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FOOD IN HEALTH AND DISEASE

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SADRŽAJ / CONTENT

INFLUENCE OF PRESERVATIVES ON QUALITY ELDERFLOWER SYRUP (*Sambucus nigra* L.)

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Original scientific paper

Summary

Elder plant lat. *Sambucus nigra* L. (*Adoxaceae*) is a shrub height up to 7 m (rarely to 10 m) with numerous vertical shoots that grow next to each other from the base. This plant has a great use in traditional medicine because it has a wide range of activities such as anti-inflammatory, especially for respiratory tract infections, antiviral, antibacterial, laxative, diuretic, antioxidant and many others. For research purposes elderflowers were collected from five different locations (Tuzla, Travnik, Tuzla, Zenica and Zivinice) and the syrup was prepared according to traditional recipes. The first analysis of samples was performed after immersing the flowers in water and left them at room temperature for 24 hours while the second analysis was performed after the addition of sugar and citric acid under the same conditions. The analysis included chemical and microbiological methods. From a microbiological point of view it has been shown that the addition of preservatives reduced the number of bacteria, and that such syrup is safe for consumption. Chemical analysis showed the syrup called after treatment reduces their significant properties but is still suitable for use.

Keywords: antioxidant activity, anthocyanins, CFU / ml, *Escherichia coli*, coagulase-positive staphylococci, sulphite reducing clostridia

Introduction

Elder plant *lat. Sambucus nigra* L. (Adoxaceae) is significant medicinal plant and natural resources of this area and has always been known in folk medicine. It is a shrub height up to 7 m (rarely to 10 m) with numerous vertical shoots that grow next to each other from the base (Atkinson and Atkinson, 2002). The leaves are opposite, odd pinnate composed, saw tooth serrated, bright green collared with unpleasant odour, up to 30 cm in length (Grlić, 2005). The flowers are yellowish-white, small, form a large thyroid inflorescence with strong and pleasant odour. Flowering time is in May and June, and the fruits ripen in September. The fruits are dark purple berries rounded juicy stone berries 3-5 mm in diameter grouped in large bunches. It grows in sunny places or shade, on the edges of the wet forest, near villages and neglected places. It favours the wetter and deeper sandy and clayey soil Godet (2000) i Grlić (2005). It is widespread in the area of Europe, Asia, North America and North America. Use of Elder plant in folk medicine and cooking has a long tradition. It is also used in horticulture, pharmacy and is a significant bio indicator. Parts of plants that are in use are the leaves, flowers, ripe fruits and bark. Medicinal properties of Elder plant has a wide range of activities such as anti-inflammatory, especially for respiratory tract infections, antiviral, antibacterial, laxative, diuretic, antioxidant and many other (Barak et al., 2002; Gorchakova et al., 2007; Zakary-Rones et al., 2004; Pool-Zobel et al. 1999). Besides juice and concentrates, elderberry fruits may be used for the industrial production of jam, jelly, desserts, wine, cakes, candies and colouring of mixed juices. Elderberry juice also contains many primary metabolites including various sugars and organic acids. Fruit extracts, containing phenolic compounds, are characterized by higher antioxidant activity than many isolated pure phenolic compounds or vitamins.

Materials and methods

Sample collection and preparation of syrup

Elder flowers (*Sambucus nigra* L.) were collected from 5 different locations and altitudes in the area of Bosnia and Herzegovina (Table 1). Samples were collected at the same amount and at the same time to ensure confidential, accurate results and stored at 4 °C for 24 hours. Elderflower syrup is prepared according to traditional recipes:

- 15-20 of flowers was immersed in 1.5 l of water and stored for 24 hours at room temperature after which time samples were taken for the first analysis.
- The rest of the syrup was treated with 500 g of sugar and 5 g citric acids and again stored at room temperature for 24 hours and the samples were taken for analysis second time.

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Table 1. Sampling localities

No.	LOCALITY	Altitude (m)
1.	Bugojno (Karadže)	550
2.	Travnik (Vlašić)	1900
3.	Tuzla (Moluška rijeka)	250
4.	Zenica (Kopilo)	320
5.	Živinice (Litve)	300

Chemical methods of analysis

Chemical analysis are generally included parameters such as pH value, which was determined potentiometrically, turbidity was determined directly in the turbidimeter and expressed in Nephelometric Turbidity Unit (NTU units), electrical conductivity was measured on the conductivity meter and the density was determined using a pycnometer. In addition to general parameters were determined and anthocyanins (total and monomeric) with pH differential method where a certain volume of the sample was transferred to a volumetric flask of 10 cm^3, which are then dilute to the mark with 0.025 mol/l buffer of potassium chloride and 0.4 mol/l buffer of sodium-acetate. After 15 minutes the absorbance was measured at 520 nm and 700 nm and the calculated value of anthocyanin expressed in mg/mL. The antioxidant activity of the samples was determined using H_2O_2 test (level of neutralizing H_2O_2 - "H_2O_2 scavenging" depending on the concentration of extracts) (Ruch et al., 1989). The solution of H_2O_2 (0.6 cm^3) with concentration of 40 mmol/l was added to extracts, after incubation for 10 minutes at a dark place absorbance was measured at 230 nm and further calculation is derived value for neutralization capacity H_20_2.

Microbiological methods of analysis

Microbiological analysis was performed before and after the addition of sugar and citric acids who served as preservatives according to traditional recipe.
The analysis was performed on the following types of bacteria:

1. *Escherichia coli*,
2. Coagulase positive *Staphylococcus aureus*,
3. Sulfitreducing *Clostridia*,
4. The total number of bacteria.

Samples for analysis were prepared under sterile conditions in a laminar with air flow. In sterile Erlenmeyer flask with 180 ml of sterile physiological solution we added 20 ml of an untreated juice, and left it on the magnetic mixer for 15 minutes to homogenize the sample. After sample preparation, under sterile conditions, with a Bunsen burner we have prepared a decimal dilution of the sample, and a nutrient medium that we used for the isolation of different types of bacteria. *Escherichia coli* was analyzed from the dilution sample by transferring 1 ml of the sample in 15 ml of sterile nutrient medium Brilliant Green Bile 2% Broth (Hi Media Laboratories), and incubated at 44 °C for 24 hours. Coagulase positive *Staphylococcus aureus* and sulfitreducing *Clostridium* are also analyzed from the 10^{-1} dilution, and seeded in salt broth and iron sulphite agar, and allowed to incubate for 24 hours at 37 °C. The total number of bacteria was analyzed in 1 ml of sample dilution on basic agar. The procedure was repeated in the same manner and under identical conditions for all samples.

Results and discussion

Results of chemical analysis

The general parameters (Table 2) chemical analysis is mainly decreasing after treatment with a preservative such a pH value before treating showed mild acidic medium while after treatment decreased considerably and shows a very acidic medium. The turbidity and electrical conductivity of samples were also decreased after treatment with sugar and citric acid. The only general parameter that was increased was the density of samples. These changes were expected due to the addition of sugar and citric acids. The higgest density was measured in a sample from the Travnik locality. We assume that the reason for that is the high altitude at which the sample was pattern is picked and low pollution in thr sampling area.

Table 2. General technological parameters of analyzed samples before and after the treatment with sugar and citric acids

No.	Locality	*pH*		*Electroconductivity (µS)*		*Turbidity (NTU)*		*Density (g/cm³)*	
		Before treatment with sugar and citric acids	***After the addition of sugar and citric acids***	***Before treatment with sugar and citric acids***	***After the addition of sugar and citric acids***	***Before treatment with sugar and citric acids***	***After the addition of sugar and citric acids***	***Before treatment with sugar and citric acids***	***After the addition of sugar and citric acids***
1.	Bugojno	6.62	3.76	3180	1019	73.5	59	1.0200	1.1868
2.	Travnik	5.89	2.92	3620	914	81	77.4	1.0135	1.1938
3.	Tuzla	5.95	3.43	5210	1646	153	150	1.0212	1.1630
4.	Zenica	5.53	3.17	3690	560	78.9	58.5	1.1830	1.1830
5.	Živinice	6.19	3.20	4140	1042	95.6	90.5	1.0200	1.1850

The amount of anthocyanin was increased after the treatment (Fig. 1). The highest amount of anthocyanins before treatment shows a sample from Bugojno while after the treatment pattern of Travnik. According to previous research, higher amounts of anthocyanins include elderberry (Galić, 2007). An important application is their antioxidant activity, which plays a key role in the prevention of neurological and cardiovascular diseases, cancers and diabetes (Ćujić et al., 2013). Anthocyanins can act an antioxidant, anti-inflammatory, anticancer, antidiabetic, oedematous, have the effect of capillary permeability, acting hepatoprotective, antibacterial, radioprotective, immunomodulatory, inhibit herpes virus, influenza virus, the protective function of endothelial cells in comparison to the various stress agents (Pascual and Sanches, 2008; Croizer and Cliffprd, 2010).

