed homogenization speed-dependent catalase activity, at the lowest rate in the extract prepared at 2.1 m/s and at the highest rate in the extracts prepared at 6 m/s. Statistically significant difference ($p < 0.02$) between this two homogenization speed was determined at all homogenization times, except for 40 s and 180 s of homogenization. Such results obtained for catalase activity imply that optimal homogenization speed for mycelia extract preparation is 6 m/s. When the homogenization time was examined, activity was increased with increase of time of homogenization. However, results showed that maximum activity was achieved at 120 s of homogenization, both at speed of 4 and 6 m/s (Fig. 1). At the applied speed of 6 m/s, almost double catalase activity was determined.

The results of protein content in the prepared extracts of *A. flavus* mycelia showed proportional dependence on the speed and time of the homogenization (Fig. 2), as it was determined in the case of catalase activity (Fig. 1). There is an observed speed-dependent protein release in extracts, at the lowest rate in the extract prepared at 2.1 m/s and at the highest rate in the extracts prepared at 6 m/s. Statistically significant difference between ($p < 0.03$) this two homogenization speed, visible in released protein content, is determined until 120 s of homogenization.

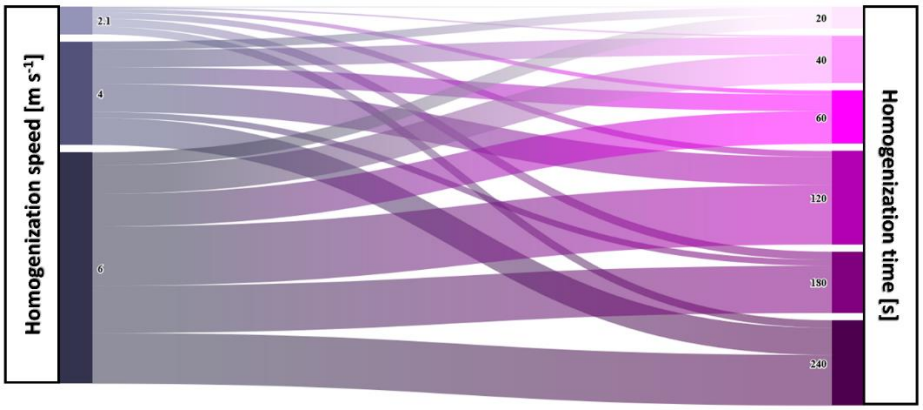


Fig. 1. Catalase activity (U/mL) in *A. flavus* NRRL 3251 extracts prepared by bead mill disintegration at different homogenization speed (● – 2.1, ● – 4 and ● – 6 m/s) and time-points (○ – 20, ○ – 40, ● – 60, ● – 120, ● – 180 and ● – 240 s). The line thickness represents the quantitative value of the catalase activity

Such results of protein content imply that optimal homogenization speed for mycelia extract preparation is 6 m/s. In the case of optimal homogenization time, trend of increase of protein content is observed until 120 s of homogenization, it can be said both for speed of 4 and 6 m/s. However, at the applied speed of 6 m/s, there was higher protein content determined in extracts (Fig. 2).

The release of aflatoxins (sum of AFT B_1 and B_2) from mycelia into extracts during optimization of mycelia homogenization process is showed in Fig. 3. There were no observed statistically significant difference ($p > 0.05$) between applied speed and time of homogenization. Accordingly, all the applied conditions resulted with release of aflatoxin at satisfying level.

A. flavus is the most important mycotoxigenic fungus which is since its discovery subject of numerous scientific researches (Kovač et al., 2018b). The most of them imply use and manipulation with intracellular content (Fountain et al., 2018; Šarkanj et al., 2018b; Kovač et al., 2018c), which require application of certain disruption processes.

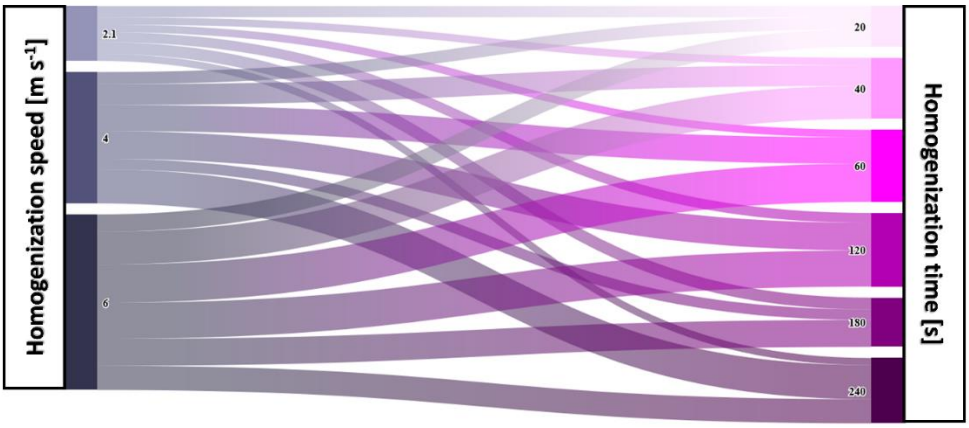


Fig. 2. Protein content (mg/mL) in *A. flavus* NRRL 3251 extracts prepared by bead mill disintegration at different homogenization speed (● – 2.1, ● – 4 and ● – 6 m/s) and time-points (○ – 20, ● – 40, ● – 60, ● – 120, ● – 180 and ● – 240 s). The line thickness represents the quantitative value of the protein content

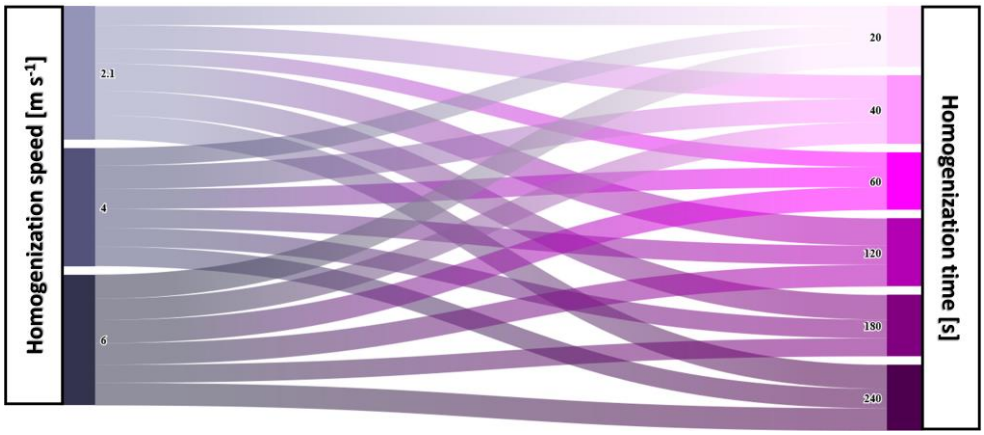


Fig. 3. Sum of aflatoxin B_1 and B_2 (ng/mL) in *A. flavus* NRRL 3251 extracts prepared by bead mill disintegration at different homogenization speed (● – 2.1, ● – 4 and ● – 6 m/s) and time-points (○ – 20, ● – 40, ● – 60, ● – 120, ● – 180 and ● – 240 s). The line thickness represents the quantitative value of the sum of aflatoxin B_1 and B_2

In this case study, bead mill homogenization was applied on cell wall disintegration and activity of catalase, release of proteins and aflatoxins in crude extracts were monitored for optimal homogenization speed and time selection. The main aim of the study was to adjust particular bead mill homogenizer,

Omni® Bead Ruptor 12 Homogenizer, to a particular strain, aflatoxigenic *A. flavus* NRRL 3251. It is known that fungal cell wall is the main factor that regulates retention of intracellular content. This is highly dynamic structure of specific composition and mechanical properties that vary between genera, even between closely related species (Durán and Nombela, 2004; Adams, 2004; Damweld, 2005; Bowman and Free, 2006; Free, 2013). Also, results presented in this study confirms that homogenization method efficiency is dependent on the particular intracellular molecule of interest location behind the fungal cell wall (Fig. 1-3).

Bead mill homogenization process efficiency can be comparable with efficiency of disintegration processes where sonification is applied, at least in the case of *Aspergillus* spp. (Klimek-Ochab et al., 2011). However, at sonification, time of exposure is critical parameter which affects intracellular compounds activity. Furthermore, the most ultrasound energy absorbed by cell suspension is appearing as a heat which also has certain effect on biological material. According to that, and based on previously reported studies (Kovač et. al., 2015; 2016; Šarkanj 2018b), assumption about bead mill homogenization method as a less time and energy consumption and more effective, at the same time, which is confirmed in this study. Despite to that, crude extract overheating still need to be prevented by sample cooling in ice-bath between cycles of homogenization.

Conclusion

To sum up, the bead mill homogenizer Omni® Bead Ruptor 12 Homogenizer can be applied for disintegration of *Aspergillus flavus* mycelia and preparation of enzymatically active cell-free extracts. It can be achieved when 100 mg of mycelia, 1 g of precooled acid washed glass beads of 0.5 mm in diameter and 1 mL of ice-cold buffer is added into homogenization tubes of 2 mL. Such mixture should be homogenized at speed of 6 m/s during 120 s, in six cycles of 20 s with cooling of samples in ice-bath between cycles.

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POMOLOGICAL PROPERTIES AND POLYPHENOL CONTENT OF CONVENTIONAL AND TRADITIONAL APPLE CULTIVARS FROM CROATIA

Ante Lončarić[1*], Martina Skendrović Babojelić[2], Tihomir Kovač[1], Bojan Šarkanj[3]

[1]Josip Juraj Strossmayer Univerity of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia
[2]University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia
[3]University North, University center Koprivnica, Department of Food Technology, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia

original scientific paper

Summary

Pomological properties and polyphenol content of six traditional and six conventional apple varieties from Croatia were studied. The highest fruit weight, height and width were measured in conventional apple cultivar, 'Red Delicious', and the lowest in traditional apple cultivar, 'Adamčica'. The highest content of soluble dry matter was measured in 'Zlatna Zimska Parmenka' (16.70%) and the lowest in 'Mutsu' (11.30%). The pH (3.90) value was highest in 'Red Delicious' and the lowest (3.12) in 'Zlatna Zimska Parmenka'. Total polyphenol content and antioxidant activity were on the highest level in traditional apple cultivar, 'Adamčica' 499.58 mg/100 g FW and 418.83 mmol trolox/L, respectively. Traditional apple varieties grown in local areas have so far been largely unexplored considering pomological and physicochemical properties. However, they might represent an important source of bioactive compounds and constitute the basis for further breeding. This research showed that traditional apple varieties are rich in polyphenols and have high antioxidant activity even higher than those found in conventional varieties. Due to the diversity of pomological properties and polyphenol content, it is important to preserve traditional apple varieties as a source of genetic variability as well as a factor of biodiversity of the area where they grow.

Keywords: traditional apple cultivars, pomological properties, polyphenols, antioxidant activity

Introduction

Apples have been grown for many centuries what is described in early legends, poems, and religious books. Current apple cultivars are developed from *Malus pumila* which originates from southwestern Asia (Cornille et al., 2012). From this cultivar, apple has been developed in many different varieties, with different sensory and organoleptic properties. Today the apple is one of the widely grown fruits with annual world production of 83 million metric tons in 2017 (FAOSTAT). Many apple cultiars have been developed over time but only some are grown for conventional use. The range of apple cultivars in the European market has been significantly reduced to no more than 12 cultivars (Jemrić et al., 2013). Planting only a small range of apple cultivars could endanger the biodiversity and lead to worldwide epidemics of certain pest and pathogens (Šavkin et al., 2014). Some of the most grown conventional apple cultivars are 'Idared', 'Jonagold', 'Golden Delicious', 'Red Delicious', 'Granny Smith' and 'Mutsu'. Traditional apple varieties are mostly cultivated in individual orchards, mainly in the marginal areas, and they show a good adaptability to the local environment and represent a valuable source for the crop genetic variability. Many traditional apple varieties carry genes for resistance to pests and diseases, drought tolerance, winter hardiness and unique fruit quality.

One of the first determinants of apples is pomological characterization. Defining pomological properties represent the basis for future scientific research work on the standardization of autochthonous genetic pool and creation of new cultivars adapted to given conditions with certain resistance and predisposition for the commercial and ecological production (Salkić et al., 2017). Traditional apples are not represented on the global market mainly because they usually do not meet some of the appearance standards (Šavkin et al., 2014). However, some of the earlier studies showed that traditional cultivars are more nutritious than newer cultivars (Jakobek et al., 2013; Balík et al., 2012; Donno et al., 2012; Iacopini et al., 2010). Apples are generally considered "healthy food", and like in other fruits and vegetables, polyphenols are one of the main compounds that are considered to have a positive impact on health. Polyphenols has several positive effects like their anticancerogenic properties, prevention of cardiovascular diseases and cancer, regulation of plasma cholesterol metabolism, antiviral properties, inhibition of *Helicobacter pylory* growth and staphylococcal enterotoxin A toxicity (Jakobek and Baron, 2016; Hyson, 2011; Valdenegro et al., 2010; Boyer and Liu, 2004). Studies showed that phytochemical composition of apples varies greatly between different apples varieties (Panzella et al., 2013), during the maturation and ripening (Kevers et al., 2011; Mainla et al., 2011) and even within different apple parts (Lončarić and Piližota, 2014;

*Corresponding author: ante.loncaric@ptfos.hr

Lončarić et al., 2014). There are plenty of data on polyphenol content in apples; however they are often confined to a few cultivars. One of the more comprehensive evaluations of the polyphenol content and profile of 104 European apple varieties was conducted by Ceymann et al. (2012). Regarding the traditional apple cultivars in Balkan there are a few studies dealing with polyphenol content and antioxidant activity (Jakobek and Barron, 2016; Šavikin et al., 2014).

Due to the insufficient information in the literature, the aim of this study was to compare six conventional and six traditional apple varieties regarding their pomological properties, polyphenol content and antioxidant activity. New knowledge in traditional apple cultivars can help with diversification of the apple market, preventing potential disappearance of these cultivars and enabling the preservation of apple biodiversity.

Materials and methods

Apples Used for Experiment

Conventional apple cultivars, 'Idared', 'Jonagold', 'Golden Delicious', 'Red Delicious', 'Granny Smith' and 'Mutsu' were purchased from local market in Osijek and the traditional apple cultivars, 'Lijepocvjetka', 'Bobovec', 'Adamčica', 'Zlatna Zimska Parmenka', 'Božićnica' and 'Kanadska Reneta' were have been collected from OPG Horvatić, Cvetkovac, 48312 Rasinja. All studied apples are presented in Fig. 1.

Fig. 1. Conventional (first row) and traditional apple cultivars (second row)

Pomological Properties

The pomological determination has been carried out at Faculty of Agriculture of the University of Zagreb, where only healthy fruits were separated and then photographed and analyzed. The analysis of pomological properties (weight, height and width of fruit, number and mass of seeds) was performed on 5 fruits of each variety. The weight of the fruit was determined by a digital two-decimal laboratory scale (OHAUS Corporation, USA) and expressed in grams (g). The height (V) and the width (s) of the fruit are measured by the digital displacement meter (Somet, Czech Republic), and the values are expressed in millimeters (mm). From the height and width data obtained, the fruit shape index is represented by the ratio of height:width. The number of the healthy seeds from the cross-section of the apples was determined. The weight of the healthy seeds was determined by the four-decimal analytical scales (KERN® Analytical balance AES-C / AEJ-CM) expressed in miligrams (mg). The content of soluble solids of apples was measured with a table top Abbe refractometer and given in Brix (°Brix). pH was measured with table top pH meter (Mettler–Toledo GmbH, Giessen/D).

Determination of Total Polyphenol Content

The total phenols content was determined by the modified colorimetric Folin-Ciocalteu method (Lončarić et al., 2014). A 0.6 mL of apple extract was mixed with 3 mL of Folin-Ciocalteu reagent (1:10) and 2.4 mL of 7.5% of sodium carbonate (Na_2CO_3) solution in the test tubes. The colour was developed during 120 min, and the absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, UK). The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as mg of gallic acid per 100 g of sample equivalents, (mg GA/100 g) of fresh weight (FW) sample. Gallic acid calibration solutions were prepared in 5 point range from 0 to 500 mg of gallic acid per 100 g of solution.

Antioxidant Activity Determination

Antioxidant activity was measured by using DPPH method; 0.2 mL of the apple extract was diluted with methanol (2 mL), and 1 mL of DPPH solution (0.5 mM) was added. After 15 min the absorbance was measured at 517 nm. The results were expressed as mmol trolox equivalents/100 mL of sample. Additional dilution was needed if the measured DPPH value was over the linear range of the standard curve (Lončarić et al., 2014).

Statistical analysis

All measurements were done in at least triplicate and data were expressed as mean ± standard deviation. Normal distribution and homogeneity of cultivars for the experimental data were established with Shapiro-Wilkovim and Levenovim testom after which the experimental data were subjected to a one-way analysis of variance (ANOVA). Fisher's LSD was calculated to detect significant difference ($p \leq 0.05$) between the mean values within each group (traditional and conventional) separately. MS Excel (StatPlus, AnalystSoft Inc.) statistical program was used for statistical analysis. Pearson's correlation coefficient was calculated using Microsoft Excel 2016 (StatPlus, AnalystSoft Inc.) in order to determine correlation between total phenol content and antioxidant activity in conventional and traditional apple cultivars.

Results and discussion

Fruit quality

Market criterion for the first class fruit regarding the weight is from 160 to 180 g (Salkić et al., 2017). From the results presented in Table 1, it can be seen that all conventional apples belongs to the first class fruits. Regarding the weight of traditional apple cultivars, only 'Lijepocvijetka' and 'Kandaska Reneta' belongs to the first class fruits (Table 2). The fruit weight of traditional apple cultivars was in agreement with the results obtained by other studies (Jemrić et al., 2013; Balík et al., 2012; Mratinić and Fotirić-Akšić, 2012). The cultivars 'Red Delicious' and 'Gold Delicious' had highest fruit height 81.64 mm and 81.25 mm, respectively with no difference between those two cultivars (Table 1). 'Kanadska Reneta' had the highest fruit height (66.00 mm) regarding the traditional apple cultivars (Table 2). There was significant difference ($p < 0.005$) comparing the fruit height between conventional and traditional apple cultivars indicating that traditional varieties do not have an attractive appearance which puts them in the other plan at choice of customers.

Table 1. Pomological property of conventional apple cultivars

Apple cultivars	'Idared'	'Jonagold'	'Golden Delicious'	'Red Delicious'	'Granny Smith'	'Mutsu'	Average
Fruit weight (g)	212.53 ± 4.55^{b}	213.92 ± 14.81^{bc}	248.49 ± 17.38^{b}	352.85 ± 35.48^{a}	205.64 ± 6.96^{c}	246.68 ± 25.91^{b}	264.69 ± 54.58
Fruit height (mm)	68.40 ± 2.52^{b}	68.67 ± 0.80^{b}	81.25 ± 3.73^{a}	81.64 ± 6.02^{a}	70.18 ± 1.46^{b}	73.87 ± 5.55^{b}	74.00 ± 6.58
Fruit width (mm)	82.18 ± 2.36^{b}	79.33 ± 4.18^{b}	82.67 ± 3.42^{b}	97.51 ± 4.84^{a}	77.95 ± 2.50^{b}	79.83 ± 1.85^{b}	83.24 ± 7.34
Fruit shape index	0.83 ± 0.04^{c}	0.87 ± 0.04^{bc}	0.98 ± 0.05^{a}	0.84 ± 0.08^{bc}	0.90 ± 0.03^{abc}	0.92 ± 0.05^{ab}	0.89 ± 0.07
Total number of seeds	10.00 ± 1.00^{b}	7.00 ± 1.00^{c}	8.00 ± 1.00^{bc}	7.67 ± 0.58^{bc}	12.67 ± 2.52^{a}	7.00 ± 1.00^{c}	8.72 ± 2.37
Number of non-healthy seeds	4.67 ± 0.58^{ab}	4.00 ± 1.00^{bc}	5.67 ± 0.58^{a}	4.33 ± 1.15^{abc}	3.00 ± 1.00^{c}	4.33 ± 0.58^{abc}	4.33 ± 1.08
Number of healthy seeds	5.33^{b}	3.00^{c}	2.33^{c}	3.33^{bc}	9.67^{a}	2.67^{c}	4.39 ± 2.79
Mass of healthy seeds (mg)	350 ± 80^{ab}	160 ± 60^{b}	160 ± 40^{b}	220 ± 70^{ab}	550 ± 80^{a}	100 ± 40^{c}	260 ± 160
Mass of one seed (mg)	70 ± 00^{a}	50 ± 00^{c}	70 ± 10^{ab}	70 ± 10^{ab}	60 ± 0.00^{bc}	40 ± 10^{d}	60 ± 10

Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD)

Table 2. Pomological property of traditional apple cultivars

Apple cultivar	'Lijepocvjetka'	'Bobovec'	'Adamčica'	'Zlatna Zimska Parmenka'	'Božićnica'	'Kanadska Reneta'	Average
Fruit weight (g)	179.13 ± 33.15^{b}	104.57 ± 1.90^{c}	95.60 ± 0.00^{c}	116.59 ± 45.64^{bc}	155.52 ± 46.73^{bc}	293.01 ± 13.81^{a}	157.64 ± 68.95
Fruit height (mm)	64.18 ± 3.45^{ab}	54.13 ± 0.06^{bc}	43.81 ± 0.00^{d}	52.18 ± 7.07^{cd}	52.33 ± 4.50^{cd}	66.00 ± 4.55^{a}	55.26 ± 8.74
Fruit width (mm)	75.51 ± 4.78^{bc}	63.37 ± 1.11^{c}	63.27 ± 0.01^{c}	64.98 ± 8.08^{c}	79.31 ± 7.90^{ab}	90.67 ± 0.34^{a}	72.99 ± 10.74
Fruit shape index	0.85 ± 0.01^{ab}	0.85 ± 0.01^{a}	0.69 ± 0.00^{cd}	0.80 ± 0.02^{b}	0.66 ± 0.00^{d}	0.73 ± 0.01^{c}	0.76 ± 0.07
Total number of seeds	8.00 ± 1.41^{b}	13.50 ± 2.12^{a}	8.00 ± 0.00^{b}	9.50 ± 0.71^{b}	8.00 ± 0.00^{b}	8.00 ± 0.00^{b}	9.85 ± 2.60
Number of non-healthy seeds	2.00 ± 0.00^{b}	4.00 ± 1.41^{a}	0.00^{c}	3.50 ± 0.54^{ab}	3.00 ± 2.82^{ab}	3.00 ± 2.83^{ab}	2.65 ± 2.12
Number of healthy seeds	6.00^{a}	9.50^{a}	8.00^{a}	6.00^{a}	5.00^{a}	5.00^{a}	7.18 ± 2.40^{a}
Mass of healthy seeds (mg)	380 ± 40^{a}	330 ± 40^{a}	350 ± 10^{a}	70 ± 40^{b}	250 ± 20^{a}	260 ± 110^{a}	334 ± 118
Mass of one seed (mg)	60 ± 10^{a}	30 ± 0.00^{b}	40 ± 00^{ab}	50 ± 20^{a}	50 ± 10^{a}	50 ± 10^{a}	48 ± 11

Each value is expressed as mean ± standard deviation (n = 3). Within the same row, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD)

Fruit width as well as axis height is important from the agronomic and market point of view (Salkić et al., 2017). The largest fruit width had conventional apple cultivar 'Red Delicious' (97.51 mm) followed by traditional apple cultivar 'Kanadska Reneta' (90.67 mm). Generally traditional apple cultivars have smaller fruit width compared to the conventional apple cultivars. Based on fruit width and criteria in the European market (65-90 mm first class (Salkić et al., 2017) it can be concluded that most of the investigated cultivars, except 'Bobovec and 'Adamčica', belong to the first class fruits. The fruit shape index is represented by the ratio of height and width and it was used for detection of the fruit shape by the following scale; less than or equal to 0.90 - flattened fruit, from 0.90 - 1.00 elliptical fruit and 1.0 and higher elongated fruit (Salkić et al., 2017). In our study fruit index ranged from 0.66 – 0.90 for almost all studied apples whereby they can be classified as flattened fruit except 'Golden Delicious' (0.98) and 'Mutsu' (0.92) which have elliptical shape. Investigation of apple seeds showed that traditional apple cultivars had healthier seeds compared to the conventional apple cultivars (Tables 1 and 2). The highest total soluble solids (TSS) and lowest pH of all investigated cultivars had traditional apple cultivar 'Zlatna Zimska Parmenka'. Generally the traditional apple cultivars had higher total soluble solids 14.45% comparing to the conventional 12.92% and lower pH 3.19 compared to conventional apple cultivars 3.55 (Fig. 2). Both observed parameters are in the range of those reported for other conventional and traditional apple cultivars (Piegentini and Pirovani, 2017; Lončarić and Piližota, 2014; Jha et al., 201).

