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FOOD IN HEALTH AND DISEASE

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Editor's Letter

Dear readers,

I am honoured to be a guest editor of the scientific-professional journal's fifth issue *Food in Health and Disease*, which is the result of a scientific cooperation in the field of food technology and nutrition science between Osijek and Tuzla over many years. On this occasion I would like to express my gratitude to the professor Midhat Jašić who is the founder and chief editor of the journal, for presenting me the opportunity to edit this anniversary issue as well as for putting an effort into starting the journal. It goes without saying that this journal deals with the most up-to-date topics. We are all aware of the importance of healthy diet and physical activity on health as well as of numerous factors influencing food and diet quality. Agricultural resources application in solving energy issues on global level, climatic changes, (uncontrolled) overuse of chemicals in food production, world population growth and migrations are only some of the factors that influence food production in the world at the present and in the future (regarding quality, quantity and price). When discussing *healthy diet* issues, several questions are raised concerning consumers' level of information, quality of healthy diet and sources of information. Awareness is a key factor that can help to resolve these issues. I am convinced that articles published in this journal can and will contribute to understanding of food quality and potentials for diet improvements, or at least raise questions to be resolved in the future. I hope that articles will be read not only by professionals, but by everybody interested in gaining knowledge in this field.

Your sincerly,

Ph.D. Drago Šubarić, full professor

Hrana u zdravlju i bolesti/Food in Health and Disease
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SADRŽAJ/CONTENTS

Influence of the immobilized yeast cells technology on the presence of biogenic amines in wine

Borislav Miličević[1], Drago Šubarić[1], Jurislav Babić[1], Đurđica Ačkar[1], Antun Jozinović[1*], Emil Petošić[2], Anita Matijević[3]

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original scientific paper

Summary

Biogenic amines are basic nitrogenous low molecular weight compounds with biological activity. Biogenic amines are important because they contain a health risk for sensitive humans. Biogenic amines in the wine can be formed from their precursors by various microorganisms present in the wine, at any stage of production. The aim of the present work was to study the changes of the content of biogenic amines in wines made from grape variety *Frankovka* and *Pinot noir* (*Vitis vinifera* L.) from Kutjevo vineyards, located in the east part of continental Croatia, vintage 2012, produced with cold maceration and use of fermentation method with immobilized yeast cells. Biogenic amines were quantified using a reversed-phase high performance liquid chromatography (RP-HPLC). Histamine was the most abundant biogenic amine followed by 2-Phenylethylamine. Total amount of biogenic amines ranged from 8.81 mg/L in wines produced with immobilized yeast cells up to 9.91 mg/L in wines made in classical fermentation process. From the results obtained in this study, it can be concluded that immobilized yeast cells technology can influence on the formation of biogenic amines.

Keywords: biogenic amines, immobilized yeast cells

Introduction

Red wine is a fickle mistress, we swirl, we sniff, we sip, but, unfortunately for our heads, most of us don't spit. Main suspect behind the red wine headache are biogenic amines. Consequently the presence of biogenic amines in wine is becoming increasingly important to consumers and producers. Biogenic amines are important because they contain a health risk for sensitive humans. Beside headaches, symptoms include nausea, respiratory discomfort, hot flushes, cold sweat, palpitations, red-rash, high or low blood pressure.

Biogenic amines (BA) are nitrogenous low molecular weight organic bases that can have an aliphatic, aromatic or heterocyclic structure. They are widely present in fermented beverages, mostly as a consequence of the decarboxylation of their free precursor amino acids (Vincenzini et al., 2009). Until now, different analytical methods have been developed to determine BA in foodstuffs samples. Concerning wine analysis, most analytical methods used for the determination of BA are based on chromatographic methods. HPLC technology has been the most popular analytical approach for the analysis of wines. A number of different detection systems have been used for this purpose, most often assays employ fluorescence detection or UV detection with precolumn or postcolumn derivatization techniques with a wide range of derivatizing agents. The most frequently used derivatizing agents, which reacts with either primary, or secondary amino groups, or even tertiary amines and in extreme reaction conditions, providing very stable derivatives are: dansyl-chloride; fluorescein isothiocianate; o-phthaldehyde and fluorescamine (Busto et al., 1997; Mafra et al., 1999). Regarding their detection, the most frequently found BA in wine are histamine, cadaverine, putrescine, 2-phenylethylamine and tyramine (Košmerl et al., 2013, Čuš et al., 2013). Wines are usually characterized by content of BA, red wines compared to white being generally characterized by higher BA content (Soufleros et al., 2007, Marcobal et al., 2005). Soufleros et al. (2007) reported up to 2.11 mg/L histamine, 3.65 mg/L tyramine and 5.23 mg/L putrescine in red wines from Greece, while Marcobal et al. (2005) found 3.62 mg/L histamine, 1.40 mg/L tyramine and 7.06 mg/L putrescine in red Spanish wines. European Union (EU) has not established regulations for the wine industry, but only suggested the *„Safety threshold values"*. Generally, the toxic dose in alcoholic beverages is considered to be between 8 and 20 mg/L for histamine, 25 to 40 mg/L for tyramine, but as little as 3 mg/L phenylethylamine can cause negative physiological effects (Karovičova

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and Kohajdova, 2005). A wide variety of viticultural and oenological factors may have an impact on the levels of biogenic amines in wine. Some amines may be already present in grape berries (Bover-Cid et al., 2006, Kiss et al., 2006). While some factors can increase the precursor amino acid concentration in the grape and wine, other factors can potentially decrease production of the biogenic amines. Among the factors that have been suggested as favouring the abundance of amines in wine, some winemaking practices seem to play a major role because they can directly affect the content of the precursor amino acids of BA (Martin-Alvarez et al., 2006, Alcaide-Hidalgo et al., 2007, Vincenzini et al., 2009). Therefore, precaution should be taken in production process. The aim of the present work was to study the changes of the content of biogenic amines in wines made from grapevine variety *Frankovka* and *Pinot noir* (*Vitis vinifera* L.) from Kutjevo vineyards, located in the east part of continental Croatia, vintage 2012, produced by cold maceration and use of fermentation method with immobilized yeast cells.

Materials and methods

Wine production

The wines was produced from the grapes varieties: *Frankovka* and *Pinot noir* (*Vitis vinifera* L.). The cold-maceration was carried out controlling the skin contact time for 4 days at temperature below of 15 °C. After the cold-maceration period was completed mash was drawn off to remove the skins and other solid parts, and left to finish the fermentation.

Sample nb. (1 - 2) of wines were produced using classical technological fermentation procedure; with selected yeast *Feromol-Bouqet 125*, and controlled thermal regime, lead trough outer chilling of fermentors with running water, with the aim of keeping the average temperature in intervals of 16 - 22 °C. The average duration of the fermentation of all grape varieties under these conditions was 40 days.

Sample nb. (1^*- 2^*) were produced using technological procedure of fermentation as shown in Fig. 1: Fermentation with immobilized yeast cells /selected yeast *Feromol-Bouqet 125*, immobilized in Ca-alginate gel (Gaserod, 1998, Poncelet et al., 2001) / in internal loop gas-lift fermentor with alginate beads as yeast cariers and controlled thermal regime using outer refrigeration of fermentors with running water, with the aim of keeping the average temperature in intervals of 16 - 22 °C. The average duration of fermentation under these conditions was 14 day for each set.

The samples of young wine were exempted at the end of fermentation and before filtration so the wine was insufficiently clear, slightly dull.

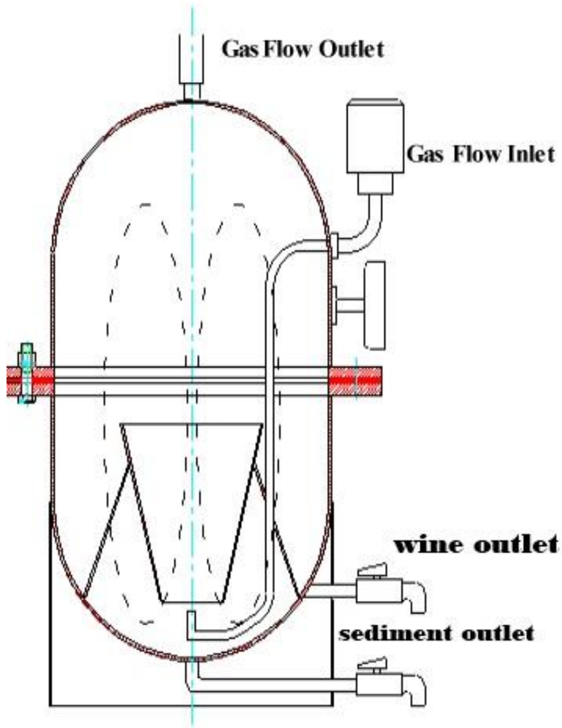

Fig. 1. Reactor for fermentation with immobilized yeast cells

Chemical analysis of wine

For the evaluation of the quality of wine fundamental analytical techniques were applied. In industrial control laboratories these techniques represent the basis for the determination of quality parameters, defined by O.I.V. (2001), Anonymous (1996) and AOAC (1995).
Chemical analysis of wine included specific mass, alcohol, total extract, total sugar, total acidity, total and free SO_2, total nitrogen analysis and the analysis of ash.

HPLC analysis of biogenic amines in wine

The biogenic amines content was determined by HPLC method according to Paris Soleas et al. (1999). Biogenic amines were separated using a liquid chromatograph HP 1100 (Agilent Technologies, Waldbronn, Germany), with an auto-sampler and UV/VIS detector with variable wavelength, and a fluorescence detector. The separation after *o*-phthaldialdehyde (OPA) derivatisation was performed on a reversed-phase column Zorbax Eclipse XDB C8 (150 mm × 4.6 mm, particle size 5 μm) equipped with a guard column Meta Guard Inertsil C18. The biogenic amines standard were obtained from Sigma-Aldrich, Steinheim, Germany and OPA application for fluorescence detector were purchased from Merck, Darmstadt, Germany.

Statistical analysis

One-way analysis of variance (ANOVA), and LSD comparison test, with a confidence interval of P < 95 %, was run to evaluate statistical differences on the measured chemical and physical parameters.

Results and discussion

The results obtained in the chemical analysis of wine reported in Table 1 show chemical and physico-chemical properties for samples of new unclarified wine. The obtained results showed that all wine samples produced using technological procedure as shown in Fig. 1 (fermentation with immobilized yeast cells) had a slightly raised amount of alcohol, ranging from 12.83 – 13.47 %, in relation to the amount of 12.76 – 13.22 % in wines which were produced using classical technological procedure. The quantity of alcohol corresponds to the requirements of Regulations of wine (Anonymous, 1996). It is important to stress that the immobilized cells gave wines with lover contents of total extract (19.30 – 22.10 g/L). The amount of total extract in wines produced using classical technological procedure was within the recommended values, from 19.60 – 24.62 g/L. The differences in the amount of total extract in wine sample are in conformity with the characteristics of quality wines obtained from examined grape varieties (Ribereau-Gayon et al., 1998, O.I.V., 2001). In wine samples produced with immobilized cells, slightly lower amount of total acids was noted (5.10 - 5.88 g/L), than it was in wines which were produced using classical technological procedure (5.30 – 6.08 g/L), which correspondents to Yajima and Yokotsuka (2001). The amount of total sugars (2.75 - 3.40 g/L) was significantly higher in all-new wines produced using classical technological procedure in relation to the value in wine samples produced using immobilized yeast cells (2.30 – 2.53 g/L). Markedly high amount of total sugars in the wine results thereby with smaller content of ethanol in this new wine (Delfini et al., 2001). The presence of free SO_2 in all wine samples, ranging from 5.90 - 7.74 mg/L, corresponds to results of Antonelli et al. (1999). By the analysis of obtained wines, it has been found that there were significant differences among the determined properties.

Table 1. Results of chemical analysis of wine

Determinate characteristics	Pinot noir	Pinot noir*	Frankovka	Frankovka*
Specific mass (20/20 °C) (g/mL)	0.9918 ± 0.10	0.9914 ± 0.30	0.9940 ± 0.20	0.9930 ± 0.25
Alcohol (%vol.)	13.22 ± 0.15	13.47 ± 0.25	12.76 ± 0.20	12.83 ± 0.30
Total extract (g/L)	19.60 ± 0.05	19.30 ± 0.25	24.62 ± 0.42	22.10 ± 0.40
Total sugar (g/L)	2.75 ± 0.10	2.53 ± 0.30	3.40 ± 0.32	2.30 ± 0.35
Total acidity (g/L)	5.30 ± 0.08	5.10 ± 0.35	6.08 ± 0.35	5.88 ± 0.40
Ash (g/L)	1.64 ± 0.10	1.74 ± 0.18	2.10 ± 0.40	1.80 ± 0.25
Free SO_2 (mg/L)	7.24 ± 0.18	7.74 ± 0.25	5.90 ± 0.18	6.60 ± 0.10
Total SO_2 (mg/L)	118.55 ± 0.20	119.40 ± 0.20	115.60 ± 0.10	116.33 ± 0.18
Total nitrogen (mg/L)	260.50 ± 0.20	250.00 ± 0.10	240.50 ± 0.10	220.00 ± 0.10

*immobilized yeast cells

According to obtained results vinification method significantly influenced the concentration of some biogenic amines (Table 2). Total amount of biogenic amines ranged from 8.81 mg/L in wines made with immobilized yeast cells up to 9.91 mg/L in wines made in classical fermentation process. In general, histamine was the major biogenic amine found in wines (3.21 - 3.49 mg/L) and histamine producing capability may be considered widespread among various oenological factors (Halasz et al., 1994, Bauza et al., 1995, Gerbaux and Monamy, 2000, Alcaide-Hidalgo et al., 2007). As shown in Table 2 putrescine concentration was relatively similar in *Frankovka* and *Pinot noir* (0.39 - 0.41 mg/L), and low (Gerbaux and Monamy, 2000) probably because fermentation was conducted by commercial pure strain culture *Feromol-Bouqet 125*. In tested wines histamine was the most abundant biogenic amine followed by tryptamine and 2-phenylethylamine (Gloria et al., 1998). In summary, from the results obtained in this study, it can be concluded that technology with immobilized yeast cells can influence the formation of biogenic acids.

Table 2. Results of HPLC analysis of biogenic amines in wine

Biogenic amine (mg/L)	Pinot noir	Pinot noir*	Frankovka	Frankovka*
Putrescine	0.41 ± 0.19	0.39 ± 0.08	0.41 ± 0.06	0.40 ± 0.04
Cadaverine	0.35 ± 0.05	0.34 ± 0.05	0.42 ± 0.05	0.40 ± 0.05
2-Phenylethylamine	2.37 ± 0.15	2.33 ± 0.15	2.64 ± 0.18	2.30 ± 0.18
Spermidine	0.58 ± 0.11	0.53 ± 0.09	0.65 ± 0.09	0.61 ± 0.09
Tryptamine	1.74 ± 0.10	1.63 ± 0.12	1.89 ± 0.17	1.69 ± 0.17
Serotonine	0.18 ± 0.05	0.15 ± 0.04	0.16 ± 0.05	0.13 ± 0.05
Tyramine	0.23 ± 0.01	0.19 ± 0.01	0.25 ± 0.02	0.20 ± 0.02
Histamine	3.36 ± 0.06	3.25 ± 0.09	3.49 ± 0.06	3.21 ± 0.06
Σ Biogenic amines	**9.22**	**8.81**	**9.91**	**8.94**

*immobilized yeast cells

Conclusions

The obtained results showed that fermentation with immobilized yeast cells had significant influence on presence of biogenic amines in wine. Moreover, the compounds that mostly contribute to the typical biogenic amines profile of wine, such as histamine, cadaverine, putrescine, 2-phenylethylamine and tyramine are affected by the fermentation process with immobilized yeast cells.

It seems that the fermentation with immobilized yeast cells is a promising approach in the wine-making process, with a reduced content of biogenic amines in wine.

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Antioxidant properties of pollen

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review

Summary

Today, bee pollen is commonly used in folk medicine and its pharmacy effects have not yet been scientifically proven. The composition and chemistry of bee pollen are not yet standardized nor defined in pharmacopoeia, and may vary due to its botanical and geographical origin, the plant species, environmental conditions, age and status of plants. Because of this, the type of bee pollen depends on the available bee pasture and types of plant species visited by bees. Bee pollen contains nutritional and essential substances and also significant amounts of polyphenolic substances, mainly flavonoids, that are considered as the main ingredients of pollen and its antioxidant properties. Researches show that pollen has significant antioxidant activity and mostly depends on phenol compounds. Large deviations of the this antioxidant activity are considerable, as well as content of phenolic compounds between pollen grains taken from different plant species and different geographical regions. The pollen antioxidant activity is usually expressed as the antioxidant capacity, and primarily depends on its botanical and geographical origin that is the subject of many scientific and research papers. This article gives an overview of bee pollen, its chemical composition and botanical origin, antioxidant properties and its capacity.

Keywords: honeybee pollen, antioxidant capacity, polyphenols, flavonoids, botanical origin

Introduction

Honey bee-derived apicultural products such as pollen have been applied for centuries in traditional medicine as well as in food diets and supplementary nutrition due to their nutritional and physiological properties, above all in regard to their health effects on the human organism (Kroyer & Hegedus, 2001). Pharmacological effects of pollen are not yet scientifically based and its chemical composition depends on the available bees pasture and species of plants visited by bees. Pollen is a considerable source of compounds with health protective potential, certain concentration of phytosterols, and is also rich in phytochemicals such as phenolic compounds, which are considered beneficial to human health. Researches show that pollen has significant antioxidant activity that mostly depends on phenolic compounds. However, large deviations of the antioxidant activity are considerable and content of phenolic compounds between pollen grains taken from different plant species and different geographical regions are remarkable. The pollen antioxidant activity is usually expressed as the antioxidant capacity, and primarily depends on its botanical and geographical origin that is the subject of many scientific and research papers. The aim of the present work was an overview of various pollen properties such as the chemical composition, the botanical origin, its collecting, phenolic compounds and an antioxidant activity.