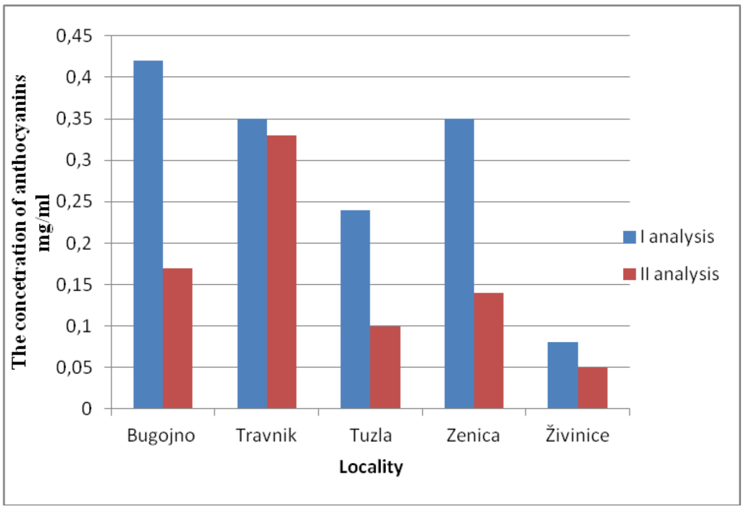

Fig. 1. Comparative display of anthocyanin content before and after the treatment of syrup with sugar and citric acids

Antioxidants as important parameters are the name of the syrup is decreased after the treatment, which indicates that a greater amount of antioxidants in the syrup have no sugar and citric acids (Fig. 2).

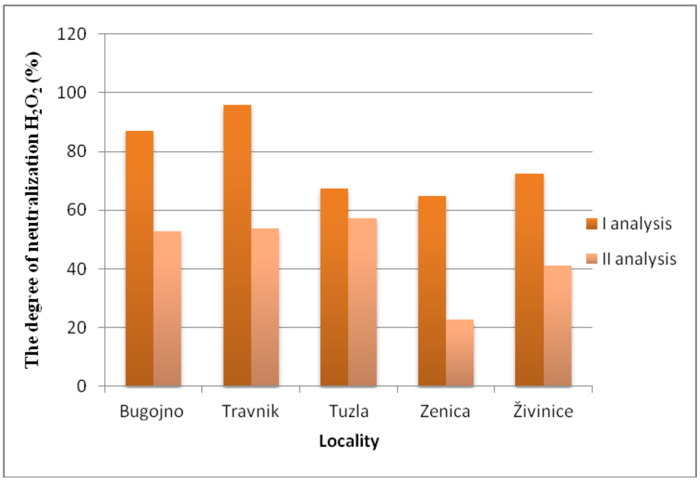

Fig. 2. Impact of sugar/citric acid treatment on antioxidant activity of analyzed syrups

Results of microbiological analysis

The results of microbiological analysis of *Sambucus nigra* L. flowers before and after addition of sugars and citric acids in water are shown in Table 3. Based on what we can see that the acidification of the environment with a citric acids and added sugar caused the lysis of bacterial cells, which resulted that in the samples completely left out the growth and development of microorganisms.

From Table 3 we can clearly see that all the samples before treatment with sugar and citric acids were a suitable medium for the growth and development of all tested species of bacteria.

Table 3. Comparison show CFU / ml (Colony Forming Units) of the tested bacterial strains in syrup Sambucus nigra syrup

No.	Locality	***E.coli***		***Sulphite reducing clostridia***		***The total number of bacteria***		***Coagulase-positive Staphylococcus aureus***	
		Determined of CFU/ml							
		Before treatment with sugar and citric acids	***After the addition of sugar and citric acids***	***Before treatment with sugar and citric acids***	***After the addition of sugar and citric acids***	***Before treatment with sugar and citric acids***	***After the addition of sugar and citric acids***	***Before treatment with sugar and citric acids***	***After the addition of sugar and citric acids***
1.	Bugojno	91	0	48	0	672	0	0	0
2.	Travnik	323	0	3	0	504	0	0	0
3.	Tuzla	103	0	0	0	689	0	0	0
4.	Zenica	78	0	0	0	394	0	0	0
5.	Živinice	52	0	0	0	323	0	0	0

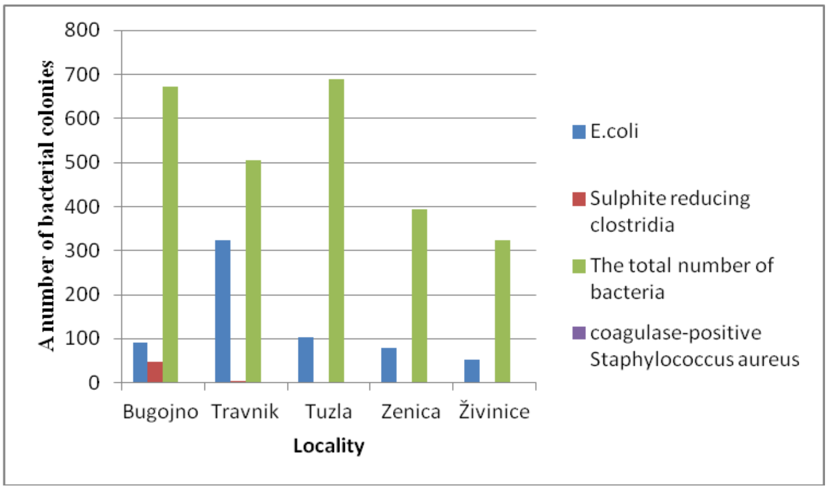

Fig. 3. Number of bacterial colonies from the syrup before treatment with sugar and citric acids, determined on selective nutrient media

From Fig. 3 we can clearly observe that samples from the Travnik locality have the largest number of *E. coli* colonies grown on VRBG media. In samples from the area of Tuzla were established the total number of bacterial colonies in most, even 689 CFU / ml. With increasing colonies of *E. coli* that have a characteristic purple colour on VRBG medium, colonies are piqued under sterile conditions and biochemical characteristics were analyzed. In Klinger's double sugar piqued colonies of *E. coli.* were sown by pricking and zigzag movements on the slant agar. Simmons citrate, and peptone for the reaction with an indole were also inoculated with the isolated bacterial colonies and left to incubate for 24 hours at 37 °C.

In the analyzed samples were not detected increase of *Staphylococcus aureus* colonies. T test has proven the greatest statistically significant difference (p) in the total number of bacteria in the amount of 0.004659973 when we compared the number of bacterial colonies which grew after incubating in the different nutritive media, from samples taken before treatment with the sugar and citric acids and after treatment. It has been found that there is a significant difference at the level of 0.05 in the number of bacterial species grown before and after the treatment of juice and pH value established after the first and the second analysis ($p = 0.000308725$).

In recent years, more and more we talk about natural production, healthy food, local cuisine and the preservation of traditional recipes. Particularly noteworthy is the consumption of natural juices and syrup prepared from fresh plant parts without adding any chemicals and preservatives in order to maintain the quality and medicinal properties. Originally it was thought that the natural acidity of certain juices enough to prevent the possible harmful effects of the consumption of these juices (Mihajlović et al., 2013). But studies have shown that for example *Salmonella* can survive in the juice of the orange and up to 3 weeks (Hodgson, 2001). The fact is that microorganisms are everywhere around us, and so are the plants we use in nutrition source of potentially harmful microbes that are the natural or adopted. *E. coli*, which is pathogenic to man is a natural inhabitant of the intestine through which faeces animals are transferred to the fruit which is why it is important to take care of hygiene, sterilization equipment and methods of keeping the product when creating natural remedies. Our analysis confirmed that untreated juice is not the safest for consumption. Some of the traditional methods of preservation are adding sugar, citric acid or pasteurization (Smith & Stratton, 2007). Microbiological analysis of samples after the addition of sugar and citric acids gave negative results showing the efficient preservation of the desired product and its safe use.

Conclusions

Results of chemical analyzes have shown that the general parameters decreased after treatment as well as antioxidant activity while the amount of anthocyanin increase. If we take into account all the parameters, syrup that is prepared from a flowers picked in Travnik (Vlašić) showed the best properties. It is assumed that the reason for this is higher altitude and less contamination of the plant itself. Based on the results of microbiological analysis, a statistically significant difference was found in the number of bacterial species grown before and after treatment. Microbiological analysis proved that the syrup after the addition of sugar and citric acids is safe for consumption. From the aspect of the chemical analysis of the syrup after treatment reduces its significant properties but is still suitable for use.

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SOLVENT EXTRACTION AND CHROMATOGRAPHIC DETERMINATION OF POLYPHENOLS IN OLIVE POMACE

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Original scientific paper

Summary

Olive oil mill waste obtained after two-phase olive oil extraction process was subjected to conventional liquid solvent extraction under different pH/temperature/duration conditions using different types of food-grade solvents. The independent variables were: solvent type (ethanol percentage), extraction temperature (20-90 °C), extraction time (30 min-24 h) and pH (2-10.3) of extraction solvent while the response variables were total phenolic content and antioxidant activity of obtained extracts. The optimum solvent extraction conditions for phenols were 120 min at 70 °C using 60% ethanol as extraction solvent, at solvent to sample ratio 5:1 (v/w). For quantification of major bioactive olive polyphenols hydroxytyrosol, tyrosol and oleuropein in obtained extracts, fast and simple RP-HPLC-DAD method was developed and validated. Oleuropein was presented in highest amounts with average value of 115.14±0.19 mg/kg of fresh olive pomace.