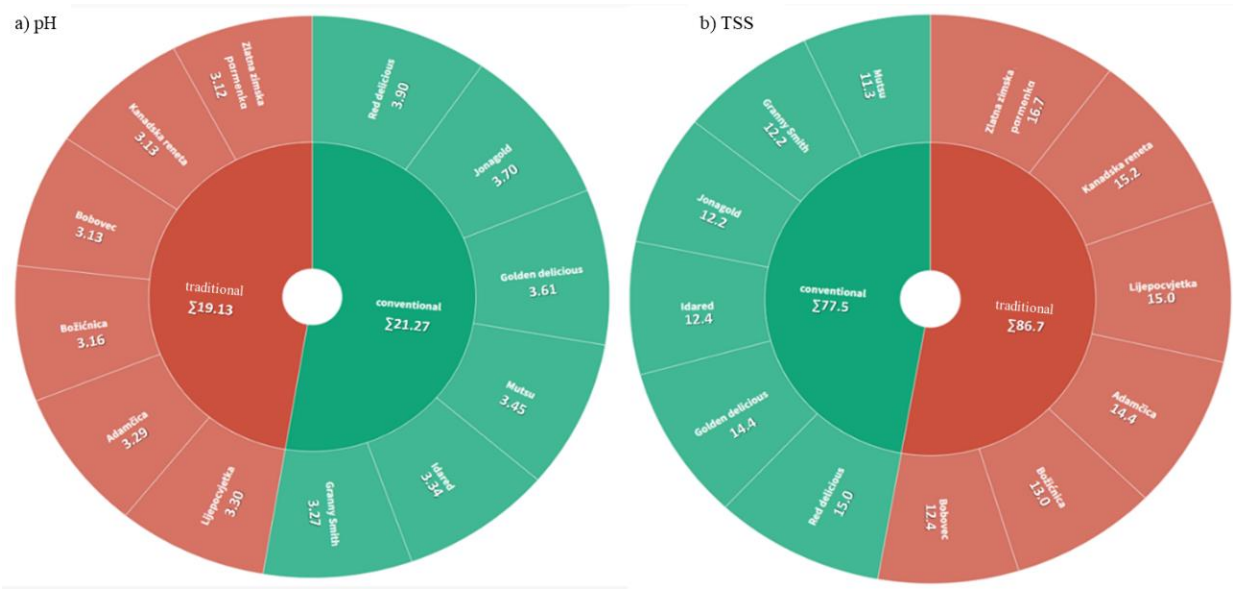

Fig. 2. Total soluble solids (b - TSS) and a - pH of activity of traditional and conventional apple cultivars

The total polyphenol content and antioxidant activity

The polyphenol content of fruits and vegetables depends on a number of intrinsic (genus, species, and cultivar) and extrinsic (agronomic, environmental, handling and storage) factors (Khanizadeh et al., 2007; Qing et al., 2007). Many studies pointed that intrinsic factors affects much more the polyphenol content of apples than extrinsic factors (Ceymann et al., 2012; Neveu et al., 2010; Wojdyło et al., 2008; Matthes and Schmitz-Eiberger, 2008; Łata, 2007; Łata and Tomala, 2007; McGhie et al., 2005; Kondo et al., 2002). The reasons for determination the total phenol content and antioxidant activity of traditional apple varieties was to enable the recognition of those varieties as a valuable source of polyphenols.

Table 3. Total phenol content and antioxidant activity of traditional and conventional apple cultivars

Apple cultivar	TPC (mg GA/100 g FW)	AOA (mmol trolox /100 mL)
Idared	160.59 ± 6.62^{cd}	384.22 ± 1.11^{b}
Jonagold	169.07 ± 1.83^{c}	398.74 ± 2.75^{a}
Golden Delicious	187.08 ± 5.50^{b}	370.91 ± 6.26^{b}
Red Delicious	252.75 ± 3.67^{a}	336.07 ± 13.74^{c}
Granny Smith	155.30 ± 8.41^{d}	398.98 ± 3.84^{a}
Mutsu	139.41 ± 3.18^{e}	373.09 ± 0.42^{b}
Lijepocvjetka	260.17 ± 0.00^{f}	402.37 ± 2.75^{b}
Bobovec	289.83 ± 3.67^{e}	368.01 ± 6.17^{d}
Adamčica	499.58 ± 8.00^{a}	418.83 ± 2.75^{a}
Zlatna Zimska Parmenka	387.29 ± 6.36^{c}	391.97 ± 1.51^{c}
Božićnica	402.12 ± 1.83^{b}	407.70 ± 8.08^{b}
Kanadska Reneta	330.08 ± 3.18^{d}	389.79 ± 3.27^{c}

Each value is expressed as mean ± standard deviation (n = 3). Within the same column, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD)

Total phenol content (TPC) of convention apples were as in the previously reported range (Kschonsek et al., 2018; Piagentini et al., 2017; Ceymann et al., 2012; Drogoudi et al., 2008). The highest TPC have 'Red Delicious' followed by 'Golden Delicious', 'Jonagold', 'Idared', 'Granny Smith' and 'Mutsu', respectively (Table 3). Determination of total phenol content of traditional apple cultivars showed that all traditional apple cultivars have higher content of total polyphenols compared to conventional apple cultivars. The highest TPC of traditional apple cultivars have 'Adamčica' followed by 'Božićnica', 'Zlatna Zimska Parmenka', 'Kanadska Reneta', 'Bobovec' and 'Lijepocvjetka', respectively. The highest antioxidant activity was measured in traditional apple cultivar 'Adamčica' 418.83 mmol trolox /100 mL and the lowest in 'Red Delicious' 336.07 mmol trolox /100 mL. Person's correlation coefficient showed that there is no significant correlation between total phenol content and antioxidant activity in conventional apple cultivars (r = -0.806, $p < 0.05$). However, correlation between total phenol content and antioxidant activity was found in traditional apple cultivars (r = 0.667, $p < 0.05$).

Conclusion

The results of pomological properties showed that all conventional apple cultivars belong to the first class fruits. Regarding the traditional apple cultivars, results showed that they do not have satisfactory appearance except 'Kanadska Reneta'. Despite to that, traditional apple cultivars are rich in polyphenols and have high antioxidant activity; even higher than those found in conventional varieties. The results showed that due to the diversity of pomological characteristics and polyphenol content, traditional apple varieties represent a good source of genetic variability as well as a factor of biodiversity of the area where they are grown. With satisfactory appearance, high total polyphenol content and antioxidant activity 'Kanadska Reneta' might be suitable cultivar for selective growth in order to produce varieties with higher content of bioactive compounds and preservation of biodiversity of M. pulmila.

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THE GENERAL NUTRITION KNOWLEDGE OF PROFESSIONAL ATHLETES

Ivan Miškulin[1*], Anja Šašvari[2], Albina Dumić[1], Vesna Bilić-Kirin[1], Željko Špiranović[2], Nika Pavlović[1], Maja Miškulin[1]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine Osijek, Josipa Huttlera 4, Osijek, Croatia
[2]Health institution dr. Spiranovic for occupational and sports medicine, Stjepana Radića 54, Osijek, Croatia

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Summary

Proper nutrition and nutrition knowledge are necessary for health benefits both for general population and athletes, professional and non-professional ones. Of course, proper nutrition is not the same for all populations and should be adapted depending on gender, age, physical activity, health status and other needs. The physical condition, training and athletes' success also depend on their diet. The aim of this study was to examine nutrition knowledge of professional athletes and compare it with the nutrition knowledge of their peers not professionally engaged in sports. This cross-sectional study was conducted with a specially designed anonymous questionnaire that was distributed to 211 participants (110 professional athletes and 101 non-athletes) by a specialist of occupational and sports medicine in Osijek, Eastern Croatia during September 2017. The median age of the participants was 20.0 years. There were 74.4% males and 25.6% females. The overall proportion of accurate answers among all participants was 27.6% with no statistically significant differences between professional and non-professional athletes as well as between females and males (p=0.584 and p=0.904, respectively). Likewise, there were no statistically significant differences in proportion of accurate answers regarding to educational level, socioeconomic status and body mass index (p=0.547, p=0.491, p=0.459, respectively). One participant (0.5%) had good nutritional knowledge, 9 (4.2%) had medium nutritional knowledge while most of the participants, 202 (95.3%), had poor nutritional knowledge with no statistically significant differences compared to whether they were professional athletes or not. According to the results in this study, the unsatisfactory level of nutrition knowledge is evident in both groups of participants. Obviously, additional education is needed how for professional athletes also as for non-professional ones.

Keywords: nutrition knowledge; professional athletes; non-professional athletes; proper nutrition; Eastern Croatia

Introduction

Nutrition, as one of the most important factors in training and exercise, affects athletes in many ways by playing a significant role in achieving and maintaining better health. Optimal nutrition can reduce fatigue and thus allow athlete longer training, competition and faster recovery between training. Also, it is important to emphasize how nutritional status of the athlete directly affects the level of physical efficiency (Sedek and Yun Yih, 2014; Arazi and Hosseini, 2012). Most athletes today realize how optimal nutrition is an important and integral part of the training program, but still most of them are not familiar with healthy nutrition practices (Spendlove et al., 2012). Nutrition of athletes is application of nutrition knowledge to a practical diet plan that would provide energy for physical activity, performing body processes, improving sports performance in competitions, and providing health and well-being. Unhealthy food habits not only affect competition performance but also negatively effect general health. Oppose to that, healthy eating habits provide energy needs and maintenance of body mass as well as body fat at a suitable level (Sedek and Yun Yih, 2014). Consumers should be familiar with the principles of proper nutrition and nutrition guidelines in order to achieve enough nutrition knowledge and to be able to apply it properly (Grunert et al., 2012). Inadequate dietary knowledge in combination with improper food intake is still present in many athletes (Abood, Black and Birnbaum, 2004; Fox et al., 2011; Rosenbloom, Jonnalagdda and Skinner, 2002). Proper nutrition as an element of athlete training, especially young ones, is necessary due to strong connections between nutritional deficiency, growth, development, injury prevention and sports performance (Petrie, Stover and Horswill, 2004). Recent studies compared athletes and non-athletes' nutrition knowledge and discovered how athletes have equal or slightly greater nutrition knowledge than non-athletes (Dragičević and Šatalić, 2015). A priority for athletes' nutrition is a satisfaction of daily energy needs. Energy balance is achieved when energy input (energy from food, liquids and supplements) becomes equal to the energy utilization. Basal metabolism, the thermal effect of food and the thermal effect of the body activity are the components of energy utilization (Poehlman, 1989). Recommended daily energy intake differ individually

*Corresponding author: ivan.miskulin@mefos.hr

and depends on gender, age, height, weight and physical activity during the day. In a negative energy balance, the fatty tissue and muscles serve as a source of energy. Lack of muscle mass will result in loss of strength and endurance, as well as compromised immunity, endocrine and musculoskeletal function (Burke, Loucks and Broad, 2006). Long-term reduced energy intake could lead to significant micronutrient deficiency that could cause metabolic dysfunction (Howarth et al., 2010). Ensuring adequate energy intake is a key step for all athletes who want to achieve optimal results. The first and most important condition for optimal exercise efficiency is an appropriate energy intake that will enable delivery of required fuels not only during exercise period, but also in periods of recovery (Kerksick, 2019). The main difference between athletes' nutrition and the nutrition of general population is that athletes require additional fluid to compensate loss of sweat as well as additional energy intake to perform physical activity. There is also a disproportion between the increased need for additional energy and the additional need for individual nutrients. As most of the extra energy comes from carbohydrates, it is highly recommended to intake food rich in these components (bread, cereals, milk, fruits, vegetables). The timing of nutrient intake is performed individually for each athlete, depending on his gastrointestinal characteristics and the intensity of the training. In extraordinarily hard training or more training sessions, athletes should eat three main meals and three snacks. For increasing muscle mass, eating at the end of the training is proposed and to have more than one afternoon snack as well as additional snack before bedtime. Utilization of maximum energy during training will be enabled by intake of 200-300g of carbohydrates 3-4 hours before training. The athletes diet before the competition must be individually adjusted. Athletes should experiment with different types of food and drink during the holiday season in order to find which food best suits them before training (Howarth et al., 2010). Optimal hydration is an important factor for athlete success. Opposite to that, dehydration is considered as liquid deficiency in the amount of 2-3% of body mass, and is responsible for compromising the performance of aerobic exercises while increasing the risk of potentially lethal heat states such as heat stroke. Athletes should therefore seek adequate fluid intake before, during and after the training or competition. It is important to consume the fluid long enough before the body activity allowing it to absorb and to achieve optimal hydration, along with excretion of excess urine fluid through the kidneys. Today it is no longer recommended taking too much fluid before training or so-called hyperhydration due to increasing the extracellular and intracellular spaces what significantly increases the risk of discharge during the competition and is not more beneficial than euhydration (Bemben and Lamont, 2005). The required amount of fluid for compensation and rehydration depends on the amount of individually extracted sweat, training duration as well as drinking conditions. During the extreme efforts, sport drinks are preferred because they contain electrolytes and carbohydrates which allow fluid replenishment and electrolyte balance. Drinks that contain potassium and sodium help in correcting electrolyte loss, stimulate thirst and kidney fluid retention (Groeneveld et al., 2005). The role of nutrition on growth, development and health is well known. To obtain better performance advantages, athletes often use various supplements like creatinine, caffeine and different multivitamins. Creatinine provides ergogenic effects while its long-term effects in young athletes are poorly investigated and that is why the American College of Sports Medicine Roundable states how "creatinine monohydrate is not recommended for children under 18 years of age". Caffeine on the other hand, has impact on performance endurance and vigilance while higher doses are associated with adverse effects. Also, given the differences in maturity, children are more vulnerable to the adverse effects of caffeine. Regarding multivitamins usage during a well-balanced diet applied in athletes, it is unlikely to have any additional benefits (Smith and Jeukendrup, 2013). The aim of this study was to evaluate the level of nutritional knowledge between young professional athletes and to determine whether there is a difference in knowledge compared to their peers, non-athletes.

Materials and methods

Methods and Participants

The cross-sectional study was conducted at the Department of Health Care Dr. Spiranovic for occupational health and sports in September 2017. From 212 volunteerely participants, 111 were professional athletes from the Osijek area, aged 19-24 and 101 participants also from the Osijek area, the same gender, age and level of education as well as professional athletes, with the condition that they are not professional athletes and that they have physical activity for up to 5 hours a week.

Participants have previously signed informed consent for participation in the study. The special anonymous

questionnaire was used as a research instrument. The questionnaire was taken from the previous study (Sedek and Yun Yih, 2014) and consisted from two parts. The first part of the questionnaire was with general data about the participant (age, gender, degree of education, economic status, body weight, body height, body mass indeks-BMI) while the second part measured nutrition knowledge so that participants had to choose one of the four offered answers. It took them about 10 minutes to complete the questionnaire. The Ethic Commitee of the Faculty of Medicine Osijek approved this study.

Statistical analysis

Category data were represented by absolute and relative frequencies. The differences between the category variables were tested by a $\chi 2$ test and if necessary also by Fisher's exact test. The numerical data were described by the median and the interquartile range. Normality of the distribution of numeric variables was tested by the Shapiro - Wilkinson test. Differences between numeric variables between the two independent groups were tested by Mann-Whitney U test and between three or more groups by Kruskal Wallis test (Ivanković et al., 1988). All p-values were two-sided. The level of significance was set to α (alpha) = 0.05. For statistical analysis, statistical software MedCalc Statistical Software version 17.8.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017) was used.

Results

The study included 212 participants, of which 111 (52.4%) were professional athletes while 101 (47.6%) participants were non-professionally engaged in sports. There were 83 (39.2%) those who were football players, 15 (7.1%) were basketball players, 8 (3.8%) were kickboxing players, (0.5%) were volleyball and archery players, one in each sport, while 3 (1.4%) were handball players. According to Fisher's Exact Test, men are more professionally engaged in sports 106 (95.0%) ($p<0.001$). According to educational level, 31 (15.0%) of participants have uncompleted or completed elementary school, 152 (73.0%) have completed secondary school, and 25 (12.0%) have completed college or faculty. A bit worse estimated economic status than the average was reported by 5 (2.0%) of the participants, average economic status by 118 (57.0%), slightly better than average by 75 of them (36.0%), while much better than the average was reported by 10 (5.0%) of participants (Table 1).

Table 1. Participants according to their basic features

	Number (%) of participants by sport mode			p
	Professional	**Unprofessional**	**Total**	
Gender				
Men	106 (95)	52 (51)	158 (75)	<0.001*
Women	5 (5)	49 (49)	54 (25)	
Total	111 (100)	101 (100)	212 (100)	
Level of education				
Uncompleted or completed elementary school	15 (14)	16 (16)	31 (15)	0.920†
Secondary school completed	81 (74)	71 (72)	152 (73)	
A completed college or faculty	13 (12)	12 (12)	25 (12)	
Total	109 (100)	99 (100)	208 (100)	
Estimated economic status				
A bit worse than the average	3 (3)	2 (2)	5 (2)	0.920†
Average	53 (49)	65 (66)	118 (57)	
A bit better than average	45 (41)	30 (30)	75 (36)	
A much better than the average	8 (7)	2 (2)	10 (5)	
Total	109 (100)	99 (100)	208 (100)	

*Fisher's exact test; †$\chi 2$ test

The median age of the participant was 21 years (interquartile range 19 to 24 years). Body height was considerably higher in subjects who were professionally engaged in sports, median 183 cm (interquartile range 178 cm to 188.3 cm) as well as body weight, median 80 kg (interquartile range 73 kg to 85 kg) ($p<0.001$) when compared to non-professional ones. The BMI was 23 kg/m^2 (interquartile range 21 kg/m^2 to 24.8 kg/m^2), without significant differences between the groups.

According to the BMI, we divided the participants into three groups: malnourished participants

(BMI<18.5), those with normal body weight (BMI 18.5-24.6); overweight participants (BMI 25.0-29.9) and obese participants (BMI 30.0 and above). There were 9 (4.0%) of the participants who were malnourished, 152 (75.0%) were with normal body weight, 38 (19.0%) were overweight and 5 (2.0%) of them were obese.

Nutrition knowledge

The fact that skipping the breakfast could negatively affect the sport performance is known to 139 (65.6%) of the participants, while only 13 (6.2%) of them knew how proteins are not the best and most efficient sources of energy. That nutrition affects mental performance 121 (57.3%) answered correctly, 118 (55.7%) that a meal should be eaten 3 to 4 hours before the performance while only 29 (13.9%) of the participants knew how calcium excretion grows with alcohol consumption. According to the pyramid of healthy diet, that 6-11 portions of bread, cereals, rice and pasta is not necessary to consume per day answered 55 (25.9%) of participants while 97 (45.8%) responded correctly how it is necessary to eat 2-4 fruit portions per day. The most accurate answers gave 158 (75.2%) of the participants about fact how breakfast consumption improves concentration, while 64 (30.6%) of the participants claimed how to much vitamin supplements may be poisonous. Participants showed poor knowledge about the fact how 60% of total calories should come from carbohydrates, 31 (14.9%), while the fact that it is necessary to eat 2-3 portions of dairy per day knew 39 (18.7%) of the participants. That carbohydrates are less dangerous for gaining weight than food rich in fat knew 38 (18.3%) of the participants.

That anemia is iron deficiency knew 105 (51.5%) of them while 74 (35.2%) of participants thought how athletes tend to consume twice as much protein as recommended. 64 (30.5%) of the participants confirmed how the best sources of iron are meat products and fish while 29 (14.3%) of the participants agreed about the average percentage of fat tissue in women to be 20-25%. Only 19 (9%) of the participants knew that excessive protein consumption is not useful for athletes.

The fact how dehydration does not mean thirstiness answered 65 (31.4%) of the participants and how cereals or breads enriched with iron should be eaten with vitamin C to improve the absorption of iron 35 (17.1%) of participants knew. Most participants, 80 (38.6%) of them knew how proteins work on repairing and building muscle tissues and on creating hormones to boost the immune system. The lowest number of participants, 24 (11.4%) of them knew how the recommended amount of fiber is 25 grams per day.

Of 29 statements, the mean value number of correct answers was 7 (interquartile range 5 to 10 exact answers) ranging from 0 to 24 exact answers, ie 24% of correct answers (interquartile range from 17% to 34% of correct answers), without significant differences between the groups (Table 2).

Table 2. Median number of correct answers by group

	Median (interquartile range) according to the mode of sport			**p***
	Professional	Unprofessional	Total	
Number of correct answers	8 (5 – 11)	7 (5 – 10)	7 (5 – 10)	0.750
Correct answers (%)	28 (17 – 38)	24 (17 – 34)	24 (17 – 34)	0.750

*Mann Whitney U test

Only one participant (0.5%) had good knowledge about nutrition while 9 (4.2%) participants had medium knowledge. Poor knowledge showed 202 (95.3%) of participants with no significant difference compared to whether they were in sports professionally or not (Table 3).