Pollen

Pollen grains are microscopic structures found in the anthers of stamens in angiosperms (de Arruda et al., 2013), they constitute the male reproductive cells in plants (Basim et al., 2006), and their purpose is to transmit their gametes to the female sex organ of the flower (Arruda et al., 2013). Bees, other insects, wind and water pollinate plants by transferring pollen from the stamen to the stigma of another plant (LeBlanc et al., 2009). According to the regulations of honey and other bee products (Službeni glasnik BiH, br. 37/09), pollen is a product that worker bees collected in nature adding to its own specific matter which forms the pellets, and place them in the honeycomb cells. Special group of worker bees that collect pollen is called pollen-bees (Dolovac, 1997).

Pollen is very important in apiculture as a source of proteins, fats and minerals to bees and as an excess produced from the apiary (de Arruda et al., 2013). The quantity and quality of pollen collected by honeybees affects reproduction, brood rearing and

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longevity, thus ultimately the productivity of the colony (Human & Nicolson, 2006).
The significance of pollen for bee's community is priceless and is associated with its survival. Bees use it as food for all the developmental stages in the hive (Almeida-Muradian et al., 2005; Morais et al., 2011). Apart from small quantities in nectar honeybees obtain all the proteins, lipids, minerals and vitamins they need for brood rearing and adult growth and development from pollen (Human & Nicolson, 2006). The bees place the pollen in honeycombs with their legs and cover this pollen with honey. This pollen reserve is referred to by beekeepers as ‘‘bee bread”. It was determined that an average value of 145 mg of pollen is required to rear just one worker bee (LeBlanc et al., 2009).

The chemical composition of pollen

Flower pollens' composition can vary due to their botanical and geographic origin (Almaraz-Abarca et al., 2004). The major components of bee pollen are carbohydrates, crude fibers, proteins and lipids at proportions ranging between 13 % and 55 %, 0.3 % and 20 %, 10 % and 40 %, 1 % and 10 %, respectively (Villanueva et al., 2002). Other minorcomponents are minerals and trace elements, vitamins and carotenoids, phenolic compounds, flavonoids, sterols and terpenes (Bogdanov, 2011). Proline, aspartic acid, phenylalanine and glutamic acid are the primary amino acids in pollen (Roldán et al., 2011). However, the composition of bee pollen depends strongly on plant source, together with other factors such as climatic conditions, soil type and beekeeper activities (Morais et al., 2011).
Few studies on the active enzymes in bee pollen have been published (Xue et al., 2012). According de Arruda et al. (2013), bee pollen is rich in B complex vitamins (thiamine, niacin, riboflavin, pyridoxine, pantothenic acid, folic acid and biotin) and carotenoids, which can be provitamin A. However, according to the same author, there is no significant amount of vitamin C or lipid soluble vitamins.
There are numerous reports of bioactive substances in the pollen such as phenols, flavonoids, anthocyans, phospholipids and proteins. The main bioactive flavonoids are naringenin, isorhamnetin3-*O*-rutinoside, rhamnetin3-*O*-neohesperidoside, isorhamnetin, quercetin3-*O*-rutinoside, quercetin3-*O*-neohesperidoside, kaempferol and quercetin and their total amounts are in the range 0.3-1.1 % w/w (Han et al., 2012).
Phenolic compounds are one of the most critical ingredients related to antioxidant activity in pollen. Usually, it contains vanillic acid, protocatechuic acid, gallic acid, *p*-coumaric acid, hesperidin, rutin, kaempferol, apigenin, luteolin, quercetin, and isorhamnetin (Bonvehi et al., 2001).

Pollen collection

The collection of this natural product is a relatively recent development, dependent primarily on the basic concept of scraping pollen off of the bees’ legs as they enter the hive (Feás et al., 2012). Honey bees collect pollen by adding sugars from nectar and their own secretions to bind the grains together (Cheng et al., 2013) and then transfer them back to the colony by packing them into hairs on the corbiculae (hind legs) of bees (LeBlanc et al., 2009).
For the commercial bee pollen collection, indoor or outdoor pollen collectors can be used. There are different versions of these collectors depending on the type of the hive and the principle of the pollen subtraction is the same. Bee with pollen must scrape through small openings in the pollen collector where it passes and the balls of pollen fall into the prepared drawer. The advantage of outdoor pollen collectors is cleaner pollen but its deficiency is smaller amount in comparison with indoor pollen collectors.
The collected raw pollen with about 20 % moisture content is subject to microbial spoilage and kept in a frozen state at - 18 °C up to a certain analysis or dried to 7-8 % moisture content and kept in a cool, dark place. For pollen analyzing, its extracts in different solvents and their mixtures are prepared, and most commonly used solvents are methanol, ethanol and water.
Kroyer & Hegedus (2001) used ethanol, methanol/water and water as pollen solvents, and reported that the content of polyphenolic compounds in pollen extract was significantly increased in absolute ethanol.

Botanical origin of pollen

Botanical origin of pollen is determined by palynological (microscopic) analysis respectively by the microscopic identification and counting of pollen grains. Each plant species has its own characteristic pollen grain that can be used to determine its botanical origin i.e. determining the plants that bees visited by gathering the pollen.
Pollen grains vary in terms of their morphological characteristics such as form, size, openings/apertures and ornamentation, as well as in terms of color and appearance. Color and other characteristics of pollen grains can be used to identify the genus of plants and, sometimes, the plant species (Bačić, 1995; de Arruda et al., 2013).

Pollen analysis allows the identification of the major pollen sources used by the bees, as well as the periods of pollen production in the field and possible times of shortage (de Arruda et al., 2013). Microscopic examination showed that each pellet of honeybee-collected pollen was largely homogeneous, confirming the observations of Almaraz-Abarca et al. (2004) who observed that pollen pellets predominantly consist of pollen grains from one species.

Research by de Arruda et al. (2013) indicates that bees use a variety of flora for the production of bee pollen and other bee products. When collecting pollen, bees generally visit the same type of plants to make pollen grains, and that pollen is mainly monofloral origin, with minor additions of pollen grains of other species of plants. According to de Arruda et al. (2013), pollen samples that have amounts exceeding 45 % of a botanical taxon in their composition can be considered as unifloral pollen. Morais et al. (2011) proved that pollens with same color belong to the same family. According to Luz et al. (2010) the pollen types observed in the pollen pellets can vary according to the region where they are offered, a factor which depends on the available surrounding bee pasture in the apiary vegetation, as well as on the climate conditions for flowering. Therefore, the composition of the pollen may vary due to its botanical and geographical origin (Almaraz-Abaraca et al., 2004) and according to Szczesna et al. (2002), the chemical composition of bee pollen varies according to the plant species, environmental conditions (different locations, seasons and years), age and status of the plant (when the pollen is developing).

For microscopic analysis, homogeneous pollen sample is taken in the amount of 2 g, which corresponds to the number of 300 pollen grains (Almeida-Muradian et al., 2005), which are classified into groups with grains of the same color (Mărghitaş et al., 2009), determining their percentage participation in the main sample. The colour of the pollen can be estimated according to the tables elaborated by Hodges (1984) and Kirk (1994) and identified by colour and microscope observations of pollen grains (Warakomska, 1962). For the determination by the palynological analysis also some others standardized taxons for the specific area or country may be used.

Antioxidant properties of pollen

In the literature, the term "antioxidant" is defined in many ways. The word antioxidant, as the same name indicates, means "something that is opposite to oxidation." Antioxidant opposes oxidation or inhibits reactions induced by oxygen or peroxides. Thus, the presence of antioxidants in the pollen reduces the harmful effects of the free radicals in the cell and can slow oxidation reactions in food.

Antioxidant ability has usually been attributed to the activity of antioxidant enzymes (mainly superoxide dismutase, peroxidase and catalase) as well as to the content of low-molecular antioxidants such as carotenoids, tocopherols, ascorbic acid, phenolic substances (Leja et al., 2007). Antioxidants are considered as possible protection agents reducing oxidative damage to important biomolecules, including lipoprotein and DNA (deoxyribonucleic acid) from ROS (reactive oxygen species). Oxidative stress, the consequence of an imbalance between ROS generation and antioxidants in the organism, initiates a series of harmful biochemical events which are associated with diverse pathological processes which can lead to various cellular damages and diseases (Mărghitaş et al., 2009).

It is believed that the bee products are large sources of antioxidants. According to Nagai et al. (2001) there is significant antioxidant activity in pollen and other bee products.

Bee pollen, like other bee products (honey, propolis), is due to the abundant and qualitatively and quantitatively different contents of phenolic and flavonoid antioxidants related to botanical species and origin, valuable sources of these healthy beneficial constituents characterized by high antioxidant activity (de Arruda et al., 2013). This various mechanisms of antioxidant activity permit a wide range of free radicals scavenging and lipo-peroxidation assays in order to evaluate the complete antioxidant potential (Mărghitaş et al., 2009).

Many of the present studies are concerned with determining the antioxidant activity of pollen samples of different geographical origin and establishing a correlation with the content of phenolic and other compounds.

Antioxidant capacity

The measure of antioxidant activity can be expressed by antioxidant capacity. Many factors may affect accurate determination of antioxidant activity (Kukrić at al., 2013). A number of methods based on different mechanisms of antioxidant defense system, are developed to determine antioxidant capacity, such as the removal or inhibition of free radicals or chelating metal ions, which otherwise may lead to the formation of free radicals (Greblo, 2009). The antioxidant properties of the pollen extracts cannot be evaluated by just one method due to the complex nature of their constituents. Recent investigations show differences between the test systems in determining antioxidant capacity. Use of at least two methods is recommended to assess and compare the antioxidant capacity of a sample (Sakanaka & Ishihara, 2008). There are various methods available in the assessment of the antioxidant capacity of samples. They provide useful data, however, they are not sufficient to estimate a general antioxidant ability of the sample (Filipiak, 2001). These methods differ in terms of their assay principles and experimental conditions. Consequently, in different methods, particular antioxidants have varying contributions to total antioxidant potential (Mărghitaş et al., 2009).

The enzymatic and non-enzymatic methods are used to determine the antioxidant capacity. Of the non-enzymatic method, indirect methods (DPPH, $ABTS^+$, FRAP) and direct methods (ORAC method) (Kukrić at al., 2013) are used mostly.

The DPPH method (Brand-Williams, Cuvelier, & Berset, 1995) principle is the reaction of DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical, which accepts an electron or hydrogen radical to become a stable molecule, and, accordingly, is reduced in presence of an antioxidant. DPPH radical is widely used for the preliminary screening of compounds capable to scavenge activated oxygen species since they are much more stable and easier to handle than oxygen free radical (Tominaga et al., 2005). The absorbance changing is monitored at 517 nm (Parkash, 2001).

The TEAC assay is based on the inhibition of the absorbance of the radical cation of ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) by antioxidants. Due to its operational simplicity, the TEAC assay has been used in many research laboratories for studying antioxidant capacity, and TEAC values of many compounds and food samples are reported.

The FRAP assay measures the ferric-to-ferrous iron reduction in the presence of antioxidants and is very simple and convenient in terms of its operation (Mărghitaş et al., 2009).

The antioxidant capacity determination results of an extract depend greatly on the methodology used, that is the oxidant and the oxidisable substrate used in the measurement. Therefore, it is important to compare different analytical methods varying in their oxidation initiators and targets in order to understand the biological activity of an antioxidant and to obtain accurate data for a better comparison with other literature. On the other hand, different antioxidants respond differently in various measurement methods which involve specific reaction conditions and mechanisms of action. This may explain the various results for DPPH, FRAP and TEAC assay, in regard with the antioxidant content of bee pollen samples analysed. In conclusion, future analysis is required, not only in testing other different systems of evaluating the antioxidant activity, but also in separation and identification the specific bioactive compounds in bee pollens with different botanical origin, in order to elucidate the differences between various samples (Mărghitaş et.al., 2009).

Phenolic compounds in pollen

Bee-collected pollen contains significant amounts of polyphenol substances mainly flavonoids which furthermore are regarded as principal indicating ingredient substances of pollen and can be used for setting up quality standards in relation to their nutritional-physiological properties and for quality control of commercially distributed pollen preparations (Kroyer & Hegedus, 2001).

In addition to testing the total phenolic compounds in pollen its constituents are tested, such as flavonoids, anthocyanins, fenilpropanoids and others. There are many studies that explore the contents of phenolics and flavonoids and their common relationship to antioxidant activity. Significant and mutual dependencies between these components and antioxidant capacity, botanical and geographical origin are established.

The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in neutralizing free radicals, quenching oxygen, or decomposing peroxides (Nijveldt et al., 2001).

According to Carpes et al. (2007), the pollen collected by bees generally shows characteristic amounts of total polyphenols due to its botanical and geographical origin.

Antioxidant activity is not necessarily correlated with high amounts of phenolic compounds and total phenolic content, measured by the Folin–Ciocalteu procedure, and does not give a full idea of the nature of the phenolic constituents in the extracts (Mărghitaş et al., 2009).

Studies by Almaraz-Abarca et al. (2004) and Mărghitaş et.al. (2009) show that the polyphenol composition of pollen, can be a factor in its determination. Mărghitaş et al. (2009) require the detailed examination of phenolic composition in bee pollen extracts for the comprehensive assessment of individual compounds exhibiting antioxidant activity.

The results in most studies show large variations and significant differences in the amount and content of phenolic compounds in pollen from different geographical destinations and different botanical origin.

The most important and largest group polyphenols are flavonoids that appear in almost all parts of the plant and today approximately 4000-5000 various types of flavonoids are known (Kukrić et al., 2013).

Flavonoids are pigments responsible for the coloration of flowers and leaves and are important for normal growth, development and defense of plants. Each type of pollen has its own specific system of flavonoids (Crane, 1990).

Recent studies have shown that flavonoids derived from the pollen of different geographical and botanical origin containing compounds of different nutritional significance. The reactions of free radicals and scavenging capacity to reactive species of oxygen in the pollen may be due to differences in atmospheric and environmental conditions, soil or plant physiology. Flavonoids have different structural features and show several biological activities. It appears that they may strongly influence antioxidant activity, gene expression, drug-metabolizing enzymes, such as cell signaling or cytochrome P450 (CYP) enzymes, express phytoestrogenic potential, protect against toxicity of the environmental contaminant dioxin (Šarić et al., 2009).

It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule, determine antioxidant properties. In general, these properties depend on the ability to donate hydrogen or electron to a free radical (Mărghitaş et al., 2009).

The high ability of phenolic constituents to neutralize the active oxygen species is strongly associated with their structure, such as the conjugated double bonds and the number of hydroxyl groups in the aromatic ring, mostly attributed to flavonoids and cinnamic acid derivatives (Leja et al., 2007).

In addition, the redox properties of polyphenol compounds, especially flavonoids, play an important role in absorbing and neutralising free radicals, quenching oxygen and decomposing peroxides (Damintoti et al., 2005).

The antioxidant activity of flavonoids is reflected in the inhibitory effect on lipid peroxidation and increasing the activity of antioxidant enzymes (Kukrić et al., 2013). Flavonoids have different structural features and show several biological activities (Šarić et al., 2009).

The best-described property of almost every group of flavonoids, which are the predominant phenolic class present in honeybee-collected pollen, is their capacity to act as antioxidants (Kroyer & Hegedus, 2001). One way is the direct scavenging of free radicals. Flavonoids are oxidised by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical (Nijveldt et al., 2001).

Total phenols is usually determined spectrophotometrically (Moreira et al., 2008; Kroyer & Hegedus, 2001; Mărghitaş et al, 2009), by modified Folin-Ciocalteu method which is based on phenol coloured reaction with the Folin-Ciocalteu reagent, measuring the resulting intensity of coloration (Kukrić et al., 2013), and total flavonoids by colorimetric tests with reference standards (Kim et al., 2003).

Health effects of pollen

Recently, increasing evidence suggests its potential therapeutic benefits, including antioxidant (Leja et al., 2007), bioactive (Kroyer & Hegedus, 2001; Roldán et al., 2001), and antimicrobial properties (Basim et al., 2006; Carpes et al., 2007; Morais et al., 2011), suggesting that it could be useful in prevention of diseases in which free radicals are implicated (Pascoal et al., 2013).

It is considered to be a natural health food which constitutes a potential source of energy and functional components for human consumption (Silva et al., 2006), with a wide range of therapeutic properties, such as antimicrobial, antifungal, antioxidant, anti-radiation, hepatoprotective, chemoprotective and/or chemopreventive and anti-inflammatory activities (Pascoal et al., 2013), free radical scavenging activities (Leja et al., 2007; Silva et al., 2006), inhibition of lipid peroxidation and suppressing the cellular and humoral response (Xu et al., 2009). These therapeutic and protective effects have been related to the content of polyphenols by Almeida-Muradian et al., (2005) and flavonoids by Šarić et al., (2009).

The daily ingestion of bee pollen can regulate the intestinal functions, effectively reduce capillary fragility and has beneficial effects on the cardiovascular system, vision and skin (Pietta, 2000). In addition, it has been reported to trigger beneficial effects in the prevention of prostate problems, arteriosclerosis, gastroenteritis, respiratory diseases, allergy desensitization, improving the cardiovascular and digestive systems, body immunity and delaying aging (Estevinho et al., 2012).