Keywords: olive pomace, extraction, HPLC-DAD, hydroxytyrosol, tyrosol, oleuropein

Introduction

The production of olive oil, the second most important agro-sector in Europe, has increased in recent years and, as the consequence, larger quantities of waste products associated with olive oil production are being disposed in the environment (olive leaves, olive pomace and olive mill waste water). They contribute significantly to excessive nutrient burdens in local ecosystems and represent ecological hazards (Aberg et al., 2004). The exploitation of olive waste from an environmental point of view may be approached in several ways: it can be used for energy generation; as fertilizer or soil conditioner; as herbicide or pesticide, as animal feed or in human consumption; for residual oil recovery; for production of various products (alcohols, biosurfactants, biopolymers, activated carbons) and for organic compounds recovery (pectin, phenolic antioxidants). Most studies dealing with biological activity of olive derived products have been focused on polyphenols as major active constituents. Olive pomace contains different phenolic compounds that can be divided in several classes: simple phenols (e.g., tyrosol (TS) and hydroxytyrosol (HTS)) cinnamic acid derivatives; flavonoids (e.g., apigenin, luteolin and rutin (quercetin-3-rutinoside)); and secoiridoids (e.g., oleuropein (OLE), oleuropein aglycone and de(carboxymethyl) oleuropein aglycone isomers) (Obied et al., 2007).
The potential of olive biophenols (OBPs) has already been recognized by competent authorities, such as EFSA (European Food Safety Authority). Extracts obtained from olives and olive mil wastes are generally regarded as safe (Soni et al., 2006; Obied et al., 2012) and reported pharmacological properties of particular OBPs include antioxidant, anti-inflammatory, cardiovascular, immunomodulatory, gastrointestinal, respiratory, autonomic, central nervous system, antimicrobial, anticancer and chemopreventive action (Obied et al., 2012). Pharmacologically active OBPs are abundant in different types of olive mill wastes, however the exact qualitative and quantitative composition of obtained extracts is significantly affected by the type of extraction (Kumar et al., 2006; Aliakbarian et al., 2011) and extraction conditions (time, temperature, pressure, solvent).
The increasing use of bioactive compounds in pharmaceutical, food and chemical industries sector such as points out the need of finding adequate extraction method for bioactive components from plant materials (Sasidharan et al., 2011). Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing (Azmir et al., 2013). The major challenges of conventional extraction techniques are longer extraction time, large solvent consumption, evaporation of the huge amount of solvent during the process, low extraction selectivity and thermal decomposition of thermo labile compounds (Luque de Castro and Garcia-Ayuso, 1998). Therefore, nonconventional extraction techniques such as ultrasound assisted extraction, enzyme-assisted

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extraction, microwave-assisted extraction, pulsed electric field assisted extraction, supercritical fluid extraction and pressurized liquid extraction have been introduced during the last two decades (Azmir et al., 2013). Those techniques have numerous advantages, especially in terms of sustainability and pollution prevention but can be conducted only in well equipped laboratories and in many cases, are demanding or not suitable for scale-up. Therefore, there is a constant need for further optimization of conventional extraction techniques in terms of efficiency, selectivity and sustainability. The aim of this work was to optimize the extraction of olive pomace polyphenols, using simple solvent extraction and only food-grade solvents with special emphasis set on HTS, TS and OLE as the major bioactive polyphenols in live. The additional goal was to develop and validate simple, fast and accurate procedure for their quantification in obtained extracts.

Materials and methods

Reagents and chemicals

Folin-Ciocalteu reagent, gallic acid, ABTS (2,2’-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), tyrosol (4-hydroxyphenylethanol), hydroxytyrosol (3,4-dihydroxyphenylethanol) and oleuropein were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium carbonate and all solvents used throughout the experiments were obtained by Merck (Darmstadt, Germany). All other chemicals were from Kemika (Zagreb, Croatia).

Stock standard solutions

Stock standard solutions of TS, HTS and OLE were prepared in HPLC-grade water at 1 mg/mL level. Working standards were prepared prior to analysis by diluting appropriate volumes of stock solutions with HPLC grade water at 3, 9, 27, 81 and 243 μg/mL level.

Instrumentation

For chromatographic analysis, we used Agilent Life Sciences 1220 LC Gradient System equipped with a dual-channel gradient pump with degasser, autosampler, column oven and an additional Agilent 1260 Infinity Diode Array Detector (Agilent, Santa Clara, CA, USA). Chromatographic separation was performed on an Zorbax Eclipse Plus C18 reversed-phase column (250 x 4.6 mm, ID 5 μm) (Agilent, Santa Clara, CA, USA). Spectrophotometric analysis was conducted on UnicamUV4 UV-VIS spectrometer.

Chromatographic analysis of HTS, TS and OLE

For the chromatographic separation of HTS, TS and OLE a gradient elution program was used. A linear gradient was run with flow rate of 1 ml/min. The column was maintained at 40 °C throughout all experiments with the aid of an electronically controlled oven. All mobile phases were vacuum filtered through 0.45 μm membrane filter and degassed in an ultrasonic bath prior to HPLC analysis. For the validation of analytical method, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use recommend the accomplishment of linearity, accuracy, precision, and sensitivity.
To evaluate the linearity of the method, standard solutions were prepared at five concentration levels containing 3, 9, 27, 81 and 243 ppm of TS, HTS and OLE. Five replicates at each concentration were analyzed. The linearity of the data was checked by performing linear least-squares regression analysis. Accuracy of the method was assessed by three quality control standards at three concentration levels, and was evaluated relative percentage error. The assay precision was evaluated by performing the assay at three levels (9, 27 and 243 ppm) in five replicates and calculating the RSD values. Intermediate precision was demonstrated by preparing standard solutions at three levels (9, 27 and 243 ppm) in three replicates on different days and calculation of RSD values. Recovery was evaluated as the ratio of the peak area for every substance in the spiked sample against that of the standard. Olive pomace samples were spiked at three different concentration levels. LOD was determined by preparing a solution that produced a response of about 3 and 10 times the baseline noise. The solution was injected three times, and the S/N (signal/noise) ratio recorded for each injection. Solution concentration is considered LOD if S/N ratio is between 3-10. LOQ was determined in the same manner but with an S/N ratio of 10-20.

Total phenolic content

Total phenolic content (TPC) was determined spectrophotometrically according to the method of Singleton and Rossi (1965) with some modifications. Briefly, adequatelly diluted olive pomace extracts (200 μL) were mixed with 1.35 mL of distilled water and 150 μL of Folin Ciocalteu reagent. After 5-minute incubation 1.5 mL of 6% Na_2CO_3 was added to each reaction mixture and obtained solutions were incubated at 50 °C for 30 minutes. Absorbance readings were conducted at 725 nm and results were expressed as gallic acid equivalents (GAE).

TEAC assay

The TEAC (Trolox Equivalent Antioxidant Capacity) assay reflects the ability of hydrogen or electron-donating antioxidants to scavenge the ABTS•+ radical cation compared with that of Trolox. As described by Re et al. (1999). ABTS radical was prepared by mixing the equal volumes of 7 mM ABTS and 2.45 mM solution of $K_2S_2O_8$ and leaving the mixture overnight allowing the complete development of the chromophore radical. The reaction mixture was prepared by mixing 2.5 mL of adequately diluted $ABTS•^+$ and 300 µL of adequately diluted sample and the absorbance was measured after 3 minutes. The quenching of initial absorbance was plotted against the Trolox concentration and obtained results were expressed as Trolox equivalents (TE).

Experimental design

Fresh olive pomace, obtained by the two-phase extraction process was delivered from the local olive-mill plant. Pomace samples (containing approximately 65% water, low amounts of pomace oil, olive pulp and pits) were homogenized, frozen immediately in the form of thin plates and used for the development and optimization of polyphenol extraction procedure. The independent variables were solvent type (ethanol percentage), extraction temperature, extraction time and pH of extraction solvent, while the response variables were total phenolic content (TPC) and antioxidant activity (TEAC) of obtained extracts (Table 1).

Table 1. Variables used in the process of the optimization of the extraction procedure

OPTIMIZATION VARIABLES	
INDEPENDENT VARIABLES	RESPONSE VARIABLES
SOLVENT TYPE (*water, 40% EtOH, 60% EtOH, 80% EtOH, 96% EtOH*)	TOTAL PHENOLIC CONTENT (*mg GAE/g*)
TIME OF EXTRACTION (*30 min, 60 min, 120 min, 300 min, 24 h*)	
TEMPERATURE (*room temperature, 20 °C, 50 °C, 70 °C, 90 °C*)	TEAC ANTIOXIDANT ACTIVITY (*mg TE/g*)
pH (*2, 6, 8.5, 10.3*)	

Extracts obtained under optimized conditions were analyzed for total phenolic content, tyrosol, hydroxytyrosol and oleuropein content, and TEAC. Chromatographic method for determination of hydroxytyrosol, tyrosol and oleuropein in obtained extracts was optimized and validated according to ICH guidelines (ICH, 1996). For HTS, TS and OLE determination, olive pomace extracts were freeze-dried immediately after extraction, dissolved in adequate volume of HPLC-grade water, filtered through 0.45 µm membrane filter and subjected to HPLC analysis.

Statistical analysis

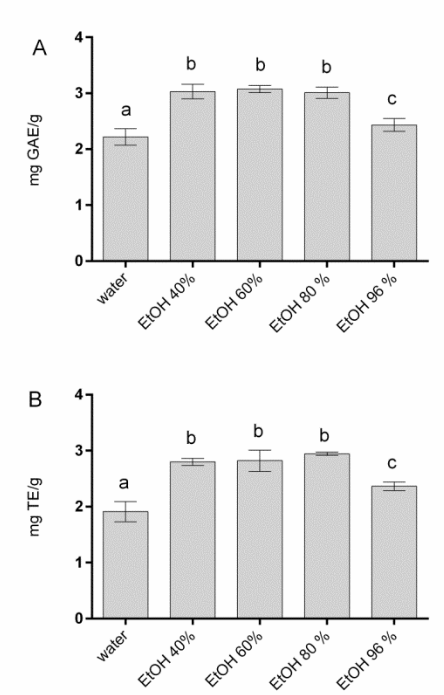
All analytical measurements were conducted at least in triplicates; the results were averaged and presented as means ± standard deviation. Analyses of variance ANOVA and post-hoc Tukey's test were used to compare significant differences in the values of response variables depending on the solvent composition and pH, extraction time and extraction temperature. Statistically significant influences were expressed using p values (Tukey's post hoc test, $p < 0.05$). Analyses were conducted using Prism GraphPad software (GraphPad Software, Inc., USA).