Table 3. Distribution of participants according to dietary knowledge in the relation to whether they are involved in sport professionally or not

Knowledge	**Number (%) of participants according to the mode of sport**			**p***
	Professional	Unprofessional	Total	
Good	1 (0.9)	0	1 (0.5)	>0.990
Medium	5 (4.5)	4 (4.0)	9 (4.2)	
Poor	105 (94.6)	97 (96.0)	202 (95.3)	
Total	111 (100)	101 (100)	212 (100)	

*Fisher's exact test

Discussion

Nutrition plays an important role in human health by affecting long-term health and due to the fact that it is a risk factor for chronic diseases. It can be influenced in order to improve the efficiency of exercise and training. Optimal health and sports nutrition strategy are today subject of numerous research. However, the recommendations may be controversial and may be misunderstood due to different opinions of the sports and fitness industry as well as too many articles and online materials that can provide unfounded claims (Furber, Roberts and Roberts, 2017). Many studies have shown how athletes often follow poor dietary habits that can jeopardize their sporting performance but, more importantly, their health (Sorić, Mišigoj-Duraković and Pedišić, 2006). One of the primary strategies for helping athletes to consume proper diet is to provide them a nutrition education. Although it is agreed how adequate nutrition knowledge does not always have to include appropriate dietary practice, it is claimed how even a small amount of nutritional knowledge is a key to healthier eating habits. Individuals who know more about nutrients have nearly twenty-five times more chance of satisfying the existing recommendations for fruits, vegetables, and fat intake than those who know less about it. Although athletes can prevent practical obstacles in applying appropriate nutritional strategies, there are also studies on limited athletes' knowledge about proper nutrition (Spendlove et al., 2012). Apart from the fact that proper nutrition improves the effectiveness of training and sports results, it also affects the athlete's health and helps to maintain the ability through sports career. Sports nutrition should be tailored to an individual athlete, so that it complies with the athletes age and gender as well as the specificity of the sports discipline, life habits and constitutional characteristics of athletes. It has also been shown how adequate nutrition also prevents injuries among athletes (Cigrovski et al., 2012). A recent review reported poorly positive correlation between nutritional knowledge of athletes and their nutritional intake (Folasire, Akomolafe and Sanusi, 2015). One participant (0.5%) showed good nutritional knowledge, 9 (4.2%) of the participants showed average nutritional knowledge while poor nutritional knowledge showed most of the participants 202 (95.3%), without any significant difference compared to whether they were in sports professionally or not. Dragičević and Šatalić (2015) also found similar results in their research, showing how the total knowledge of football players about proper nutrition was poor (42.6%) and was not significantly different from the nutritional knowledge of a group that was not professionally engaged in sports (44.8%). Often the difference in general nutritional knowledge between professional athletes and non-athletes usually cannot be noticed although the intakes differ. It is also not possible to notice differences in energy and nutrient intakes, distribution of macronutrients and quality of nutrition in general (Nikić et al., 2014). One study (Heaney et al., 2011) refers to seven different articles comparing nutrition knowledge of professional athletes from various sports with a group that is not professionally engaged in sports (Heaney et al., 2011; Barr, 1987; Collison, Kuczmarski and Vickery, 1996; Cupisti et al., 2002, Federick and Hawkins, 1992; Guinard et al., 1995; Raymond-Barker, Petroczi and Quested, 2007). Five of seven articles showed average results about nutrition knowledge but higher than 50%. Total nutrition score was equal or even greater than those in the comparison group. One study showed slightly higher result in professional athletes about proper nutrition (athletes 20%, comparative group 17%) and a slightly lower result on general nutrition knowledge (38% for athletes and 41% for comparative group, respectively). Three studies showed how athletes have significantly higher nutritional knowledge than the comparative group. Athletes also showed significantly better results than a comparative group in a questionnaire based on general nutrition. Heaney (2011) also summarizes 22 studies about nutrition knowledge in athletes only without a comparative group. Most of those studies (n=19) showed the results of 50-70%, while in three of them the results were 40-50% for proper nutritional knowledge. In this study, out of 29 statements, the median value of correct answers was 7 (interquartile range 5 to 10 exact answers) ranging from 0 to 24 exact answers, ie 24% of the correct answers (interquartile range 17% to 34% responses), without significant differences between the groups. Opposed to our research, Sedek and Yun Yih (2014) showed good general nutrition knowledge in most of the participants, without significant differences between gender and between the groups. They also mention Paugh's study which pointed how female runners showed better knowledge of proper nutrition than the male basketball players, and also Sowell's study which showed how women who do not practice professional sport have greater nutritional knowledge than men who also do not deal with sport professionally. According to those observations, only 31 (14.9%) of the participants were familiar with the recommendations by pyramid of healthy diet on how 2-3 dairy portions should be consumed daily (Sedek

and Yun Yih, 2014). The recommended daily intake of protein is known to be 1.2 g/kg of body mass, or 10-15% of the daily energy intake (Barr, 1987), while intake from more than 2.8 g/kg of body mass can lead to kidney function disorders in trained athletes (Worme et al., 1990). The liver inability to degrade too much substances increase the concentration of waste products such as ammonia and urea. Urea excretion results with a special need for water intake so it can be said how dehydration could actually be very dangerous consequence associated with too high protein intake. This study showed how only 19 (9%) of participants in this research knew that excessive protein consumption is not beneficial to athletes. The recommendations according to the pyramid of healthy diet to consume 2-4 fruit portions per day knew 97 (45.8%) of the participants according to this study, while 55 (25.9%) of the participants were familiar with the recommendations how it is not necessary to consume 6 to 11 portions of cereals, rice and pasta per day. As for carbohydrates, daily intake recommendations are 6-10 g/kg of body mass and the amount of intake differs depending on the intensity of the exercise as well as the type of activity, the gender and the environmental conditions (Elango et al., 2010). 31 (14.9%) of the participants new that 60% of total calories should come from carbohydrates. The recommended daily fat intake is 20-35% of the total daily intake (Devlin and Belski, 2015). Single and multiple saturated fatty acids should be taken equally (about 10%). Only 79 participants (37.8%) knew that fats are necessary in daily diet. Vitamins, minerals, and antioxidants are considered as protective substances. Vitamins play an important role in energy production, hemoglobin synthesis, bone and immune function maintenance, and body defense against oxidative stress. Micronutrients on the other hand help in synthesis and recovery of muscle tissue after training. Long-lasting training leads to biochemical muscle adaptation, thus increasing the need for vitamins and minerals. Daily exercise also causes loss of micronutrients due to sweating. As a result of increased need and loss, athletes are advised to intake additional vitamins and minerals. Except the fact the proper nutrition help athletes in exercise and to achieve the best performance, proper nutrition can also affect physiological adaptation to training, recuperation, immune function and general health. There are many reasons why dietary advices are not followed. This may be due to lack of knowledge and interest to change the nutrition or certain perceived barriers that can prevent people from eating healthier, such as lack of money, time or even taste of food. Athletes can often rely on nutritionists in certain sports so their knowledge is also important. Nutrition knowledge could be gained through regular and wide-ranging educational programs as well as self-education (Arazi and Hosseini, 2012). It has been anticipated how improved nutrition knowledge would help athletes to achieve and maintain a proper diet. Although knowledge is one of the few factors that are needed to change nutritional behavior, there are evidences suggesting how adequate nutrition knowledge plays a small but crucial, influential role in daily eating habits (Devlin and Belski, 2015). Nowacka et al. (2015) evaluated the intake of energy, basic nutrients and supplements with daily nutrition by professional slalom canoeists without dieticians control, before and after the nutritional education and concluded how nutritional education might improve eating habits. Carvalhais et al. (2018) evaluated the association between urinary incontinence and bad nutrition habits in elite female athletes. They discovered how those who had bad nutritional habits were three times more likely to have urinary incontinence than women without bad nutrition habits. Mitchell et al. (2018) investigated the level of general nutrition knowledge in registered exercise professionals and compared it with community members and university trained dietitians. They found how total nutrition knowledge in registered exercise professionals is limited and suggested to encourage them for collaboration with multidisciplinary team to accomplish best results. Philippou et al. (2016) proved the impact of nutrition education on nutrition knowledge and adherence to the Mediterranean Diet in adolescent competitive swimmers. The improvement was done by interactive nutrition education workshops. They also suggested parental participation in nutrition education.

There are several limitations in this study. First of all is a small sample of participants. Also, the fact that study was voluntary, probably had influence on how much athletes' and general population nutrition knowledge really is. Guidelines about proper nutrition for athletes are of great importance to ensure that physically active individuals and athletes to be more effectively trained, and thereby to reduce the risk of injury and disease as well as to improve the effectiveness of exercise (Cupisti et al., 2002). Proper nutrition habits will not turn an average athlete into a winner, but poor eating habits can be an explanation for his failure despite his great potential (Dragičević and Šatalić, 2015).

Conclusions

It can be concluded how both groups of participants have shown poor general nutrition knowledge without any significant difference in overall

knowledge between groups of professional athletes and non-athletes. It has also been discovered that professional athletes, despite the importance of sports nutrition for their profession, show lack of proper nutrition knowledge. Educational interventions considering nutrition are needed.

Conflicts of Interest: The authors declare no conflict of interest.

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DIETARY HABITS AND ESTIMATION OF SALT INTAKE IN CROATIAN SCHOOLCHILDREN

Maja Miškulin[1*], Danijela Periš[2], Nika Pavlović[1], Ivan Miškulin[1], Vesna Bilić-Kirin[1], Albina Dumić[1], Gabrijela Dumančić[3]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine Osijek, Josipa Huttlera 4, 31000 Osijek, Croatia
[2]Non profitable organization Health Center "Vaga", Ulica Hrvatske Republike 19C, 31000 Osijek, Croatia
[3]Institute of Public Health for the Osijek-Baranja County, Franje Krežme 1, 31000 Osijek, Croatia

original scientific paper

Summary

High salt intake is the major cause of hypertension and accordingly leads to cardiovascular diseases. The intake of the so-called "hidden salt" through some food stuffs is an important public health issue. This cross-sectional population based study examined dietary habits and evaluated the salt intake through daily snacking among Croatian schoolchildren. The study included 1077 schoolchildren, aged 8.6±1.2 years (range 6 to 11 years old), 48.9% boys and 51.1% girls. The self-administered questionnaire was used for data collection on dietary habits and laboratory determination of salt content in snack meals was performed. Study revealed several unhealthy dietary habits in studied population such as skipping breakfast (32.0% of children), unhealthy snacking (51.6% of children) and consumption of away-from-home meals (46.7% of children) whereas excessive salt intake through unhealthy snacking could pose a serious public health issue in a studied population. In order to improve dietary habits of this population, targeted dietary public health interventions are needed.

Keywords: children; diet; nutrition; salt; unhealthy snacking; Croatia

Introduction

Hypertension is the most significant risk factor for the global burden of disease (Correia-Costa et al., 2016). High salt intake is the major cause of hypertension and accordingly leads to cardiovascular diseases (Cotter et al., 2013; Ma et al., 2015; Ohta et al., 2016). Diets high in salt are now recognized as one of the leading risks to cardiovascular health in the world as they increase blood pressure in both, children and adults (Campbell et al., 2012). Salt restriction is important for the prevention and treatment of hypertension (Ohta et al., 2016).

Reducing salt content of processed foods has been recognized as a feasible and more effective strategy for reducing daily salt intake than simply reducing the amount of salt added during cooking or on the table (Neal, 2007; Nwanguma and Okorie, 2013). This is based on the realization that processed foods are major contributors to the daily salt intake of populations. The top five saltiest processed foods were processed meat, bread and bakery products, dairy and cereal products (Nwanguma and Okorie, 2013; Ni Murchu et al., 2011; Woodward et al., 2012; Gillespie et al., 2009). Unfortunately, consumers are often unaware of the salt content of some of these processed foods that they consume regularly. This so-called 'hidden salt' has been reported to contribute up to 95% of the salt intake of some people, especially in countries where processed foods are widely consumed (Nwanguma and Okorie, 2013; Anderson et al., 2010). The study of the sodium content of processed foods in the United Kingdom reported sodium concentrations of up to 1200 mg per 100 g (equivalent to 3.0 g of salt per 100 g of bread) in some brands of bread (Ni Mhurchu et al., 2011). Thus, because of their popularity with both children and adults, bread and other bakery products have been reported to contribute up to 30.5% of the dietary salt intake in some countries (Nwanguma and Okorie, 2013; Ni Mhurchu et al., 2011; Woodward et al., 2012; Grimes et al., 2011; Villani et al., 2012; Gaitán et al., 2015).

Data from around the world suggest that the population average sodium consumption is well above the minimal physiological needs, and it is above the recommended value of 2 g sodium/day (equivalent to 5 g salt/day) in many countries (World Health Organization, 2012). In children, excessive salt intake by overeating or consuming fast food has also been reported (Ohta et al., 2016; Kar and Khandelwal, 2015). Establishing healthy eating habits early during the childhood can reduce the risk of diet related chronic diseases across the lifespan (Johnsom et al., 2017). If interventions to lower blood pressure levels through healthy dietary choices are initiated in children, then the development of high blood pressure may be suppressed. Therefore, improvements in dietary habits, including salt reduction in childhood, are considered important for

*Corresponding author: maja.miskulin@mefos.hr

the prevention of hypertension (Ohta et al., 2016). Understanding the contribution of daily snacking to the intake of salt will enable the design of specific preventative interventions in children directed toward the improvement of diet quality and consequently toward the improvement of children's health in general (Johnson et al., 2017). Thus, the aim of this study was to examine dietary habits and to evaluate the salt intake through daily snacking in a population of schoolchildren from the Osijek area in Eastern Croatia.

Subjects and methods

Participants

This cross-sectional population based study was conducted during 2009/2010 school year among elementary schoolchildren aged between 6 and 11 from the Osijek area in Eastern Croatia. Six out of 20 elementary schools founded by the city of Osijek (30.0% of elementary school situated in the Osijek area) were randomly selected for the conduction of this study. The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of the Institute of Public Health for the Osijek-Baranja County approved the study (ethical approval code: 6002/09) and informed consents were obtained from parents of schoolchildren who participated in the study.

Questionnaire

Through self-administered questionnaire the data of the age and gender of children and their dietary habits, such as eating breakfast every day, number of meals per day, type of daily snack meals and frequency of eating at least one cooked meal at home daily, were collected. Response rate was 90% (1077/1200).

Determination of salt content in bakery products

The value of salt content in 42 bakery products available at the bakery shops around six schools included in this study, was established in the Institute of Public Health for the Osijek-Baranja County. Among these 42 bakery products, there were 22 most frequently sold pastries and 20 most frequently sold stuffed bakery products.

Determination of salt content in bakery products was based on titrimetric determination of chloride ions with silver nitrate, and calculation of corresponding sodium chloride content. Titration was performed using the visual method for determining the end point, enabled by the presence of potassium chromate as an indicator. The procedure for determining sodium chloride content in bakery products was as follows: test sample was finely comminuted and thoroughly mixed. Visual determination: 10.0 g of test sample was weighed into a 100 ml volumetric flask and diluted to volume with distilled water. After mixing and filtration, 20 ml aliquot was transferred to titration flask and potassium chromate indicator was added. The solution was titrated with silver nitrate standard volumetric solution (0.1 M) to the characteristic yellow-orange end point. Salt content was calculated according to the following equation: NaCl (%) = mL $AgNO_3$ × 0.05844 × 5 × 100 / g of test sample. All the results were expressed as percentage of sodium chloride as described as an appropriate methodology within the research of Paplović et al., (2015).

Statistical analysis

Statistical analysis included data obtained by the laboratory analysis (determination of salt levels in the selected bakery products available at bakery shops in the Osijek area) and data on dietary habits of the schoolchildren from the same area collected through the self-administered questionnaire.

Upon confirming normality of data distribution by Kolmogorov-Smirnov test, all data were processed by the methods of descriptive statistics. The categorical variables were described in absolute and relative frequencies. The $\chi 2$-test and the Fisher's exact test were used for the comparison of categorical variables between the groups. The level of statistical significance was set at $p < 0.05$. Statistical analysis was done using SPSS Statistical Package for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

The study included 1077 children aged 8.6±1.2 years (range 6 to 11 years old) from the Osijek area in Eastern Croatia. Among the study subjects there were 48.9% boys and 51.1% girls. The study revealed that there were 68.0% children who eat breakfast every day, 27.5% children who eat breakfast sometimes and 4.5% children who never eat breakfast. Considering the number of meals consumed daily, the study revealed that 32.7% children consume five meals per day, 51.9% children consume three to four meals per day and 15.4% consume one or two meals per day. Regarding the type of snack meal consumed each day the study established that 48.4% children consume fruit as a snack meal each day, 32.0 % children consume some

kind of bakery product as a snack meal each day while 19.6% children consume some kind of sweets as a snack meal each day. Finally, the study revealed that there were 53.3% children who eat at least one cooked meal at home daily, 29.6% children who eat at least one cooked meal three to four times a week and 17.1% children who eat at least one cooked meal one or two times a week, during weekends only.

Regarding the habit of eating breakfast, the study determined some differences between boys and girls but these were not statistically significant (χ2-test; p=0.280) (Fig. 1).

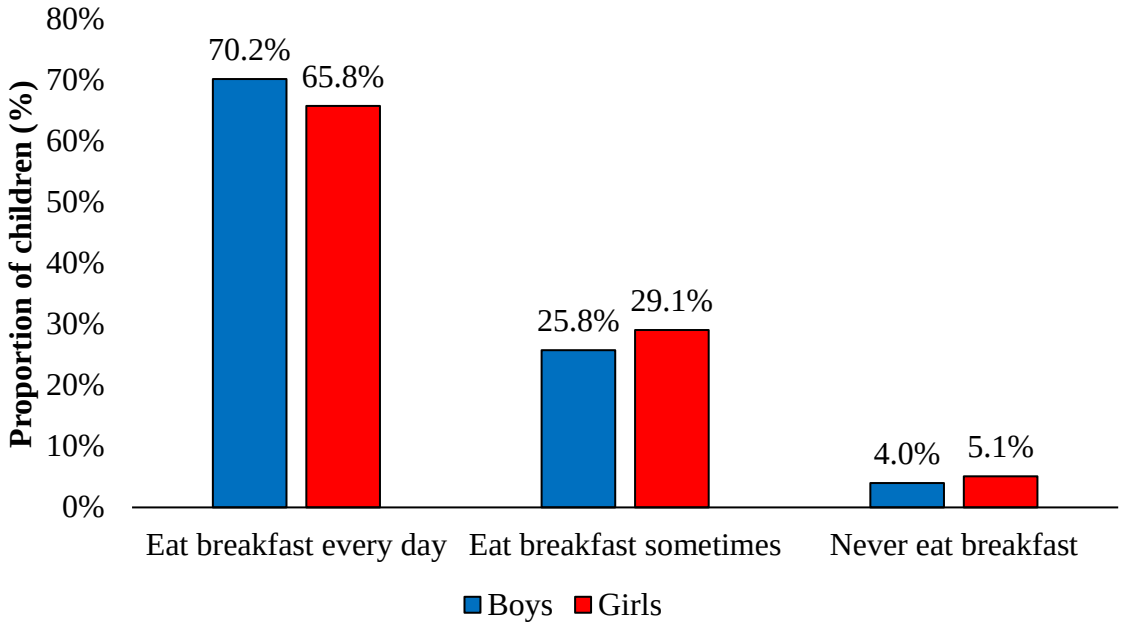

Fig. 1. The habit of eating breakfast among schoolchildren from the Osijek area in Eastern Croatia (χ^2-test; p=0.280)

The study further found some differences between boys and girls in the number of meals consumed every day but these were not statistically significant (χ2-test; p=0.366) (Fig. 2).

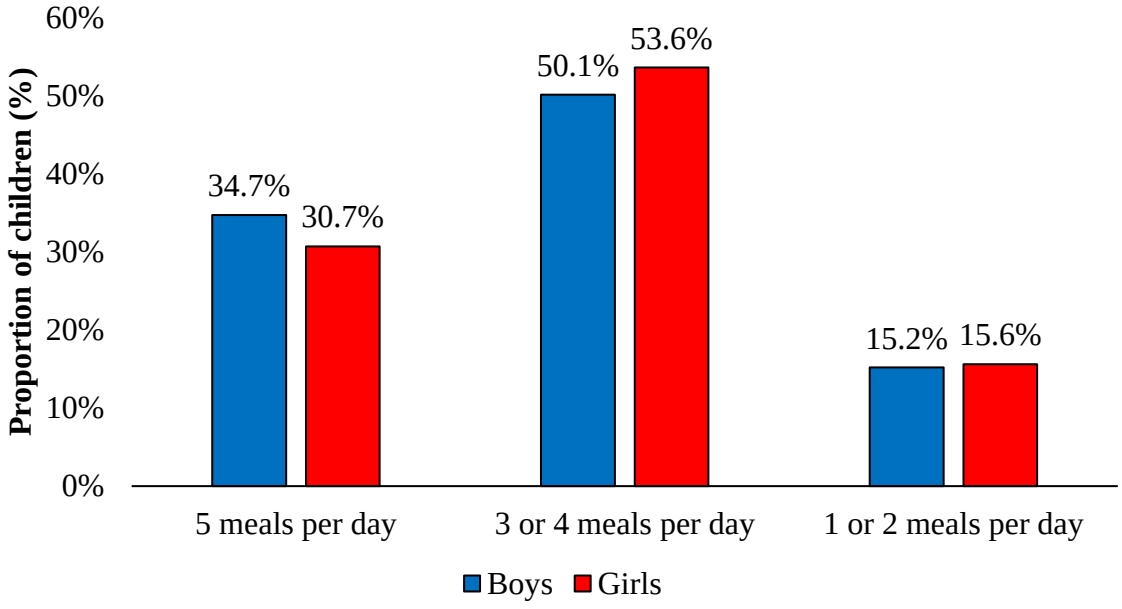

Fig. 2. Number of meals consumed every day among schoolchildren from the Osijek area in Eastern Croatia (χ2-test; p=0.366)

Considering the type of snack meal consumed each day the study revealed statistically significant differences between boys and girls (χ2-test; p=0.002) (Fig. 3).

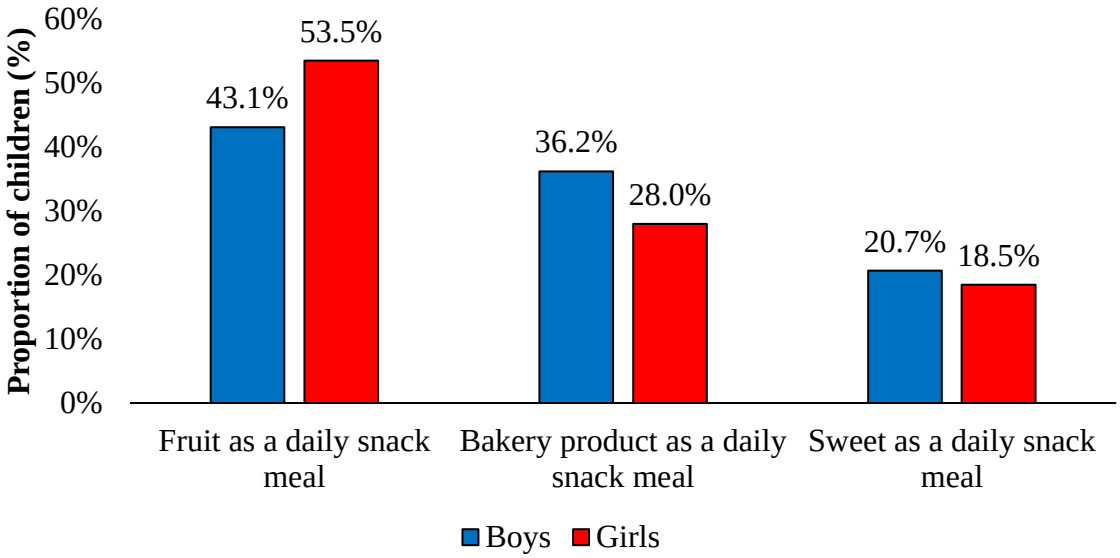

Fig. 3. The type of daily-consumed snack meal among the schoolchildren from the Osijek area in Eastern Croatia (χ2-test; p=0.002)

Finally, the study established differences between boys and girls regarding the frequency of eating at least one cooked meal daily at home but these differences were not statistically significant (χ2-test; p=0.716) (Fig. 4).

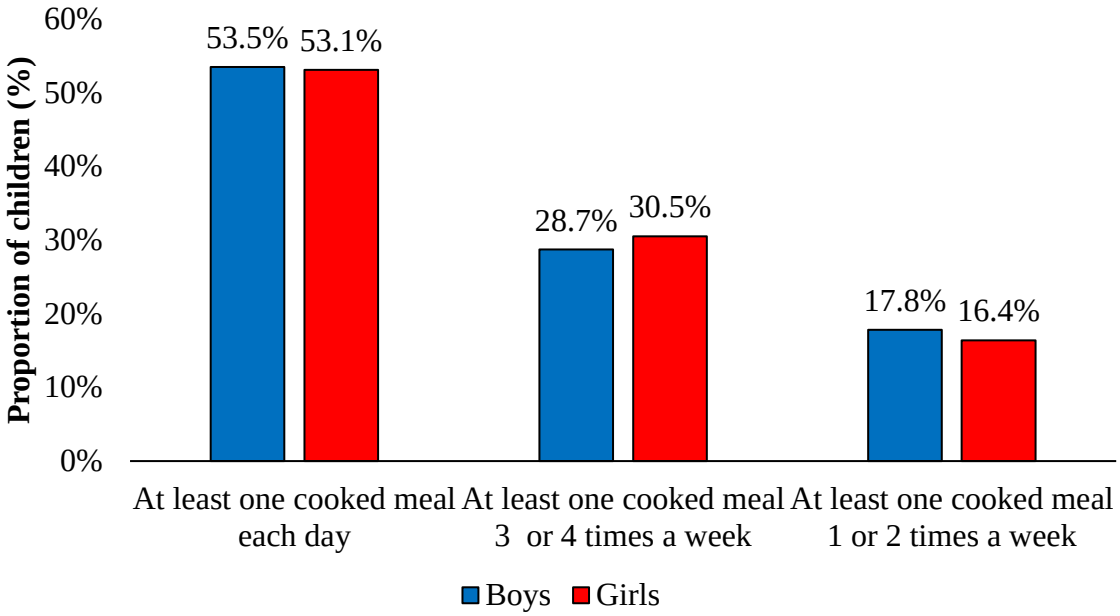

Fig. 4. The schoolchildren from the Osijek area in Eastern Croatia according to the frequency of eating at least one cooked meal daily (χ2-test; p=0.716)

The mean proportion of salt in all 42 bakery products was 2.4±0.9 %. In pastries, this value was 2.8±1.0 % while in stuffed bakery products it was 1.9±0.5 %. This means that, if a child eats only one salty pretzel per day (~ 70 gram), he or she will intake around 2 grams of salt. So if we know that recommended intake for this age group is 5 grams of salt per day it is clear that children who eat bakery products as a daily snack consume through such product almost 50.0% of recommended salt intake.