Phytochemicals, such as phenolic compounds are considered beneficial for human health since they decrease the risk of degenerative diseases by reducing oxidative stress and inhibiting macromolecular oxidation. They have been shown to possess free radical-scavenging and metalchelating activity in addition to their reported anticarcinogenic properties (Morais et al., 2011).

Conclusions

Effects of pharmacological bee pollen still have not been scientifically based and is commonly used in folk medicine. The composition and chemistry of pollen are not yet standardized nor defined with pharmacopoeia, and may vary due to its botanical and geographical origin, the plant species, environmental conditions, age and status of plants. Because of this, the type of bee pollen depends on the available bee pasture, types of plant species visited by bees and the period of flowering for characteristic plant species.

Antioxidant capacity of bee pollen, as well its other physico-chemical properties primarily depend on its botanical and geographical origin of and that is the subject of many scientific and research papers. Studies have generally shown significant association between antioxidant capacity and total content of polyphenols or flavonoids.

Further studies of bee pollen for certain geographic region can be directed to the determination of its botanical origin, phenolic constituents and to determine the quality of the characteristic pollen types from the point of antioxidant capacity, and as a result, to provide recommendations of applying pollen into functional, nutritional and pharmaceutical purposes.

Pollen should be used preventively in enrichment of everyday food or as a natural dietary supplement, due to it contains all essential amino and fatty acids and all the ingredients for a healthy and normal development of the organism.

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Brain food: how nutrition alters our mood and behaviour

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review

Summary

Studies have been showing the food we eat affects chemical composition of our brain and alters our mood. Nutrition affects cognitive possibilities, including alertness and the production or release of neurotransmitters, the chemical messengers that carry information from one nerve cell to another. Foods are made up of more than one nutrient, and their interaction is going to affect the production and release of neurotransmitters. Neural impulses are largely resulting from sodium-potassium exchange, but numerous others such as complex carbohydrates, amino acids (tryptophan and tyrosine), fatty acids, particularly omega-3 fatty acids, affect permeability of cell membrane, neurotransmitter metabolism and glial cells. The delicate brain chemical balance is somewhat controlled by the blood – brain barrier. Still, brain remains highly susceptible to changes in body chemistry resulting from nutrient intake and deficiency. The direct connection between nutrition, brain function and behaviour exists, without any doubt. It can be seen through brain's capability of receiving, storing and integrating sensory information, while initiating and controlling motor responses. These functions correspond to mental activities and form the basis for our behaviour. Constant rise in number of evidence from epigenetic studies confirms that specific nutrients alter our brain development and susceptibility to diseases. Still, specific combination of foods can be extrapolated to a dietary regime, like the Mediterranean diet which has shown its positive impact on maintaining brain function and lower incidence of neurodegenerative diseases. This is of special importance since elderly population (people of 65 years and older) is on the rise all over the world, and the quality of life becomes a priority.

Keywords: nutrition, food composition, neurotransmitters, mood, behaviour

Nutrition and cognitive performance

All that we experience affects synapses (junctions of neurons), and these changes are responsible for memory and other mental abilities. According to Thurston primary mental abilities are (set in 1938): verbal fluency (eloquence), verbal comprehension, visual and spatial (physical) abilities, memory, numerical ability, perceptual speed spotting, inductive reasoning (from individual to general) and deductive reasoning (from general to specific). Practically, when something that we are going to remember happens electric signal occurs, causing chemical and structural changes in the neurons. These changes are possible due to a series of reactions involving various molecules, including calcium, some enzymes and neurotrophins, aiming for synapses activation. Healthier brain produces more neurotrophins, which reinforce links between neurons in the part of the brain responsible for learning and memory. Parts of the brain where specific memory is stored have been discovered. For semantic memory, which concerns facts are responsible multiple cortical areas, while procedural memory involved in motor learning depends on the other parts of the brain, including basal ganglia (Fig. 1). Nutrition in the first years of life can have a significant impact on development; the ability to learn, communication, analytical thinking, successful socialization and adaptation to new situations (Isaacs and Oates, 2008; Budson and Price, 2005).

Proper nutrition and health are closely interrelated throughout life, but probably the highest importance is expressed in the first years of life. Inadequate nutrition causes lower cognitive development, reduced attention and concentration and reduces performance in later life. Also, foetal programming in utero should not be neglected, for its proven influence on the later development of a child (Langley-Evans, 2008). As nicely illustrated by Vanhees et al. (2014) we are what we eat, and so are our children. Their extensive review on epigenetic studies clearly illustrates the importance of balanced diet of both, mother and father. Besides macronutrient composition of the diet (high-fat diets, protein restricted diet, diet high in carbohydrates), intake of specific micronutrients, especially those involved in one-carbon metabolism (folic acid, vitamin B_2, B_6 and B_{12}) day by day shows more potential in programming offspring's epigenome (Vanhees et al., 2014).

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At birth, the brain reaches 70 % size and 25 % weight of an adult brain. In the subsequent period, are created new nerve cells (neurons) that travel to their final destination. Brain changes throughout life. It normally makes fiftieth part of human body weight (average weighs between 1000-1500 grams), in adolescence reaches its definite size (Benton, 2008). Brain is a very dynamic organ, showing high plasticity. Due to this characteristic, altering our diet in terms of having a balanced nutrition without any deficiency or over-nutrition can preserve our brain from deterioration. For example, one study showed that high-dose supplementation with folic acid during early pregnancy shows association with increased neurodevelopment, resulting in enhanced vocabulary development, communicational skills and verbal comprehension at 18 months of age (Chatzi et al., 2012). Similar findings have been shown for boosting cognitive performance and intake of iron (after correcting iron deficiency anaemia) (McCann and Ames, 2007; Black et al., 2011; Goergieff, 2011).

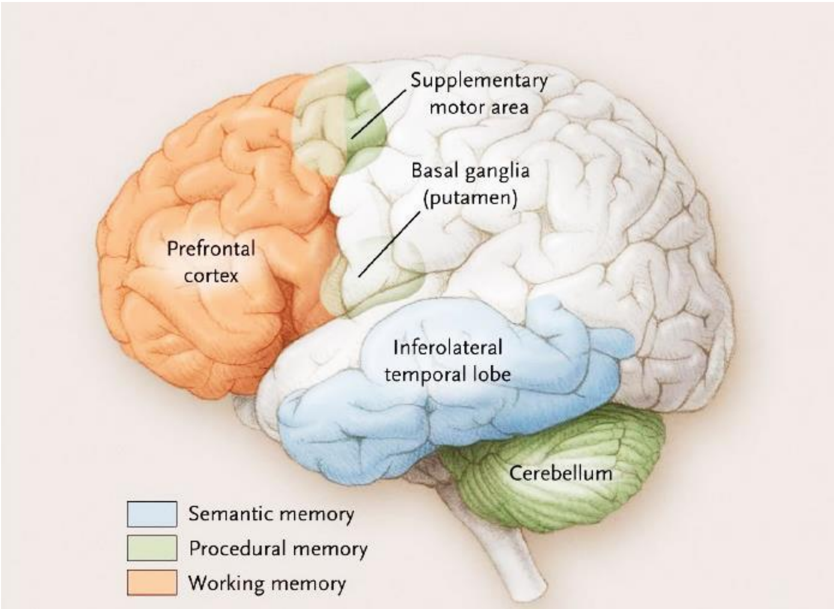

Fig. 1. Memory systems and parts of the brain (Budson and Price, 2005)

Food and neurotransmitters

Neurotransmitters are produced in our brain from numerous nutrients originating from our diet by means of a many-step process. First, nutrients (marked as 1 in Fig. 2), such as amino acids, carbohydrates, fats, and peptides, are extracted and absorbed from the food we eat and transported out of the arterial blood supply to the brain. They are actively carried through the blood-brain barrier and transported into neurons. Enzymes (2) convert these nutrients into different neurotransmitters. Neurotransmitter molecules are actively transported into synaptic vesicles (3). The arrival of an action potential (4) at an end of the axon induces entry of calcium ions, which initiate release of neurotransmitters (5) into synaptic cleft. The neurotransmitter molecule briefly interacts or binds with a protein, i.e. receptor (6), on the neuron surface on the other side of the synapse. Consequence of this binding action is that some ions, such as calcium or sodium, move into the downstream neuron to induce secondary biochemical processes (7), which may have long-term consequences on the neuron's behaviour. Meanwhile, after interacting with the receptor, neurotransmitter's actions must be terminated by reabsorption (8) back into the neuron that originally released it, which is called reuptake. A secondary method of neurotransmitter inactivation is by enzymatic conversion (9) into a chemical that can no longer interact with brain. Once inactivated by enzyme, neurotransmitter is removed from the brain into the bloodstream (10). Such byproducts can be easily monitored in body fluids, and used to determine whether our brain functions normally. Nutritional composition of our diet can interact with any of these previously described processes and impair, or even enhance, the production of neurotransmitters, as well as impair their storage into synaptic vesicles, alter their release from neurons, modify their interaction with receptor proteins (11), slow their reuptake, and possibly even stop their enzymatic inactivation (Wenk, 2010).

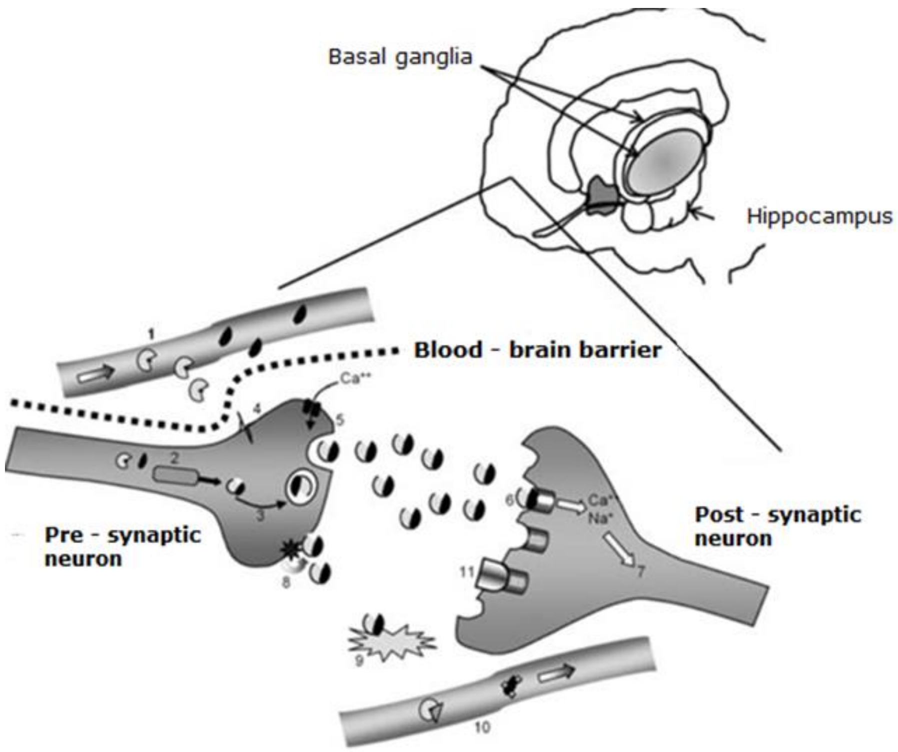

Fig. 2. The absorption of nutrients and their effect on neurotransmitters (Wenk, 2010)

Carbohydrates and the brain

Brain needs two times more energy than other cells in our body, and glucose is the only fuel that can be used directly by the brain (Coimbra, 2014). Neurons are always in a state of metabolic activity and have constant demand for energy, even during sleep. Most of the neuron's energy demand goes on bioelectric signals responsible for communication of neurons; they consume one-half of the brain's energy which is nearly 10 % of total human energy requirements (Coimbra, 2014). Because neurons cannot store glucose, they depend on the bloodstream to deliver a constant supply of this primary fuel. Different sugars have different effects on the brain. While glucose has an impact on regions like insula and ventral striatum, controlling appetite, motivation and reward processing, fructose does not (Page, 2013; Purnell and Fair, 2013).

Therefore, it is important to control the amount of carbohydrate in our diet, as well as the type of food we combine. This is where we get to the glycaemic index (GI) concept. GI is a ranking system categorizing the food according to its impact on blood glucose levels, so GI indicates whether certain foods raise blood sugar levels dramatically, moderately or slightly. The intake of foods made from white flour and white sugar should be limited for the above reasons. Potatoes also have a high GI value (Ek et al., 2012). The best choices are fibre-rich foods. Complex carbohydrates take longer to digest, causing a slower and more gradual release of glucose into bloodstream, leading to a feeling of fullness for longer period of time. A fibre-rich diet, besides its proven effect in the prevention of type 2 diabetes and cardiovascular diseases, probably helps improving memory and cognition (Kendall et al., 2010; Kaczmarczyk et al., 2012). The glycemic response depends on the combination of consumed food. Complex, varied meal that contains complex carbohydrates, proteins and adequate types of fats, rich on dietary fibers will provide a moderate GI and supply the brain for a long time with glucose. Combining foods with high GI and those with a low GI balances the response of the organism (Jenkins et al., 2013).

Fats and the brain

Fatty acids are present in membranes of every cell of our body and make 60 % of the brain’s dry weight, half of which are omega-6 fatty acids, while the other half consists of omega-3 fatty acids. Dietary fats alter the composition of nerve cell membrane and myelin sheath, and that, in turn, influences neuronal function. Fatty acids are involved in the development and growth of the brain, they affect cognitive abilities (attention, reasoning, memory, and learning), vocabulary and intelligence (Gogus and Smith, 2010). Humans cannot synthesize essential fatty acids from simple carbon precursors so they must be acquired through diet. There are two essential fatty acids, both polyunsaturated fatty acids, linoleic acid (LA) which is a precursor of omega-6 fatty acids and alpha-linolenic (ALA), which is a precursor of omega-3 fatty acids. Arachidonic acid (AA) is synthesized from LA, while from ALA eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are synthesized (Davis and Kris-Etherton, 2003; Vannice and Rasmussen, 2014). Long-chain omega-3 and omega-6 fatty acids compete for the same enzymes cyclooxygenase and lipoxygenase and therefore a diet is considered to be the best way to maintain balance between omega-3 and omega-6 fatty acids. It is believed that this ratio should not be greater than 5:1 in favour of omega-6, and it is known that the Western diet has a ratio of 12:1, or even worse. The importance of this ratio is supported by the fact that inflammatory eicosanoids are formed by the metabolism of omega-6 fatty acids, while EPA and DHA products are thought to be relatively anti-inflammatory (Gogus and Smith, 2010; Vannice and Rasmussen, 2014; Kidd, 2007; Shaikh and Brown, 2013).

Omega-3 fatty acids are critical for foetal and newborn neurodevelopment. During the third trimester of pregnancy, approximately 50-70 mg of DHA per day is delivered to foetus via placental transfer. DHA is accumulated in the central nervous system (CNS) before birth, and therefore considered to play a critical role in the development of cognitive functions (Langley-Evans, 2008; Greenberg et al., 2008; Montgomery et al., 2013). Nutrient deficiencies during development may have long-lasting consequences on neurone outgrowth (Greenberg et al., 2008; Montgomery et al., 2013). A positive correlation has been observed between DHA in red blood cells and visual acuity, as well as other indexes of brain development in newborns (Jensen et al., 2005). Nutritional guidelines during pregnancy recommend an additional intake of omega-3 fatty acids, i.e. 250 mg/day EPA and DHA (Vannice and Rasmussen, 2014; WHO, 2008; EFSA, 2010; FAO, 2010). Essential fatty acids are necessary for the child's normal growth and development, which is an approved health claim by the European Food Safety Authority. Beneficial effect has been proven by taking 1 % of the total energy of LA and 0.2 % ALA per day (EFSA, 2008). DHA is essential for the growth and functional neurodevelopment of newborns, and required to maintain a normal function of adult brain. Intake of DHA during pregnancy and lactation of at least 200 mg per day, contributes to the normal brain development of foetus and infant (EFSA, 2009). Low brain DHA is associated with age-related cognitive decline, as well as the early development of Alzheimer's disease. On the other hand, increased dietary intake of DHA can result in improved cognitive abilities due to the fact that a lack of essential fatty acids has been linked to deficits in learning and memory (Cunnane et al., 2009; Yurko-Mauro et al., 2010; Cunnane et al., 2013).

Interesting results shows a study by Conklin et al. (2007). The study involved fifty-five healthy adults who completed two 24 h dietary recall interviews. Based on an intake of EPA and DHA, the respondents were divided into three groups: low intake (0-20 mg/day, 16 respondents), medium intake (25-70 mg/day; 21 respondents) and high intake of EPA and DHA (80-1600 mg/day, 18 respondents). Magnetic Resonance Imaging (MRI) scans of respondent's brains revealed a positive correlation between increased intake of these two fatty acids and the volume of gray matter in the anterior cingulate cortex, the right hippocampus and the right amygdala. Since mentioned areas are responsible for mood, scientists believe that increased intake of EPA and DHA has a positive effect on mood, but also on memory functions (Conklin et al., 2007).

The best dietary sources of omega-3 fatty acids are oily fish (sardines, mackerel, tuna, anchovies), cold water fish (herring, salmon), algae, zooplankton and seafood as well as seeds and nuts. Nutritional supplements containing purified and concentrated fish oil are also a valuable source of omega-3 fatty acids in the diet of a modern man (Shaikh and Brown, 2013; Bradbury, 2011). The amount of EPA and DHA in fresh fish varies depending on a species. Oily fish is particularly useful for pregnant women, and it is recommended to be consumed once a week. But the special attention is needed with canned tuna for possible intoxication with mercury. Species of fish that are long-lived and high on the food chain tend to have higher levels of methylmercury which has negative impact on the nervous system of a foetus. A total amount of methylmercury in fish remains relatively unchanged after cooking (WHO, 2007; WHO, 2010; Brown, 2010).