Results and discussion

Influence of different extraction parameters on TPC and TEAC

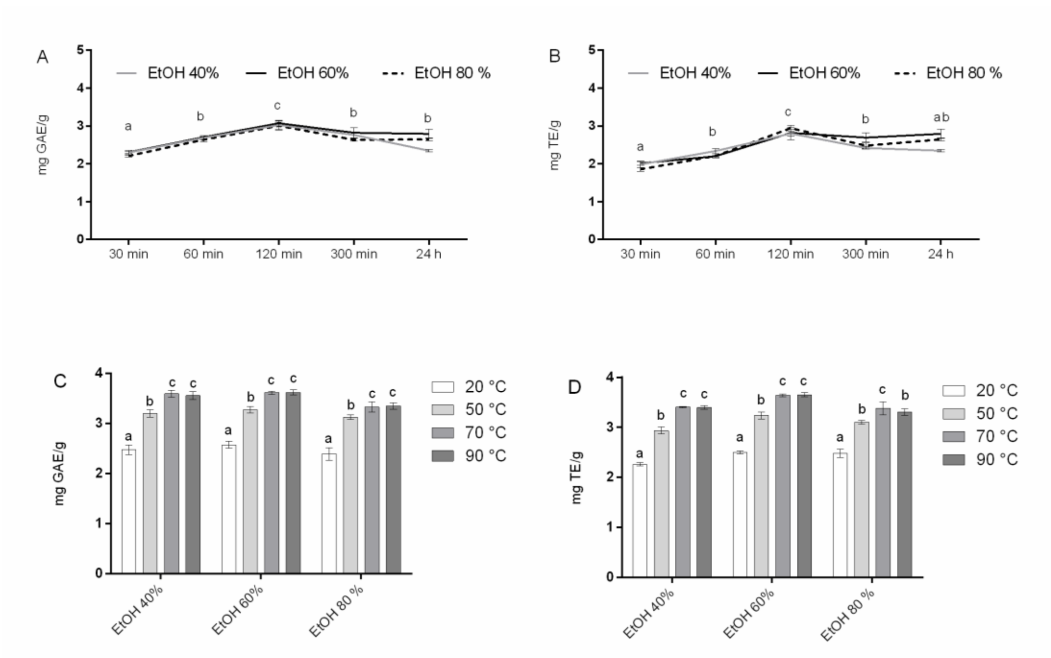
All olive pomace extracts contained significant amounts of polyphenolic compounds and showed antioxidant activity; however, obtained TPC and TEAC values differed significantly depending on the solvent polarity, pH and the duration and temperature of extraction. The influence of solvent on TPC and TEAC is presented in Fig. 1. Obtained results showed that significantly higher TPC and TEAC were obtained by using ethanol-water mixtures in comparison to pure water or 96% ethanol ($p<0.05$). Observed differences between TPC and TEAC content in extracts with 60% 40% or 80% ethanol were not statistically significant ($p>0.05$).

Extractions using different volume fractions of ethanol in water were used for the subsequent optimization of extraction procedure in terms of time of extraction and applied temperature. Obtained results are presented in Fig. 2.



[a,b,c]columns marked with the same letter belong to the same statistical group (p>0.05). Extractions were performed at pH=6, by shaking the mixtures at 70 °C (100 rpm) for 120 min. Sample to solvent ratio was 1:4.

Fig. 1. Impact of the solvent type on the TPC (A) and ABTS antiradical activity (B) of olive pomace extracts

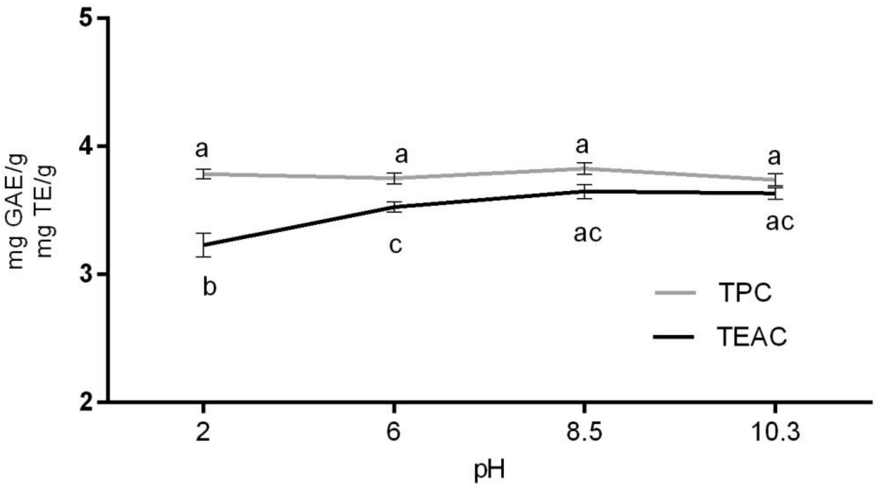


[a,b,c]data marked with the same letter belong to the same statistical group (p>0.05). Extractions were performed at pH 6, by shaking the mixtures at 70 °C (A, B), for 120 min (C, D). Sample to solvent ratio was 1:4.

Fig. 2. Impact of extraction time (A, B) and temperature (C, D) on the recovery of total phenols and ABTS antiradical activity of pomace extracts

By increasing the time of extraction from 30 to 120 min resulted with significant increase of polyphenolic content and antioxidant activity of obtained extracts for each investigated solvent. Further elongation of the extraction process to 300 min did not contribute to the efficiency of the extraction process, while after 24 h extraction significant decrease of TPC and TEAC was observed (Fig. 2A, Fig. 2B). Observed decrease in the extraction yield is probably due to degradation of polyphenolic compounds caused by hydrolysis, internal redox reactions and polymerization (Alonso-Salcez et al., 2001). Under the same extraction conditions, TPC and TEAC were comparable for all investigated extraction solvents (40%, 60% and 80% ethanol). Obtained results are consistent with the trends reported by Aliakbarian et al. (2011) who concluded that the prolongation of extraction time from 15 to 90 min significantly improves extraction of polyphenolic compounds from olive pomace in high-pressure-high temperature reactor, while longer extraction times promote degradation of polyphenols and negatively influences extraction yields. Similar trends were observed by Jerman et al. (2010) who optimized ultrasound-assisted solid liquid extraction of polyphenols from olive fruit.

Extraction temperature was also found as significant factor affecting the TPC and TEAC (Fig. 2C, Fig. 2D) and the higher extraction yields were obtained at the temperatures of 70 °C and higher. However, increasing the temperature above 70 °C did not produce any additional benefit; therefore 70 °C has been chosen as the optimal temperature for the extraction of olive pomace polyphenols. At 70 °C, significant differences were observed between the efficiency of tested extraction solvents; the efficiency of 80% ethanol was significantly lower in comparison to 40% and 60% ethanol. Obtained results indicate that the major polyphenolic compounds in olive pomace are thermostabile and/or that possible thermal degradation of polyphenols generates new polyphenolic compounds that retain antioxidant activity. Observed results are consistent with data of Herrero et al. (2011), who optimized the pressurized liquid extraction of olive pomace polyphenols using food-grade solvents and obtained the highest yields at high temperatures (150 °C and 200 °C for water and ethanol, respectively). Similarly, Aliakbarian et al. (2011) investigated extraction of olive pomace polyphenols by high-pressure-high temperature reactor and observed higher polyphenol yields at higher temperatures (optimal temperature was 180 °C). pH can also significantly influence the efficiency of extraction procedure, because polyphenolic compounds in plant material are often part of high molecular mass complexes that can be partially degraded under acidic or alkalic conditions enhancing in that way the extractability of phenolic compounds. On the other hand, extreme pH values can cause the degradation of phenolic compounds resulting in lower extraction yields. Therefore, the outcome depends on the nature of plant material and physicochemical characteristics of particular polyphenols. In case of olive pomace, the change of pH of the extraction solvent did not produce significant changes in TPC of obtained extracts; however significantly lower TEAC values were recorded in extracts obtained under acidic conditions (Fig. 3).



[a,b,c]data marked with the same letter belong to the same statistical group (p>0.05). Extractions were performed by shaking the mixtures at 70 °C (100 rpm for 120 min, using 60% ethanol as extraction solvent. Sample to solvent ratio was 1:4.