Discussion

Present study showed that schoolchildren from Eastern Croatia exhibit several bad dietary habits. These habits include skipping breakfast (32.0% of children), eating less than five meals per day (67.3% of children), choosing unhealthy snack meal every day such as bakery products or sweets (51.6% of children) and not eating at least one cooked meal at home per day (46.7% of children). The percentage of schoolchildren from Eastern Croatia who eat breakfast every day is similar yet larger than among Lithuanian and Czech schoolchildren (Smetanina et al., 2015, Voráčková et al., 2015). According to experts' recommendations breakfast should supply for 20% of a child's daily energy. Furthermore, there are scientific evidences that proved how not having breakfast has negative effects on alertness, concentration, memory, sight complicated processes, problem solving, and comprehending mathematics (Hosseini et al., 2015). Considering all the above one can say that the results obtained from Eastern Croatia are quite concerning.

This study also revealed that only 32.7% of children consume five meals each day, meaning there are a large proportion of children who skips meals. These data are much lower than among Lithuanian and Norwegian schoolchildren (Smetanina et al., 2015; Stea et al., 2015). Skipping meals, especially skipping breakfast is connected with poor academic performance and obesity in children (Stea et al., 2015; McIsaac et al., 2015) and thus poses a serious threat to health and well-being of children. The consequences of such bad influence can adversely affect the children in adulthood.

Present study further found that 51.6% of children from Eastern Croatia choose some sort of unhealthy snack every day. Besides poor academic performance, unhealthy snacking is also a strong predictor of childhood obesity (Baygi et al., 2013; Correa-Burrows et al., 2015). This study showed that 32.0% of schoolchildren from the Osijek area consume some of the bakery products as a snack meal every day and thus intake additional amount of salt. Such percentage, although lower than the one determined among children from Bosnia and Herzegovina, still presents a major concern (Hasanbegović et al., 2010). There is also increasing evidence that obesity and high salt intake are not only important risk factors for hypertension but also the two most important modifiable risk factors (Correia-Costa et al., 2016). Based on the previously mentioned one can say that this study reveals an important possibility for specific public health interventions among the studied population in Croatia.

Regarding the frequency of eating at least one cooked meal daily at home this study established that 53.3% of the studied population actually eat at least one cooked meal at home each day. Such finding is rather disturbing because intake of away-from-home meals are associated with greater overall intake of energy, total fat, saturated fat, sugar and salt in children and adolescents putting them at risk of various health disorders in childhood but also in adulthood (Fulkerson et al., 2012; Appelhans et al., 2014). With respect to the above mentioned experts recommend limiting eating out and encourage more frequent home meal preparation (Fulkerson et al., 2012). This is even more important in childhood due to the known fact that dietary habits, which are important for one's long term health status, develop early in life (Kudlová and Schneidrová, 2012). Moreover, some studies indicate that children naturally prefer higher levels of sweet and salty tastes than adults do which makes them especially vulnerable to the modern diet and today's diet differs greatly from the diet of our past, when salt and sugars were once rare and expensive commodities (Mennella et al., 2014). Considering all that it is obvious that excessive intake of salt during childhood directly predisposes a child for such intake later in life.

Present study revealed statistically significant differences between boys and girls considering the type of snack meal consumed each day, where girls made healthier choices more often and consumed less bakery products in comparison to boys. A simple explanation for the latter is that boys have greater energy requirements than girls which leads to a greater total food consumption, therefore, higher salt intake (Marrero et al., 2014). This explanation is confirmed in this study through fact that boys more frequently consumed five meals per day and that they consumed breakfast more regularly in comparison to girls. On the other hand, this study did not find difference between boys and girls regarding the consumption of at least one cooked meal per day at home. This finding confirms the fact that dietary choices and habits of children, especially of younger children such as children in this study, largely depends on diet behaviors and choices of their parents (Kudlová and Schneidrová, 2012).

This study has several limitations and its results must therefore be interpreted with caution. The first limitation is connected with the fact that this study did not include the determination of salt in actual whole daily meals of the study subjects; thus it is not possible to exactly elaborate the amount of salt attributable to some bakery product consumed as a snack meal within the particular child's whole daily meal. Furthermore, the study did not include performance of anthropometrical measurements of children and because of that, it is not possible to exactly elaborate the connection between weight and salt consumption in a studied population that could certainly be important for deeper analysis of the obtained results. Consequently, these issues should certainly be addressed in future studies.

Conclusions

Present study points to the fact that an excessive salt intake through diet in a population of Croatian schoolchildren could pose a serious public health issue. The study further emphasizes the role of daily consumption of bakery products as a snack meal in the overall intake of salt in the studied population. Understanding children's salt consumption is vitally important given the known associations between salt consumption and life-course progression to hypertension and all of its serious consequences. Because of that, these findings represent valuable information from public health point of view. Namely, bearing in mind that dietary habits, important for one's long-term health status, develop early in life and that parents certainly have an important role in the formation of such habits, it is necessary to strengthen both the parents and the children with additional knowledge dealing with this issue. This can be achieved by designing and implementing targeted preventive activities focused on dietary habits in the study population that need to involve parents, but other interested stakeholders as well. This is important because consequences of poor dietary habits, seen through the morbidity and mortality patterns in the Croatian adult population, negatively affect the whole society and can be changed by focusing on relatively simple issues such as this one that was investigated in this study.

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PRODUCTION AND STABILIZATION OF PEANUT OIL

Tihomir Moslavac[1*], Drago Šubarić[1], Jurislav Babić[1], Antonija Šarić[1], Dubravka Vitali Čepo[2], Antun Jozinović[1]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia
[2]University of Zagreb, Faculty of Pharmacy and Biochemistry, Ante Kovačića 1, 10000 Zagreb, Croatia

original scientific paper

Summary

The colour of peanut oil is pale-yellow, it has a neutral scent and it is rich in oleic and linoleic acid. The aim of this study was to investigate the effect of peanut kernel conditioning (25 °C, 35 °C, 45 °C, 55 °C), the addition of sunflower shells (3%, 6%, 9%), and the usage of oil during pressing. Peanut pressing is performed with a continuous press. Following the pressing process, a decision is made on the volume and the temperature of crude oil, as well as on pressing time. The additional steps include the natural precipitation of crude oil and the vacuum filtration of crude oil to obtain cold pressed oil. Some of the basic parameters for oil quality that are determined using standard methods are: peroxide number, free fatty acids, the proportion of insoluble impurities, and moisture content. Additionally, the results of the addition of natural and synthetic antioxidants on the oxidation stability of cold pressed peanut oil were examined. Finally, the results of the research show that conditioning and the addition of sunflower shells affect oil recovery during the pressing process. Conditioning peanuts at higher temperatures and the addition of a larger proportion of sunflower hulls resulted in greater amounts of produced oil. The natural antioxidant rosemary extract and synthetic propyl gallate achieve greater protection from oxidative degradation for the oil.

Keywords: peanut oil, cold pressing, oxidative stability, antioxidants

Introduction

Peanut is an important oilseed crop in Taiwan and one third of these seeds is processed for edible oil (Chu and Hsu, 1999). Peanut oil is the main natural edible oil consumed without additives in the middle Mediterranean region south of Turkey. Peanut oil is one of the most stable vegetable oils in relation to oxidation. The oxidative stability of oils may be influenced by many factors, such as light, metal ions, oxygen, temperature, and enzymes (Nawar, 1985). The effect of fatty acid composition on the oxidative stability of oil for vegetable oils has been studied by a number of investigators (OKeefe et al., 1993; Liu and White, 1992). Peanut oil is composed of ≈80% of unsaturated fatty acids, with oleic acid (18:1, ω-9) comprising an average of ≈50% and linoleic acid (18:2, ω-6) 30% of the total fatty acid composition (Mercer et al., 1990). Because of the polyunsaturated fatty acids, peanuts are susceptible to lipid oxidation (Braddock et al., 1995). The production of edible cold-pressed oil is done with a continuous screw press without the use of organic solvents. The processing of oil yielding plants through the cold pressing procedure ensures the maximum retention of active compounds in oil, like essential fatty acids, phenolic and flavonoid compounds, tocopherols, tocotrienols, phytosterols, and others (Teh and Birch, 2013), as well as the preservation of characteristic sensory properties of oil. This pressing procedure results in crude oil, which must undergo the procedure of removing insoluble solid particles (through sedimentation, filtration, centrifuge) in order to obtain cold pressed oil. One of the by-products in the process of pressing oil yielding plants is cake, which retains a certain amount of oil, significant proteins, minerals, fibre, and other ingredients (Zubr, 1997; Quezada and Cherian, 2012). Jokić et al. (2014) investigated the optimisation of the production of cold pressed walnut oil with a screw press and determined that the processing parameters of the pressing process have an effect on the utilisation of oil. Moslavac et al. (2014) indicated that the process parameters of cold pressing have an effect on the utilisation of camelina oil (*Camelina sativa* L.). Cold-pressed pumpkin seed oil is made through the process of mechanical pressing of an industrial variety of pumpkin (field pumpkin) using screw presses, and it is consumed as salad oil (Fruhwirth, 2008). Cold-pressed pumpkin seed oil belongs to the group of edible oils with high nutritional value, due to the favourable composition of fatty acids and other ingredients that yield a positive effect in the organism (Murković and Pfannhauser, 2000; Neđeral, 2006). Tocopherols and tocotrienols are non-glycerol ingredients in plant oils with antioxidant properties that protect the oil from oxidative degradation. Edible plant oils very quickly become sensitive to negative changes (chemical reactions, enzymatic and microbiological processes), which results in the spoiling of the oil. Auto-oxidation can occur faster or slower, depending on the production process, oil composition, storage conditions, the presence of compounds that accelerate

*Corresponding author: tihomir.moslavac@ptfos.hr

(pro-oxidants) or decelerate (antioxidants) this oxidation reaction (Martin-Polvillo, 2004). Primary and secondary oxidation products are created in the process of oxidative degradation of oil (Gray, 1978; Rovellini, 1997). The small quantities of these products of oil degradation deteriorate the sensory properties of oil (Broadbent and Pike, 2003). It is important to know the sustainability or stability of plant oils, in order to be able to determine the time required to preserve the oil from the more significant effects of oxidation and to determine the time period in which the oil can be used. A significant amount of research into oxidative degradation of plant oils has shown that their sustainability depends on the type of oil or the composition of fatty acids and the type and share of the active compounds with antioxidative properties in oil. Frega et al. (1999) determined that free fatty acids in plant oil act as pro-oxidants, accelerate the oxidative degradation of oil, and in larger shares reduce the stability of oil. Matthaus (1996) indicated that specific ingredients affect the stability of sunflower oil, rapeseed oil, and walnut oil. Various methods based on the accelerated oxidation of oil are used today to determine the oxidative stability of plant oil: Oven test, AOM test, and the Rancimat test (Shahidi, 2005; Suja, 2004; Abramović, 2006; Farhoosh, 2008). The stability of plant oils can be improved with the addition of antioxidants, compounds that decelerate the auto-oxidation process. There are synthetic and natural antioxidants which are applied for the stabilisation of edible plant oils, i.e. increasing their resistance to oxidation (Yanishlieva and Marinova, 2001; Merrill, 2008). Recently, various plant materials, particularly herbs, are investigated due to their active compounds (phenolic compounds) which are showing significant antioxidative properties in plant oils (Berra, 2006; Bandoniene, 2000). The stabilisation of cold-pressed oils is focused toward the application of various plant extracts (rosemary, green tea, sage, oregano, and others) for the purpose of protection against oxidative degradation (Pan, 2007; Ahn, 2008). Erkan et al. (2008) investigated the antioxidant activity of rosemary extracts and other compounds for the purpose of oil stabilisation. Gramza et al. (2006) reported high antioxidant activity, measured as an induction period, in the ethanol extract of green tea, regarding the activity of BHT and the extract of black tea in sunflower oil. Hraš et al. (2000) tested and determined that rosemary extract and alpha tocopherol have antioxidant and synergetic effects for the stabilisation of sunflower oil.

The aim of this research was to investigate the effect of conditioning peanut kernels (25 °C, 35 °C, 45 °C, 55 °C), as well as the effect of adding sunflower hulls (3%, 6 %, 9%) on the utilisation of oil during pressing. The effect of adding natural and synthetic antioxidants to the produced cold-pressed peanut oil on the changes of the oxidative stability of the oil was tested.

Materials and methods

Materials

Peanut kernels purchased in the store were used in this research. The antioxidants used in the research are natural antioxidants: rosemary extract (type Oxy Less CS), sage extract, DOPE-dry olive pomace extract (type hpDOPE and ramDOPE) in the concentration of 0.2%, alpha tocopherol and mixtures of tocopherols in the concentration 0.05%, and synthetic antioxidants: propyl gallate and butylhydroxyanisole in the concentration of 0.01%. Rosemary extract (type Oxy Less CS) and sage extract were supplied from Naturex (France). Alpha tocopherol and the mixtures of tocopherols were supplied from DSM Nutritional Products Ltd. (Switzerland). Dry olive pomace extract type hpDOPE is extract prepared with hydroxypropyl-β-cyclodextrin and ramDOPE is extract prepared with randomly-methylated-β-cyclodextrin. Propyl gallate (PG) and butylhydroxyanisole (BHA) were purchased from the company Danisco (Denmark). All other chemicals and reagents were of analytical reagent grade. The peanut oil was obtained by pressing, using different process conditions. The peanut kernels were pressed in a screw expeller (firm Gorenje, power of the electric motor 650 W). The produced crude oil was collected in a graduated cylinder and the volume and temperature were measured. After the precipitation of crude oil, vacuum filtration was carried out to remove insoluble particles from the oil. Cold pressed oil was produced in this way. Part of the peanut kernel press samples were heated in an oven to a temperature 25 °C, 35 °C, 45 °C, 55 °C for 30 minutes and sunflower hulls were added to some samples (3%, 6%, 9%) to influence the change of oil consumption during pressing.

Determination of initial oil and water content

The initial oil content in peanut kernels was measured using the automatic extraction system Soxterm by Gerdhart with *n*-hexane (Aladić et al., 2014). The measurement was performed in duplicate. The average of the initial oil content for two replicates was 51.82 ± 0.12 %. Cake residual oil (CRO) was also determined by the automatic extraction system Soxterm. The moisture content of the peanut kernels (2.81 ± 0.09 %) was determined according to the AOAC Official Method 925.40. The determination of moisture in the defatted cake was done using the modified standard HRN ISO 6496:2001.

Oil quality parameters

The peroxide value (PV) of oil samples was determined according to ISO 3960. The PV was expressed as mmol O_2/kg of oil. Free fatty acids (FFA) in oil were determined using ISO 660. Insoluble impurities (II) and moisture and volatile matter content of oil were determined according to ISO 663 and ISO 662. All these determinations were carried out in duplicate.

Determination of oxidative stability

Oxidative stability was determined using the rapid oil oxidation test - sustainability test at 98 °C during 20 days. The influence of the addition of natural antioxidants, namely rosemary extract, sage extract, olive pomace extract in concentration of 0.2%, alpha tocopherol and mixtures of tocopherols (0.05%), and synthetic antioxidants propyl gallate and butylhydroxyanisole in the concentration of 0.01%, on the oxidative stability of cold pressed peanut oil were monitored. The result of the oil oxidation was expressed as peroxide value (PV) during the 20 days of the test. All determinations were carried out in duplicate.

Results and discussion

Table 1 shows the results of determining the basic quality parameters of peanut kernels for pressing. The resulting values for the oil content are 51.82% and for the moisture content 2.81%.

Table 1. Peanut composition

Parameters	Content
Oil (%)	51.82%
Moisture (%)	2.81%

The testing results regarding the influence of the conditioning temperature of peanut kernels (25 °C, 35 °C, 45 °C, 55 °C) on the efficiency of crude oil and cold pressed oil production are shown in Table 2. After pressing the peanuts (0.5 kg) at room temperature (25 °C), 78 mL of crude oil was obtained, with the temperature of 29 °C. After 7 days of sedimentation and vacuum filtration of the crude oil, 15 mL of cold pressed peanut oil was obtained. An analysis was conducted to determine the share of oil cake waste (pressing by-product) and it was 47.87%. When conditioning the peanut kernels at the temperature of 35 °C before pressing, 110 mL of crude oil was obtained, and 32 mL of cold pressed oil was obtained after sedimentation and filtration. The share of oil cake waste was 47.42%. By increasing the peanut conditioning temperature to 45 °C and pressing, a larger quantity of crude oil (144 mL) and cold pressed oil (71 mL) was obtained, with even less oil cake waste (42.71%). After increasing the peanut heating temperature even more, to 55 °C, even more crude oil (146 mL) and cold pressed oil (80 mL) was obtained. The analysis determined a lower share of oil cake waste (42.37%). The results achieved in this research have shown that the peanut kernel conditioning temperature affects the level of utilisation of oil in the pressing process.

Table 2. The influence of the conditioning of peanut kernels (0.5kg) on the efficiency of the production of crude oil and cold pressed oil

Conditioning temperature (ºC)	Crude oil (mL)	Temp. crude oil (ºC)	Cold pressed oil (mL)	Mass of cakes (g)	Oil in cakes (%)	Moisture in cakes (%)
25	78	29	15	404.22	47.87	2.54
35	110	29	32	393.15	47.42	2.44
45	144	34	71	386.09	42.71	2.62
55	146	39	80	379.99	42.37	2.59

The sedimentation and filtration of crude oil lasted for 7 days

Table 3 shows the results of the testing regarding the utilisation of oil after the addition of sunflower hulls (3%, 6%, 9%) to the peanut kernels. After the addition of sunflower hulls (3%) to the peanut kernels, a larger quantity of crude oil (111 mL) and cold pressed oil was obtained, when compared to the peanut oil obtained without adding the sunflower hulls (control sample). By increasing the share of sunflower hulls to 6% and pressing the peanuts, the quantity of the obtained crude oil (139 mL) and cold pressed oil (55 mL) increased. The share of oil cake waste was determined by analysis at 41.81%. By increasing the share of the added sunflower hulls even further, to 9%, the amount of the obtained crude oil (150 mL) and cold pressed peanut oil (61 mL) increased even more, after 7 days of sedimentation and filtration. This research indicates that sunflower hulls have a significant effect on the utilisation of peanut oil in the cold pressing process.

Table 3. Effects of the addition of sunflower hulls (3%, 6%, 9%) to peanuts (0.5 kg) on the production of cold pressed oil

Sunflower hulls (%)	Conditioning temperature (°C)	Crude oil (mL)	Temp. crude oil (°C)	Cold pressed oil (mL)	Mass of cakes (g)	Oil in cakes (%)	Moisture in cakes (%)
0	25	78	29	15	404.22	47.87	2.54
3	25	111	35	44	402.00	44.24	2.72
6	25	139	30	55	398.10	41.81	2.98
9	25	150	33	61	412.88	39.30	3.13

The basic quality parameters for the cold pressed peanut oil were determined according to the Regulation on Edible Oils and Fats (Official Gazette of the Republic of Croatia 11/2019), which is shown in Table 4. The obtained results show that the peroxide value (PV), free fatty acids (FFA), and the moisture value conform to the regulation values. The share of insoluble impurities in oil (0.2%) is somewhat higher than indicated in the Regulation (max. 0.05%) and the sedimentation time of the crude oil should be increased, in order to separate the solid particles from the oil.

Table 4. The basic quality parameters of the produced cold pressed peanut oil

Oil quality parameters	Content
Peroxide value (PV), mmol O_2/kg	0.70
Free fatty acids (FFA), %	0.73
Moisture, %	0.0082
Insoluble impurities, %	0.20

Table 5 shows the results of adding antioxidants (natural and synthetic) on the oxidation stability of cold pressed peanut oil. The oxidation stability test was conducted at the temperature of 98 °C in a thermostat, during a period of 20 hours. The obtained results show that the peroxide value (PV) of the oil gradually increases during the testing, due to increased oxidative degradation. According to research done by Chu and Hsu (1999) efficient peanut oil stabilization is achieved by adding catechins in combination with other antioxidants. The cold pressed peanut oil (control sample) had the PV of 3.57 mmol O_2/kg of oil after 20 hours of testing. With individual additions of natural antioxidants, the oil was stabilised, i.e. its resistance to oxidative degradation increased. Apart from the addition of α- tocopherol (0.05%), where a higher PV (4.08 mmol O_2/kg) was observed when compared to the control sample after 20 hours of testing. With the addition of rosemary extract (type OxyLess CS) to the oil in the share of 0.2%, the highest protection of the oil from oxidative degradation was achieved, after 20 hours of testing the PV was the lowest, at 1.75 mmol O_2/kg. Ozcan (2003) investigated the effect of antioxidants on the oxidative stability of peanut oil during storage and determined their positive effect on oil stabilization. A satisfactory protection of the oil from oxidative degradation was also achieved with the addition of dry olive pomace extract (type ramDOPE) in the share of 0.2%, where the PV was 2.02 mmol O_2/kg after 20 hours of testing. Sage extract (0.2%) and olive pomace extract (type hpDOPE) in the share of 0.2% showed equal antioxidation effects, where the PV remained approximately equal during 20 hours of testing. The use of a tocopherol mixture (0.05%) resulted in a very low level of oil protection against oxidative degradation. The PV was 3.40 mmol O_2/kg after 20 hours of testing, only slightly lower than the control sample (3.57 mmol O_2/kg). The use of synthetic antioxidants propyl gallate (PG) and *butylated hydroxyanisole* (BHA) in the share of 0.01% also achieved the protection of peanut oil from oxidative degradation. The application of PG resulted in a significantly higher level of oil protection from oxidation (PV was 1.58 mmol O_2/kg), when compared to BHA (3.40 mmol O_2/kg) after 20 hours of testing.

Table 5. The influence of the addition of antioxidants (natural and synthetic) on the oxidative stability of cold pressed peanut oil

Sample	Share of antioxidants (%)	PV (mmol O_2/ kg)								
		0 h	1 h	2 h	3 h	4 h	6 h	8 h	12 h	20 h
Oil without antioxidant addition	-	0.70	0.97	1.55	1.74	1.79	2.24	2.50	2.67	3.57
Rosemary extract (type Oxy' Less CS)	0.2	0.70	0.72	1.19	1.33	1.43	1.45	1.51	1.56	1.75
hpDOPE	0.2	0.70	1.15	1.43	1.28	1.48	1.58	1.73	2.03	2.53
ramDOPE	0.2	0.70	0.95	1.30	1.36	1.40	1.52	1.58	1.66	2.02
α- tocopherol	0.05	0.70	1.27	1.57	1.70	1.96	2.26	2.39	3.13	4.08
Tocopherol mixture	0.05	0.70	1.25	1.65	1.66	1.75	2.32	2.41	2.81	3.40
Sage extract	0.2	0.70	1.26	1.63	1.64	1.66	1.53	1.75	1.85	2.48
PG	0.01	0.70	0.87	1.02	1.36	1.35	1.36	1.46	1.51	1.58
BHA	0.01	0.70	1.54	1.79	1.69	1.80	1.84	2.13	2.89	3.40

DOPE-dry olive pomace extract; hpDOPE-extract prepared with hydroxypropyl-β-cyclodextrin; ramDOPE-extract prepared with randomly methylated-β-cyclodextrin; BHA-butylhydroxyanisol; PG-propyl-gallate

Conclusions

The peanut conditioning temperature before pressing affects the utilisation of oil. With the increase of the peanut conditioning temperature, the production of crude oil and cold pressed oil increases. The addition of sunflower hulls to peanut kernels before pressing affects the utilisation of crude oil. As the quantity of added hulls increases, the amount of obtained crude oil and cold pressed oil increases as well, with the reduction of oil cake waste. The tested natural antioxidants are efficient in protecting peanut oil from oxidative degradation, apart from α-tocopherol (0.05%). The addition of rosemary extract (type OxyLess CS) resulted in higher oil protection when compared to other tested natural antioxidants. The use of dry olive pomace extract (type ramDOPE) resulted in significant oil protection from oxidative degradation. The addition of sage extract and dry olive pomace extract (type hpDOPE) resulted in approximately equal levels of antioxidant activity. The tocopherol mixture has a negligible effect on the reduction of oxidative degradation in peanut oil. The synthetic antioxidant propyl gallate is more efficient in protecting peanut oil from oxidation when compared to butylated hydroxyanisole.