Amino acids and neurotransmitters

The most common neurotransmitters are: acetylcholine, glutamate, gamma-aminobutyric acid (GABA), glycine, serotonin, dopamine, norepinephrine, epinephrine and histamine produced by our brain directly from nutritional components of our diet. Activity and levels of these neurotransmitters depend on food intake and change in nutrient intake can significantly affect behaviour, sleep and energy levels (Sommer, 1995; Gustafson, 2008).

Serotonin is produced from the amino acid tryptophan, which is found in protein-rich food, such as chicken, dairy products, eggs and legumes. Ironically, consumption of high-protein foods decreases levels of tryptophan and serotonin in the brain, while the consumption of carbohydrate-rich foods has the opposite effect. After consumption of high-protein foods, tryptophan competes with other amino acids in order to pass the blood-brain barrier, which results in a lower increase in brain serotonin. When large amounts of carbohydrates are eaten, insulin is released, causing the absorption of the majority of amino acids into the bloodstream while giving advantage to tryptophan for brain access, leading to increased level of brain serotonin. The resulting increase in brain serotonin promotes the feeling of calmness, improves sleep, increases pain tolerance and reduces food cravings (Sommer, 1995; Fernstrom, 2013; Parker and Brotchie, 2011).

Dopamine and norepinephrine are synthesized from the amino acid called tyrosine, with the assistance of folic acid, magnesium and vitamin B_{12}. Unlike tryptophan, tyrosine level raises after consuming a protein-rich foods which leads to increased levels of dopamine and norepinephrine, both affecting alertness and mental energy (Sommer, 1995; Fernstrom, 2013; Parker and Brotchie, 2011; Daubner et al., 2011). Acetylcholine is synthesized from choline and unlike other amino acids that have to compete for brain access, choline does not need to. The best source of choline is egg yolk. Acetylcholine is important for memory and general mental ability. Reduced levels of acetylcholine are associated with memory loss, decreased cognitive function and Alzheimer's disease at old age. Choline deficiency induces neuronal death and mental fatigue, a person cannot think clearly, is depressed and forgetful (Sommer, 1995; Holmes et al., 2002; McCann et al., 2006).

Mediterranean diet

When speaking about brain food we must not forget about one specific dietary regime which shows immense potential in maintaining and boosting brain functioning. This is the Mediterranean diet (MD). Despite several differences between Mediterranean regions, they all have something in common. The specific combination of foods make it so simple and yet so complicated at the same time (Banjari et al., 2013). Yet this is exactly the perfect combination of macro and micronutrients, making it a number one choice for health and longevity. Health benefits of the MD go well beyond preventing cardiovascular diseases, lover mortality and morbidity (Banjari et al., 2013), as shown by the Lion Diet Heart Study (De Lorgeril, 2013), study by Trichopoulou et al. (2003) in Greece, or the PREDIMED study conducted in Spain (Estruch et al., 2013). Protective effect of the MD has been determined for number of degenerative diseases, like cancers dementia, and the risk of Alzheimer’s disease (Shah, 2013; Lourida et al., 2013; Sofi et al., 2013). Furthermore, Skarupski et al. (2013) showed its potential in reducing depression among people of 65 years and older. Also, rising interest of the non-Mediterranean countries, firstly Scandinavian countries, resulted in vast number of evidence showing the MD potential in protecting from premature death (Hodge et al., 2011; Gardener et al., 2011; Hoevenaar-Blom et al., 2012; Martínez-González et al., 2012; Hoffman and Gerber, 2013; Tognon et al., 2013), and cerebrovascular diseases (Misirli et al., 2012).

Food and mood

We can boost our mood by retaining available neurotransmitters in the gap between nerve cells as long as possible and it seems possible, but yet-to-be-tested, that expressions of foods in art can also serve to improve mood. Regulation of three key neurotransmitters responsible for mood (dopamine, noradrenaline and serotonin) by modulating food intake impacts durability of their stimulation of nerve cells, thus impacts mood and behaviour (Privitera et al., 2013; Hamburg et al., 2014).

Chocolate and caffeine

A study of 8000 people has shown that people who consume chocolate live longer compared to those who never eat chocolate. Positive effect of a chocolate lies in its flavonoid content. Chocolate flavonoids reduce the amount of low-density lipoprotein (LDL) cholesterol and reduce blood pressure. They also show the potential to slowdown growth of cancer cells (Engler and Engler, 2004; Paoletti et al., 2012). Due to chocolate production processes, it is believed that only dark chocolate products with a cocoa content of approximately 70 % or higher truly offer a significant benefit of flavonoids (Goldoni, 2004; Rawel and Kulling, 2007). Cocoa beans contain 61 % of cocoa butter, tannin, catechin and alkaloids theobromine and caffeine, which have different effects on our brain and emotions. Cocoa beans are also rich in hydrolysis products of polyhydric phenols such as quercetin, caffeic and p-hydroxycinnamic acid (Jalil and Ismail, 2008; Smit et al., 2004; Parker et al., 2006). It is known that chocolate contains over 300 substances, but the key ingredient is phenylethylamine. Most phenylethylamine is metabolized in the body, but some reaches the brain where it leads to dopamine increase. After consumption of chocolate, phenylethylamine is released into the human system producing the arousing effects of an intense emotional stimulus leading to euphoria. Some antidepressants have a similar effect, because they inhibit monoamine oxidase (MAO inhibitors) and prevent the degradation of phenylethylamine. Therefore, chocolate can have antidepressant effect. Chocolate contains anandamide, a substance that is an endogenous cannabinoid and occurs naturally in the brain where stimulates positive feelings. Anandamide targets the same brain structure as tetrahydrocannabinol (THC), the active ingredient in cannabis. Chocolate also contains tryptophan. The release of endorphins is stimulated with chocolate generating feeling of pleasure and promoting a sense of well-being. Alkaloids in chocolate, as well as in wine and beer improve mood (Smit et al., 2004; Parker et al., 2006).

Some researchers believe that women crave chocolate prior to menstruation because it contains high levels of magnesium. Magnesium deficiency increases the intensity of premenstrual syndrome. Even 91 % of women have cravings for chocolate in the second half of their menstrual cycle, with greater desire in the afternoon and early evening, and magnesium intake could significantly improve premenstrual mood changes (Ghalwa et al., 2014).

Another CNS stimulant is caffeine, which shows positive and adverse effects depending on a dose and frequency of administration. Caffeine is a chemical methylxanthine, first isolated from coffee beans, which is the major source of caffeine, but is also found in other drinks such as green and black tea, Guaraní, cocoa and soft drinks, especially Cola and energy drinks (Persad, 2011). The amount of caffeine present in products depends on the type of a product, serving size and preparation method. Chocolate also contains small amounts of caffeine, but for the sake of comparison it can be said that a cup of cocoa contains 20 mg of caffeine, while a cup of tea contains 40 mg on average, and a cup of coffee contains 155 mg of caffeine (Heckman et al., 2010).

Caffeine acts as an antagonist to adenosine receptors. Adenosine is a substance produced in the body as a product of increased metabolism and signals fatigue and the need for rest (Higgins et al., 2010). Caffeine therefore acts as a psychostimulant in the brain: enhances attention, causes alertness, improves memory and increases the ability to process degraded stimuli. At the same time also raises heart rate, increases force of myocardial contraction, secretion of urine and secretion of gastric juice. The most notable behavioural effects of caffeine occur 15 minutes after drinking caffeinated beverage (Persad, 2011).

Due to caffeine effects, including increased alertness, energy, ability to concentrate and wakefulness, it is primarily used as a stimulant in fatigue and somnolence. Consumption of caffeinated coffee in a dose-dependent was found to reduce the incidence of dementia, particularly Parkinson's disease (Fredholm, 2011).

Scientists believe that caffeine consumption is safe up to 200 mg per day and has beneficial effects on the body even in people with hypertension (Cano-Marquinaa et al., 2013).

Ingestion higher than 400 mg of caffeine, especially in caffeine-sensitive individuals, pregnant women and children, may have adverse effects like insomnia, excessive excitement, nervousness, increased heart rate and increased gastric acid secretion (Persad, 2011; Higgins et al., 2010; Nehlig et al., 1992; Snel and Lorist, 2011).

Conclusions

Studies have shown that food can promote proper functioning of the brain. In order to improve our mental abilities, concentration, memory and vigilance, proper nutrition is of great importance. By affecting neurotransmitters, substances that activate different regions of the brain, actively participate in the creation of nerve impulses and thereby regulate our mental abilities, emotions and mood. Cognitive performance and maintenance of mental health, especially among elderly may be improved with proper diet consisting of complex carbohydrates, polyunsaturated fatty acids, especially omega-3 fatty acids, proteins and specific foods containing specific nutrients, like flavonoids. In addition, mood and concentration as well as alertness can be affected by moderate consumption of chocolate and caffeinated beverages. Keeping in mind the risk factors for loss of mental abilities, by proper nutrition we can potentially prevent or delay neurodegenerative changes in the brain including Parkinson's and Alzheimer's disease. The conclusion arising from the compiling evidence elaborated in the text says that in order to improve cognitive performance and maintain brain vitality the Mediterranean diet should be chosen. The Mediterranean diet poses itself as a possible solution via its specific combination of foods which are, if separately analysed for nutrient composition, the ideal combination to maintain and keep proper brain function through old age.

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Food industry by-products as raw materials in functional food production

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review

Summary

Western civilization problems nowadays are overweight, obesity, diabetes, cardiovascular diseases, cancer and different disorders closely linked to unbalanced diet. Since it is extremely difficult to influence nutritional preferences of consumers, food industry is now increasingly developing new products, such as bread, pasta, snack products and other highly consumed products by all groups of consumers enriched with ingredients that are lacking in every day nutrition (fiber, polyphenols, antioxidants, vitamins, ß-glucan…) and functional products which have scientifically proven beneficial effect on human health. Food industry by-products, such as apple pomace, by-products from sugar industry and brewers spent grains are rich source of polyphenols, fiber and ß-glucan. Grape pomace is rich in polyphenols, tomato pomace in lycopene and carrot pomace in β-carotene. These are just some examples of by-products with great potential of application in enriched and functional food production. In addition to natural substances which are produced in this manner, problem of large quantities of waste disposal is also resolved.

Keywords: food industry by-products, functional food, fiber, ß-glucan, antioxidants

Introduction

The modern problems of Western civilization are overweight, obesity, diabetes, cardiovascular diseases and various disorders that are closely related to improper diet. As it is difficult to affect eating habits of consumers, today's food industry develops new products consumed by the wide population, enriched with ingredients that are poorly represented in the daily diet (fiber, antioxidants, polyphenols, vitamins, ß-glucan, minerals,...) and functional products, which are scientifically proven to have a beneficial effect on health. Modern trend is a demand and production of food products with the specific taste and health benefits. All of these requirements of consumers pose a major challenge for food technologists and all those involved in the food production chain.

By-products of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used because of their favourable technological or nutritional properties (Schieber et al., 2001b). At present up to one third of fruit and vegetables in the form of peels, pips, kernels and skins can be discarded during preparation and processing, therefore creating a ‘waste’, while also decreasing the maximum nutritional potential of the fruit or vegetable (O'Shea et al., 2012).

This review illustrates nutritional value of some food industry by-products and their application in production of various type of new products.

Table 1. The content of various compounds in the fruit and vegetable by-products

Compound	Content [% w/w, db]	Source	References
Pectin	13 – 39	Apple pomace	Renard et al. (1996)
	15 – 30	Sugar beet pulp	Yapo et al. (2007)
Total dietary fibre • **Insoluble** • **Soluble**	51.1 • 36.5 • 14.6	Apple pomace	Sudha et al. (2007)
	57 • 47.6 • 9.41	Orange peel	Chau and Huang (2003)
	63.6 • 50.1 • 13.5	Carrot pomace	Chau et al. (2004)
Protein	27.5	Kernels of peach	Rahma (1988)
	20 – 25	Bitter apricot seeds	Tunçel et al. (1998)
	16.1	Cauliflower	Stojceska et al. (2008a)
	20	Brewer's spent grain	Mussatto et al. (2006)

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Fruit by-products

Because of its high quantity in fruit processing industry and their nutritive value (dietary fibers, polyphenols, pectins,...) in this chapter are presented some of the most investigated fruit industry by-products.

Apple

The major product from apple processing is apple juice. The entire fruit is usually pressed in a cold press to extract the juice from the fruit. This can result in much waste, which is termed apple pomace (O'Shea et al., 2012). Apple pomace, inexpensive and primary by-product of apple juice and cider production is used as a source of pectin (Hwang et al., 1998), as animal feed (Sandhu and Joshi, 1997), as dietary fibres (Leontowicz et al., 2001) or as a source of phenolic compounds (Schieber et al., 2004).

Since apple pomace is rich in pectins, between 13 and 39 % of pectins (Renard et al., 1996), Royer et al. (2006) showed that it is possible to obtain jellies with apple pomace without incorporating gel additive. Production of pectin is considered the most reasonable way of utilizing apple pomace both from an economical and from an ecological point of view (Endreß, 2000, Fox et al., 1991). In comparison to citrus pectins, apple pectins are characterized by superior gelling properties. However, the slightly brown hue of apple pectins caused by enzymatic browning may lead to limitations with respect to their use in very light-coloured foods (Schieber et al., 2001b).

Gorinstein et al. (2001b) investigated the dietary fibre levels of a whole apple, its pulp and its peel. Interestingly, they found that the majority of the total fibre was located in the peel of the apple (0.91 % fresh weight [FW]). The percentage of insoluble (0.46 % FW) to soluble fibre (0.43 % FW) was found to be well balanced in terms of receiving a health benefit. Dried apple pomace is considered as a potential food ingredient, having dietary fibre content of about 36.8 %, and has been used in apple pie filling and in oatmeal cookies (Carson et al., 1994).

Apple pomace has been shown to be a good source of polyphenols which are predominantly localized in the peels and are extracted into the juice to a minor extent. Major compounds isolated and identified include catechins, hydroxycinnamates, phloretin glycosides, quercetin glycosides, procyanidins, chlorogenic and caffeic acid, and phloridzin (Foo and Lu, 1999, Lommen et al., 2000, Lu and Foo, 1997, 1998, Schieber et al., 2001a, Garcia et al., 2009, Schieber et al., 2003).

Masoodi et al. (2002) studied cake making from apple pomace wheat flour blends at 5, 10 and 15 %, so as to enrich the cake with fibre content. Sudha et al. (2007) also investigated the addition of apple pomace in wheat flour at 5, 10 and 15 % levels and studied rheological characteristics and cake making. These authors concluded that the cakes prepared with apple pomace had pleasant fruity flavour, and had higher dietary fiber and phenol contents.

Recently, apple pomace is tried to be incorporated into other products, such as "snacks", which are highly consumed products by all groups of consumers (Karkle et al., 2012).

Grape

Grape (*Vitis* sp., Vitaceae) is one of the world's largest fruit crop with more than 60 million tons produced annually. About 80 % of the total crop is used in wine making and pomace represents approximately 20 % of the weight of grapes processed. From these data it can be calculated that grape pomace amounts to more than 9 million tons per year (Schieber et al., 2001b). Viniculture is an important agricultural activity in a lot of countries in southern Europe like in Spain, Italy and France and produces huge amounts of grape marc. This by-product consists mainly of skins and in certain case of seeds and some stalks. After extraction in the distilleries of wide range of products (ethanol, grape seed oil, anthocyanins and tartrate), the remaining pomace is currently not upgraded but used for composting or discarded in open areas potentially causing environmental problems. Considering the growing demand for green materials and components, agricultural by-products like pomace have an obvious potential as a renewable starting material (Rondeau et al., 2013). It is also used in the production of citric acid, methanol, ethanol and xanthan gum as a result of fermentation. The nutritional and compositional characteristics of grape pomace are known to vary, depending on the grape cultivar, growth climates and processing conditions (Deng et al., 2011).

Grape pomace has been shown to be a rich source of dietary fibre; its components mainly comprise of cellulose, small proportions of pectins and hemi-celluloses (Kammerer et al., 2005, González-Centeno et al., 2010).

Furthermore, grape pomace has also been evaluated as a source of antioxidants because of its high contents of polyphenols (Negro et al., 2003). Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols and stilbenes are the principal phenolic constituents of grape pomace (Schieber et al., 2001b). Ruberto et al. (2007) carried out a study on the polyphenol content of Sicilian red grape pomace. The authors found that anthocyanins, flavonols and the phenolic acid, gallic acid, were the main polyphenols present.

In recent years grape pomace was used for production of different types of products. Altan et al. (2009) investigated the functional properties and *in vitro* starch digestibility of barley-based extrudates from fruit and vegetable by-products (tomato and grape pomace), and concluded that increasing level of both tomato and grape pomace led to reduction in starch digestibility.

Graphical optimization studies resulted in 155-160 °C, 4.47-6.57 % pomace level and 150-187 rpm screw speed as optimum variables to produce acceptable extrudates and the results suggest that grape pomace can be extruded with barley flour into an acceptable snack food (Altan et al., 2008b).

Peach and apricot

Peaches and apricots contain significant quantities of phenolics and carotenoids, components with various health benefits (Campbell and Padilla-Zakour, 2013).