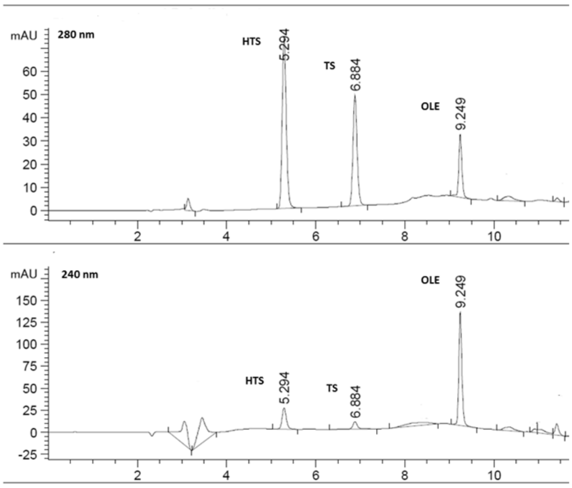
Fig. 3. Impact of pH on the recovery of total phenols and ABTS antiradical activity of pomace extracts

Development and validation of RP HPLC-DAD method for determination of HTS, TS and OLE in olive pomace extract

A

Time (min)	Solvent A[a] (%)	Solvent B[b] (%)	Flow-rate (ml/min)
0	90	10	0
4	80	20	4
8	40	60	8
11	30	70	11
15	90	10	15
20	90	10	20

B



C

Component	Regression equation	Correlation coefficient (r^2)
Hydroxytyrosol	y = 10.184x + 70.179	0.9859
Tyrosol	y = 11.709x - 4.3663	0.9998
Oleuropein	y = 21.527x - 75.091	0.9996

[a]Solvent A: acetate buffer; [b]Solvent B: acetonitrile

Fig. 4. RP HPLC-DAD method for determination of HTS, TS and OLE in olive pomace extracts: gradient elution program (A); chromatograms of reference standards (27 ppm) (B); regression equation and correlation coefficients (C)

For the chromatographic separation of HTS, TS and OLE a gradient elution program was used as shown in Fig. 4A. Solvent A was 0.05 M Na-acetate buffer (pH=5) and solvent B was acetonitrile.
UV spectra of all substances were recorded by diode array detection system and the maximum of absorbance were determined to be 240 nm for OLE and 280 nm for HTS and TS (Fig. 4B). Identification of the eluting peaks was performed by comparing their retention time values and the corresponding UV spectra with those of the standards. The linearity of the method was evaluated by linear regression analysis using five concentrations of tyrosol, hydroxytyrosol and oleuropein. Good linearity was achieved for all the analytes, as shown in Fig. 4C. For accuracy determination, three standard solutions at concentration levels 27, 81 and 243 ppm were analyzed. Obtained results were expressed as relative percentage error ranging from 1.33% - 4.04% for HTS; 1.99% - 3.42% for TS and 0.64% - 2.35% for OLE. Precision analysis was conducted by analyzing 5 replicates of standard solution at three concentration levels (9, 27 and 243 ppm). RSD values were ranged from 0.84 - 2.99 for HTS; 0.04 - 0.13 for TS and 0.86 - 3.45 for OLE. Intermediate precision was determined by analyzing three replicates of prepared standard solutions at three concentration levels on different days. Obtained RSD values were from 1.12 - 2.60 for HTS; 1.47 - 2.79 for TS and 0.67 - 2.38 for OLE. For recovery determination olive pomace samples were spiked with standard solutions of hydroxytyrosol, tyrosol and oleuropein at three concentration levels (27, 81 and 243 ppm). The obtained recovery was 100.9% - 102.4% for HTS; 99.3% - 101.3% for TS and 99.9% - 100.5% for OLE. The sensitivity of the method has been assessed by determining LOD values for HTS (1.5 ppm), TS (1.0 ppm) and OLE (2.0 ppm).
Applied method was found to be suitable for the analysis of olive pomace extracts due to simple sample preparation (it does not require any sample pre-treatment), short time of analysis and satisfying validation parameters. The only disadvantage of the method was its relatively low sensitivity especially inthe case of OLE; however, it was high enough for the analysis of obtained olive pomace extracts.

The content of HTS, TS and OLE in olive pomace extracts obtained under optimized conditions

Olive pomace extracts obtained under optimized extraction conditions (continuous shaking at 70 °C for 120 min) using 40% and 60% ethanol as extraction solvents were subjected to HPLC analysis in order to compare the efficiency the two extraction solvents. Namely, there were no significant differences between the two solvents regarding total phenol yields or antioxidant activity of obtained extracts. Namely, despite the wide variety of polyphenolic compounds present inolive oil and pomace HTS, TS and OLE are considered to be the main polyphenolic bearers of the health-promoting properties of olive oil.

The only authorised health claim for olive oil, listed in the Regulation 432/2012 (EC, 2012), relates to the level of olive phenolic compounds and the impact on the protection of blood lipids from oxidative stress. The conditions of use of the claim are that it "may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. OLE complex and TS) per 20 mg of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 mg of olive oil (EFSA, 2011).

Obtained results are presented in Table 2, and clearly emphasize that using 60% ethanol (instead of 40% ethanol) results in small but statistically significant increase in HTS, TS and OLE content of obtained extracts.

Table 2. TPC, AA, HTS, TS and OLE content of ethanolic pomace extracts obtained under optimized conditions

Compound*	**40 % EtOH**	**60 % EtOH**
TPC *(mg GAE/g)*	3.59±0.07[a]	3.62±0.03[a]
AA *(mg TE/g)*	3.41±0.01[a]	3.64±0.03[a]
HTS *(mg/kg)*	75.46±0.33[a]	81.8±0.41[b]
TS *(mg/kg)*	82.08 ±0.13[a]	86.05±0.34[b]
OLE *(mg/kg)*	110.37±0.07[a]	115.14±0.19[b]

**values are expressed per g(kg) of fresh olive pomace. Differences between values in the same row marked with different letters are statistically significant* (*p*<0.05).

The amounts of HTS, TS and OLE are generally comparable to levels found in olive pomace by other authors. The content of bioactive polyphenols in olive pomace is variable and depends on numerous factors: olive cultivar, the olive oil extraction process and the type of pomace that remains as the by-product (traditional extraction, two-phase process or three-phase process, continuous combined percolation-centrifugation etc.) (Dermeche, 2013). However, our results show that OLE is the most abundant among analysed polyphenols in olive pomace which is consistent with observations of other authors (Cioffi et al., 2010; Rubio-Senet et al., 2013). They investigated more advanced extraction methods, using hydrothermally treated pomace, different types of organic solvents or applying higher temperatures or pressures.It is hard to compare the absolute yields of HTS, TS and OLE to ours since results obtained by different authors are often expressed in different ways (per g of dry extract, per g of dry pomace, per g of fresh pomace) without sufficient data that would allow re-calculation and comparison.

Conclusions

Extracts with different antioxidant (phenolics) concentrations and activities were obtained from fresh olive pomace by changing the conditions of conventional solvent extraction. For this purpose, RP HPLC-DAD method was validated and found to be suitable for the analysis of olive pomace extracts due to simple sample preparation (it does not require any sample pre-treatment), short time of analysis and satisfying validation parameters. Among different food-grade solvents, 60% ethanol was selected as the most appropriate solvent for the extraction of phenolic compounds from olive oil pomace under optimized conditions (120 min with shaking, 70 °C, 120 min). Satisfactory phenolic (antioxidant) yields prove that oil mill waste is a low-cost, renewable and abundant source of phenolic antioxidants and that simple solvent extraction which uses only food-grade solvents can be successfully applied to olive pomace.

Aknowledgement

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UTJECAJ UNOSA JODA SA SOLI I GOITROGENA IZ HRANE NA POREMEĆAJ FUNKCIJE ŠTITNE ŽLIJEZDE

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Pregledni rad

Sažetak

Uvod: Poremećaj funkcije štitne žlijezde je relativno često oboljenje s većom prevalencom pojavljivanja kod žena nego kod muškaraca, a rizik se povećava sa starosnom dobi. Uzroci mogu biti različiti, a jedan od uzroka je nedovoljan ili preveliki unos joda kao i goitrogena iz hrane.
Cilj rada: Cilj rada je bio prikupiti i analizirati dostupne znanstvene informacije o utjecaju prekomjernog unosa joda sa soli i goitrogena iz hrane na poremećaj funkcije štitne žlijezde.
Rezultati i rasprava: Jod se u najvećoj mjeri unosi sa soli iz kruha i tjestenine, zatim direktnim dosoljavanjem i konzumiranjem industrijskih gotovih proizvoda. Stoga ne čudi što se hranom unosi i 2 do 5 puta više joda nego što su trenutne preporuke (za odrasle je približno 150 µg/dan). Podnošljiva gornja granica unosa joda za odrasle iznosi 1100 µg/dan. Brojne biljne vrste sadrže goitrogene ili antitiroidne supstance koje ometaju sintezu hormona štitne žlijezde. Goitrogeni djeluju na različite načine, uzrokujući gušavost i poremećaje povezane sa jodom kao što su hipotireoza, hipertireoza i tiroiditis. Posebno osjetljive na goitrogene su osobe sa genetskom predispozicijom.
Zaključci: Dugotrajna izloženost unosu joda sa soli kao i goitrogenima nije kvalitetno istražena. Međutim, postoji vjerovatnost da jod iz soli i goitrogeni iz hrane imaju uticaj na funkciju štitne žlijezde mijenjajući proizvodnju i izlučivanje njenih hormona.