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CHEMICAL COMPOSITION OF JAM FROM TRADITIONAL APPLE CULTIVARS FROM BOSNIA AND HERZEGOVINA

Senahid Mujkanović, Midhat Jašić, Martina Andrejaš*, Marizela Šabanović, Damir Alihodžić

University of Tuzla, Faculty of Technology, Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina

original scientific paper

Summary

Jam (traditionally called pekmez) is a product produced by concentrating of fresh thick apple juice. In this study, 20 jam samples, from different apple cultivars, were analyzed, including: 'Samoniklica', 'Paradija', 'Habikusa', 'Zuja', 'Srebrenicka' and mixed cultivars. The aim of this study was to determine physicochemical properties of jam from different apple cultivars and mixed jam. The analyses have shown that the average value of the dry matter was 77.38%, the ash 1.26%, pH value 4.25, electrical conductivity 2.90 mS/cm. The nitrogen content was 685.10 mg/100 g. The relative density was 1.36 g/mL. The potassium content (K) was 430.70 mg/100 g, sodium (Na) 99.31 mg/100 g, calcium (Ca) 43.65 mg/100 g, magnesium (Mg) 30.80 mg/100 g, iron (Fe) 5.61 mg/100 g, zinc (Zn) 1.07 mg/100 g, copper (Cu) 0.41 mg/100 g, manganese (Mn) 0.26 mg/100 g. The total polyphenols were 0.78 g/kg. Hydroxymethylfurfural (HMF) was 162.02 mg/kg. Apple jam is recommended in recovery from many diseases because of its special nutritional value, especially for people who suffer from *anaemia sidropenica* and similar diseases, and for the athletes as well.

Keywords: jam, apple, chemical composition

Introduction

Apples represent very important part of human nutrition as they are source of sugar, acids and many other biological active compounds such as phenolic compounds which are responsible for antioxidant activity (Wu et al., 2007). Besides sugar and organic acids, phenols also determine apple quality (Nogueira et al., 2006).

According to the some authors (Drake and Eisele, 1997), there is a significant difference between chemical composition among different cultivars of apples. The factors which are affecting chemical composition of apples, as well as the content of some components, are: cultivar, climate, harvesting period, soil, storage and others. Bosnia and Herzegovina is known for its valuable domestic fruit cultivars. Simultaneously, there is a trend of inscreasing demand for fruit which has been organically farmed and has not been or is in a small degree chemically treated.

The apple jam (traditionally called pekmez) can be defined as a concentrated and shelf-life extended form of apple juice produced by boiling without adding sugar or any other food additives (Yogurtcu and Kamisli, 2006). The production of jam from traditional apple cultivars has a long tradition in Bosnia and Herzegovina. There is 46 accounted and recognised traditional apple cultivars so far. The jam is produced from numerous traditional apple cultivars: '*Paradija*', '*Samoniklica*', '*Žuja*', '*Šarenika*', '*Srebrenička*', '*Sladica*', '*Tankokora*' and others (Beširović, 2009). Jam consumption in Bosnia and Herzegovina has not been systematically monitored, but more and more importance is given to the jam as a traditional product. For centuries, the jam has been used as the unique permanent product from apples and also for preparing many Bosnian delicacies (Lakešić, 1999).

In folk medicine of Bosnia and Herzegovina this product has been used as a medicine for anemia, cough, inanition and boosting immunities. The nutrition rich in fruits and vegetables is directly connected with lower risk of chronic, non-communicable diseases. A concept of functional foods is based upon these findings. Characteristics of traditional products are being discovered but the new functional foods are being designed also (Miletić et al., 2008).

Nutritional properties of food products are including energy value, compounds composition, nutrient utilization, antioxidant capacity, the influence of bioactive compounds on human health etc. Nutritional attributes are measurable parameters. Based on nutritional properties it can be concluded whether the specific product fills the conditions of functional food. Many definitions show that functional food is a food which contains components with beneficial effects on one or more functions of the human organism and in that way it affects improvement of general organism condition and health or significantly reducing the risk of diseases (FAO, 2007).

The jam is produced from royal apple juice and it can be classified as functional food due to the components such as polyphenols, flavonoids, dietetic fibres,

*Corresponding author: martina.andrejas@hotmail.com

minerals, as well as other active ingredients present in traces: alkaloids, glycosides, phytosterols, polyunsaturated fatty acids and other phytochemicals.
Functional properties of jam can also be determined by nutritional attributes such as glycemic index (GI) of food. Besides positive parameters which affect nutritional and functional properties of the jam, there are also parameters which affect negatively such as Maillard reaction and caramelisation products, present microorganisms and their toxins, different ecological contaminants and others.
Hydroxymethylfurfural (HMF) represents Maillard reaction product. HMF is a product of simple sugars degradation (most often fructose) in the presence of acids and it occurs in the moment when the proportions of acids and simple sugars are favourable for the reaction. HMF can generate during the food storage, but it is usually formed at higher temperatures during the processes such as baking, cooking and drying. Although epidemiological studies have not showed adverse effects on health, laboratory studies showed that HMF has cytotoxic and genotoxic effects.
The aim of this study was to determine chemical composition of the jam from traditional apple cultivars in Bosnia and Herzegovina.

Materials and methods

Production of traditional jam

The traditional jams was produced by concentrating of fresh thick apple juice. Traditional way of production is carried out by boiling a juice until a concentration reaches values between 67% and 75%. The concentrating on the temperature up to 70 °C is more modern way of a jam production.
20 jam samples were used as follows: 10 jam samples from 'Samoniklica' cultivar and 10 samples from mixed apple cultivars which are: 'Paradija', 'Šarenika', 'Žuja', 'Sladica' and 'Tankokora' (Table 1).

Table 1. Materials used in this study

The jam samples	The jam from apple cultivar	Jam production method	The year of production
1	'Samoniklice'	On the temperature up to 70 °C	2011
2	'Samoniklice'	On the temperature up to 70 °C	2011
3	'Samoniklice'	On the temperature up to 70 °C	2011
4	'Samoniklice'	On the temperature up to 70 °C	2011
5	'Samoniklice'	On the temperature up to 70 °C	2011
6	'Samoniklice'	Traditional	2011
7	'Samoniklice'	Traditional	2011
8	'Samoniklice'	Traditional	2011
9	'Samoniklice'	Traditional	2011
10	'Samoniklice'	Traditional	2011
11	Mixed cultivars	Traditional	2011
12	Mixed cultivars	Traditional	2011
13	Mixed cultivars	Traditional	2011
14	Mixed cultivars	Traditional	2011
15	Mixed cultivars	Traditional	2011
16	Mixed cultivars	Traditional	Before the year 2006
17	Mixed cultivars	Traditional	Before the year 2006
18	Mixed cultivars	Traditional	Before the year 2006
19	Mixed cultivars	Traditional	Before the year 2006
20	Mixed cultivars	Traditional	Before the year 2006

Determination of the content of soluble dry matter

For this measurement refractometry method was used (measuring the light refraction index of different liquid materials with Abbe refractometer by Metller Toledo - USA).

Determination of pH value

This determination is based on the measuring of potential differences between two electrodes that are immersed in a solution. Reference electrode has a permanent potential, while the glass electrode has the potential, which is function of activity of H+ ions in the solution. pH value of samples is determined by using a digital pH-meter MP 225 manufactured by Metller Toledo (USA), with combined glass electrode.

Determination of the total and reduced sugars in the jam

Reduction of Fehlings solution by a solution of reduced sugars in the jam, using methylene blue as an indicator

were used. The content of reducing sugars is determined by volumetric method upon Luff-Schoorl (Trajković et al., 1983).

Determination of the total ash

Burning on 600 C in burning furnace (Instrumentaria, Zagreb) was used. The total ash includes an inorganic leftover that remains after the burning and represents the total mineral content of the sample. During the burning, all the cations, beside ammonia cations, turn into carbonates or into others anhydrous inorganic salts. The total ash content was determined by standard gravimetric method (Trajković et al., 1983).

Determination of the concentration of total nitrogen

Kjeldahl method on the Kjeltec TM 2300 device (Foss Tecator, Sweden) was used. Determination of the concentration of total nitrogen was conducted in three fases: wet burning of the sample, distillation and titration. In a cuvette the jam sample was pipetted and two catalyst tablets were placed and the sample was burned on the unit for burning. After the total burning, cuvette was transfered to the unit for a distillation. The strong base was added which caused a release of NH_3. NH_3 was transfered through the cooling system and treated with 1% boric acid which caused an increase of pH value in a solution. This solution was titrated with 0,1 N HCl. Bromine cresol and methil red were used as indicators. The concentration of ammonium ion was determined based on a volume of HCl that was needed for a titration.

Mineral analysis

The following minerals have been analyzed: iron (Fe), manganese (Mn), copper (Cu), potassium (K), magnesium (Mg), zinc (Zn), sodium (Na) and calcium (Ca), using methods of atomic spectometry on instruments A Analist 100 and HGA-800 (Perkin Elmer, Waltham, MA, SAD). 2 g of a sample were weighed into a ceramic vessel for annealing. The vessel was put into a cold annealing furnace by Instrumentaria (Croatia). The temperature was adjusted to 550 °C and maintained for 4 hours. After 4 hours vessel was cooled down and the sample inside was treated with 5 mL of 3N HCl. Then, the vessel was covered with a watch glass and the content was cooked for 10 minutes. After 10 minutes, the content was cooled down and filtered to a volumetric flask. Volumetric flask was made up to the mark with distilled water. From the prepared solution were determined contents of Fe, Mn, Cu, K, Mg, Zn, Na and Ca. The blank was also prepared with the same method, only without the sample.

Determination of the total polyphenols

Folin-Ciocolteu method was used for total phenols determinatiom using spectrophotometer by ThermoFisher Scientific (USA) at wavelength of 756 nm. Gallic acid was used to prepare the standard curve. 0,1 mL of diluted jam solution was pipetted to the test tubes after which 1.9 mL of a distilled water, 10 mL of Follin-Ciocolteu reagent and 8 mL of sodium carbonate solution were added. Apsorbance was measured after 2 hours. The blank was prepared with distilled water instead of the sample.

Determination of hydroxymethylfurfural (HMF)

Hydroxymethylfurfural reaction with barbituric acid and p-Toluic acid were applyed, where the pink color is produced which is measured on the wavelenght of 550 nm. 10 g of the sample was dissolved in 20 mL of distilled water. This solution was transfered to the measuring flask of 50 mL and was made up to the mark with distilled water. 2 mL of this solution was pippeted into each of two tubes. Into one tube was added 1 mL of distilled water and into other 1 mL of barbituric acid. The tube with distilled water was used as a blank. Apsorbances in these samples were measured. Content of HMF is expressed in mg/kg.

Results and discussion

As shown in Fig. 1, the content of dry matter of jam samples was from 64.27% to 77.33%. The average value was (72.38 ± 3.62 %). As the process of concentration of juice is the main factor which determine the content of dry matter, that means that the cultivar of apple does not have an impact on the dry matter content of the jam. Apple jam samples had the similar content of dry matter as the commercial apple jam (65.7%) (Mendoza et al., 2002). Fig. 2 shows a coefficient of correlation between dry matter content and relative density.

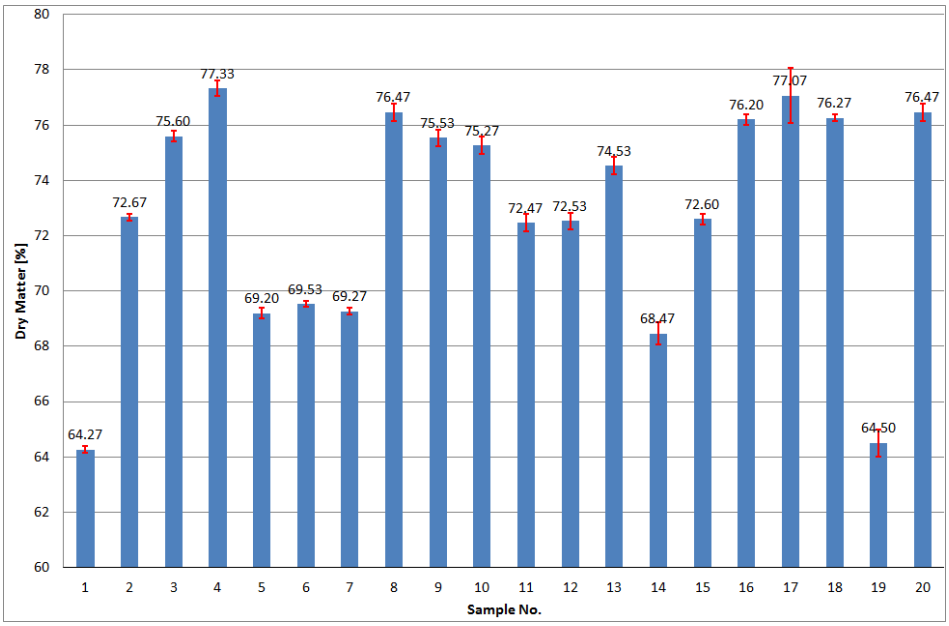


Fig. 1. The content of dry matter in the jam

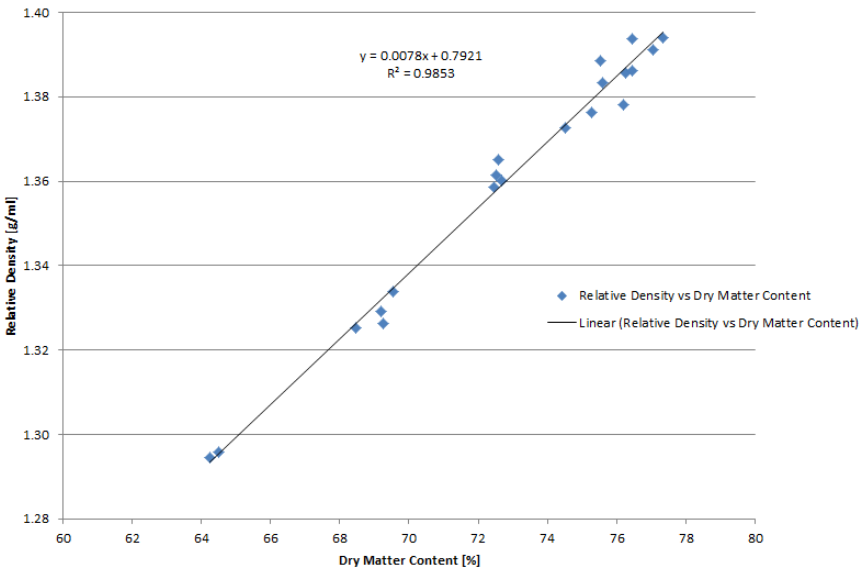


Fig. 2. Coefficient of correlation between dry matter and relative density

The presence of acids in apples affect the pH value of the jam. Research results of the eight apple cultivars have showed that the pH value is 3.87, while the pH value of the jam was from 4.05 to 4.70, as shown in Fig. 3, and the average value was (4.25±0.15). Apple jam samples had the similar pH value as the commercial apple jam (3.54) (Mendoza et al., 2002).
As shown in Fig. 4, the total sugars in jam samples were between 53.49 g/100 g and 67.32 g/100 g, while the reducing sugars were between 51.50 g/100 g and 65.55 g/100 g. Apple jam samples had the similar total sugars values as the grape, apricot, strawberry and blueberry jams (65.99 - 67.65 g/100 g) (Mohd Naeem et al., 2015). The content of sucrose was from 0.72 g/100 g to 1.99 g/100g. The reducing sugars make 97.42% compared to the total sugars.

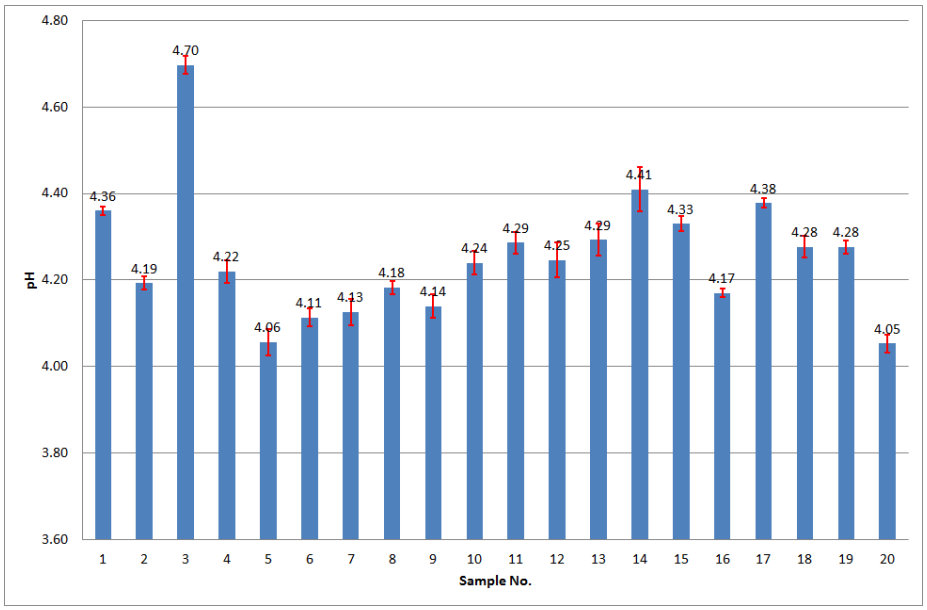


Fig. 3. pH values of the jam samples

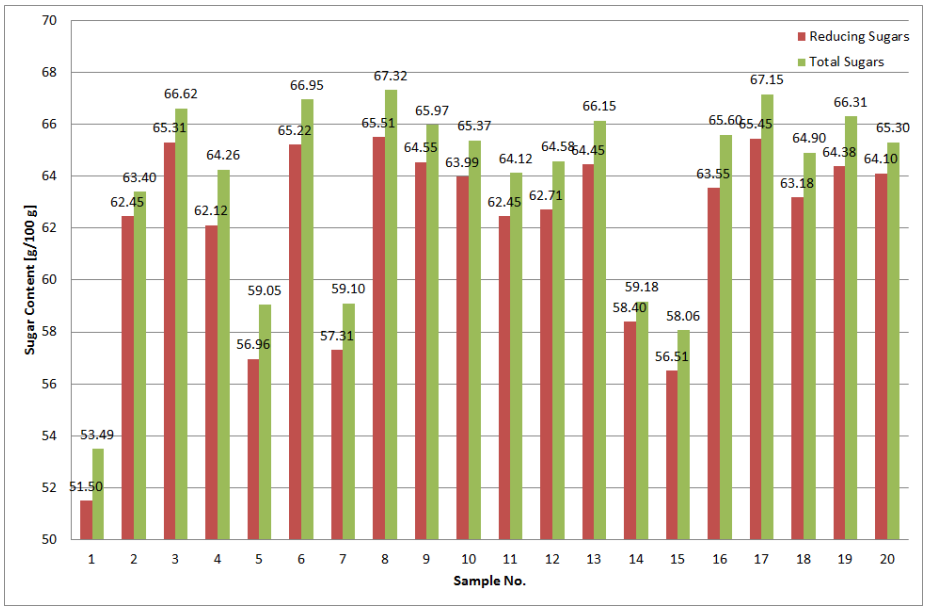


Fig. 4. Sugar content in the jam samples

As shown in Fig. 5, the ash content in the jam samples was between 0.93% and 1.60%. The average value was (1.26 ± 0.21 %). Ash in the jam does not represent just valuable substances. Ash content of the apple jams was significantly higher to the one reported for the grape, apricot, blueberry and strawberry jams (0.12 - 0.25 %) (Mohd Naeem et al., 2015). However, the presence of some metals in the concentrations above allowed values can cause toxicity of the jam.

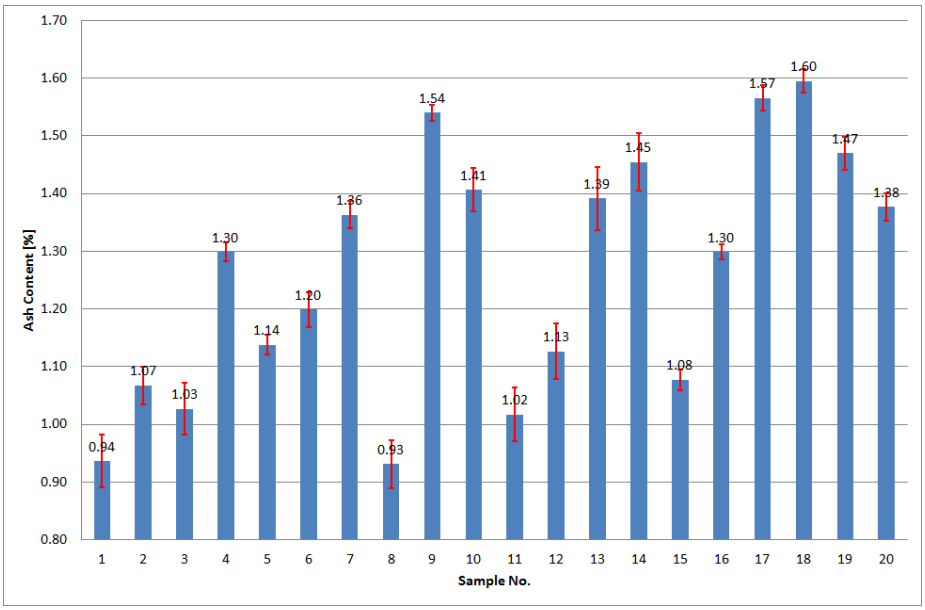


Fig. 5. The ash content in jam

As shown in Fig. 6, the total nitrogen in the jam samples was from 301.80 mg/kg to 1472.60 mg/kg. The average value was 685.10 ± 300.80 mg/kg. The sample No. 2 had significantly bigger nitrogen content compared with the sample No. 18.

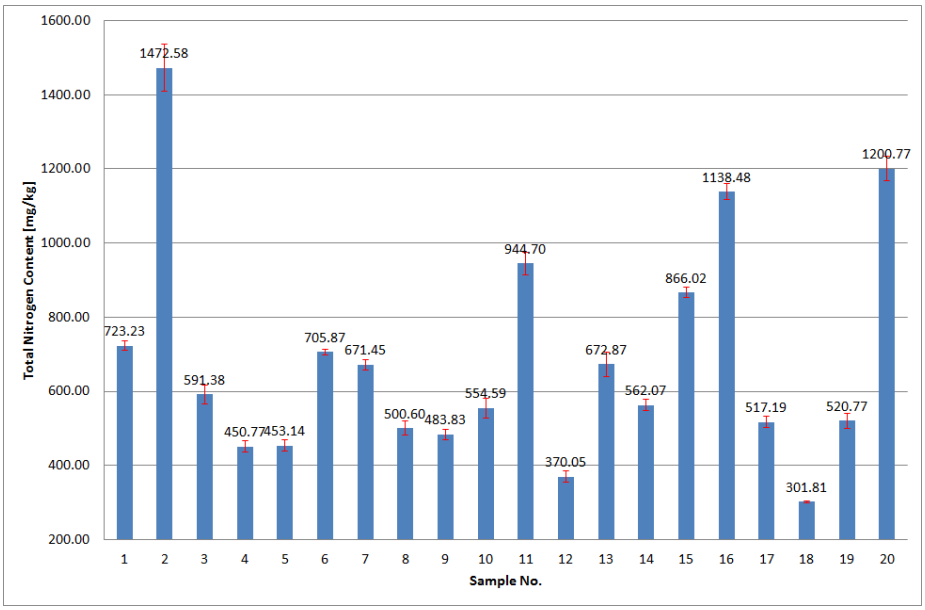


Fig. 6. Total nitrogen content of the jam samples

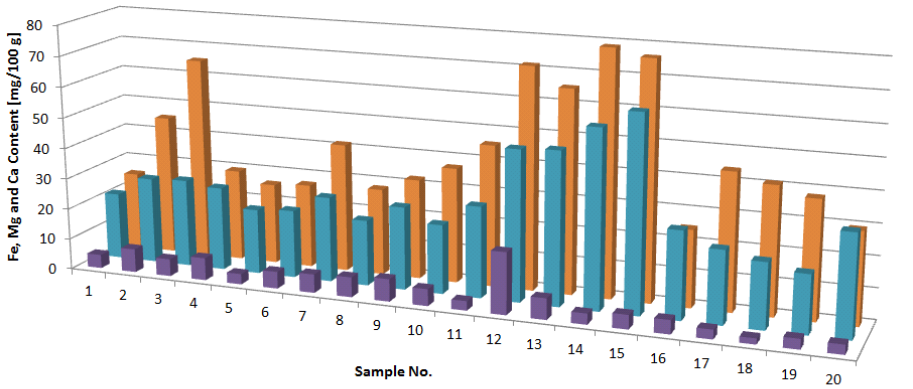
For the determination of nutritional grade of jam, 20 jam samples have been analysed and the content of: iron (Fe), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), copper (Cu) and manganese (Mn) have been determined.