Peach has been widely used around the world in the form of peach slices in syrup or just eaten as a dessert. The remnants from peach processing usually include the kernel and the peel. Over the years, these remnants have been used for their pectin as a thickener in jams; nowadays they are used commercially as a general thickener in foods (O'Shea et al., 2012). Págan and Ibarz (1999) described the recovery of pectin from fresh peach pomace**.** It is concluded that peach pectin is highly methoxylated and has favourable gelling properties (Págan et al., 1999). Kurz et al. (2008) characterized the cell wall polysaccharides of peaches and concluded that the main polysaccharides found were in the form of pectin.

Kernels of peach fruits contain 54.5 % and 27.5 % oil and protein, respectively, but ash and total carbohydrates were quite low (Rahma, 1988). Because of this high content of oil, Sánchez-Vicente et al. (2009) used peach seed as raw material for supercritical fluid extraction of oil. Furthermore, peach seeds may be used for the production of persipan (Schieber et al., 2001b).

Apricot is one of the most delicious and commercially traded fruits in the world. The plant is rich in mono- and polysaccharides, polyphenols, fatty acids and sterol derivatives, carotenoids, cyanogenic glucosides, and volatile components due to its appealing smell (Erdogan-Orhan and Kartal, 2011).

More than 650 metric tonnes of bitter apricot seeds are produced in Turkey per year as a by-product from the fruit canning industry (Tunçel, 1995). They are used as a substitute for bitter almonds to produce persipan for the bakery industry. The oil (53 % in the seed) is used, in e.g. cosmetics, as a cheaper substitute for bitter almond oil. The seeds can also be of interest as a food or feed ingredient because of their high crude protein content (20-25 % w/w, dry weight basis). The main problem is that bitter seeds contain approximately 50-l50 μmol/g (dry weight basis) of potentially toxic cyanogenic glycosides, mainly amygdalin and prunasin (Tunçel et al., 1998). Because of that, before using, seeds must be debittered by hydrolysis of amygdalin (Schieber et al., 2001b), and there are various researches about this (Tunçel et al., 1990, 1995, 1998, Nout et al., 1995).

Lemon and orange

Approximately 50 % of the original whole fruit mass, after citrus processing for juice, consist of the peel, membranes and seeds. Citrus residues consist mainly of insoluble fiber (celluloses) and a small proportion of soluble fiber (hemicelluloses and pectin). For this reason, citrus residues could be considered as a potential high fiber ingredient that is used for food industry (García-Méndez et al., 2011). Residues of citrus juice production are a source of dried pulp and molasses, fiber-pectin, cold-pressed oils, essences, D-limonene, juice pulps and pulp wash, ethanol, seed oil, pectin, limonoids and flavonoids (Schieber et al., 2001b).

Comparison of some biochemical characteristics of different citrus fruits investigated Gorinstein et al. (2001a). These authors concluded that lemons possess the highest antioxidant potential among the studied citrus fruits and are preferable for dietary prevention of cardiovascular and other diseases. The peels of all citrus fruits are rich in dietary fibres and phenolic compounds and suitable for industrial processing. García-Méndez et al., (2011) found that extrusion is a process that has the capability to transform insoluble fiber to soluble fiber in lemon residues. The highest content of soluble fiber was 50 %, when operating conditions were high in temperature (100 °C), low in moisture content (40 %) and low in screw speed (10 rpm).

85 % of oranges are processed into some form of orange juice, leaving behind tonnes of by-product after production. As a result of the functional and nutritional characteristics of orange peel, it may be

considered to be a viable ingredient for a wide variety of products such as meat pastes, baked goods and yoghourt (O'Shea et al., 2012). Chau and Huang (2003) found that the orange peel contain 57 % DW total dietary fibre; of this 47.6 % DW was the insoluble fraction and 9.41 % DW was the soluble fraction.
Larrea et al. (2005) investigated the effects of some operational extrusion parameters on selected functional properties of orange pulp and its use in the preparation of biscuit-type cookies. They concluded that biscuits of good technological quality and with a good level of acceptance were obtained by means of replacing up to 15 g/100 g of the wheat flour with extruded orange pulp.

Vegetable by-products

As a rich source of lycopene (tomato), β-carotene (carrot) and dietary fiber (cauliflower), and because of their high quantity in vegetable processing industry in this chapter are presented these three nutritive valuable vegetable industry by-products.

Tomato

Tomato (*Lycopersicon esculentum*) is one of the most popular vegetables and an integral part of human diet worldwide. Significant amounts are consumed in the form of processed products such as juice, paste, puree, ketchup, sauce and salsa (Altan et al., 2008a). During tomato processing a by-product, known as tomato pomace, is generated. This by-product represents, at most, 4 % of the fruit weight, and mainly consists of fibre; it can represent up to 50 % of the by-product on a dry weight basis (Del Valle et al., 2006). Furthermore, this by-product can still contain many nutrients and phytochemicals (O'Shea et al., 2012). The skin, important component of pomace, is source of lycopene. Lycopene is an excellent natural food color and also serves as a functional ingredient with important health benefits beyond basic nutrition (Kaur et al., 2005). It has been associated with various health benefit claims including immune system modulation, as a free radical scavenger and as having anticarcinogen properties (Dehghan-Shoar et al., 2010).
Nowadays, there are many researches about using tomato pomace as a novel ingredient in different types of food products. Dehghan-Shoar et al. (2010) investigated the addition of tomato derivatives to traditional starchy extruded snacks to improve their nutritional properties. These authors concluded that lycopene retention was higher in products containing tomato skin powder and significantly lower when wheat flour was used to make the snacks. Increases in the processing temperature improved the physicochemical characteristics of the snacks but had no significant effect on lycopene retention ($P > 0.05$) and texture of the product. Calvo et al. (2008) incorporated tomato powder (from tomato peel) into fermented sausages. Besides, tomato peel was successfully added to hamburgers to improve their nutritional content via the presence of lycopene (García et al., 2009). Tomato pomace can be extruded with barley flour into an acceptable and nutritional snack. Extrudates with 2 % and 10 % tomato pomace levels extruded at 160 °C and 200 rpm had higher preference levels for parameters of color, texture, taste and overall acceptability (Altan et al., 2008a).

Carrot

The carrot (*Daucus carota*) is a root vegetable, usually orange, purple, red, white or yellow in color, with a crisp texture when fresh. It is a rich source of β-carotene and contains other vitamins, like thiamine, riboflavin, vitamin B-complex and minerals. Carrot pomace is a by-product obtained during carrot juice processing. The juice yield in carrots is only 60-70 %, and even up to 80 % of carotene may be lost with left over carrot pomace (Kumar et al., 2010). The total dietary fibre content of the carrot pomace was found to be 63.6 % DM, with 50.1 % DM being the insoluble fraction and 13.5 % DM the soluble fraction (Chau et al., 2004).
Because pomace received from carrots doesn't contain kernels and seeds, it can easily be added to a product without introducing negative functional or flavour issues while still retaining a lot of its phytochemicals (Chantaro et al., 2008). Various attempts were made at utilizing carrot pomace in food such as bread, cakes, dressing and for the production of functional drinks (Schieber et al., 2001b). Upadhyay et al. (2010) investigated the optimization of carrot pomace powder (CPP) incorporation on extruded product quality. The study demonstrated that an acceptable extruded product can be prepared by CPP incorporation, and optimum incorporation level of CPP was found to be 5 %. Kumar et al. (2010) found that carrot pomace could be incorporated into ready-to-eat expanded products up to the level of 8.25 %.

Cauliflower

Cauliflower has a very high waste index and is an excellent source of protein (16.1 %), cellulose (16 %) and hemicellulose (8 %). It is considered as a rich source of dietary fibre and it possesses both antioxidant and anticarcinogenic properties. Encouraging characteristics such as its pale colour, bland taste and high nutritional content make it an attractive novel ingredient (Stojceska et al., 2008a). Llorach et al. (2003) analysed the antioxidant capacity of cauliflower by-products and found that flavonoids and hydroxycinnamic acids were the main phenolics present. Similar to some of the fruit and vegetables, cauliflower by-products (such as the stem) have been shown to contain a significant amount of phytochemicals (O'Shea et al., 2012), and can be good novel ingredient for production of various food products. Stojceska et al., (2008a) used cauliflower by-products in production of cereal based ready-to-eat expanded snack and found that increasing the cauliflower to levels of 5-20 % increased dietary fibre in the finished product by over 100 %, increased protein content and water absorption index. Sensory test panel indicated that cauliflower could be incorporated into ready-to-eat expanded products up to the level of 10 %.

Sugar beet by-products

A third of the world production of sugar comes from sugar beet (*Beta vulgaris*). One ton of sugar beet (sucrose content 16 %) provides a dried weight of around 130 kg of sugar and 50 kg of a by-product, sugar beet pulp (SBP) (Rouilly et al., 2006). Molasses represents the runoff syrup from the final stage of crystallization. It mainly consists of fermentable carbohydrates (sucrose, glucose, fructose), and of nonsugar compounds which were not precipitated during juice purification. Molasses is used as feed and as a source of carbon in fermentation processes, e.g. for the production of alcohol, citric acid, L-lysine and L-glutamic acid (Schieber et al., 2001b).

Sugar beet pulp (SBP), a major by-product of the sugar refining industry, is a potential feedstock for biofuels. It contains 20-25 % cellulose, 25-36 % hemicellulose, 20-25 % pectin, 10-15 % protein, and 1-2 % lignin content on a dry weight basis (Zheng et al., 2013). Due to highly digestible fiber it is valued as an excellent food complement for animal feed and energy source. Raw pulp has been proposed as cultivation substrate, as well, for divalent cations complexation, as source of polyols for the production of urethanes and polyurethanes, as source of fiber in biodegradable composites or for paper manufacture (Rouilly et al., 2009). Addition of sugar beet fiber to semolina increased dietary fiber content but adversely affected colour and cooking loss of spaghetti (Özboy and Köksel, 2000). Owing to its high pectin content (15-30 %) on dry weight basis, and its availability in large quantities, sugar beet pulp (SBP) is another source, after apple pomace and citrus peels, for commercial pectin production (Yapo et al., 2007). Because of that, there are many researches about extraction of pectin from SBP (Li et al., 2012, Yapo et al., 2007, Lv et al., 2013, Ma et al., 2013). Pectins from SBP have poor gelling properties compared to citrus and apple pectins due to their high degree of methylation and low molecular weight and they are not extensively used in traditional applications in the food industry (Mata et al., 2009). In many parts of the world, utilization of SBP is an economically marginal part of beet sugar processing due to the low feed value and high drying cost. In certain areas, dehydrating and pelletizing SBP contribute 30-40 % of the overall energy cost of sugar beet processing. Therefore, the beet sugar industry seeks to add value to SBP via a process that does not require drying. In light of this, converting SBP into fuel ethanol through biological pathways, including hydrolysis and fermentation, is an attractive option (Zheng et al., 2012).

Brewer's spent grain

Brewer's spent grain (BSG) is the major by-product of the brewing industry. BSG is a lignocellulosic material containing about 17 % cellulose, 28 % non-cellulosic polysaccharides, chiefly arabinoxylans, and 28 % lignin. BSG is available in large quantities throughout the year, but its main application has been limited to animal feeding. Nevertheless, due to its high content of protein and fibre (around 20 and 70 % dry basis, respectively), it can also serve as an attractive adjunct in human nutrition (Mussatto et al., 2006). According to these authors, BSG is good for the manufacture of flakes, whole-wheat bread, biscuits and aperitif snacks, but it must be first converted to flour. Nevertheless, there are some limitations in the use of the flour as a protein additive or as a partial replacement for presently used flours, due to its colour and flavour. However, ß-glucan from BSG has a significant positive impact on health and because of that BSG is excellent raw material for the production of functional products.

Recent researches show that BSG contains a significant content of polyphenols (Moreira et al., 2013, Meneses et al., 2013, McCarthy et al., 2012). Stojceska and Ainsworth (2008) added BSG in wheat flour in bread production. Increasing the level of dietary fibre increased dough development time, dough stability and crumb firmness but decreased the degree of softening and loaf volume. Ktenioudaki et al. (2013) investigated sensory properties and aromatic composition of baked snacks containing brewer's spent grain. They found that addition of BSG altered the odour profile of the snacks, however sensory results indicated that BSG-containing snacks at a level of 10 % were highly acceptable and highlighted the possibility of using BSG as a baking ingredient in the formulation of enhanced fibre baked snacks. Stojceska et al. (2008b) incorporated BSG into ready-to-eat expanded products and concluded that addition of BSG significantly increased protein content, phytic acid and bulk density. Furthermore, Ainsworth et al. (2007) found that addition of BSG in maize extrudates has no significant effect on the total antioxidant capacity (TAC) and total phenolic compounds (TPC) values, but increase phitic acid (PA), protein in vitro digestibility (PIVD) and resistant starch (RS) values.

Conclusions

The food processing industry produces large quantities of waste products. These by-products are sources of components of high nutritive value, and can be used as raw materials for other purposes. Furthermore, they are inexpensive and available in large quantities. This paper clearly demonstrates the high nutritional value that many by-products possess, and their application in production of various new products.

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The application of Herzegovinian herbs in production of tea mixes

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review

Summary

Bosnia and Herzegovina (B&H) is a country that is biologically diverse in its rich and varied landscape surroundings. Due to the rapid development of chemistry in the last decade it is assumed that the synthetic substances will obtain advantage over herbs. However, there was a sudden increase in demand for products obtained from medicinal plants in western European countries. Special significance for human life had a plant species that can be used for production of herbal remedies in the pharmaceutical industry in the form of mono component herbal teas or tea mixes, which have extensive use in traditional medicine. Tea is having a share in highly competitive field - the global beverage market. A wide range of tea products are still evolving to be developed. The tea industry must follow the challenges facing the future with confidence. The pharmaceutical industry each year produced more herbal products, which are sold in pharmacies and traditional specialized herbal pharmacies. Research on the effects of tea on human health has been linked by the growing need to provide naturally healthy diet products that include plant-derived polyphenols. In line with this, there is need to explain how well known functional components in foods could extend the role of diet in disease prevention and treatment. This paper will highlight the application of tea mixes in human everyday use with emphasis on their therapeutic effects and the factors which affect the quality of herbs included in selected herbal tea mixes. The market analysis of herbs in B&H focused on different area of Herzegovina with diversity of plant species will be reviewed.

Keywords: Herzegovinian herbs, tea mixes, market analysis, tea application

Introduction

Every day we use whole plants, their parts or plant extracts in different fields: food, medicine, pharmaceutical and cosmetic industry, and in many other purposes. World Health Organization (WHO) estimates that the majority of the world's population, especially those in developing countries relies on traditional health care based on the use of medicinal plants (Lange, 1998). According to the definition of the WHO, the herbs are among those plant species of which one or more elements contain biologically active substance that can be used for therapeutic purposes or for pharmaceutical chemical synthesis (Šiljež et al., 1991). It is known that between 40,000 and 50,000 plant species (WHO compiled a list of more than 21,000 plant species) are used in traditional and modern medicine worldwide. More than half of the drugs in the world are produced from plants or represent synthetic copies of plant chemicals (Lange, 1998).

Most important Herzegovinian herbs

According to the florist analyzes, on our Planet is growing about 350,000 plant species of which about 12,000 can be used for obtaining biologically active substances which are used for healing. In B&H for this purpose are utilize around 500 species. Among them 160 - 170 types of species are native plants. Constantly discovering the new plant species occurs, but at the same time the production of synthetic preparations increase. Medicinal and aromatic plants and products (drugs, essential oils, extracts, tinctures and pharmaceutical products) made from natural substances are desirable goods especially in world developed markets (Šiljež et al., 1991). As a source for getting the drugs, wild plants from nature or cultivated grown plants can be used (Mihaljev et al., 2011). Special significance is in plant species that can be used for obtaining herbal remedies in the pharmaceutical industry in the form of mono component herbal teas/infusions or tea mixes, which have extensive application in traditional medicine (Tucakov, 1986).

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According to long experience in the area of herb collection our company Vextra (Mostar, B&H) gave in Table 1 the data for the most important Herzegovinian herbs and the way of their collecting.

Table 1. The most important plants in the Herzegovinian area of and the way of collection

Herb name	Latin name	Croatian name	Wild plant	Cultivated plant
Basil	*Ocimum basilicum* L.	Bosiljak		+
English ivy	*Hedera helix* L.	Bršljan	+	
Horsechestnut	*Aesculus hippo- cast anum*	Divlji kesten	+	
White mistletoe	*Viscum album* L.	Imela	+	
Iceland moos	*Cetraria islandica* L.	Islandski lišaj	+	
Primorse	*Primula officinalis* Jacq.	Jaglac	+	
Sage	*Salvia officinalis* L.	Kadulja	+	
Chamomile	*Matricaria chamomilla* L.	Kamilica		+
St. John's wort	*Hypericum perforatum* L.	Kantarion	+	
Juniper	*Juniperus communis* L.	Kleka	+	
Netle wort	*Urtica dioica* L.	Kopriva	+	+
Milfoil	*Achillea millefolium* L.	Stolisnik	+	
European linden	*Tilia cordata* M.	Lipa	+	
Lavender	*Lavanda angustifolia* L.	Lavanda		+
Balm beaves	*Melissa officinalis* L.	Matičnjak		+
Dandelion	*Taraxacum officinale* W.	Maslačak	+	
Mother of thyme	*Thymus serpyllum* L.	Majčina dušica	+	
Marjoram	*Origanum vulgare* L.	Mažuran	+	+
Mint	*Mentha piperita* L.	Paprena metvica		+
Calendula	*Calendula officinalis* L.	Neven		+
Rosemary	*Rosmarinus officinalis* L.	Ružmarin		+
Helichrysum	*Helichrysum arenarium* D.C.	Smilje	+	+
Gentian	*Gentiana lutea* L.	Srčanik / lincura		+
Brier hip	*Rosa canina* L.	Šipak	+	
Bearberry	*Arctostaphylos uva ursi*	Uva	+	+
Heather	*Calluna vulgaris* L.	Vrijesak	+	
Elder	*Sambucus nigra* L.	Bazga	+	

Cultivation of medicinal and aromatic plants, until recently, had no significance due to insufficiently explored domestic and international market of these products. There was no interest in plantation farming according to not enough researched production technology, and especially because of lack of mechanized harvesting and processing. The interest in plantation cultivation of medicinal and aromatic plants, as well as alternatives to wild plants, encourage the demand of manufacturing industry whose end products are based on medicinal and aromatic plants, and on the raw materials of consistent quality and quantity. In addition, targeted cultivation of medicinal and aromatic plants could reduce uncontrolled harvesting of wild plants and thus prevent its extinction (Šiljež et al., 1991).