Ključne riječi: unos joda, goitrogeni, štitna žlijezda, poremećaj funkcije

Uvod

Jod je mikroelement koji se nalazi u Zemljinoj kori i priobalnim područjima (Leung i Braverman, 2014). Nedovoljan unos joda Svjetska zdravstvena organizacija (WHO) svrstava među vodeće svjetske zdravstvene probleme i procjenjuje se da više od dvije milijarde ljudi živi u krajevima s nedostatkom joda (ZZJZ, 2006).
Preporučeni unos joda ovisi o tjelesnoj masi, starosnoj dobi, fizičkoj aktivnosti, spolu, zdravstvenom stanju i drugim faktorima. Preporučeni unos joda za dojenčad je 50 µg/dan, za djecu 1-6 godina 90 µg/dan, za djecu 7-12 godina 120 µg/dan, te za odrasle i djecu iznad 12 godina 150 µg/dan. Za trudnice i dojilje preporuka je 200 µg/dan (WHO, 1996a). Vjerovatno, sigurna gornja granica unosa joda za nedonoščad je 100 µg/kg/dan, dojenčad do 6 mjeseci 150 µg/kg/dan, dojenčad od 7-12 mjeseci 140 µg/kg/dan, djeca od 1-6 godina 50 µg/kg/dan, školska djeca od 7-12 godina 50 µg/kg/dan, adolescenti i djeca preko 12 godina 30 µg/kg/dan, a za trudnice i dojilje 40 µg/kg/dan (FAO, 2002). Podnošljiva gornja granica unosa joda (tolerable upper intake level-ULI) za odrasle osobe iznosi 1100 µg/dan (Gardner i sur, 1988).
Ukoliko se kroz duži vremenski period unosi količina joda koja je blizu granica podnošljivog ili toksičnog, postoji opasnost od štetnih efekata na zdravlje organizma.
Najniža doza zapaženog štetnog učinka (LOAEL) je 1500-1800 µg/dan, a najviša doza bez štetnog efekta (NOAEL) od 1000-1200 µg/dan i to su vrijednosti za odrasle osobe (Gardner i sur, 1988). U cilju objezbjeđivanja unosa 150 µg/dan joda putem jodirane soli, WHO i Međunarodni dječji fond Ujedinjenih naroda za hitne potrebe (UNICEF) su 1996. godine preporučili da sadržaj joda u soli bude 20-40 mg joda/kg soli. Ova preporuka je zasnovana na podacima koji ukazuju na gubitak joda od mjesta proizvodnje do domaćinstva, te da se 20 % gubi kuhanjem prije upotrebe (ZZJZ, 2006). Tijekom prošlog stoljeća sol je bila predmetom intenzivnih znanstvenih istraživanja vezanih uz povišen krvni tlak i kardiovaskularne poremećaje (Sung Kyu Ha, 2014). Podaci iz velikog broja istraživanja o unosu soli ukazuju da se širom svijeta dnevni unos najčešće kreće u obimu 9-12 g/dan (Brown i sur., 2009). Smanjenje unosa soli hranom sa sadašnjih 9-12 g/dan u odnosu na preporučenu količinu od 5-6 g/dan dalo bi pozitivne učinke na zdravlje kardiovaskularnog sustava. WHO i Organizacija za prehranu i poljoprivredu (FAO) preporučuju

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konzumaciju manje od 5 g/dan soli kao cilj kojem se treba težiti (Sung Kyu Ha, 2014). U ljudskom organizmu nalazi se 15-20 mg joda od kojeg je čak 70-80 % koncentrirano u štitnoj žlijezdi. Od ukupne količine unesenog joda, 15 % odlazi u štitnu žlijezdu u toku 24 h, a ostatak se izlučuje preko bubega u urinu (Ristić-Medić, 2009). Štitna žlijezda luči trijodtironin (T_3), tiroksin (T_4) i klacitonin. Biosinteza hormona štitne žlijezde zavisi od unosa egzogenog joda putem hrane i vode. Gotirogeni iz hrane pokazuju negativan utjecaj na metabolizam štitne žlijezde. Štetne učinke pokazuju prvenstveno smanjujući raspoloživu koncentraciju joda u organizmu (Rohner i sur., 2014). Poremećaji funkcije štitne žijezde su registrirani u više od 110 zemalja svijeta s 1.6 milijardi ljudi u opasnosti koji trebaju suplementaciju joda. Većina od njih su u zemljama u razvoju, Aziji, Africi i Latinskoj Americi. Poremećaji štitne žlijezde se javljaju zbog nepravilnosti u obavljanju njenih funkcija i povećanja štitne žlijezde (Khan i sur., 2002). Više od 12 % populacije u Sjedinjenim Američkim Državama ima tendenciju za pojavom nekog od poremećaja funkcije štitne žlijezde, što bi značilo da 20 milijuna ima neki od ovih poremećaja. Žene obolijevaju i do osam puta češće od muškaraca (ATA, 2017).

Vrste i sastojci hrane koji utječu na funkciju štitne žlijezde

Unos soli i joda

Upotreba kuhinjske soli u pripremi jela uveliko premašuje dnevnu potrebu organizma, koja je 5 g/dan soli (WHO, 2006). Izvori unosa kuhinjske soli u prehrani mogu se grubo svrstati u dvije skupine. Kao začin kuhinjska sol se dodaje u malim količinama da bi pojačala okus jela (Yee i sur., 2011). Drugi izvor unosa kuhinjske soli je u obliku konzervansa. Značajni izvori skrivene soli nalaze se u polugotovoj i gotovoj hrani kao što su: suhomesnati proizvodi, paštete, tvrdi sirevi, sirni namazi, grickalice (čips, štapići, kikiriki, pistacije), gotovi umaci, senf, majoneza, hamburgeri, juhe iz vrećice. Kukuruzne i žitne pahuljice predstavljaju izvor skrivene soli. Pekarski proizvodi, osobito kruh, koji je zastupljen u svakodnevnoj prehrani, izvor je kuhinjske soli (Ugarčić-Hardi i sur., 2010) (Tablice 1 i 2). Prema istraživanju koje je rađeno u Hrvatskoj unos kuhinjske soli u oba spola je čak, dvostruko veći od preporučene količine prema WHO. Najveći dnevni unos kod muškaraca je 29.9 g, a kod žena 19.4 g. U cijeloj skupini ispitanika uočena je statistički značajna korelacija između indeksa tjelesne mase i unosa kuhinjske soli (Đurić i sur., 2011). Preporuke za dnevni unos soli za odrasle osobe je 5 g, s tim da bi dalje smanjenje unosa na 3 g dovelo do značajnog smanjenja rizika za nastanak kardiovaskularnih bolesti i sniženja troškova zdravstvene zaštite (Bibbins-Domingo i sur., 2010).

Tablica 1. Udio kuhinjske soli u dnevnom unosu u pojedinim namirnicama (BGK, 1989)

Proizvod	Udio kuhinjske soli u dnevnom unosu (%)
Kruh i pekarski proizvodi	34
Meso i mesni proizvodi	28
Masti, slatkiši, napici	11
Sir, vrhnje, jaja	10
Riba i riblji proizvodi	7
Mlijeko i mliječni proizvodi	5
Voće i prerađevine	5

Potrebe za jodom u organizmu su male, međutim on mora da se unosi svakodnevno. Skupština WHO-a 1990. godine je usvojila univerzalnu metodu jodiranja soli (USI) kao metodu izbora za eliminaciju jodnog nedostatka - IDD (Benoist i sur., 2004). Rezultati pokazuju da je jodirana sol efikasno sredstvo za poboljšanje jodnog statusa. Varijacije u nivou joda u soli ukazuju na to da se posebna pažnja treba obratiti na osiguravanje kvalitete prilikom proizvodnje i skladištenja (Clar i sur., 2002). Za procjenu koncentracije joda u organizmu koriste se četiri metode, a to su: određivanje koncentracije joda u urinu, određivanje stope gušavosti i koncentracije u krvi tireostimulirajućeg hormona štitne žlijezde i tireoglobulina. Ovi pokazatelji su komplementarni, s tim da je urinarni jod osjetljiv pokazatelj nedavnog unosa joda (dani), tireoglobulin pokazuje srednji odgovor (nedjelja do mjesec dana), dok promjene u stopi gušavosti odražavaju dugoročnu manjkovost joda u ishrani (mjeseci do godina dana) (Zimmermann, 2008).

Tablica 2. Izvori joda iz hrane (Pennington i Spungen, 2010)

Namirnice	Unesena količina	Količina joda
Bakalar	85 g	99 µg
Kukuruzna krupica	1 šalica	68 µg
Mlijeko, 2% mliječne masti	240 ml (1 šalica)	56 µg
Mlijeko, nemasno	240 ml (1 šalica)	51 µg
Bijeli kruh	50 g (2 komada)	46 µg
Goveđa jetra, kuhana	85 g	36 µg
Grahorice, kuhane	90 g (1/2 šalice)	35 µg
Krumpir, pečen	110 g	34 µg
Kruh od cjelovitog pšeničnog zrna	50 g	32 µg
Jaje, kuhano	50 g	24 µg
Zobena kaša, kuhana	1 šalica	16 µg

Jod je prisutan u koncentraciji do nekoliko tisuća puta većoj od preporučene u lijekovima, dodacima prehrani i u kontrastnim sredstvima koja se koriste za radiološke studije (Leung i Braverman, 2014). Akutna trovanja jodom rijetko se javljaju i to samo u dozama od nekoliko grama unešenog joda. Simptomi akutnog trovanja uključuju žarenje usta, grla i želuca, vrućicu, mučninu, povraćanje, dijareju, slab puls, cijanozu i komu (Zimmermann, 2012). Kronična izloženost jodu uzrokuje jodizam. Simptomi nalikuju prehladi, kao što je oticanje pljuvačnih žlijezda, gastrointestinalne iritacije, metalni ukus u ustima, gingivitis, konjuktivitis i edem vjeđa (Goodman i Gilman, 1970).