As shown in Fig. 7, the content of iron in apple jam samples was from 1.88 mg/100 g to 19.82 mg/100 g. The average value was 5.61 ± 3.74 mg/100 g. In the jam sample No. 12 the content of iron was 19.82 mg/100 g. Fe content of the apple jams was significantly higher to the one reported for the grape,

apricot, blueberry and strawberry jams (0.200-0.267 mg/100 g) (Mohd Naeem et al., 2015). The reason for this high content of iron is the equipment used during grinding, percolating and concentrating. The jam from 'Samoniklica' cultivar had the iron content 5.88 mg/100 g, while the jam from mixed apple cultivars had the iron content 5.34 mg/100 g. When the results of the sample No. 12 were excluded, the average value for the jam from mixed apple cultivars was 3.82 mg/100 g. Based on the results it can be concluded that the jam from 'Samoniklica' had bigger content of iron compared with jam from mixed cultivars.

The content of magnesium (Mg) in the jam was present in much less amount compared with the other products of plant. The content of magnesium in jam, which can be seen in Fig. 7, was from 18.79 mg/100 g to 62.86 mg/100 g. The average value was (30.80 ± 12.91) mg/100 g. Mg content of the apple jams was significantly higher to the one reported for the grape, apricot, blueberry and strawberry jams (2.717- 6.783 mg/100 g) (Mohd Naeem et al., 2015). Unlike iron which has to be taken by food, the organism effectively process Mg and stored it in the kidneys, as well as excrete all of the excesses Mg. This is the reason why magnesium deficiency is rare. The results of magnesium content indicate that analysed samples from 1 to 10 had approximate content and those were the samples of the jam from 'Samoniklica'cultivar. The magnesium content was slightly higher for other ten samples (samples 11-20) and the differences between the samples were significant.

Calcium (Ca) has an important role as a macro-element in the organism, where the lack of calcium can lead to diseases such as osteoporosis. The content of calcium in jam samples, which can be seen in Fig. 7, was from 24.80 mg/100 g to 78.82 mg/100 g. The average value was 43.64 ± 18.03 mg/100 g. Ca content of the apple jams was significantly higher to the one reported for the grape, apricot, blueberry and strawberry jams (6.517- 18.100 mg/100 g) (Mohd Naeem et al., 2015). The present amount of Ca in the jam can not satisfy minimum daily intake nor 10% of organism needs. The low content of Ca in the jam is a reflection of insufficient amount of Ca in the soil which confirms that the soil on this territory is acidic. Often for intensive fruit-growing, the whitewash is added for the neutralisation of the acidic soil. The low calcium content in the jam is a good base for absorption of iron in the human organism, because high values of calcium prevent absorption of iron.



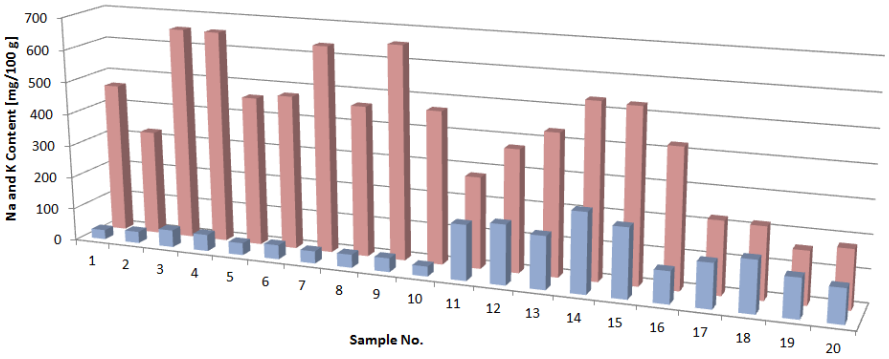
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Fe	4.42	7.6	5.6	7.28	3.39	5.46	6	6.43	7.24	5.43	3.03	19.82	6.81	3.5	4.56	4.46	3.04	1.88	3.27	3.09
Mg	21.51	27.77	28.35	27.02	20.99	21.86	27.4	21.14	26.67	22.23	29.35	48.43	49.15	57.23	62.86	28.13	23.48	21.09	18.79	32.63
Ca	25.2	45.29	65.38	29.56	26.1	27.06	41.5	27.98	32.24	37.11	45.63	71.52	65.41	78.82	76.45	24.83	44.04	41.07	38.05	29.58

Fig. 7. The content of iron, magnesium and calcium in the jam

Fig. 8 shows that sodium content (Na) in the jam samples was from 29.10 mg/100 g to 244.19 mg/100 g. The average value of sodium in 20 analyzed samples was 99.31 ± 68.89 mg/100 g. Na content of the apple jams was significantly higher to the one reported for the grape, apricot, blueberry and strawberry jams (1.367 - 9.233 mg/100 g) (Mohd Naeem et al., 2015). From the results of the Na content in the jam from Samoniklica it can be seen that it can satisfy up to 10% of organism needs, regarding the fact that the recommended minimum daily intake of Na is 500 mg. The content of sodium in the 'Samoniklica' jam was approximately equal and it was not bigger than 50 mg/100 g, while the content of sodium in the jam from mixed cultivars was much higher.

Potassium (K) belongs to the group of macro-elements and it has a significant effect on the balance of liquids in the organism. Regarding the obtained results, it is in considerable amount represented in the

jam. As shown in Fig. 8, the content of potassium was from 160.10 mg/100 g to 656.00 mg/100 g. The average value was 430.70 ± 159.70 mg/100 g. It can be seen from the results that the jam had a high content of potassium because of the high content of potassium in the soil. The content of K was determined on the territory of Gradačac by Faculty of Agriculture in Sarajevo in collaboration with „Proizvodno marketinška grupa voće i povrće poljoprivredna zadruga Gradačac“ (PZ „PMG“ Gradačac) and results showed that the content of K in the soil was from 30.00 mg/100 g to 100.00 mg/100 g. Recommended minimum daily intake of potassium is 2000.00 mg, and it can be concluded that 100 g of jam can satisfy 25% of total needs for the human organism. The jam from ‘Samoniklica’ contains more potassium in comparison with the jam from mixed apple cultivars.



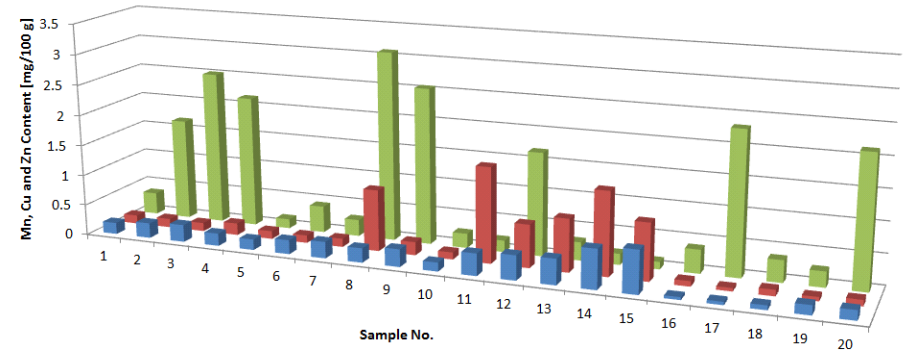
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Na	29.2	35.79	52.8	51.09	37.62	43.88	37.76	40.33	42.35	30.45	169.6	182.9	161.4	244.2	211.9	97.7	135.2	157.5	120	104.5
K	463.1	323.8	656	654.9	460.6	475.4	636.6	462.9	656	468.2	279.7	374.7	433.9	535.6	530.4	424.2	221.9	217.8	160.1	178.1

Fig. 8. The content of sodium and potassium in the jam

Manganese content (Mn) in the jam samples was between 0.05 mg/100 g and 0.70 mg/100 g, which can be seen in Fig. 9. The average value was (0.26 ± 0.17) mg/100 g. The jam samples from ‘Samoniklica’ had similar manganese content, while that was not a case with mixed cultivars. The highest manganese content was observed in the sample No. 15. Recommended minimum daily intake for manganese is 2.50-5.0 mg. It has the important role in the formation and maintenance of bones, cartilage and connective tissue. Also, it contributes to the protein synthesis and genetic material, helps generating energy from food, works as antioxidant and helps normal blood coagulation. The nutrient composition of foods can satisfy recommended intake of manganese.

Copper content (Cu) in the jam samples was between the values 0.05 mg/100 g and 1.55 mg/100 g, as shown in Fig. 9. The average value was 0.41 ± 0.48 mg/100 g. The highest content of Cu was recorded in the sample No. 11. Copper is important for human health because it helps creating haemoglobin in blood, facilitates iron absorption so that the red blood cells can transfer oxygen to the tissue. It affects the blood pressure and heart beats, helps strengthening blood vessels, bones, tissues and nerves, improves fertility and provides healthy skin and hair pigmentation. Also, it protects the tissue from free radical damages, strengthens immune system and has a part in cancer suppression. Cu content of the apple jams was significantly higher to the one reported for the grape, apricot, blueberry and strawberry jams (0.003 - 0.023 mg/100 g) (Mohd Naeem et al., 2015). It was determined that jam samples 8, 11, 12, 13, 14 and 15 had higher copper content compared with all the other samples. The reasons for high copper content in samples number 8, 11, 12, 13, 14 and 15 can be the: presence of large amount of copper in the soil, as well as the production method of jam. Traditionally, the jam was produced by concentrating the juice done in cupric kettles or tavulji which are not appropriate for the production of jam.

Zinc content (Zn) in the jam samples was between values 0.12 mg/100 g and 3.09 mg/100 g. The results showed in Fig. 9 were pointing out that there were no significant differences between the jams produced from 'Samoniklica' and from mixed apple cultivars. The average value was 1.07 ± 1.05 mg/100 g. Zn content of the apple jams was significantly higher to the one reported for the grape, apricot, blueberry and strawberry jams (0.010 - 0.070 mg/100 g) (Mohd Naeem et al., 2015). Zinc is significant element but its lack in the organism is very common due to insufficiently food intake. Compared with 15.00 mg of recommended daily dose, 100.00 g of the jam can satisfy 10 - 20 % of daily needs of the organism, and it can be significant for kids, vegans and elders.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mn	0.187	0.246	0.282	0.205	0.174	0.23	0.27	0.235	0.288	0.145	0.361	0.403	0.419	0.644	0.7	0.045	0.051	0.074	0.156	0.16
Cu	0.139	0.143	0.137	0.194	0.13	0.124	0.136	1.001	0.216	0.115	1.554	0.701	0.86	1.36	0.931	0.087	0.054	0.102	0.067	0.1
Zn	0.357	1.668	2.512	2.154	0.152	0.434	0.27	3.092	2.555	0.236	0.189	1.682	0.298	0.18	0.121	0.386	2.324	0.36	0.256	2.139

Fig. 9. The content of manganese, copper and zinc in the jam

The highest difference in the content of Ca, Mg, Na, Mn and Cu was observed between the samples 11, 12, 13, 14 and 15. Many factors can influence the minerals content, for instance: the soil, precipitation, technological processes and the equipment.

Beside minerals which are present in the jam, biological active compounds like polyphenols are also present. As shown in Fig. 10, the content of total polyphenols in jam samples was in range from 0.36 g/L to 1.00 g/L. The average value of polyphenols in the jam was 0.75 g/L. Samples 10, 13 and 14 had highest polyphenol content which was not influenced with apple cultivar. Some of the jam samples, such as sample No. 20, had low polyphenols content which can be result of low exposure to sunlight which leads to unripe apples.

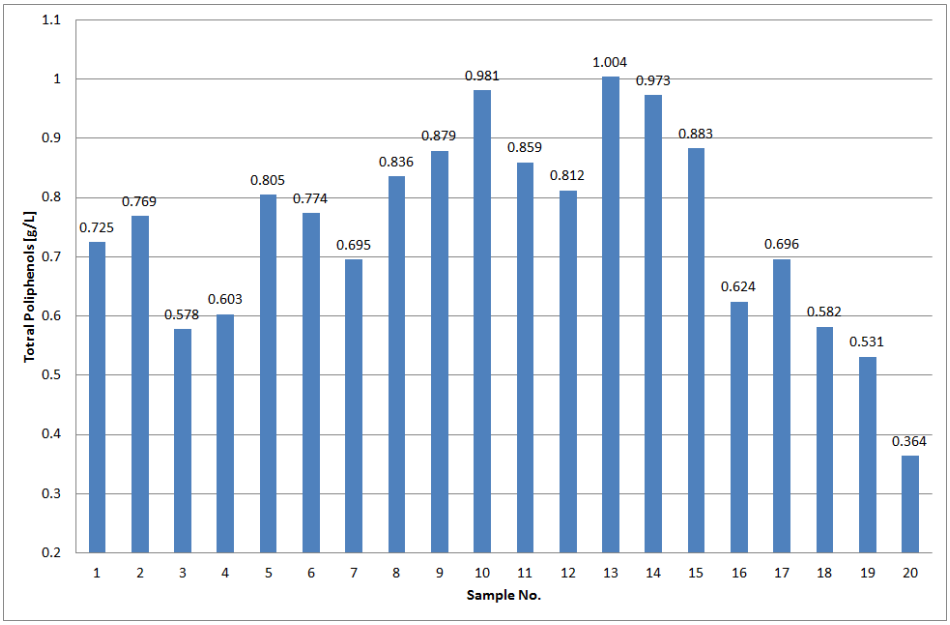


Fig. 10. The content of polyphenols in jam

The results of HMF content analysis in the jam were divided in three groups. The first group represented results of jam sample which were produced at lower temperatures up to 70 °C. The second group included the samples which were produced in traditional way, while the third group included the samples which were produced before the year 2006.

As shown in Fig. 11, the HMF content in jam samples produced at lower temperatures was between values 0.63 mg/kg and 4.4 mg/kg, while the average value was

2.86 mg/kg. Besides temperature as the main influence factor on the high HMF content, the significant effect can have the juice amount which needs to be concentrated, as well as the evaporable surfaces.

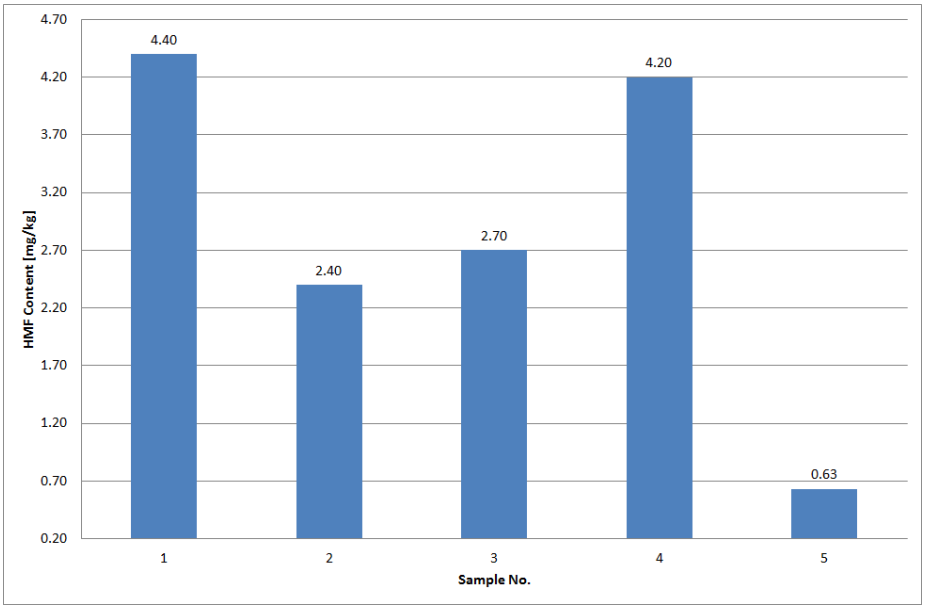


Fig. 11. The HMF content of jam produced at temperature up to 70 °C

The second group included the results of samples analyses which were produced in traditional way, which can be seen in Fig. 12.

Their HMF content was from 3.40 mg/kg to 30.90 mg/kg, while the average value was 24.79 mg/kg. The jam samples of the second group were produced in the year 2011 and the process of concentrating of juice was carried at controlled temperature. The sample No. 14 had significantly lower HMF content compared with other samples. This result indicates that the sample No. 9 is concentrated at lower temperature compared with the traditional process of concentration, as well as the evaporable surface was bigger. The sample No. 9, according to the HMF content can be classified within the first group of samples which were concentrated at the temperatures up to 70 °C which leads to the conclusion that the jam produced in traditional way can have low HMF content.

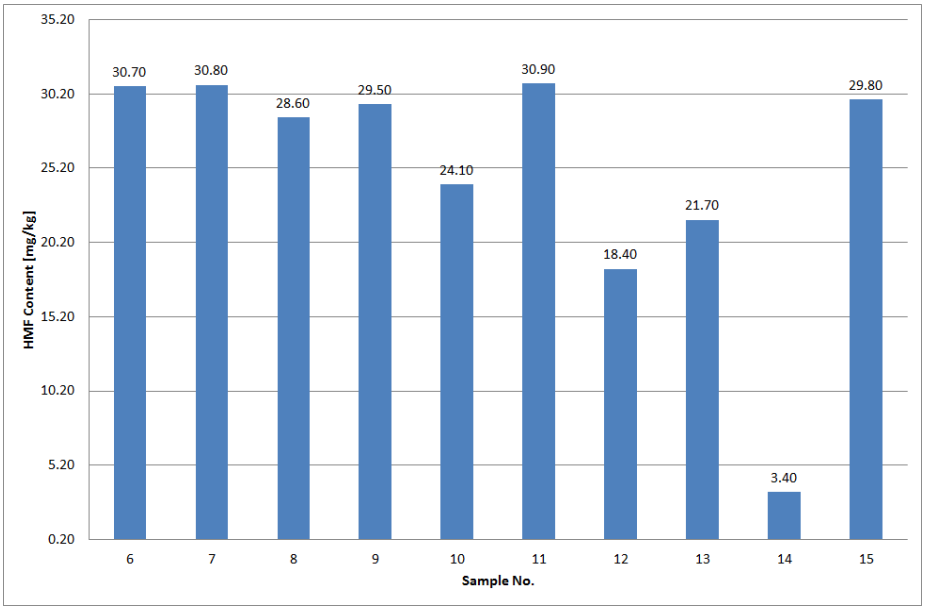


Fig. 12. HMF content of jam produced in traditional way

The third group included samples which were also produced in traditional way but the jam was produced before the year 2006. HMF content in the jam produced before the year 2006, which can be seen in Fig. 13, was between 67.67 mg/kg and 1646.03 mg/kg, while the average value was 595.66 mg/kg. The big differences ocurred among the results of samples belonging to the third group. The high HMF content in the samples of third group can be caused by the year of production of the jam and bad juice percolation (presence of fruit parts) which lead to increase of the HMF content at high temperatures. The findings were not comparable to the HMF content of apple commercial jam (0.98 mg/199 g), which had a significantly lower HMF content (Mendoza et al., 2002).

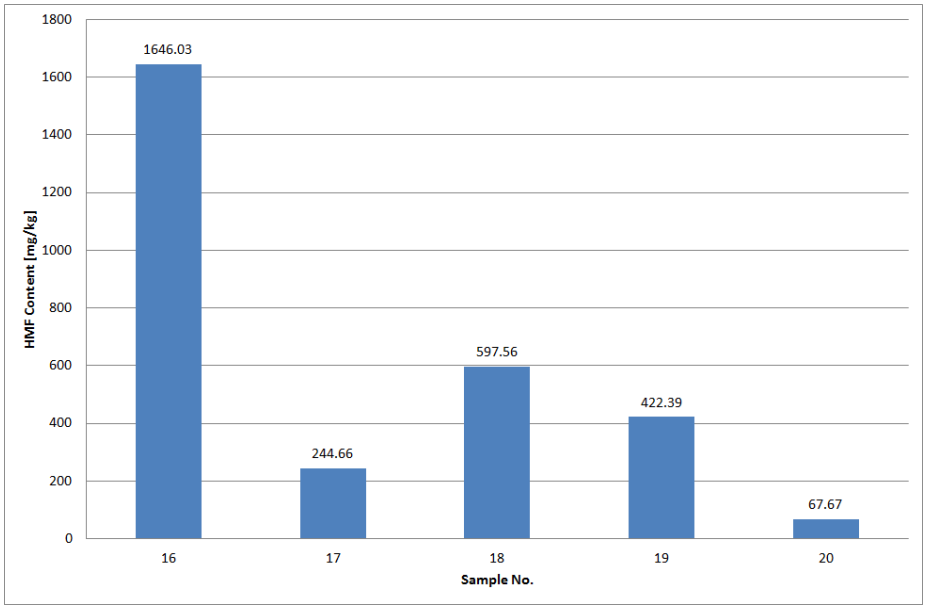


Fig. 13. HMF content in jam produced before the year 2006

The apple cultivar does not have the effect on the HMF content. The results showed that the traditional way of the jam production can satisfy standards when it comes to the HMF content, along with quality production control. The dry matter content in the jam had a low coefficient correlation with the HMF content.

Conclusions

According to the obtained results it can be concluded that the jam from traditional apple cultivars can be used as functional food. Carbohydrates make 85% of the entire dry matter of the jam which indicates that the jam can be classified as high energy food and as such can be recommended to the sport players. The recommended daily intake of potassium is 2000 mg, which means that 100 g of jam can satisfy 25% of daily organism requirements. The content of zinc in 100 g of jam compared to the recommended daily intake of 15 mg, can satisfy 10 - 20 % of daily organism requirements, which can be significant for little children, vegans and elderly. 100 g of the jam can satisfy 35% of daily organism requirements for iron. The high HMF content can be caused by the higher temperatures, so it leads to a conclusion that jam needs to be prepared in controlled temperature.

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REINVENTING THE TRADITIONAL PRODUCTS - THE CASE OF BLACKBERRY WINE

Darko Velić[1], Natalija Velić[1*], Daniela Amidžić Klarić[2], Vlatka Petravić Tominac[3], Ilija Klarić[4], Mara Banović[3]

[1]University of Osijek, Faculty of Food Technology Osijek, F. Kuhača 20, 31000 Osijek, Croatia
[2]University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovačića 1, 10000 Zagreb, Croatia
[3]University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia
[4]Public Health Brčko DC, Department of Health Ecology, R. Dž. Čauševića 1, 76000 Brčko DC, Bosnia and Herzegovina

review paper

Summary

Blackberry wine is traditionally produced in the continental parts of Croatia and consumed in moderate quantities as a dessert wine. It is often used as a popular remedy for anaemia and iron deficiency, as well as for alleviating sleep disorders, because of its mineral composition comprising among other elements, iron and magnesium. Besides minerals, blackberry wine is a good natural source of strong antioxidants, such as phenolic acids, anthocyanins, flavonols, catechins and other flavonoids. For a long time, fruit wines in Croatia were mainly produced by traditional methods in small scale. Therefore, the wines were of inconsistent characteristics and often lower quality. Small and medium scale producers are now implementing the more appropriate modern production technologies to achieve good fermentation control and produce high-quality blackberry wine. Consumers are looking for a fruit wine with traditional, geographically unique characteristics, as well as appropriate enological properties, sensory attributes and high added value (*e.g.* wine rich in bioactive compounds). This is in accordance with the trends in the food industry and food markets (functional, organic) that are geared towards the development of new products with the high added value associated with health or well-being of environment and society. This work aims at reinventing the traditional product blackberry wine, through the prism of claims and requirements for functional and organic foods.