The impact of environmental factors on plant growth

Plant life and its environment are closely linked. Environmental conditions have influence on the life of plants, while plants affect the environment (Šiljež et al., 1991). Day by day, the knowledge of plant metabolism are growing. For biosynthesis of plants environmental factors are extremely important. It should be particularly taken into account in plantation cultivation of medicinal plants, which are the most important ecological conditions that will allow the plant species forming the maximum amount of useful biologically active substances. Agriculture is one of the sectors, which are both sensitive to global warming (e.g. on atmospheric temperature, precipitation, soilmoisture, sea level and humidity) and contributes to climate change. In response to changes in climate, through practicing adaptation options it is important to protect both market and nonmarket benefits from damages. Naturally, plants have their own mechanism to tolerate a certain level of increased temperature. As soil temperature increase, the decomposition rate of organic matter will increase, and then nutrient mineralization and availability for plants uptake become increased at presence of sufficient water if other conditions are unchanged (Amedie, 2013).

Temperature

Temperature affects the distribution, the method of growing plants and basically, the production of biomass. Various secondary plant constituents are result of the following biochemical synthesis and each of them claims the optimum temperature. Therefore, the content of active substance increases or decreases depending on the temperature optimum for a particular type (Šiljež et al., 1991). The uptake of minerals, nutrient and water, absorption of light energy for the formation of carbohydrate through photosynthesis reactions as well as the breakdown and burning processes of carbohydrate for growth and development of the plant (respiration) is highly dependent on the amount of atmospheric CO_2 concentration and ambient temperature (Amedie, 2013). Temperature can affect photosynthesis through modulation of the rates of activities of photosynthetic enzymes and the electron transport chain and indirectly through leaf temperatures defining the magnitude of the leaf–to-air vapor pressure difference. Unlike the temperature sensitivity of processes like flowering and fruiting many other physiological processes have small genotypic variations, although some genetic adaptation have been observed (Lloyd and Farquhar, 2008).

Geographic location

Geographic location is important because of air temperatures and sunny periods during the year. A typical example of the importance of latitude is the synthesis of fatty acids (Šiljež et al., 1991). However, the acclimation potential varies a lot across taxa and biogeographic origin. With a rise in temperature at any particular location, species adapted to warmer climates would be expected to tend to increase in abundance compared to those adapted to relatively cooler conditions. If this process carries through to its logical conclusion, this would lead to a general shift in distributions to higher altitudes and latitudes (in the hottest regions, species would presumably persist if they could survive, possibly with selection for increased high-temperature tolerance). A typical example of the importance of latitude is the synthesis of fatty acids (Šiljež et al., 1991). However, it is important to recognize that the climate of a location includes not just period mean conditions (annual, monthly, decade) and normal seasonality, but also the typical variability in conditions, such as extremes of temperature and internal variation in precipitation regime (Viner, et al., 2006).

Soil

The soil can greatly affect qualitatively and quantitative properties of plants. Altitude highly affects the amount and the quality of the active substance. Studies have shown that the lowest altitudes can reduce the amount of active substances and medicinal plants. This phenomenon is observed in herbs such as lavender, wormwood, mint and thyme. In the processes of plant growth, many processes and reactions directly affected by rising temperature, decomposition, weathering and mass flow diffusion may hasten in the soil under optimum soil moisture condition. At low temperatures, the reaction processes become slower, temperature can indirectly affect plant morphology, growth, roots turn over etc., if it is both beyond and under the optimum level for the plants. In addition, soil moisture, availability of nutrient and minerals together with other processes will play an important role in plant growth and development (Amedie, 2013).

Market analysis of herbs in B&H

The history of the collection of medicinal and aromatic plants in B&H is not sufficiently researched and documented, although is traditionally a very important sector. People have been collecting herbs for their personal use or to provide income for their families or members of herb community. During the former Socialist Republic of Yugoslavia, as well as today, B&H is a supplier of mainly unprocessed medicinal and aromatic plants. It is impossible to find reliable data on the quantities of purchased, sold or exported plant material that are originated from B&H (USAID, 2010).

Currently, about 50 small and medium enterprises in B&H operate in this sector (Fig. 1) and are engaged in the collection and sale of wild medicinal and aromatic plants (USAID, 2010).

B&H has over 700 species of medicinal and aromatic plants, of which about 200 are exploited (Gatarić et al., 1988). A relatively small number of medicinal and aromatic plants are grown (Kala, 2000). It is therefore necessary to start with the cultivation of medicinal and aromatic plants in order to provide raw material for the industry and for people who are interested in the traditional system of medicine. Conservative estimation of the value of total annual trade herbs in the world, done by The World Conservation Union (IUCN) ranges between 40 and 60 billion dollars. China is the world's largest manufacturer of herbs and medicines, followed by India. According to the data of Foundation for revitalization of Local Health Traditions – FRLHT, with a significant increase in demand for medicinal plants in the international market, it is expected that trade in medicinal plants will grow to 5 trillion by 2050 (Šiljež et al., 1991). This sector in B&H

represents a significant share of trade in medicinal plants in the world, and it is estimated that about 8 % of exports of medicinal and aromatic plants are originate from the Balkans (USAID, 2010).

In the postwar period, a significant number of companies were involved in the production and processing of medicinal and aromatic plants into products with added value, such as, essential oils, various medicinal applications and cosmetic products, spices and teas. Unfortunately, essential oils are exported to the international market mainly packaged in aluminum bottles or drums of 0.1 - 50 kg. Only a small number of companies in B&H exported essential oils as a final product, packaged in small vials (10 ml).

According to the Final Report of the EU (Analysis and presentation of the distribution of the value chain) annual harvesting of medicinal and aromatic plants in B&H ranges between 1,500 and 9,000 tons (depending on the demand and climatic conditions). They are packed and sold, mainly as a dried raw material, in bags of 25 kg. According to available data, the Foreign Trade Chamber of B&H in the field of medicinal plants and wild fruits export of medicinal plants in the 2012 year amounted to 654 995,38 kg, which is a slight increase compared to the year 2011 when it was 627 464,30 kg.

International market has high demands for significant quantities of various medicinal and aromatic plants (herbs, other forest fruits, mushrooms, etc.). Despite the fact that the majority of medicinal and aromatic plants which are harvested in B&H are intended for export, often domestic production cannot meet the demands of foreign markets in terms of quantity / quality control of raw material of medicinal and aromatic plants, or any other product of medicinal and aromatic plants.

Experts in this field, as well as manufacturing organizations, claim that Bosnia trades with only 20 % of the total number of plants collected. Approximately 85 % of these plants are exported, mainly dried and packed in bulk in cotton or paper bags or cardboard boxes, mostly into the European countries. Collectors (around 100,000) are associated with these companies in B&H. They collect raw materials mainly on land owned by the state, where they have free access. Only a small part is grown on private land (USAID, 2010).

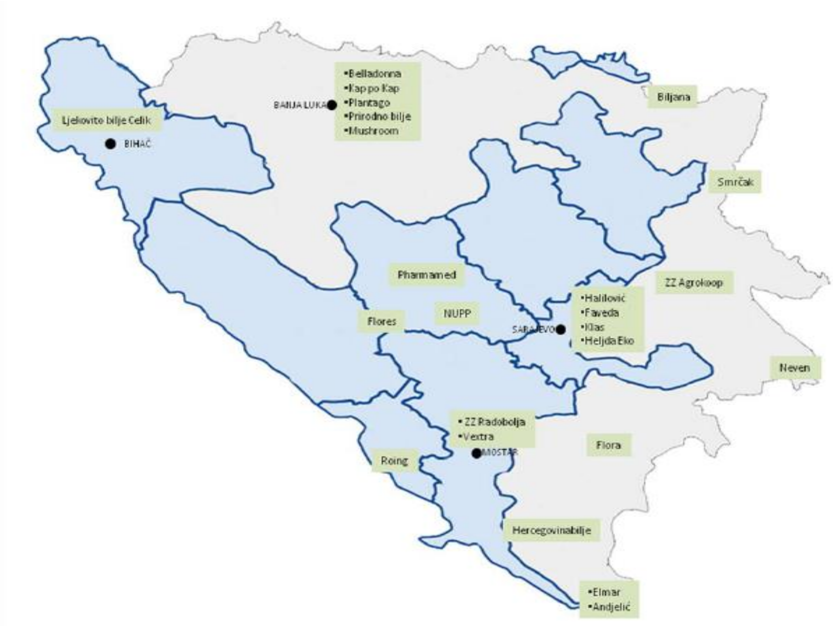

Fig. 1. Companies dealing with medicinal plants in B&H (USAID, 2010)

Tea consummation in B&H

Tea is infusion of the leaves of *Camellia sinensis* plant and is not to be confused with so-called herbal teas (Higdon, 2007). After water, tea is the most popularly consumed beverage worldwide with a per capita consumption of 120 mL/day. Every day, 800 million cups or glasses of tea are consumed globally (Natarajan, 2009). It is one of the most popular and the lowest cost beverages and is consumed by a wide range of age groups in all levels of society (Hicks, 2009). As tea is already one of the most popular beverages worldwide, future studies, designed to accurately assess tea consumption and tea polyphenol status, should be directed to quantifying its role in the primary and secondary prevention of chronic diseases (McKay and Blumberg, 2002).

The tradition of drinking tea and healing herbs in Herzegovina goes far away in the past where people were only relied on nature. The proof of that is written in many Herbal manuals. Herbal manuals had been written in the monasteries, and one extremely valuable can be found in monastery in Humac, Ljubuški (Herzegovina). Herbs had been long known as human's reliable helper in his fight against various diseases and health problems. It's really amazing how many different medicinal substances can be found in certain parts of the plant (leaves, flowers, roots). It is a well-known fact that the teas are predecessor of drugs. Hippocrates left records of 400 medicinal plants and their use in the treatment as originally acted agents for the treatment. Like every other science, knowledge of action and the use of medicinal plants are also constantly evolving. Today teas come on the market in three forms: in bulk, in filter bags or instant tea.

The biggest challenges of the tea industry today are: maintaining "healthy" products, the possibilities of finding new products, promoting a healthy lifestyle, cultivation of medicinal and aromatic plants and discovering the better production technologies for other plant species (USAID, 2010).

Antioxidant capacity of teas

The complex chemical composition of teas includes polyphenols, alkaloids (caffeine, theophylline and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, trace elements and other unidentified compounds. Among these, polyphenols are the most interesting group and are the main bioactive molecules in tea (Cabrera et al., 2003). The scientists are particularly interested in the potential health benefits of a class of compounds in tea known as flavonoids. Flavonoids in tea can bind nonheme iron, inhibiting its intestinal apsorbation. Nonheme iron is the principal form from iron in plant food, dairy products, and in iron supplements. The consumption of one cup of tea with a meal has been found to decrease the apsorption of nonheme iron in meal by 70 %. In many cultures tea is an important source of dietary flavonoids. Tea is also good source of another class of flavonoids, called flavonols. Flavanols are the most abundant class of flavonoids in tea. All teas contain caffeine, unless they are deliberately decaffeinated during processing (Higdon, 2007).

Tea active ingredients are of interest to functional foods markets (Hicks, 2009). Clinical studies have revealed several physiological responses to tea which may be relevant to the promotion of health and the prevention or treatment of some chronic diseases. Some apparent inconsistencies between studies on tea and health now suggest improved research approaches which may resolve them (McKay and Blumberg, 2002). Being an old and traditional beverage, tea was first grown in China and then spread to other countries and has always been liked by people all over the world. Despite the increasing market share of modern drinks such as soft drinks and alcohol drinks, tea has never lost its popularity, especially in recent years, when people are increasingly aware of the importance of organic foods and drinks, tea is being considered one of the most natural and healthy drinks which is promoted by more and more people around the world (Wang, 2011).

Application of tea mixes produced in Vextra company

Many plants through the history and in everyday use have shown exceptional healing properties. These properties, specially its versatility in the oral intake of tea are confirmed by lot of old books, knowledge and experience.

Vextra company was established in 1989 and since then engaged in education, collection and production of herbal preparations of medicinal herbs. For many years has been making the product line and now in daily sales has available about 200 species of herbs, 90 tea mixes with the therapeutic effect, and a number of herbal drops, oils and balms that are used to supplement therapy. Production takes place according to the rules of good manufacturing practice and the manufacturer's specification. The annual production capacity is about 15,000 production units. Educated staff working on improving the use of herbs with therapeutic effect and contributes to the development of medicinal plants in the service of science. The best healing property in application to human health showed herbal tea mixes. Tea mixes are composition of several species of plants in different percentages ratios. The concept of traditional recipes geared to the target action on certain problems to the specification and the chemical composition of plants.

Tea mixes that are in our sales in last two decades show extremely effective and therapeutic effects usually intended for the regulation of the cardiovascular system, digestive system, musculoskeletal system and the nervous system. Also therapy is often focused on specific diseases or where

the cause is unknown or is associated with a combination of several disorders.
Therapeutic stronghold, the old recipes and empirically proven effects, are found in plants with medicinal properties of the Herzegovinian region. The most characteristic plants that are in our recipes are Mulberry Black (*Morus nigra* L.), Sage (*Salvia officinalis* L.), Nettle wort (*Urtica dioica* L.), Milfoil (*Achillea millefolium* L.), Lady's mantle (*Alchemilla vulgaris* L.), St. John's wort (*Hypericum perforatum* L.), Helichrysum (*Helichrysum arenarium* D.C.), Lavender (*Lavanda angustifolia* L.), Calendula (*Calendula officinalis* L.), Chamomile (*Matricaria chamomilla* L.), Mint (*Mentha piperita* L.), Heather (*Calluna vulgaris* L.), Elder (*Sambucus nigra* L.), Rosemary (*Rosmarinus officinalis* L.), Golden fern (*Asplenium ceterach* L.), etc. In addition to these plants for better therapeutic effect, we added plants which are not typical for our region, but with characteristic chemical composition such as Sweet flag (*Acorus calamus* L.), Red eyebright (*Euphrasia officinalis* L.), Club moss (*Lycopodium clavatum* L.), etc.
According to long experience in our company, we had concluded that recipes which possess excellent activity are:

1. Tea mix “Antidiabetic”,
Major components: Mulberry Black (*Morus nigra* L.) and Gentian (*Gentiana lutea* L.).
2. Tea mix “Regulation of blood lipids”,
Major components: Golden fern (*Asplenium ceterach* L.) and Ramson (*Allium ursinum* L.).
3. Tea mix “Arthritis”,
Major components: Goldenrod (*Solidago vigra urea* L.) and Club moss (*Lycopodium clavatum* L.).
4. Tea mix “Psoriasis”,
Major components: Calendula (*Calendula officinalis* L.) and Yellow bedstraw (*Galium verum* L.).
5. Tea mix “Herb relaxation”,
Major components: Lavender (*Lavanda angustifolia* L.) and St. John's wort (*Hypericum perforatum* L.).
6. Tea mix “Function of the liver”,
Major components: Club moss (*Lycopodium clavatum* L.) and Dandelion (*Taraxacum officinale* W.).

Also there are tea mixes that are characteristic for Herzegovinian area, but not important for medicinal therapeutic use. All tea mixes are made from high quality herbs according to traditional recipes and contain certain percentages of herbs combination that has a specific and targeted action.
Tea mixes which we preferred for everyday use are tea mix “Herb Detox” and “Elixir”. In its structure they include key aromatic plants for Herzegovina region. Traditional recipes tea mix „Elixir“ contains the following plants: White birch (*Betula alba* L.), Chamomile (*Matricaria chamomilla* L.), Nettle wort (*Urtica dioica* L.), Garden raspberry (*Rubus ideaus* L.), Balm beaves (*Melissa officinalis* L.), Dandelion (*Taraxacum officinale* W.), Blackberry (*Rubus fruticosus* L.), Mint (Mentha piperita L.), Calendula (Calendula officinalis L.), Coltsfoot (*Tussilago farfara* L.), Mother of thyme (*Thymus serpyllum* L.), European linden (*Tilia cordata* M.), Shave grass (*Eqoisetum arvense* L.), Sweet flag (Acorus calamus L.), High mallow (*Malva silvestris* L.), Brier hip (*Rosa canina* L.) and Heather (*Calluna vulgaris* L.). „Elixir“ has special aromatic taste because of the combination of Mother of thyme (*Thymus serpyllum* L.), Mint (*Mentha piperita* L.), Heather (*Calluna vulgaris* L.) and European linden (*Tilia cordata* M.). The composition of this mix is designed to encourage complete detoxification, increases concentration and strengthens the immune system. Tea mix “Herb Detox“ has the purpose of detoxification of the body. It is intended for the elimination of toxic substances and cleaning the liver. The emphasis is on diuretic components. This effect is realized through the pronounced effect of following plants: Basil (*Ocimum basilicum* L.), Sage (*Salvia officinalis* L.), Marjoram (*Origanum vulgare* L.) and Glyeyrrhise (*Glycyrrhiza glabra* L.), and with the addition of other diuretic plants that support this action.