Unos goitrogena

Faktori okoliša kao i neki lijekovi ometaju funkciju štitne žlijezde, što predstavlja opasnost za pojavu bolesti. Zagađivači koji uzrokuju gušavost poznati su kao goitrogeni za zaštitu okoliša koji mogu djelovati izravno na štitnu žlijezdu, ali i neizravno mijenjanjem njenih regulatornih mehanizama, perifernog metabolizma kao i promjenama u izlučivanju hormona. Antitiroidni spojevi mogu ući u vodu, zrak i putem hranjivih tvari, postajući tako važan faktor okolišnih goitrogena kod čovjeka. Prirodni i antropogeni agensi mogu djelovati kao goitrogeni, kao i neki lijekovi udruženi s nedostatkom prehrambenog joda, dovodeći do pojave gušavosti i sličnih povezanih poremećaja (Gaitan, 1988). Izvori goitrogena u hrani se nalaze u kupusnjačama, a u manjoj mjeri i u drugim namirnicama. Njihov utjecaj kod zdravih osoba nema nikakvog značaja, ali u stanju manjka joda u organizmu ne bi trebalo pretjerivati u njihovom konzumiranju. Kupusnjače su: brokula, prokulica, kupus, karfiol, raštika, hren, kelj, koraba, rotkvice, repa, radić itd. Važni izvori goitrogeni su soja, proso, kikiriki, kao i neke vrste voća i povrća (HSH, 2014).

Poremećaji funkcije štitne žlijezde vezani za unos joda i goitrogena iz hrane

Bolesti štitne žlijezde se mogu svrstati u endokrine, nutricijske i metaboličke i sve tri su podjednako prisutne. Osobe s povećanim rizikom za pojavu poremećaja funkcije štitne žlijezde su one s pozitivnom obiteljskom povijesti, disfunkcijom žlijezde nakon poroda ili operacije, žene starosti od 55 godina, te osobe koje dolaze iz područja s endemskim nedostatkom joda. Bolesti koje su blisko povezane s poremećajima funkcije štitne žlijezde su: dijabetes melitus, autoimune bolesti, endokrini nedostaci, Downov sindrom, Turnerov sindrom, hipofizna ili hipotalamusna abnormalnost. Kao posljedica uzimanja lijekova (amiodaron, litij) i kontrastnih sredstava, također se mogu pojaviti neki od poremećaja funkcije štitne žlijezde (Stockigt, 2003). Uzroci nastanka poremećaja funkcije štitne žlijezde uključuju upalu kao što su akutni ili subakutni tiroiditis, autoimune bolesti kao što su Hashimotov tireoiditis i Gravesova bolest, nasljedne metaboličke nedostatke kao što je nedostatak mono- i dijodotirozin dejodinaze, malignost i nedostatke u prehrani (Walker, 1990). Poremećaji koji nastaju usljed nedostatka joda pokrivaju spektar patoloških stanja koja dovode do oboljenja vezanih za njegov nedostatak, a uključuju gušavost, hipotireoidizam, kretenizam, patuljasti rast, mrtvorođenost, kongenitalne anomalije i povećan perinatalni mortalitet (WHO, 1996b). Glavni izravni učinci prekomjernog unosa joda na endokrini sistem se odražavaju na štitnoj žlijezdi i regulaciji proizvodnje i izlučivanja njenih hormona. Učinci viška joda na štitnu žlijezdu mogu se svrstati u tri tipa: hipotireoza, hipertireoza i tiroiditis (ATSDR, 2004). Hipotireoza se odnosi na smanjenu proizvodnju hormona štitne žlijezde dovodeći do kliničkih manifestacija insuficijencije štitne žlijezde. To može biti praćeno s ili bez gušavosti, povećanjem žlijezde koja se javlja kao odgovor na povišene razine cirkulirajućeg hormona hipofize TSH tijekom razdoblja supresije proizvodnje hormona štitne žlijezde. Tipični biomarker hipotireoza je smanjenje cirkulirajućih razina tiroksina (T_4), a kada je zatajenje štitne žlijezde daleko naprednije, trijodotironina (T_3). To je uvijek praćeno povećanjem TSH, osim ako uzrok hipotireoze ne leži u hipofizno-hipotalamusnoj regulaciji (ATSDR, 2004). Većina bolesnika ima primarnu hipotireozu kao rezultat bolesti štitne žlijezde koja uništava sposobnost proizvodnje adekvatnih hormona. Povremeno hipotireoza može da bude sekundarna, uzrokovana bolestima hipofize ili hipotalamusa, što rezultira neadekvatnom produkcijom tireostimulirajućeg hormona štitne žlijezde (Brams, 2005). Hipotireoza također može biti posljedica operacijskog odstranjenja štitne žlijezde ili kemijskog razaranja tkiva tijekom liječenja hipertireoze. Za proizvodnju tjelesne energije potrebne su određene količine hormona štitne žlijezde. Pad koncentracije hormona dovodi do smanjene proizvodnje energije. Čest uzrok hipotireoze je Hashimotov tireoiditis, autoimuni poremećaj u kojem bijela krvna zrnca potpuno istiskuju tkivo štitne žlijezde koje tada napadaju protutijela (Baum i sur., 1996).

Hipertireoza je prekomjerna proizvodnja i/ili izlučivanje hormona štitne žlijezde. Klinička manifestacija abnormalno povišenih razina hormona T_4 i/ili T_3 u cirkulaciji je tireotoksikoza. Tiroiditis se odnosi na upalu žlijezde, koja je često sekundarna pojava (ATSDR, 2004). Jodom inducirana hipertireoza je poremećaj nedostatka joda koji se pojavljuje kod starijih osoba, kada populacije s iznimnim deficitom joda povećaju njegov unos, čak i kada je ukupna količina u prihvatljivim granicama od 100-200 μg/dan. Hipertireoza inducirana jodom predstavlja prolazno povećanje učestalosti hipertireoizma, koje se može smanjiti uz ispravku deficita joda. Hipertireoza uzrokovana jodom se javlja kod osoba koje imaju već postojeću autoimunu nodularnu gušavost. Broj ljudi u rizičnoj skupini za hipertireozu direktno je proporcionalan broju pacijenata s nodularnom gušavošću. Ne postoji nivo joda u soli koji omogućava potpunu zaštitu od nekih povećanja učestalosti hipertireoze u populaciji koja je prethodno imala veliki nedostatak joda (WHO, 1996b). Hipertireozu karakterizira povećana aktivnost štitne žlijezde s prevelikom biosintezom i lučenjem hormona štitne žlijezde, što dovodi do tireotoksikoze koju karakterizira gubitak težine, povećanje apetita, otežano disanje, nervoza, slabost i umor (WHO, 1996b). Uslijed autoimunih poremećaja pojavljuje se Basedowljeva bolest uz otpuštanje viška hormona. Toksični adenomi (čvorovi) rastu u štitnoj žlijezdi i luče hormone štitne žlijezde, mijenjajući normalan tok kemijskih tvari i narušavajući tjelesnu ravnotežu. Upala štitne žlijezde u subakutnom tireoditisu izaziva otpuštanje hormona, ima za posljedicu prolaznu hipetireozu. Iako rijetko, hipertireoza može biti posljedica poremećaja hipofize ili rasta raka štitne žlijezde (Baum i sur., 1996). Laboratorijskim testovima se očitavaju vrijednosti razine hormona štitne žlijezde i tireostimulirajućeg hormona koji potiče njen rad (Baum i sur., 1996).

Visoke koncentracije joda mogu dovesti do poremećaja funkcije štitne žlijezde kod osjetljivih pojedinaca, ali općenito pokazuje dobar utjecaj na njenu funkciju. Kako bi se spriječio nedostatak joda preporučuju se dodaci prehrani (Leung i Braverman, 2014).

Dodaci se mogu uzimati zajedno uz konvencionalne lijekove, ali samo uz preporuku liječnika. Za njihov učinak su ponekad potrebni i mjeseci. Mnogi dodaci potrebni su za održavanje zdravlja štitne žlijezde. Vitamin C, vitamin E i vitamini skupine B mogu biti korisni u liječenju hipertireoze i hipotireoze. Dodatne količine cinka također su potrebne da podstaknu rad štitne žlijezde. Osobe koje imaju hipotireozu trebaju prehranom da unose dovoljne količine selena (Ashwell i sur., 1999). Dok jod predstavlja bitnu komponentu hormona štitne žlijezde, selen ulazi u sastav enzima jodotironin-dejodinaze (DIO) i neophodan je za konverziju T_4 u biološki aktivni hormon štitnjače T_3 (Schneider i sur., 2006). Visoka prevalencija nedostatka željeza kod djece u području endemske gušavosti može smanjiti efikasnost jodirane soli. Teška anemija uslijed nedostatka željeza može narušiti metabolizam štitne žlijezde na sljedeće načine: (1) mijenjanjem lučenja TSH; (2) smanjenjem aktivnosti enzima peroksidaze koja katalizira jodiranje tireoglobulina za proizvodnju hormona i (3) ograničavajući u jetri pretvorbu T_4 u T_3, povećanje cirkulirajućeg T_3 i smanjenje vezivanja T_3 na nuklearne receptore (Zimmermann, 2006). Nedostatak vitamina A kod istraživanja na životinjskim modelima utječe na aktivnost hipofize i štitnu žlijezdu: (1) povećanjem sinteze i izlučivanja hormona koji stimulira štitnu žlijezdu (TSH); (2) povećanjem veličine štitne žlijezde; (3) smanjenje udjela joda kod štitne žlijezde i smanjenje sinteze i jodiranja tireoglobulina i (4) povećanje koncentracije cirkulirajućih hormona štitne žlijezde (Zimmermann, 2007).