Keywords: blackberry wine, functional products, organic products, winemaking practices

Introduction

Among the traditional alcoholic beverages of Croatia, blackberry wine has been valued and cherished as a natural remedy for iron deficiency (anaemia). Furthermore, it is generally believed that moderate drinking of blackberry wine can help treat exhaustion and loss of appetite, regulate the digestion and blood pressure, increase the blood cell counts and improve the overall health status. Blackberry wine is obtained by the alcoholic fermentation of fresh blackberry juice or blackberry must. Alcoholic fermentation, performed by yeast, is a complex biochemical process of sugar decomposition with the formation of ethanol and carbon dioxide as the main products and several secondary products essential to the overall quality of blackberry wine. Blackberry wine proved to be an excellent natural source of dietary fibres, minerals, and many bioactive phytochemicals with strong antioxidant potential, such as flavonols, anthocyanins, phenolic acids, vitamins, and others (Amidžić Klarić et al., 2011a). Having all this in mind, it is understandable why the homemade production of blackberry wine in Croatia has been continually flourishing. However, the scale-up of blackberry wine production has been relatively slow compared to grape wines. The vast majority of blackberry wine producers in Croatia are small and medium-scale family businesses, often incorporating both blackberry cultivation and blackberry wine production. The trends in the food industry and the recommendations of nutritionists are geared towards the development of new functional products, which offer benefits beyond their nutritional value by exerting positive effects on human health. In that sense, blackberry wine can be considered as a functional product (Dey and Sireswar, 2019). Valls et al. (2013) stated that functional foods and nutraceuticals are one of the top trends in the food industry. The high portion of blackberry wine production in Croatia is based on organically cultivated blackberry. Besides functional foods, organic foods are another segment of the European and global food market that has seen a constant growth within the last couple of decades. Both organic and functional food markets aim at providing consumers with high-quality products with added value. The added value of functional foods is associated with health, while the added value of organic products, besides health, extends to the well-being of the environment and the society in general (Khal et al., 2012; Popa et al., 2018). In this way, the traditional products, such as blackberry wine, now being labelled as functional or organic, are being reinvented in order to gain full market acceptance.

This work aims to give a new insight into the traditional product, *i.e.* blackberry wine, through the prism of claims and requirements for functional and organic foods.

*Corresponding author: nvelic@ptfos.hr

From Blackberries to Blackberry Wine - Obtaining a High-Quality Product with Added Value

The essential step in high-quality blackberry wine production is the selection of berries. The blackberry fruit is a delicate and aromatic aggregate fruit, which has a refreshing acidic-sweet taste and high nutritional value. The nutritional value of blackberry and fruit products derived from its processing are given in Table 1. Fresh, high-quality blackberries contain high amounts of nutrients (sugar, organic acids and dietary fibres) and bioactive phytochemicals that play an essential role in health promotion and disease prevention, such as already mentioned minerals, flavonols, anthocyanins, phenolic acids, vitamins and others (Lenter, 2000; Amidžić Klarić, 2011c; Schulz et al., 2019). Some of them survive the winemaking process and transfer to blackberry wine unchanged, while others are subjected to changes during and after alcohol fermentation as well as maturation (Amidžić Klarić, 2011). The overall chemical composition of blackberry wine comprises mainly water and ethanol, followed by glycerol, organic acids (malic acid as the major organic acid present in blackberry), residual sugars, aroma compounds (esters, higher alcohols, acetates, aldehydes, ketones, lactones, terpenes and phenols), as well as minerals and phenolic compounds. The two latter ones being the most important when discussing the blackberry wines as functional foods.

Table 1. The nutritional value of blackberry and fruit products derived from its processing is expressed in 100 g of product (Brodarec, 1976)

Ingredient		Fresh blackberry	Frozen blackberry	Blackberry juice
Water (g)		82 – 86	74.3 – 82	85.8
Proteins (g)		0.7 – 1.3	0.8 – 1.0	0.8
Fats (g)		0.4 – 0.9	0.3 – 0.5	0.8
Carbohydrates (g)	Total	11.5 – 12.9	15.7 – 24.4	12.1
	Mono- and disaccharides	6.4	7.8	
	Dietary fibres	4.1 – 7.3	1.8 – 7.2	2.7
Total acidity (expressed as citric acid) (g)		1.5		
pH value		3.2		
Ash (g)		0.5	0.5	0.5
Vitamins				
Carotenes		100 - 230 IU 96 μg	140 IU 66 μg	150 IU
Retinol (μg)		16	11	
α-tocopherol (mg)		0.35 – 0.6		
L-ascorbic acid (mg)		20 – 21	8	5 – 10
Thiamine (mg)		0.03	0.02	0.02 – 0.03
Riboflavin (mg)		0.04	0.1	0.03 – 0.05
Niacin (mg)		0.4	0.6	1.2
Pyridoxine (mg)		0.05		0.06
Nicotinic acid (mg)		0.4		0.3
Pantothenic acid (mg)		0.25		
Biotin (μg)		0.4		
Mineral composition				
K (mg)		170 – 210	105	160 – 170
Ca (mg)		32 – 63	17	20 – 25
Na (mg)		1 – 4	1	1
Mg (mg)		23 – 30	12	22
Fe (mg)		0.55 – 1	0.6	0.4 – 0.9
Cu (mg)		0.12		
Zn (mg)		0.27		0.25
P (mg)		19 – 25	17	17 – 30
Cl (mg)		22		
S (mg)		17		
Energy value		29 – 58 kcal 121 – 253 kJ	72 – 96 kcal 300 – 400 kJ	54 kcal

Table 2 gives a list of some of the phenolic compounds and minerals determined in Croatian blackberry wines. The epidemiological studies have proved that phenolic compounds prevent diseases such as cancer, diabetes, osteoporosis, cardiovascular and neurodegenerative diseases (Arts and Hollman, 2005; Graf et al., 2005). Even though there is a lack of research on the health benefits of fruit wines, it is expected that the presence of ethanol in blackberry wine enhances the bioavailability of phenolic compounds, which can be supported by the results reported by some authors that indicate higher health benefits of grape wines than the isolated phenolic extracts alone or alcohol-free wine (Gambelli and Santorini, 2004; Cliff et al., 2007). Small amounts of minerals are required for various metabolic processes essential for the functioning of the human body (Gharibzahedi and Jafari, 2017). According to Caillot et al. (2018), some polysaccharide fractions isolated from blackberry wine markedly reduced nitric oxide and pro-inflammatory cytokine production (TNF-α and IL-1β) in lipopolysaccharide-treated cells.

Table 2. Some of the phenolic compounds and minerals determined in Croatian blackberry wines (Amidžić Klarić et al., 2011[a,b]; Amidžić Klarić et al., 2016[c]; Amidžić Klarić et al., 2017[d])

	Blackberry wine (No. of samples)[Ref.]
Total phenolics*	733 – 2698 (17)[a]
Total anthocyanins**	1.3 – 125.3 (17)[a]
Individual phenolic compounds	
Flavonol	
Quercetin	0.8 – 21.7 (15)[d]
Anthocyanins	
Cyanidin (as aglycon)	<LOD – 3.2 (15)[d]
Pelargonidin (as aglycon)	<LOD – 1.46 (15)[d]
Phenolic acids	
Gallic acid	28.1– 122.4 (17)[a]
Caffeic acid	2.0 – 4.8 (17)[a]
Chlorogenic acid	1.0 – 3.9 (17)[a]
p-Coumaric acid	01.0 – 4.4 (17)[a]
Metals	
K	564 – 2014 (32)[b,c]
Na	12 – 213 (32)[b,c]
Ca	86 – 457 (32)[b,c]
Mg	706 – 381 (32)[b,c]
Fe	0.082 – 8.4 (32)[b,c]
Cu	0.06 – 0.77 (32)[b,c]
Mn	0.7 – 11.5 (32)[b,c]
Zn	0.25 – 6.65 (32)[b,c]

Legend: all values are expressed in mg/L; *values are expressed as gallic acid equivalents; **values are expressed as malvidin-3-glucoside equivalents; LOD - Limit of Detection.

Blackberry wine as a functional product

Can blackberry wine be considered a functional product? To answer this question, it is necessary to have a distinctive, unmistakable definition of functional foods. Doyon and Labercque (2008) concluded that despite the large body of literature dealing with legislative, technological perspectives and market potential of functional foods, the commonly accepted definition of functional foods is still lacking. Therefore, they generated the following definition based on the extensive literature review and the use of Delphi technique with a group of experts in the field: "*A functional food is, or appears similar to, a conventional food. It is part of a standard diet and is consumed regularly, in normal quantities. It has proven health benefits that reduce the risk of specific chronic diseases or beneficially affect target functions beyond its basic nutritional functions*".

Blackberry wine, as a traditional product, is, by all means, conventional food product. It is not part of the standard diet, as most of the alcoholic beverages. However, in Croatia, it is often regularly consumed in small amounts by people with iron deficiency. Therefore, it fits a normal consumption pattern in a specific geographic/cultural context (Doyon and Labercque, 2008). Even though it is generally believed that blackberry wine has many health benefits, the research to date has tended to focus on

the pharmacological activity of blackberries rather than blackberry wine, so the scientific literature on health benefits of blackberry wine (or other fruit wines for that matter) beyond its nutritional functions is scarce. Functional food is still not regulated in Europe, so the formal labelling does not exist. EC-regulation 1924/2006 regulates the claims (for consumers' information) related to the positive health effects of foods. The scientific evidence for claimed health benefits should be provided (Kahl et al., 2012). Mudnić et al. (2012) made a comparison of four blackberry wines with two red and two white grape wines based on their *in vitro* antioxidant and vasodilatory effects. The antioxidant capacity of the examined blackberry wines proved to be stronger than that of the examined grape wines, despite their lower total phenolic content. The blackberry wines proved to be less potent vasodilators than their grape wine counterparts. The overall results indicate the biological potential of blackberry wines and call for further research. Ljevar et al. (2016) studied the phenolic profile of different Croatian fruit wines, including blackberry wine, and evaluated their antioxidant and biological potential. Blackberry wines contained a high amount of total phenolics with distinctive phenolic composition. Along with other examined fruit wines, they inhibited the growth of human cancer cells *in vitro* in a dose-dependent manner, with higher susceptibility in HeLa and MCF-7 cells than CaCo-2 cells. The mineral composition of 17 commercially available Croatian blackberry wines was investigated by Amidžić Klarić et al. (2011b). The results showed that tested wines could be considered as an additional source of magnesium, manganese and potassium. All being said, it can be concluded that blackberry wine could be considered as a functional food. However, the caution must be exercised when a formally labelling product as functional, since the regulation to date does not exist and proper scientific validation of health benefits must be provided for each product.

Blackberry wine as an organic product

The new lifestyle trend known as "green consumerism", with people demanding more foods that are organic and with reduced levels of chemical preservatives for food production and preservation (Leite et al., 2006), has led to a re-discovery of traditional food products (Settanni et al., 2012). At the same time, a traditional product associated with a given geographical area is positively perceived (Francesca et al., 2016). Consumers' demands for organic food is constantly increasing, since it is perceived as more valuable than conventional food, concerning its essential nutritional, sensory and safety properties. Furthermore, it is believed to be more environmentally friendly and more respectful to the welfare of the animals (Suciu et al., 2019). EU market analyses show that the food produced by organic standards is currently mostly lacking. However, the quality-quantity ratio of Croatian domestic organic products is still insufficient, and they do not match the price of products that mostly come from imports. This can also be applied to blackberry wines made from organically grown fruit.
Until recently the cultivation of blackberry in Croatia was reduced only to the exploitation of wild species. However, the cultivated farming of blackberry has started in the continental part of the country, mostly on small family plantations using traditional cultivation techniques and manual harvesting. Thornless Logan, Thornfree, Black Satin and Tayberry are the most commonly cultivated cultivars of blackberry in Croatia, with significant quantities of fresh fruit directly processed into fruit products - jam, juice or wine (Voća et al., 2008). A large portion of the cultivated blackberry farming is based on organic principles, and consequently so is the blackberry wine production. Organic food production is a unique system of sustainable management in agriculture, food industry and forestry, which includes the cultivation of plants and animals, food, raw materials and natural fibres, and the processing of primary products. The production of organic foods in the European Union has been regulated by Commission Regulations (EC) 834/2007 and 889/2008 and Commission Implementing Regulation (EU) 203/2012. Although demands related to organic production vary, some rules are applied generally, *e.g.* the use of herbicides, chemosynthetic insecticides and organic fungicides is uniformly prohibited (Velić, 2014). The purpose of such production is to protect the health and life of people and the environment. Phytopharmaceuticals (*i.e.* pesticides, herbicides, fungicides) and artificial fertilisers are replaced by natural, ecological means, thus preserving soil fertility, purity of water and air. The quality of organic products is controlled, which is of great interest to consumers. Organic food production has many advantages (increasing the overall quality of food products, environment protection, rural development), and such products can achieve higher market prices and are more profitable than those produced conventionally. In the organic food processing industry, which also includes blackberry wine production, there is a need to develop and monitor all relevant production phases trying to preserve the nutritional and sensory properties of the raw material. In this sense, the

minimal processing of the raw material is required. It is also essential to establish a comprehensive process with an emphasis on food safety and quality standards, packaging handling, traceability, control and certification systems.

Conventional and organic cultivation practices differ significantly. However, research on the impact of the cultivation method, *i.e.* organic farming on nutritional quality and health effects of the organic foods often give inconsistent results (Vinković Vrček et al., 2011). Lower levels of known toxicants (nitrates; phytopharmaceuticals, namely pesticides) have been reported in organic products, but natural toxic contaminants (*e.g.* mycotoxins) should be more closely monitored in organic production (Pussemier et al., 2006). When comparing the organic and conventional food products based on nutrients and health-related compounds, the already mentioned inconsistency of results is present. Vinković Vrček et al. (2011) evaluated the mineral and polyphenol content and antioxidant capacity of wines produced from conventionally and organically grown grapes and concluded that the values of antioxidant activity were higher in organic wines. Amidžić Klarić et al. (2017) evaluated the quercetin content, colour and selected physicochemical quality parameters of Croatian blackberry wines produced from organically and conventionally grown blackberries. Quercetin content of organic wine samples group was slightly higher than that of conventional wine samples group. Vitali Čepo et al. (2018) compared the Croatian organic and conventional grape wines based on the levels of pesticide residues and mycotoxin Ochratoxin A. The results showed that organic wines contained significantly lower levels of pesticides and that ochratoxin A positive wines mostly belong to the conventional group. The health effects of functional foods are based on the presence and concentration of specific bioactive compounds and thus, measurable and comparable to conventional foods.

On the other hand, organic food has no placebo for comparison, and it is difficult to support the health-related claims of organic foods. This, however, does not stop the consumers from buying organic food, since they perceive it as more natural and better for their health, the environment and society in general (Kahl et al., 2012). It is, therefore, expected that the Croatian organic blackberry wine would also find its way to the global organic foods market.

Traditional Production ("homemade") *vs* Modern Winemaking Practices

Blackberry wine, and all other non-grape wines for that matter, in Croatia and Europe, is covered by the regulations on fruit wines that define the fruit wines must be obtained by the fermentation of the juices of fruits other than grape (Ordinance on fruit wines, OG 73/06, 24/11, 120/12, 59/13). Furthermore, the regulations define the primary classification of fruit wines to still and sparkling, and the alcoholic strength permitted by different national regulations between 1.2% and 14% by volume (Kosseva et al., 2017).

Blackberry wine quality concept is taking into consideration both physico-chemical and sensory characteristics of wine, which are influenced by various factors: soil, climate, mode of blackberry cultivation and applied cultivation practices, selection of cultivar, agro-technical variables such as physiological state of the raw material at harvest, the proportion of relevant constituents (sugars, organic acids, bioactive compounds, aroma, colour etc.), implementation of maceration, enzymatic processing, selection of yeast and optimisation of fermentation conditions, fermentation equipment etc. (Velić et al., 2013; Petravić Tominac et al., 2013; Velić et al., 2018a).

As already mentioned, the scale-up of fruit wine production, including blackberry wine, has been slow and the majority of producers are small and medium-scale businesses. This could probably be the reason why consumers often associate fruit wines with "homemade" products, which implies lower quality. Setting the consistent standards of quality for fruit wines, as well as marketing initiatives, are needed to alter this perception (Velić et al., 2018b). Traditional homemade blackberry wine is a very heterogeneous group of products from the perspective of its oenological (and consequently functional) properties. The quality of the product depends on the specific blackberry wine recipe, used equipment that is often very simple (plastic vessels for maceration, glass demijohns or plastic barrels for fermentation) and winemaking practices that seldom include the use of maceration enzymes and sulphur. Furthermore, the use of selected wine yeasts is often omitted, and the fermentation temperature is not controlled. All this leads to inconsistent product quality and often also to wine spoilage.

Modern winemaking practices implemented in blackberry wine production tend to change this inconsistency and yield a product of consistent (high) quality. Apart from a careful selection of raw material, modern practices include the use of selected wine yeasts and modern fermentation equipment made of food-grade stainless steel (AISI 304), as well as a double-jacket fermenter for cooling (temperature control) and air-locks. The general scheme of modern blackberry wine production is given in Fig. 1.

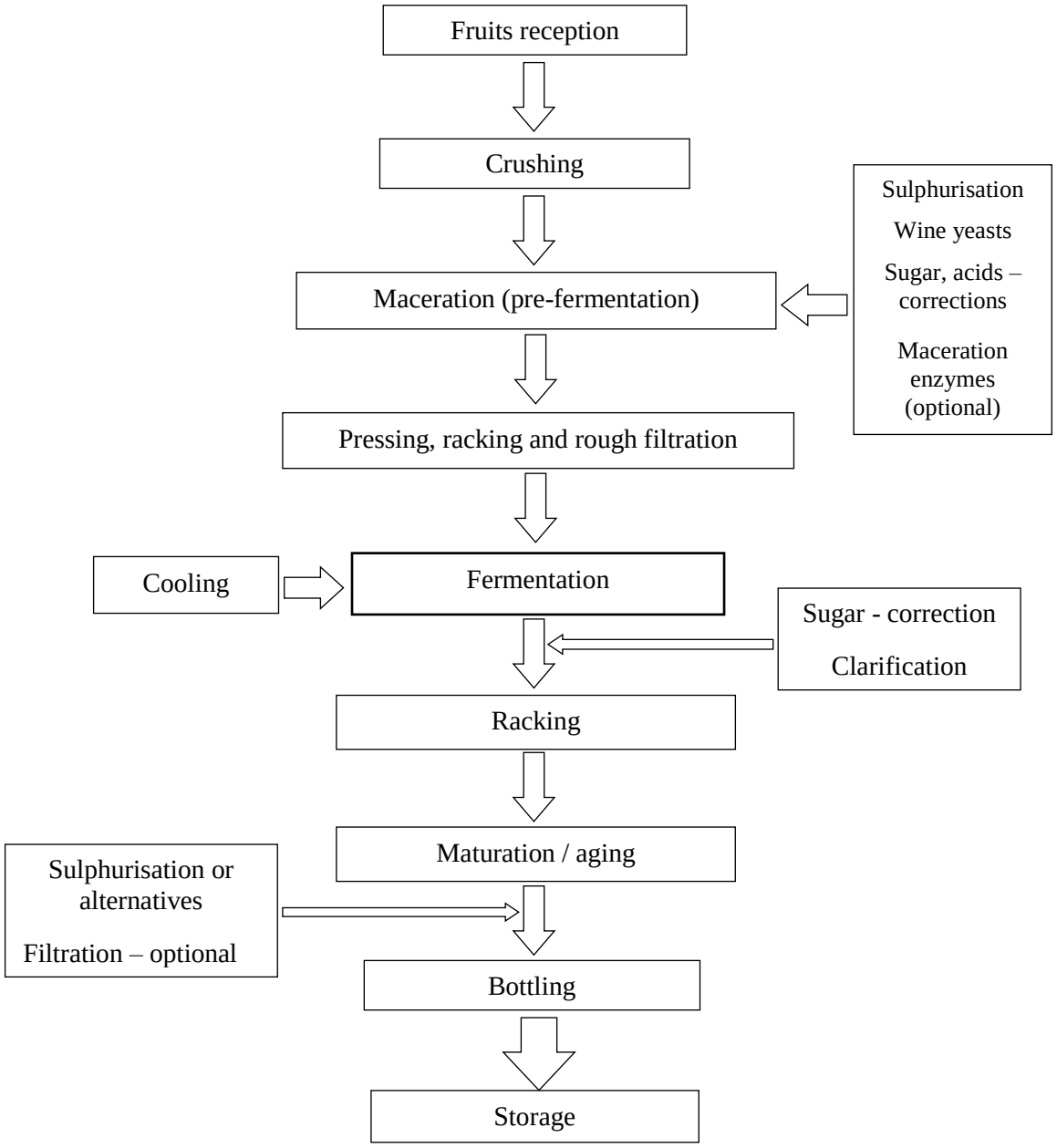

Fig. 1. General scheme of modern blackberry wine production (Velić et al., 2018b)

One of the most significant improvements in wine production in general, but also in blackberry wine production, is better fermentation control using selected yeast strains. Oenological traits of wine yeasts have been divided into two groups, *i.e.* technological and qualitative. Both groups of traits have to be considered when selecting wine yeasts (Rainieri and Pretorius, 2000). Technological traits influence the fermentation efficiency, while qualitative traits determine the chemical composition and sensorial characteristics of wines (Petravić Tominac et al., 2013; Velić at al., 2018a). Tomić et al. (2018) studied the influence of pectolytic enzymes and selected yeast strains on the chemical composition of blackberry wines of Thornfree cultivar and concluded that both enzymes and yeast significantly affected the chemical composition and quality of blackberry wines, especially the concentration of individual anthocyanins in the analysed wines.

EC 889/08 and the Ordinance on wine regulate the production of organic grape wine in conjunction with the detailed provisions of EC 203/2012, Annex VIII.a. These regulations are also applied to organic fruit wine production. It provides the list of allowed oenological additives, as well as allowed practices. According to these regulations, enological additives for organic wine production include:

- *Organic Wine Yeast* (*Saccharomyces cerevisiae*) – 20 - 30 g per 100 kg mass at the initial stage of maceration. It is necessary to provide a GMO-free declaration for yeasts on the prescribed form by EC 889/08, Annex XII.
- *Yeast nutrients* (optional) that can be added during the rehydration of yeast or in case of a stuck fermentation.
- *Potassium metabisulfite*, $K_2S_2O_5$, (5 - 7 g/hL or 30 to 50% lower than the amount recommended for the conventional wine production). K-metabisulfite degrades, among others, to sulphur dioxide (SO_2), which protects wine on several levels (essential free sulphur dioxide, SO_2). The use of permitted oenological additive (E224) is carried out by the recommendations for organic food production according to EC 889/08, Annex VIII and the Ordinance on wine related to the detailed provisions of EC 203/2012 Ecological Wine Annex VIIIa. The total free SO_2 concentration should not exceed the prescribed concentration of 50 mg/L.
- *Maceration enzymes* (optional) can be added to increase the yield, colour and stability (see Fig. 1). The so-called "maceration enzymes" include pectinases and small amounts of cellulase and hemicellulase. They provide better extraction of compounds which contribute towards aroma, colour and health benefits of wine from the fruit during maceration.
- *Brown organic sugar, certified* is added during maceration in order to adjust the sugar content of must, as well as after the fermentation is completed (see Fig. 1). Blackberry fruit is acidic and much lower in sugar content than grapes, so the sugar content has to be adjusted to make it suitable for winemaking. Unlike grape wines production, fruit wines production allows the addition of sugar, fruit juice or concentrated fruit juice in wine, as long as the content of the actual alcohol at the time of delivery to the consumer does not exceed 13% vol (Ordinance on fruit wines, OG No 73/06, 24/11, 120/12, 59/13).
- *Wine clarification agents*, such as pentagel (bentonite) or active sodium bentonite, may be added to the must (better phase separation) or wine before racking (see Fig. 1).
- *Ascorbic acid (vitamin C), certified GMO free* is added to the finished wine as a preservative and antioxidant.

Since blackberry wine has a high amount of residual sugar (initial sugar addition or addition after fermentation) and filtration is mostly omitted to prevent significant loss of colour (residual yeast cells), the main problem associated with blackberry wine production is possible refermentation of wine. This is especially the case with organic blackberry wines because the addition of conventional wine preservatives and stabilisers is not permitted (Velić, 2014).

Other problems that can lead to blackberry wine quality deterioration include inadequate storage, exposure to temperature oscillations (exposure to temperatures above ambient), mechanical packaging damage, high concentration of nitrogen compounds, low concentration of free SO_2, etc. It is recommended that organic wines be appropriately stored in a cool place (Velić, 2014).