Conclusions

The domestic market of medicinal and aromatic plants in B&H is still in development. The need for education all participants in the value chain and the consumers are very important. The tendency is that this sector is growing more and more, despite the fact that the domestic market is still not strong enough. It is time to start with the popularization of medicinal and aromatic plants in the domestic market, taking into account the trend of the popularity of aromatherapy and wellness treatments abroad and in the country. Teas are considered to be a part of the huge beverage market, not in isolation. However, 50 – 60 % of the production cost is in the labor cost. There are numerous types of teas produced in many tea-producing countries. Generally, the age of plantation workers is increasing, as the younger generations do not wish to work in plantations. Mechanization of teas is thus inevitable, along with imported labor. There is a potential for agro / eco tourism through the tea plantations but producers should be more market-oriented and aware of the value of the tea market.

The research results of health benefits of tea consumption should also be used more extensively in promoting consumption in both producing and importing countries. In addition, strategies to exploit demand in value-added market segments, including special and organic teas, should also be more aggressively pursued. In targeting potential growth markets, recognition of and compliance with food safety and quality standards is essential. Even the impact of imposing a minimum quality standard as a means of improving the quality of tea traded internationally, would by default, reduce the quantity of tea in the world market and improve prices, at least in the short to medium term.

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Virgin olive oil and nutrition

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review

Summary

Numerous medical studies (a study in seven countries, Monika study, Dart studies, etc.) have shown that olive oil is one of the most important ingredient of "Mediterranean diet" associated with a reduction in cardiovascular disease and certain types of cancerous diseases. Nutritional and health value of virgin olive oil is attributed to the large proportion of monounsaturated fatty acids (mainly oleic, 55-83 %), and precious unsaponifiable ingredients that include aliphatic and triterpene alcohols, sterols (mainly β-sitosterol), hydrocarbons (squalene), volatile compounds, tocopherols (preferably α-tocopherol), pigments (chlorophylls and carotenoids) and antioxidants. Oleic acid is the most abundant fatty acid in olive oil that is claimed to affect the increase in level of high density lipoprotein (HDL) and reducing levels of low-density lipoprotein (LDL) in the blood plasma. For this reason it is considered that oleic acid could prevent the occurrence of certain cardiovascular diseases which are still one of the major causes of death. Besides the already mentioned high level of oleic acid, virgin olive oil is characterized by a highly valuable unsaponifiable ingredients which are attributed to exceptional biological value as virgin olive oil is classified as functional food.

Keywords: virgin olive, oil, fatty acids, biological value, Mediterranean diet

Introduction

Virgin olive oil is, unlike most seed oils, obtained by using a series of mechanical operations at a specific temperature (up to 28 ºC) whose purpose is to extract the oil droplets in cells of the pulp of olives. The resulting oil is fat naturally created in olive fruit and has a unique chemical composition and specific pleasant aroma. Therefore, it can be consumed directly without any further refining treatment. Today its biological, nutritional and health effects are scientifically and professionally recognized worldwide (Boskou, 2006).

Virgin olive oil contains two main fractions: the oil or saponifiable fraction and unsaponifiable fraction. Saponifiable fraction are mainly triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids and phospholipids, and they represent nearly 98.5 %-99.5 % of the oil chemical composition. Unsaponifiable fraction consisting of hydrocarbons, tocopherols, coloring pigments, sterols, phenols, triterpenes and other compounds which contribute around 0.5 % to 1.5 % of the oil composition. The olive oil triacylglycerols is the most common monounsaturated fatty acids (oleic acid), together with small amounts of saturated and significant amounts of polyunsaturated fatty acids (mainly linoleic acid) (Aparicio et al., 2000). The quality and biological value of olive oil depends on the following factors: variety, soil management and agrotechnical procedures, climatic conditions, health of the fruit, degree of maturity, method of harvesting and transportation of fruits, handling of fruits during storage to processing period and processing method. Preserving the quality and biological value of olive oil depends on the time and conditions of storage of oil. Today, the parameters of quality and biological value of olive oil are: fatty acid composition, the ratio of polyunsaturated to saturated fatty acids, ratio of omega-6 and omega-3 fatty acids, the amount of total phenols, the ratio of total phenols and polyunsaturated fatty acids, sterol composition, free acidity, peroxide value and sensory evaluation. Extra virgin olive oil, beside a high content of oleic acid, contains polyunsaturated essential fatty acids, linoleic and linolenic. The essential ingredients of olive oil can be found in unsaponifiable part which consists of, up to now, about four hundred identified compounds. These compounds play an important role in many physiological and biochemical processes in the body. In addition to the positive impact on the health of consumers, significantly affect the sensory properties of the oil (flavor, aroma and color), and antioxidant activity due to increased resistance to oxidative deterioration of oil. Among these compounds are especially important antioxidants, particularly phenols, tocopherols, pigments, pro-vitamins and vitamins (Gugić, 2010).

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Fatty acids of virgin olive oil

Saturated fatty acids: lauric, myristic, palmitic, stearic, arachidic, behenic and lignoceric are present in olive oil. Unsaturated fatty acids are an important factor by which the olive oil is distinguished from other fats. The most common monounsaturated fatty acid in olive oil is oleic acid (18:1 n-9), and in the composition of the total fatty acids it is represented with 55-83 %. It has a great biological nutritional value and is easily digestible. That's why olive oil is a representative of the oleic acid oil group (Šarolić, 2014). In 2004 Agency for Food and Drug Administration in the United States (FDA) allowed the claim to be printed on the labels of virgin olive oil which stated "benefit of reducing the risk of heart disease and cardiovascular system while consuming about two tablespoons (23 g) of virgin olive oil daily, thanks to its high content of oleic acid " (Ghanbari et al., 2012). It is believed that oleic acid (C18:1), the most common fatty acid in olive oil increases the level of high density lipoprotein (HDL) and apoprotein A1 and reduces the level of low density lipoprotein (LDL) and apoprotein B (Ghanbari et al., 2012). Therefore, the oleic acid can prevent cardiovascular diseases which are the main cause of death today (Ranalli et al., 1996). Certain factors such as growing area, variety, altitude, climate and degree of ripeness of the fruit significantly affect the fatty acid composition of olive oil. Besides the oleic acid in olive oil there are other polyunsaturated fatty acids: palmitoleic acid (16:1, n-7), gadoleic acid (20:, n-11), which is represented in a very small quantity (up to 0.5 % of the total amount of fatty acids). The most important essential fatty acid in olive oil are linoleic (18:2, n-6), in an amount of 3.5 to 21 %, and linolenic acid (18:3, n-3) in an amount up to 0.9 % (Boskou, 2006). Polyunsaturated fatty acids (PUFA) with 18 carbon atoms known as essential fatty acids are linoleic acid (18:2, ω-6) in an amount of 3.5 to 21 % and α-linolenic (18:3, ω-3) in an amount up to 0.9 %. Since the body cannot synthesize it these should be taken in with food. These fatty acids are essential for life because they regulate membrane fluidity, permitting action of cell organelles, are included in the composition of lipoproteins that carry blood lipids, participate in biochemical processes and immunologic processes, reduce blood cholesterol levels by activating cell receptors for LDL and are precursors of long-chain polyunsaturated fatty acids such as gamma-linolenic or GLA (20:3 omega-6), arachidonic acid or AA (20:4 omega-6), EPA or eicosapentaenoic (20:5 omega-3) and docosahexaenoic or DHA (22:6 omega-3). The daily intake of EFAs should be about 6-8 % of the calories of the total ingested fats (Viola, 2009).

At the present time nutritionist recommendations for a balanced diet fit for a general input equal to 30 % of calories, following the distribution of fatty acids:

- saturated fatty acid, 6-8 %
- monounsaturated fatty acids, 12-16 %
- polyunsaturated fatty acids ω-6, 6-7 %
- polyunsaturated fatty acids ω-3, 0,5-1,5 %

Table 1. Group of fatty acids of olive oil compared to other edible oils and fats (Viola, 2009)

Type of oil/fat	saturated fatty acids (%)	monounsaturated fatty acids (%)	ω – 6 (%)	ω – 3 (%)
butter	45-55	35-55	1.5-2.5	0.5
seam	40-46	42-44	6-8	0.5-0.9
olive oil	8-14	65-83	6-15	0.2-1.5
peanut oil	17-21	40-70	13-28	-
corn oil	12-28	32-35	40-62	0.1-0.5
soybean oil	10-18	18-30	35-52	6.6-9
sunflower oil	5-13	21-35	56-66	-

The ratio of polyunsaturated and monounsaturated fatty acids

As already stated monounsaturated oleic acid (18:1, ω-9) predominates in the olive oil, while polyunsaturated fatty acids, especially linoleic and linolenic prevail, in the seed oils in varying concentrations. Discussing the importance of linoleic acid a few years ago, a classification of different seed oils which were considered better for the food since they had a higher concentration of linoleic acid was made, while olive oil was considered "neutral" to human health, because monounsaturated fatty acids weren`t given any special importance. Today's findings on this issue are quite different. It is considered enough to provide the necessary amounts of essential fatty acids, and reduce the intake of saturated fatty acids because of the risk for the cardiovascular system and the possible risk of tumors, and generally speaking that the main source of energy intake should be from monounsaturated fatty acids. The balanced diet ratio of polyunsaturated : monounsaturated : saturated fatty acid is 1:2:1, the olive oil is about 0,5:5:1, while the value of the seed oils of about 5:2:1. From the above relations of fatty acid in olive oil, its stability and resistance against oxidation change are derived, taking into account that the degree of oxidation of linoleic acid to be ten times higher than oleic acid (Viola, 2003). It is considered that the good sensory characteristics of virgin olive oil are to be expected if the proportion of oleic acid in the oil is below 73 % and linoleic below 10 %, and when the ratio of oleic acid : linoleic acid is over seven (Koprivnjak et al., 1998).

The ratio of polyunsaturated and saturated fatty acids

Relatively high proportion of monounsaturated, a small proportion of saturated and a substantial proportion of essential fatty acids give olive oil a high nutritional value. Virgin olive oil, obtained exclusively by mechanical extraction methods from olive fruits are characterized by antioxidant activity as well as beneficial effects on human health due to the presence of highly valuable minor ingredients such as phenols (Covacs et al., 2006; Covacs et al., 2008, Tuck et al., 2002). Polyunsaturated fatty acids are recommended to reduce blood cholesterol levels and to prevent the development of atherosclerosis. Saturated fatty acids increase blood cholesterol levels and act as "promotors" of development of certain cancerous diseases. Since our initial recommendations, according to which it was necessary to maintain the ratio of P/S=2, ie for each gram of saturated, there should be two grams intake of polyunsaturated, the latest findings have shown that the best ratio of P/S=1, which means that for every gram of saturated it would be good to enter a gram of polyunsaturated fatty acids. This ratio is most favorable in olive oil (Viola, 2003).

The ratio of ω-6 and ω-3 fatty acids

Linoleic acid and alpha-linolenic acid, as has already been said, cannot be biosynthesized and must be therefore, already formed, taken with food. For this reason, they are defined as "essential fatty acids" or EFA (Essential Fatty Acids) and are compared to essential amino acids, vitamins and minerals. According to LARN - in (Livelli di Assunzione Raccomandati dei Nutrienti - Italian Society of Human Nutrition) total intake of polyunsaturated fatty acids in turn should not exceed 15 % of total calories and a desired ratio between the two series for adults is 10:1 and 5:1 for childrens and an old people. Having determined that it is necessary to enter a certain amount of EFA, it is very important, as noted above, to establish the most favorable possible ratio between the two series of polyunsaturated fatty acids, because, as we have seen, excessive amounts of linoleic acid can affect the elongation of alpha-linolenic which has negative effects on the body. The World Health Organisation has recommended ratio omega-6/omega-3 valued 5:1 to 10:1. This ratio is extremely important especially during growth and development, as polyunsaturated fatty acids of omega-3 series, except participating in the construction of the brain and retina, have an important function in male sex glands, helping the child`s development and preventive effect on the development of vasculopathy and various malignant disease (Viola, 2009). Since olive oil contains about 10 % of linoleic and less than 1 % of linolenic acid this ratio is completely satisfied with olive oil, which can not be said for the seed oil, especially for corn oil, where this ratio is 50:1, while in sunflower oil reaches 150:1. Good concentration of omega-3 fatty acids is found in soybean oil, but in this oil there is a significant imbalance in the ratio of antioxidants and polyunsaturated fatty acids with an increased risk of peroxidation (Viola, 2003).

Biological mechanisms of defense against free radicals

During normal oxidation of nutrients in producing energy (respiratory chain), a small portion of oxygen escapes the normal use and leads to the creation of free radicals, highly reactive and volatile compounds. Oxygen free radicals, if not neutralized, affect some macromolecules such as DNA (responsible for the genetic code), some specialized proteins and especially polyunsaturated fatty acids, which are an integral part of the phospholipids of cell membranes (disrupting their structure and function) and lipoproteins that carry cholesterol (altering their ability to deliver cholesterol to the cells that need it). The body defends itself against harmful effects of free radicals due to enzymatic and non-enzymatic antioxidant substances that are partly innate and partly to be brought in with food. This shows how important it is to increase the number of antioxidant substances, but given that we cannot influence the innate structure, we should increase our intake of antioxidants through food and at the same time try to reduce the causes which may favor the formation of free radicals. In case that there is imbalance between oxidant and antioxidant factors, an "oxidative stress" occurs, which is a condition that leads to changes in cell function which can result in a complete disruption of cellular activities (Viola, 2009). Oxidative stress is considered one of the main factors that cause various diseases such as cancer, aging, inflammation, atherosclerosis, cardiovascular disease and certain neurodegenerative diseases such as Parkinson's disease (Jenner et al., 1996). Today it is widely accepted that the risk of oxidative damage may reduce the high intake of plant antioxidants. In this sense, olive oil phenols act as "scavengers" of oxygen free radicals (Ghanbari, 2012). It is clear, therefore, why it is so important to eat fresh fruits and vegetables as well as extra virgin olive oil. It is also clear that it would be better to avoid the intake of easily oxidizing substrate, ie, polyunsaturated fatty acids. The olive oil ratio of antioxidants and polyunsaturated fatty acids is more than satisfactory, while it can not be said for seed oil in which, as already mentioned, is dominated by gamma and delta tocopherols which organism practically does not use, while polyphenols are completely absent (Viola, 2003).

The first line of defense (present in the body)	Second Line of Defense (entered through food)
superoxide dismutase	β-carotene
catalase	nonvitamin carotenoids
glutathione peroxidase	α-tocopherol
uric acid	ascorbic acid
bilirubin	phenolic compounds
transferrin	selenium

The ratio of α-tocopherol and polyunsaturated fatty acids

Tocopherols have natural antioxidant activity and inhibit the process of oxidative deterioration of oil. Average tocopherol in olive oil is 150-330 mg/kg (Koprivnjak et al., 1998). It is believed that the recommended daily intake of tocopherol is the amount of 1 mg of α-tocopherol. However, the optimum amount depends on the composition of fatty acids in the oil. The maximum amount is represented by α-form (vitamin E), which has significant biological activity. Quantity of the predominat tocopherol α -tocopherol varies from a few mg up to 300 mg/kg. Significant concentrations of α-tocopherol in virgin olive oils support the ideal ratio of vitamin E/polyunsaturated fatty acids. This ratio is expressed as mg of vitamin E per g of polyunsaturated fatty acids. This ratio should not be less than 0.5, and it is rarely found in seed oils, but in virgin olive oils it is in the range of 1.5 to 2 (Viola, 2009). Together with α-tocopherol, β, γ, and δ forms are also found in olive oil. These forms of tocopherol are found in virgin olive oils in amounts from several to 25 mg/kg (Ghanbari et al., 2012). It is believed that the ratio of α-tocopherol/polyunsaturated fatty acid in the presence of other antioxidants is sufficient to satisfy the need for vitamin E, and the protection of the fatty acids oxidation (Covian 1988). Reduction of tocopherol in olive oil occurs during fruit ripening and the process of refining of oils.

Antioxidant and biologically valuable components of virgin olive oil

Virgin olive oil is considered an example of a natural functional food because of its active substances which contribute to prevention and treatment of various diseases (Bendini et al., 2007). Antioxidants are as their name says, substances that prevent or slow down the process of oxidation in the product and the body. According to these substances, virgin olive oil is significantly different from other vegetable oils. The most important antioxidants in virgin olive oils are phenolic compounds. As well as contributing to the health value of oil, they affect the sensory properties and increase the stability of the oil from oxidative deterioration. (Bendini et al., 2007). Many vegetable oils contain small amounts of tocopherols of natural phenolic antioxidant terpene origin. It is believed that the tocopherol (vitamin E) prevents free radicals oxidation of lipid membranes and thus slows down the aging process (Pine, 1996).

In addition to removing free radicals, tocopherols in virgin olive oil prevent photooxidation changes and so increase the oxidative stability of the oil during storage (Kamal-Eldin, et al, 1996). Tocopherols defend the body against free radical attacks, prevent atherosclerosis, skin diseases and prevent certain cancerous diseases. Tocopherols exhibit extremely synergistic effect in antioxidant activity with phenolic compounds (Hudson et al., 1983). Virgin olive oil is unique among all vegetable oils because of its high content of phenolic compounds. Their shares and composition could be the basis for assessing the quality of the oil, because phenols are the most important antioxidants that contribute significantly to the stability of the oil, and prevent many diseases (Tura et al., 2007). Purely mechanical method of processing fruits in oil contributes to the high proportion of phenolic compounds in virgin olive oil. In addition, phenol compounds have exceptional antioxidant, anti-cancer and anti-inflammatory properties and are therefore important in the prevention of these diseases. Furthermore, it is important to highlight the impact of phenolic compounds on the sensory properties of virgin olive oil. Namely, the phenolic compounds are associated with the sensation of bitterness and pungency in oil (Bendini et al., 2007). Phenolic compounds, which are often referred in the literature as the polyphenols, in olive oil include a complex mixture of compounds of different chemical structure. The concentration and composition of phenolic compounds in virgin olive oil is determined by many factors, of which it is important to emphasize the variety, farming area, degree of ripeness of the fruit and the method of processing the fruit into oil (Lerma-García et al., 2009.). According to literature, the proportion of the phenolic compounds in olive oil is in the range of 40 to 1000 mg/kg (Serville, 2002.). The hydrophilic phenols of virgin olive oil belong to different substances classified into several groups: phenolic acids, phenyl-ethyl alcohols, hydroxy-isochromans, flavonoids, lignans and secoiridoids. Secoiridoids are specific to plants of the family *Oleaceae* and the principal component of the phenolic fraction. Many agro-technical and technological factors affect the proportion of phenols in virgin olive oil. The shelf life of this oil is considerably longer than other vegetable oils due to the presence of phenolic compounds that contain a catechol group such as hydroxytyrosol and derivatives secoiridoids.

Table 2. Biological activities and potential health benefits relating to olives/olive oil phenolics

Biological Activity	**Potential Clinical Target**
Antioxidant activity	Cardiovascular and degenerative diseases
Anti-inflammatory activity	Inhibition of proinflammatory enzymes
Antimicrobial activity	Infectious diseases
Anti-atherogenic activity	Coronary heart diseases, stroke
Anti tumor activity	Various cancers
Anti platelet aggregation	Coronary heart diseases, stroke
Anti-hypertensive activity	Hypertension
Increased vitamin A and β-carotene activity	Antiaging/skin protection
Increased immune activity	Infectious diseases; various cancers
Reduction in the levels of plasma cholesterol and oxidized LDL	Coronary heart diseases

Olive oil is the main source of fat in the Mediterranean diet, which is associated with a lower incidence of heart disease and circulatory system and some cancers. Extra virgin olive oil contains a considerable amount of phenolic compounds, for example, hydroxytyrosol and oleuropein, which are responsible for its distinct taste and high stability.

Conclusions

Virgin olive oil is an example of a natural functional food ingredients for which its activities contribute to prevention and treatment of various diseases. It contains a lot of antioxidants, it is dominated by monounsaturated fatty acids, low in saturated fatty acids, and contains essential fatty acids with a balanced ratio between a series of ω-6 and ω-3 fatty acids.

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Accumulation of heavy metals in the fruit and leaves of plum *(Prunus domestica* L.*)* in the Tuzla area

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Preliminary communications

Summary

Introduction: The term heavy metals imply all metals of specific density greater than 5 g/cm^3. Anthropogenic emissions of heavy metals affect the ongoing pollution of the Tuzla area. Cultivated plants acquire large amounts of heavy metals and therefore there is a real possibility of their involvement in the food chain. *Goals:* The basic objective of this paper is to determine the content and dynamics of heavy metals: cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn) in the fruit and leaves of plum *(Prunus domestica* L.) in the Tuzla area in order to obtain information about their impact on the environment. *Materials and methods:* Research consisted of fieldwork and laboratory analysis. Plant material was sampled at nine sites in the Tuzla area and prepared for chemical analysis of heavy metals. The content of heavy metals: chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), in solutions of plant material samples was determined by atomic absorption spectrophotometry (AAS method), with the instrument "Perkin-Elmer" 3110 and graphite cuvette "Perkin-Elmer" HGA-440. Determination of heavy metals was carried out according to ASTM-E 1812-96 standard. *Results:* The determined values of cadmium and copper content in plum leaves were higher than the natural content for plants in non-polluted environments in most localities. Cadmium concentration in the fruit of plum on most sites exceeded natural values of 0.8 mg/kg. The content of zinc in the fruit of plum at all locations was within the limits of the average value. In plum leaves the identified concentrations at the site of Donji Bistarac were 2.5 times higher than the average value which is 30 mg/kg. The highest concentration of chromium in leaves of plum was found at the site Donji Bistarac (2.25 mg/kg), and lowest at the site Donji Pasci. Plum fruit has a much smaller amount of the mentioned metal than a leaf, except at the site Donji Pasci where the determined values were 3 times higher than the average. *Conclusion:* The highest concentrations of heavy metals were found on sites that are located near industrial plants. Therefore, in the industrial-urban areas there should be provided continuous monitoring of heavy metals content in order to produce healthy food and improve the quality of life of people.

Keywords: Prunus domestica, cadmium, copper, zinc, chromium

Introduction

Tuzla area is contaminated with heavy metals as a result of continuous environmental pollution by high immissions of dust containing heavy metals and other harmful pollutants.

Large amounts of heavy metals are accumulated by cultivated plants too, so there is a real possibility of their involvement in the food chain. The fruits of plum *(Prunus domestica* L.) are consumed in fresh and processed forms, and it is important to determine the contamination of the site from which they are harvested. This fruit is rich in vitamins, minerals and phenolic compounds that contribute to its antioxidant activity (Walkowiak-Tomczak et al., 2008). In addition, they are a significant source of carbohydrates, organic acids, vitamin A, vitamin B, potassium, calcium, magnesium, zinc, selenium and fiber. The content of total fiber in the plum fruit increases with drying processes (Siddiq, 2006).

Iqbal and Khan, 2010 suggest that plants that are grown in polluted areas have a higher concentration of heavy metals than those grown in unpolluted environment. Among the heavy metals Cd, Cr, Cu and Zn occupy a special place because they belong to the so-called harmful elements. The greatest amount of heavy metals plants absorb by roots. Adoption and accumulation of heavy metals in plants depends on a number of environmental factors of habitats: soil pH, absorption capacity, the amount of $CaCO_3$, the distance from the source of emission of pollutants, exposure time, etc. (Kataba-Pendias and Pendias, 1984; Csintalan and Tuba, 1992; Goletić 1998, Goletić and Redžić, 2003). If heavy metals by precipitation land on leaf surfaces, the plant then adopts them directly through stomata (Reimann et al., 2001, Lokeshwari and Chandrappa, 2006).

The basic objective of this paper is to determine the content and dynamics of heavy metals: cadmium (Cd),

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chromium (Cr), copper (Cu) and zinc (Zn) in the fruit and leaves of plum (*Prunus domestica* L.) of Tuzla area in order to obtain information about their impact on the environment, because it is known that at higher concentrations heavy metals become included in the food chain and lead to various disorders in ecosystems.

Materials and methods

Research consisted of fieldwork and laboratory analysis. Fieldwork implied sampling of plant material at nine sites in Tuzla area. Laboratory work consisted of preparation of plant material for chemical analysis and chemical analysis of heavy metals in plant material. Samples of plant material for chemical analysis were prepared as follows: 1 g of plant material was dissolved in nitric acid (HNO_3, p.a.) and hydrochloric acid (HCl, p.a.) with heating. After decomposition of the sample (clarifying the contents of the flask), the flask of 25 ml was supplemented with perchloric acid ($HClO_4$, p.a.), and the sample was heated until the occurrence of perchloric vapors and their rising to the neck of the flask. The flask content was cooled down to room temperature, and then the flask was supplemented with distilled water up to a mark and the content was well mixed. Thus the sample solution was prepared to measure the concentrations of heavy metals.

Content (concentration) of heavy metals: chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), in solutions of plant material samples was determined by the method of atomic absorption spectrophotometry (AAS method), with the instrument "Perkin-Elmer" 3110 and graphite cuvette "Perkin-Elmer" HGA-440. Determination of heavy metals was carried out according to ASTM-E 1812-96 standard. Measuring parameter was the absorbance and the results were read from the calibration curve, which is constructed on the basis of the absorbance readings and the given concentration in solutions for calibration. Parameters at which the absorbance measurement was performed were set according to the manufacturer's instructions. Cathode lamps (Hollow Cathode Lamps) were used for the determination of each metal separately.

Results and discussion

The content of cadmium (Cd) in the investigated plant species

Natural cadmium content in plants varies in the range from 0.02 to 0.50 mg/kg of dry matter (Ward, 1995), while the average values of cadmium tolerance in plants are from 0.1 to 0.8 mg/kg (Bohn et al., 1985). From the shown results (Fig. 1) it is evident that the determined values of cadmium content in the leaf of plum are larger than the natural content for plants in unpolluted environments, which is within the range of 0.02-0.5 mg/kg (Ward, 1995). The values obtained were above the limit value of cadmium in plants at the following locations: Puračić (3.5 times higher than the limit value), Donji Bistarac (3.5 times higher), Crveno Brdo (3 times higher). These sites are generally closest to the dominant sources of heavy metals and therefore suffer the greatest impacts. This confirms that the industry and the burning of fossil fuels have influence on the increased absorption and accumulation in plants, which can be risky for consumers.

The concentration of cadmium in the fruit of plum on most sites exceeded the natural value of 0.8 mg/kg. The minimum values were registered at the site Donji Bistarac (0.27 mg/kg) and the highest at the site Donja Lipnica (2 times greater than natural values) (Fig. 1).

Analyzing the determined concentrations we can conclude that in most plants the maximum value was determined at locations that are closer to industrial zones, and the least was at remote locations. Many authors have ascertained the same in the environment of different factories (Kataba-Pendias i Pendias, 1984; Yanyu and Wang 1996; Elezi, 1998; Goletić and Redžić, 2003).

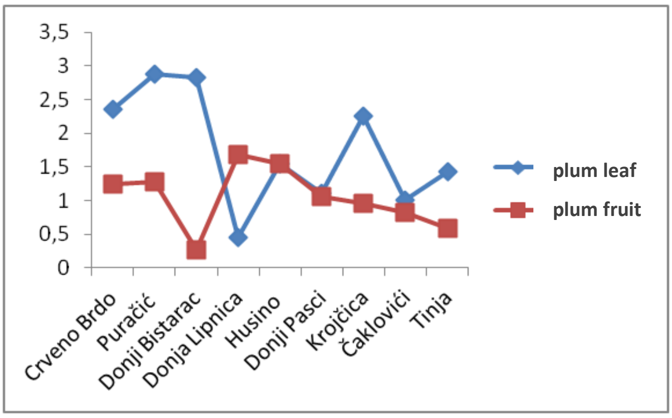

Fig. 1. The content of cadmium (Cd) in leaf and fruit of plum (mg/kg dry matter) at the investigated sites

The content of chromium (Cr) in the investigated plant species

The average chromium content in plants is in very small concentrations which vary in the range of 0.2-0.4 mg/kg of dry matter (Bogdanović et al., 1997). The highest concentration of chromium in the leaf of plum was found at the site Donji Bistarac (2.25 mg/kg), and lowest in the locality of Donji Pasci (Fig. 2). The plum fruits had a much smaller amount of the said metal than the leaves, except at the site Donji Pasci where the determined values were three times higher than the average (Fig. 2). As justification for the found condition in the above localities we may indicate the pH value of the soil. Values of soil pH indirectly affect the adoption of heavy metals, because the accessibility of certain elements in the soil depends on the pH of the soil (Kastori, 1998; Osmanovic et al., 2011).

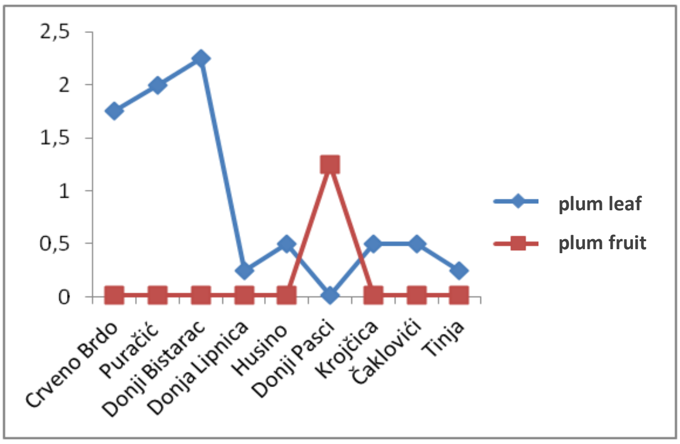

Fig. 2. The content of chromium (Cr) in leaf and fruit of plum (mg/kg dry matter) at the investigated sites

The content of copper (Cu) in the investigated plant species

Natural copper content in plants varies in the range of 1-15 mg/kg (Bašić et al., 1998) or 1-12 mg/kg (Ward, 1995), and the average content in herbaceous plants between 4 and 15 mg/kg (Bohn et al., 1985), or 2-20 mg/kg (Kastori, 1998). Copper content in the leaf of plum was higher than the limit value (20 mg/kg of dry matter) in most localities. The highest value was recorded in Donji Bistarac (27.7 mg/kg), and lowest at the site Krojčica (18 mg/kg). In the plum fruit were found significantly lower concentrations of copper than in the leaf (Fig. 3). This can be explained by the fact that copper accumulates significantly in leaves, where it accumulates most in chloroplasts, tied for plastocyanin which participates in the process of photosynthesis (Kataba-Pendias and Pendias, 1984; Udris and Neiland, 1990).

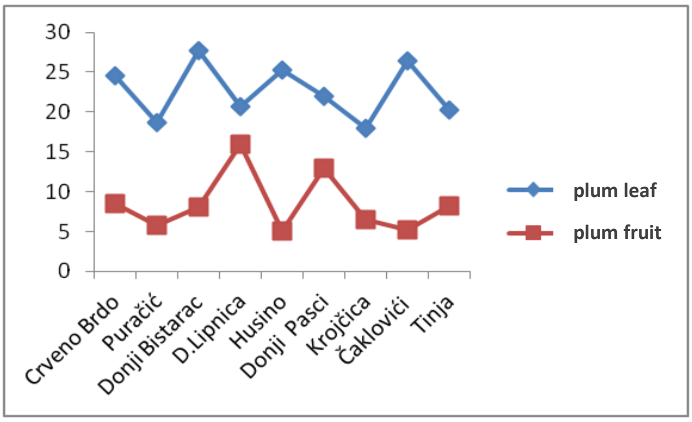

Fig. 3. The content of copper (Cu) in leaf and fruit of plum (mg/kg dry matter) at the investigated sites

The content of zinc (Zn) in the investigated plant species

The content of zinc in plants of unpolluted environments varies between 20 and 100 mg/kg (Kastori, 1998), and the average value is 30 mg/kg of dry matter (Ward, 1995). In organs of plants it occurs in an amount of 2-200 mg/kg of dry matter (Gračanin and Ilijanić, 1977), and its phytotoxic threshold is 200 mg/kg (Ivetić et al., 1991). Harmful effects on plants occur when the content exceeds 100 to 300 mg/kg (Kastori, 1998). Zinc content in the fruit of plum at all locations is within the limits of the average value. In the leaves of plum the determined concentrations at the site of Donji Bistarac are 2.5 times larger than the average value which was 30 mg/kg. The lowest concentration was registered at the site Donja Lipnica (20.5 mg/kg) (Fig. 4).

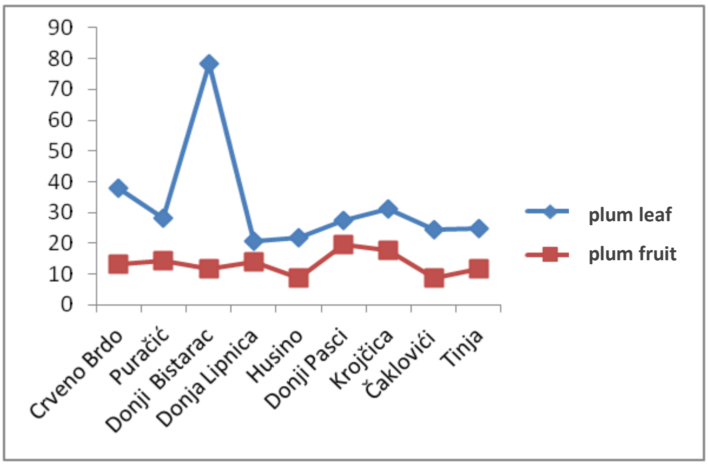

Fig. 4. The content of zinc (Zn) in leaf and fruit of plum (mg/kg dry matter) at the investigated sites

Conclusions

Based on the conducted studies during the realization of this work we came to the following conclusions:
The determined values of cadmium content in leaves and fruit of plum on most sites exceeded the natural values.
The determined chromium content in the leaf of plum did not exceed the limit values except in the locality of Donji Bistarac. The fruit of plum has a much smaller amount of chromium than the leaf, except at the site Donji Pasci where the determined values were three times higher than the average.
Copper content in the leaf of plum was higher than the threshold value at most locations. The determined value of copper in the fruit of plum was much lower than the limit value.
Zinc concentrations in plum leaves at most sites were higher than the average value. Zinc content in the fruit of plum in all localities exceeded the average value.
The highest concentrations of heavy metals were found at sites that are located near industrial plants. This should be borne in mind, especially due to the fact that environmental pollution by heavy metals can cause direct effects on human health.
By organized monitoring it is possible to control the intensity of accumulation and adoption of heavy metals, in order to create optimal conditions for the growth and development of plants, production of healthy food and improvement of the quality of people's lives because this way they consume health safer products.

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