Some of the basic physico-chemical properties of blackberry wine which determine the overall wine quality are the contents of alcohol, residual sugar, total and volatile acids, free and bound SO_2, phenolic and aroma compounds, as well as wine colour. Table 3 gives an overview of the average values of some (basic) physico-chemical properties of Croatian organic fruit wines over the ten years (2008 – 2017). The samples were obtained from small and medium-scale organic blackberry wine producers from Slavonia and Baranja region and analysed at the Faculty of Food Technology Osijek. Part of the results presented in Table 3 includes those obtained by the VIP project: "Development and Standardisation of Organic Blackberry Wine Production", funded by the Croatian Ministry of Agriculture, 2012 – 2014.

Table 3. Average values of basic physico-chemical parameters of organic blackberry wines for the period 2008 – 2017

Physico-chemical parameter	Value (range)	Unit
Total alcohol	16.1 – 19.2	% vol.
Real alcohol	10.8 – 13.0	% vol.
Reducing sugars	67.8 – 98.5	g/L
Total sugar	71.3 – 98.5	g/L
Total extract	107.4 – 148.3	g/L
Extract (total sugar excluded)	35.3 – 49.7	g/L
Ash	2.6 – 3.9	g/L
pH	3.27 – 3.68	-
Total acidity (as malic acid)	10.2 – 11.9	g/L
Volatile acidity (as acetic acid)	0.4 – 0.7	g/L
Free SO_2	5 – 28	g/L
Total SO_2	49 – 125	g/L

Conclusion

In the light of new consumers' demands, traditional food products, such as blackberry wine are being rediscovered and reinvented to reach their full market potential. In that sense, informal labelling of blackberry wine as a functional product is part of the reinvention strategy. Furthermore, organic blackberry wines are even more valued, as they reflect the need to be in harmony with the environment and society in general. To obtain the high-quality blackberry wine that can be adequately marketed and positively perceived by the consumers, the transition from homemade products of inconsistent quality to consistently high-quality products had to be done. The process scale-up and the implementation of modern winemaking practice already led to the standardisation of blackberry wine production in Croatia. Further work is needed in the field of health studies that will test the positive health claims generally believed and associated with this traditional product in Croatia, thus making blackberry wine more recognisable at the global functional and organic food market.

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NUTRITIVE COMPOSITION AND LIPID QUALITY INDICES OF COMMERCIALLY AVAILABLE FILLETED FISH

Greta Krešić[1], Ana Vulić[2], Lidija Dergestin Bačun[2], Tina Lešić[2], Darko Želježić[3], Jelka Pleadin[2*]

[1]Faculty of Tourism and Hospitality Management, Department of Food and Nutrition, University of Rijeka, Primorska 42, 51410 Opatija, Croatia
[2]Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska Cesta 143, 10000 Zagreb, Croatia
[3]Croatian Veterinary Institute, General Department, Savska Cesta 143, 10000 Zagreb, Croatia

professional paper

Summary

The importance of fish in a healthy diet is to be attributed to its low energy value, high content of proteins and essential minerals, and favourable lipid composition. Since modern consumers seek convenience, the demand for frozen fish as a nutritious food that can be quickly prepared either at home or in catering establishments is ever growing. In this study, a total of 45 fillets of three commercially important filleted frozen fish, including chum salmon *(Oncorhyncus keta)*, saithe *(Pollachius virens)* and hake *(Merluccius hubbsi)*, were analysed for their basic nutritive composition, fatty acid profile and lipid quality indices. The obtained results showed the highest average amount of fat in hake (4.20 ± 0.85 g/100 g), while chum salmon was the richest in proteins (18.74 ± 1.48 g/100 g). Hake was found to contain the highest amount of n-3 polyunsaturated fatty acids and to have the most favourable thrombogenic index, while chum salmon had the most favourable atherogenic index and the most preferable ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids. Our results could serve as the basis for widening the scope of recommendations for lean fish dietary intake.

Keywords: filleted fish, nutritive composition, fatty acids profile, lipid quality indices

Introduction

Literature data have confirmed numerous health benefits of fish consumption, since moderate-to-high fish intake is associated with the lower prevalence of chronic diseases, such as cardiovascular conditions and diabetes, as well as with the lower prevalence of some cancers (Oehlenschläger, 2012). In general, fish represents a healthy source of energy, high-quality easily-digestible proteins, vitamins (A, D, E and B12), essential minerals and amino acids. The importance of fish in a healthy diet is to be attributed to its favourable lipid composition in terms of high level of unsaturated fatty acids (60 – 84 %), especially n-3 long-chain polyunsaturated fatty acids (LC PUFA). Among the latter acids, eicosapentaenoic acid (20:5 n-3 EPA) and docosahexaenoic acid (22:6 n-3 DHA) are considered to be the most important from health aspect due to their well-recognised pleiotropic health and disease-preventative effects (Gil and Gil, 2015); of note, these acids are mainly present in fish (Murillo et al., 2014). Although the effects of fish consumption on lipid profile have generally been studied on fatty fish, several studies have confirmed that the consumption of lean fish (containing < 5% of fat) may also have a favourable impact on triglyceride levels, blood pressure and type 2 diabetes (Leaf et al., 2009; Erkkilä et al., 2008; Rylander et al., 2014).

The amount of fat and the fatty acid profile are some of the important determinants of quality and nutritive value of fish and fish products (Krešić et al., 2017). Literature data have revealed that lipid content highly varies both between and within fish species in dependence of feed used during farming, environmental conditions at the farm location, fish size and maturity, biological variations, tissue sample profile, and fish starvation (Rueda et al., 2001). Because the world's fish stocks are limited, it has recently been suggested that farmed fish might pose as an alternative suitable for consumers. Farmed seafood has an indisputable advantage over captured fishery products because it gets to be produced and harvested under controlled conditions, therefore posing as a minimal consumption-related health risk (Pleadin et al., 2017). In order to achieve the most favourable fatty acid profile, manufacturers make numerous variations in farming conditions, mainly by altering fish feed regimens (Kris-Etherton et al., 2003; Pratoomyot et al., 2010).

As with other food products, fish consumption is expected to be influenced by consumers' need for convenience (i.e. desire to save effort and time when preparing food). Carlucci and co-workers (2015) have recently documented that frozen fish has been ever more often chosen by consumers due to its convenient preparation, immediate availability and lower price. Since lean fish, especially frozen one, represents an underappreciated source of unsaturated

*Corresponding author: pleadin@veinst.hr

fatty acids, the aim of this study was to analyse three commercially available frozen fish fillets, that is to say, chum salmon *(Oncorhyncus keta)*, saithe *(Pollachius virens)* and hake *(Merluccius hubbsi)*, in order to compare their chemical, in particular fatty acid, composition. Additionally, health-related lipid indices, including the atherogenic index (AI), the thrombogenic index (TI), and the ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH), were determined and compared across the analysed species.

Materials and methods

Sampling and sample preparation

Chum salmon *(Oncorhyncus keta)*, saithe *(Pollachius virens)* and hake *(Merluccius hubbsi)* fish fillets were sampled from the Croatian market during the 2017 – 2018 timeframe. Each type of fish fillets was sampled in three replicates yielding three distinctive fillet lots sampled in five pieces. In total, 45 fillet pieces were taken (15 per fish species), yielding three mutually different 15-piece lots.

After mild defrosting, samples of the same type and lot (in groups of 5; see above) were homogenized for 15 sec at 6000 rpm using a laboratory homogenizer (Grindomix GM 200, Retsch, Haam, Germany). All samples were analysed within 48 hours and stored in plastic containers at +4 °C pending analyses of basic chemical parameters. The extracted fat was stored in a refrigerator at -18 °C pending fatty acid composition analysis, carried out within the next 48 hours.

Basic chemical composition

The water content (g/100 g) was established gravimetrically (ISO 1442:1997) at the sample-drying temperature of 103 °C using a Memmert UF75 Plus drier (Memmert, Germany). The total protein content was determined using the Kjeldahl technique (ISO 937:1978) that utilised a digestion unit (Unit 8 Basic, Foss, Sweden) and an automated distillation & titration device (Vapodest 50s, Gerhardt, Germany). The total fat content was determined using the Soxhlet technique (ISO 1443:1973); to that end, ether-mediated lipid extraction was performed using an extractor (Soxtherm 416 Automatic, Gerhardt, Germany). The total ash content was ascertained via incineration in a muffle furnace (LV9/11/P320, Nabertherm, Germany) at 550 °C (ISO 936:1998). The salt content determination made use of the multiple standard addition potentiometric technique that employs an ion-selective electrode and a Na EasyPlusTM analyser (Mettler Toledo, Germany). Based on the established sodium content, the representation of sodium chloride (salt) was determined stoichiometrically. The carbohydrate content was calculated based on the sum of water, ash, protein and fat content and the difference of up to 100%. The sugar content was determined spectrophotometrically (HACH DR/6000U, Germany) using an enzyme Sucrose/D-Glucose/D-Fructose kit (R-Biopharm, Germany) according to the kit manufacturer's instructions. All chemicals used for the analyses were of an analytical grade. The means obtained from two parallel runs in form of weight percentage (%) with the accuracy of 0.01% were considered descriptive of a target sample. Quality control of the analytical methods used was performed using the Certified Reference Material (CRM) T0149 (FAPAS, York, England).

Fatty acid profile

In order to determine the fatty acid composition, fatty acid methyl esters were obtained from the extracted fat according to the standard EN ISO 12966-2:2017 technique that involves dissolution of glycerides in isooctane and trans-esterification using potassium hydroxide methanol solution, and then analysed using gas chromatography (GC) according to the ISO 12966-4:2015 and EN ISO 12966-4:2015. For this purpose, 7890BA gas chromatographer equipped with flame ionization detector (FID), a 60-m DB-23 capillary column having an internal capillary diameter of 0.25 mm and the stationary phase thickness of 0.25 μm (Agilent Technologies, Santa Clara, USA) was used. The components were detected by FID at the temperature of 280 °C, hydrogen flow rate of 40 mL/min, air flow rate of 450 mL/min and nitrogen flow rate of 25 mL/min. The initial column temperature was 130 °C; after a minute, it was increased by 6.5 °C/min until the temperature of 170 °C was reached. The temperature was further increased by 2.75 °C/min until the temperature of 215 °C was attained. The latter temperature was maintained for 12 min and then further increased by 40 °C/min until the final column temperature of 230 °C was reached, the latter being maintained for 3 min. One mL of a sample was injected into a split-splitless injector at the temperature of 270 °C and with the partition coefficient of 1:50. The carrier gas was helium (99.9999%), flowing at the constant rate of 43 cm/sec. Fatty acid methyl esters were identified by comparing their retention times to those of fatty acid methyl esters contained by the standard mixture, as described earlier by Pleadin et al. (2015). The results are expressed as a percent-share (%) of an individual fatty acid in total fatty acids, the accuracy thereby being 0.01%. Fatty acid methyl ester values were

converted into fatty acid values per 100 g of edible fish sample part according to the FAO/INFOODS Guidelines for Converting Units, Denominators and Expressions (2012). Quality control made use of the CRM BCR 163 (Institute for Reference Materials and Measurements, Geel, Belgium) that has a specified content of seven individual fatty acids.

Lipid quality indices

Data on fatty acid composition were used for the calculation of lipid quality indices including the atherogenic index (AI), the thrombogenic index (TI) and the hypocholesterolaemic/hypercholesterolaemic ratio (HH). The atherogenic index (AI) indicates the relationship between the sum of main saturates and the sum of main non-saturates. This parameter was calculated as follows: AI = [(C12:0 + (4 x C14:0) + C16:0)] / [∑ MUFA + PUFA n-6+ PUFA n-3] (Ulbritcth and Southgate, 1991). The thrombogenic index (TI) represents the relationship between pro-thrombogenic (saturated) and anti-thrombogenic FAs (MUFA, PUFA n-6 & PUFA n-3). This index was calculated as follows: TI = (C14:0 + C16:0 + C18:0) / [0.5 x ∑MUFA + 0.5 x PUFA n-6 + 3 x PUFA n-3) + (PUFA n-3/PUFA n-6)]. The ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH) takes well-known effects of certain FAs on cholesterol metabolism into account (Santos-Silva et al., 2002). It was calculated as follows: HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0) (Ulbritcth and Southgate, 1991).

Data analysis

Results of basic chemical composition and fatty acid profile were expressed as mean values obtained from two parallel runs. Data analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013).

Results and discussion

The results presented in this study provide the nutritional profile of three lean fish species (chum salmon, saithe and hake) commercially available on the Croatian market. The aim of this study was to prove that lean fish is often underappreciated and underrepresented in a daily diet in comparison with fatty fish. However, since consumer demand for frozen fish continuously increases and given that fatty fish is prone to lipid oxidation and loss of nutritional quality during freezing and frozen storage, frozen filleted lean fish emerges as an interesting market niche.
Basic nutritive composition of frozen fillets of chum salmon, saithe and hake available on the Croatian market is shown in Table 1.

Table 1. Basic nutritive composition of commercially available frozen fish fillets

Chemical parameter (g/100 g)	Chum salmon	Saithe	Hake
Water	76.63±2.60	79.73±0.75	79.80±1.38
Ash	0.89±0.16	1.53±0.05	1.04±0.02
Fat	4.13±3.86	1.04±0.10	4.20±0.85
Protein	18.74±1.48	18.63±0.75	16.03±0.37
Carbohydrates	<0.5	<0.5	<0.5
Sugar	<0.2	<0.2	<0.2
Salt	0.21±0.06	0.35±0.05	0.19±0.01

The analysis of basic nutritive parameters confirmed that fish fillets represent a valuable source of proteins (ranging from 16.03 g/100 g in hake to 18.74 g/100 g in chum salmon). The share of fat in chum salmon and hake was similar (about 4%), but significantly higher than in saithe (about 1%). Although lean marine fish species usually have high protein and low fat content, the latter content significantly depends on fish maturity and nutritional status (Oehlenschläger, 2012). Generally speaking, lean marine fish species contain less than 5% of fat, medium fatty marine species 5 – 10 %, and fatty marine species > 10% of fat (Baltić and Teodorović, 1997). Based on the above, all three fish species under study can be categorized as lean marine fish species. The water content found in all analysed species varied dependent on fat, since the two accounts for approximately 80% of the fish fillet composition.
The fatty acid composition of commercially available frozen fish fillets investigated in this study, expressed as mean values and standard deviations relative of the total fatty acid content, is shown in Table 2.

Table 2. Fatty acid composition of commercially available frozen fish filets

Fatty acid	Mean±SD (% of total fatty acids)		
	Chum salmon	Saithe	Hake
C4:0	ND	ND	ND
C6:0	ND	ND	ND
C8:0	0.10±0.15	0.06±0.01	ND
C10:0	0.06±0.10	0.04±0.03	ND
C11:0	ND	ND	ND
C12:0	0.60±0.21	0.13±0.01	ND
C13:0	0.02±0.03	0.05±0.04	ND
C14:0	8.17±1.31	6.60±1.21	4.64±2.25
C14:1	0.06±0.10	0.18±0.05	ND
C15:0	0.85±0.15	0.96±0.15	ND
C15:1	0.06±0.11	0.30±0.06	ND
C16:0	20.90±0.84	30.29±2.61	30.25±2.60
C16:1n7t	0.34±0.10	0.48±0.03	ND
C16:1n7c	6.24±1.89	7.42±1.22	3.85±1.90
C17:0	1.32±0.17	0.92±0.06	2.61±2.81
C17:1	0.38±0.33	ND	ND
C18:0	8.26±2.39	4.14±0.17	18.64±4.69
C18:1n9t	0.78±0.32	1.04±0.26	1.36±0.32
C18:1n9c	28.87±2.40	18.56±1.47	14.03±2.64
C18:1n7	2.84±0.23	3.24±0.76	4.11±0.83
C18:2n6t	0.27±0.30	0.07±0.01	ND
C18:2n6c	1.37±0.52	1.62±0.09	1.26±1.32
C18:3n6	0.59±0.52	0.35±0.25	2.79±1.51
C18:3n3	1.55±0.77	1.26±0.38	4.78±2.66
C18:4n3	0.14±0.25	0.62±0.11	ND
C20:0	0.13±0.13	0.46±0.15	ND
C20:1n9	3.11±0.37	6.59±1.22	7.53±5.33
C20:2n6	0.21±0.19	0.24±0.02	ND
C21:0	ND	ND	ND
C20:3n6	ND	ND	ND
C20:4n6	0.04±0.08	0.16±0.16	ND
C20:3n3	0.05±0.08	0.05±0.08	ND
C20:4n3	0.13±0.22	0.26±0.04	ND
C20:5n3	0.79±0.85	1.14±0.54	ND
C22:0	ND	0.21±0.08	ND
C22:1n11	5.35±1.81	4.12±0.65	4.15±4.85
C22:1n9	3.25±2.40	0.95±1.26	ND
C22:2n6	ND	ND	ND
C23:0	ND	ND	ND
C22:5n3	0.64±0.35	0.67±0.18	ND
C24:0	0.15±0.26	ND	ND
C22:6n3	1.65±1.04	5.20±0.95	ND
C24:1n9	0.73±0.11	1.63±0.29	ND

ND – not detected; limit of detection (LOD) = 0.05%

Prato and Biandolino (2012) investigated the share of fat and the fatty acid profile of 11 commercially available fish species inhabiting the Mediterranean Sea, and determined a total of 25 C12- to C22- fatty acids. In general, fish fat mainly consists of unsaturated fatty acids (60 – 84 %), among which, when it comes to the marine fish, approximately 88% are highly unsaturated fatty acids with 5 or 6 C-double bonds. About half of fish fat is made of oleic fatty acid (C18:1n9) responsible for the soft, juicy texture (Cvrtila and Kozačinski, 2006). In this study, the fatty acid most represented in chum salmon was oleic acid, followed by palmitic (C16:0) and myristic (C14:0) fatty acid. On the other hand, the fatty acid dominating in saithe and hake was palmitic acid, followed by oleic fatty acid, while myristic acid made the least of their fatty acid content.

Since saithe is one of the most common fish in northern European inshore water, and is often to be found in the vicinity of sea cages at salmon farms, Skog and co-workers (2003) conducted an interesting research into the effects of the vicinity of salmon farms on saithe

fatty acid profile. They found the concentrations of palmitic, linoleic (C18:2n6) and α-linolenic (C18:3n3) acids to decrease as the distance from fish farms increases. Fatty acid profile of saithe retrieved from the nearby fish farm-free fjords was very similar to that of saithe samples under this study. From the nutritional standpoint, fatty acids containing C20 and C22 are more valuable than fatty acids containing C18, the highest importance thereby being attributed to EPA and DHA which are, at the same time, largely responsible for changes in n-6/n-3 ratio (Pleadin et al., 2017). In this study, the highest content of fatty acids detailed above was determined in saithe, followed by chum salmon, while none of them was found in hake.

The analyses revealed the highest amount of MUFA in chum salmon followed by hake, while the representation of MUFA in saithe was 10-fold lower as compared to chum salmon (Table 3). Among the analysed samples, hake proved itself as a filleted fish with the highest nutritional value due to its richness in n-3 polyunsaturated fatty acids. Hypotriglyceridaemic effect of n-3 LC PUFA has been proven beneficial in terms of reducing the percent-share of small pro-atherogenic low-density lipoprotein (LDL) particles, and possibly also in terms of ameliorating inflammatory processes associated with metabolic syndrome seen in patients with diabetes mellitus or cardiovascular conditions (Lopez-Huertas, 2012). Generally speaking, fatty acid profile was the poorest in saithe, especially given that only a small amount of fat was determined in that fish.

Table 3. Quantification of fatty acids in chum salmon, saithe and hake (absolute figures)

Groups of fatty acids	Chum salmon (g/100 g)	Saithe (g/100 g)	Hake (g/100 g)
SFA	1.04±1.23	0.31±0.04	1.64±0.22
MUFA	2.01±2.01	0.20±0.07	1.69±0.51
PUFA	0.29±0.29	0.05±0.02	0.44±0.11
∑ n-3	0.22±0.25	0.03±0.01	0.35±0.09
∑ n-6	0.07±0.05	0.02±0.01	0.09±0.02
n-6/n-3	0.32±0.20	0.67±1.00	0.25±0.22

SFA – saturated fatty acids, MUFA –monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, n-3 - omega-3 fatty acids, n-6 - omega-6 fatty acids

In the previously mentioned research by Prato and Biandolino (2012), the shares of saturated (SFA), polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) were 38.1 - 49.8 %; 27.6 - 34.7 %; and 17.8 - 32.4 %, respectively. SFAs predominated due to the high palmitic acid content of roughly 70%. The predominant MUFA was oleic acid (about 60 – 70 % of the total fat content).

In order to gain insight into the possible health effects of certain fatty acids present in fish, in addition to PUFA/SFA ratio, the effects of certain saturated fatty acids should be taken into account, since some of these acids (i.e. C12:0, C14:0 and C 16:0) have been evidenced to increase the total serum cholesterol (Ulbricht and Southgate, 1991). Lipid quality indices determined in commercially available frozen fish fillets analysed in this study are shown in Table 4.

Table 4. Lipid quality indices determined in analysed farmed fish species

Lipid indices	Chum salmon	Saithe	Hake
AI	0.92±0.15	1.12±0.09	1.02±0.20
TI	0.86±0.21	1.53±0.09	0.76±0.14
HH index	1.21±0.17	0.58±0.11	0.79±0.14
PUFA/SFA	0.18±0.03	0.15±0.04	0.27±0.05
n-6/n-3	0.56±0.35	0.95±0.33	0.27±0.05

AI - atherogenic index, TI - thrombogenic index, HH - ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids, SFA – saturated fatty acids, PUFA – polyunsaturated fatty acids, n-3 - omega-3 fatty acids, n-6 - omega-6 fatty acids

PUFA/SFA ratio is recommended to be higher than 0.4, so as to reduce the risk of cardiovascular, autoimmune and other chronic diseases (Simopoulos, 2002). The recommended value was not determined in any of the fish fillets analysed in this study, since the PUFA/SFA ratio determined within this study frame ranged from 0.15 in saithe to 0.27 in hake. Literature data have revealed that lower n-6/n-3 ratios allow for better utilisation of n-3 fatty acids in the human body (Wood et al., 2008). All species analysed in the study by Prato

and Biandolino (2012) had the n-6/n-3 ratio of 0.18 in salema to 0.40 in sea bass and black goby, i.e. similar to the ratio obtained in this study for hake, whereas for chum salmon, and especially saithe, higher ratios were established. According to health recommendations, n-6/n-3 ratio should be lower than 4, thereby reducing the incidence of chronic food-related illnesses (Cordain et al., 2005; Simopoulos, 2002); in this study, that was the case in all three fish species analysed. The lowest n-6/n-3 ratio was determined in hake, which also had the lowest PUFA/SFA ratio, while the highest n-6/n-3 ratio was obtained for saithe due to the lowest amount of PUFA.

The atherogenic index (AI) is the parameter descriptive of the ability of some saturates to exhibit pro-atherogenic effects due to the facilitation of the lipid adhesion onto cells the immune and the circulatory system are composed of, while non-saturates are considered to be anti-atherogenic as they inhibit the formation of plaques and diminish the levels of esterified fatty acids, cholesterol, and phospholipids, therefore preventing micro- and macro-coronary events (Ulbritcth and Southgate, 1991). The thrombogenic index (TI) shows the tendency towards blood clotting. Lipid quality indices determined in this study showed the best AI (<1) and HH (>1) values for chum salmon, while the best TI (<1) was determined for hake.

Given that this research made use of frozen fish fillets coming from the market, it is safe to assume that the determined PUFA levels, a substantial loss of nutritional value, and suboptimal values of lipid quality indices, come as a consequence of freezing and frozen storage. Namely, several studies that made use of salmon, hake and saithe fillets have confirmed that freezing and frozen storage may provoke lipid decomposition, PUFA content decrease and SFA content increase (Dawson et al., 2018; Saldana and Bragagnolo, 2007; Karsdotttir et al., 2014).

Conclusion

Modern consumers are looking for healthy, but easily available and ready-to-prepare food. In light of the foregoing, one of the possibilities is to consume fish in form of frozen fillets. Our results confirm that commercially available frozen chum salmon fillets contain the highest amounts of proteins and fat (above all MUFA) and have the most favourable atherogenic index and hypocholesterolaemic over hypercholesterolaemic fatty acid ratio. On the other hand, hake contains the highest portion of n-3 PUFA and has the most favourable thrombogenic index. Frozen saithe fillets have a four-fold lower total fat content as compared to other two species under study.

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e-mail address: ivana.laus@ptfos.hr

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