Goitrogeni pokazuju negativan utjecaj na metabolizam štitne žlijezde prvenstveno smanjujući raspoloživu koncentraciju joda u organizmu. Skupine stanovništva koje su posebno osjetljive na izloženost goitrogenima su dojenčad i mala djeca (Rohner i sur., 2014). Hrana koja uzrokuje poremećaje proizvodnje hormona štitne žlijezde kod ljudi razvrstana je u dvije široke kategorije: cijanogene i flavonoide koji su prisutni u biljakama. Brojne biljne vrste koje se konzumiraju sadrže goitrogene ili antitiroidne supstance koje ometaju sintezu hormona štitne žlijezde djelujući na različite načine uzrokujući gušavost i poremećaje povezane s jodom (Chandra, 2010). Najvažnije skupine tvari koje izazivaju gušavost su glukozinolati, izoflavoni (genistein i daidzein) te cijanogeni glikozidi. Glukozinolati i cijanogeni glukozidi tek razgradnjom daju potencijalno štetne spojeve. Šećerni dio glukozinolata odvaja se od početne molekule enzimom (mirozinaza tj. tioglukozidaza) kojeg sadrži ista biljka, ali tek oštećenjem biljnog tkiva dolazi u kontakt sa supstratom (Šarkanj i sur., 2010). Daljim reakcijama nastaju nitrili, izotiocijanati, tiocijanati i oksazolidini. Cijanogeni glikozidi imaju svojstvo oslobađanja cijanovodonika najčešće enzimskom hidrolizom (Šarkanj i sur., 2010). Krstašice sadrže tioglukozide koji se metaboliziraju u tiocijanate. Ovi spojevi inhibiraju transport joda i ugradnju jodida u tireoglobulin, čime se povećava lučenje tireostimulirajućeg hormona i proliferacija ćelija štitne žlijezde. Tiocijanat je kompleksan anion koji je snažan inhibitor jodidnog transporta i predstavlja detoksikacijski produkt cijanida i lako se može mjeriti u tjelesnim tekućinama (Erdogan, 2003).

U eksperimentima na životinjama utvrđeno je da ove supstance izazivaju karcinome štitne žlijezde (Truong i sur., 2010). Cijanid u duhanskom dimu pretvara se u tiocijanat u jetri, stavljajući pušače s niskim unosom joda pred rizik za razvoj gušavosti. Također tiocijanat utječe na transport joda u laktacijskom periodu mliječne žlijezde, što dovodi do niskih koncentracija joda u majčinom mlijeku i oslabljene opskrbe dojenčadi sa jodom (Laurberg, 2014).

Kvercetin je najobilniji flavonoid koji se nalazi u voću i povrću. On inhibira gen štitne žlijezde ograničenjem ekspresije i njene funkcije. Antitiroidni efekti kvercetina pokazuju da on može inhibirati vezanje jodida na tireoglobulin (jodidnu organifikaciju) i aktivnost tiroidne peroksidaze (TPO). Također ometa metabolizam hormona štitne žlijezde (Giuliania i sur.,2014). Gluten je na vrhu liste potencijalnih goitrogena, a osjetljivost na gluten doprinosi širokom spektru autoimunih oboljenja pored celijakije koja je direktno povezana sa glutenom (HSH, 2014). Soja sadrži goitrogeno jedinjenje poznato kao genistein. Ovo jedinjenje, kao i hormoni štitne žlijezde, vezuju molekule joda iz TPO. Genistein i slični izoflavoni iz soje mogu se natjecati s hormonima štitne žlijezde za jod ili mogu blokirati aktivnost TPO. Izotiocijanati, kao i sojini izoflavoni mogu remetiti i signalizaciju kroz membranu ćelija štitne žlijezde. Uočeno je da stres različitoga porijekla, od mehaničkih oštećenja do UV zračenja može povisiti koncentraciju glukozinolata u biljnim namirnicama nakon berbe (Šarkanj i sur., 2010).

Usitnjavanjem hrane značajno se povećava razina produkata glukozinolata u povrću. Posebno osjetljive na goitrogene su osobe s genetskom predispozicijom, koja rezultira smanjenim nakupljanjem joda u štitnoj žlijezdi ili hipotireozom (Šarkanj i sur., 2010).

Zaključci

- Poremećaji funkcije štitne žlijezde zauzimaju jedno od vodećih mjesta među oboljenjima žlijezda s unutrašnjim lučenjem.
- Hranom se unosi i do 5 puta više soli nešto sto stoji u preporukama WHO, čime se premašuju i preporuke za unosom joda kojim je sol fortificirana. Podnošljiva gornja granica unosa joda nije jasno definirana ako osoba duži vremenski period unosi više joda nego što je gornja granica podnošljivosti.
- Brojne namirnice sadrže goitrogene ili antitiroidne supstance koje ometaju sintezu hormona štitne žlijezde djelujući na različite načine, uzrokujući gušavost i poremećaje povezane s jodom.
- Dugotrajna izloženost unosu joda solju kao i goitrogenima nije kvalitetno istražena. Međutim, postoji vjerovatnost da jod iz soli i goitrogeni iz hrane imaju utjecaj na funkciju štitne žlijezde mijenjajući proizvodnju i izlučivanje njenih hormona.

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INFLUENCE OF IODINE CONSUMPTION FROM SALT AND GOITROGENS FROM FOOD ON THE THYROID GLAND DISORDERS

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Review paper

Summary

Introduction: The function of the thyroid gland is relatively frequent disease with greater prevalence among women than men, and the cause increases with age. Causes differ, and one of the factors is insufficient or too high iodine intake as well as intake of goitrogens from food.
Aim: The aim of the study was to collect and analyze available scientific information on the effect of excessive intake of iodine from salt and goitrogens from food on the thyroid gland function.
Results and discussion: The greatest contribution to total iodine intake is from salts in bread and pasta, or by direct dosing and by consuming processed foods. Therefore, the average daily intake of iodine is 2 to 5 times higher than the recommended intake (for adults is approximately 150 μg/day). The upper tolerable limit for iodine intake is 1100 μg/day. Many plants contain goitrogens or antithyroid substances that interfere with the synthesis of thyroid gland hormones. Goitrogens are acting in various ways causing iodine-related lupus and other disorders. People with genetic predisposition are particularly sensitive to goitrogens.
Conclusions: Long-term exposure to salt intake of iodine and goitrogens is not well understood. However there are indications that the iodine salts from goitrogens of food affect the thyroid function by shifting its production and secretion of hormones.

Keywords: iodine intake, goitrogens, thyroid gland, impaired function

NUTRITIONAL AND HEALTH BENEFITS OF CURCUMIN

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Review paper

Summary

Turmeric (*Curcuma longa*) belongs to the family *Zingiberaceae* and it has been traditionally used for centuries in Asian cuisine. India is the largest producer, consumer and exporter of turmeric. Its dried and ground tuber is used worldwide as a spice (the most famous spice which contains turmeric is *curry*) and as an additive for a variety of products that require medically acceptable intense yellow color. The most important part of the turmeric tuber is a group of bioflavonoids, i.e. curcumins (curcumin (77%), bisdemethoxycurcumin and demethoxycurcumin). The most common method for the isolation of curcumin is extraction with organic solvents, usually ethanol, by using Soxhlet, ultrasonic and microwave extraction, and more recently, due to increased use in the food industry (food supplements), triacylglycerol. Curcumin has significant anti-inflammatory, antioxidant, chemoprotective, anticancer, and gastroprotective properties. It affects the neurosystem and it is one of the most investigated bioflavonoids. The aim of this paper is to present the dietary and health benefits of turmeric and curcumin for both humans and animals.

Keywords: turmeric, spice, food and health benefits

Introduction

Turmeric (curcuma) - *Curcuma longa L.* (*Zingiberaceae*) is a perennial plant native to Southeast Asia; it has been used since ancient times as a spice, in medicine, and for coloring and flavoring food. In addition to *Curcuma longa L.*, the genus turmeric contains about 30 other species. The name Curcuma is derived from the Arabic word "turmeric" which means yellow. In Sanskrit, turmeric has 55 different names associated with its religious and medical use (Ravindran, 2007). The varieties *Allepey* and *Madras* are mostly cultivated in India (82%), and its yield was around 720,000 tons in 2015/2016. The part of turmeric used in food and medicine is the rhizome. The rhizome is harvested, cleaned, and boiled until it becomes soft, and then dried in the sun in a 5-7 cm thick layer for about 2 weeks. The yield is 10-30%, depending on the variety and the cultivation area (Kandiannan et al., 2008). Dried product is milled and marketed. Products prepared in this way are used in diets as spices, and as the basic raw material for the production of the bioflavonoid curcumin. Curcumin is used as an additive (E100), and its main purpose is coloring products yellow and up to red, depending on the pH of the product. Curcumin is one of the most researched bioflavonoids today and a number of studies have confirmed its antioxidant, anti-inflammatory, anti-cancer, chemoprotective, gastroprotective, and many other health properties. Studies have shown that it is not toxic, even at doses up to 12 g / day, and it is tolerated very well by the human body (Basnet et al., 2011). Most studies have shown that the biological effect of curcumin in large part comes from its ability to directly bind to different proteins (cyclooxygenase, lipoxygenase) or to modulate the intracellular redox state (Srivastava et al., 2011).
The shortfall of this bioflavonoid is its relatively poor absorption, and it is therefore necessary to conjugate it. Recent research studies considered its adoption in the form of nanoparticles.

Preparation of curcumin and its use as a food additive

Curcuma longa L. contains about 2-9% of curcuminoids. The most important of those are curcumin (77%), dimethoxy-curcumin (17%), and *bis*-demethoxy-curcumin (3%). These three compounds differ in the substitution of methoxy groups in the aromatic ring (Fig. 1).

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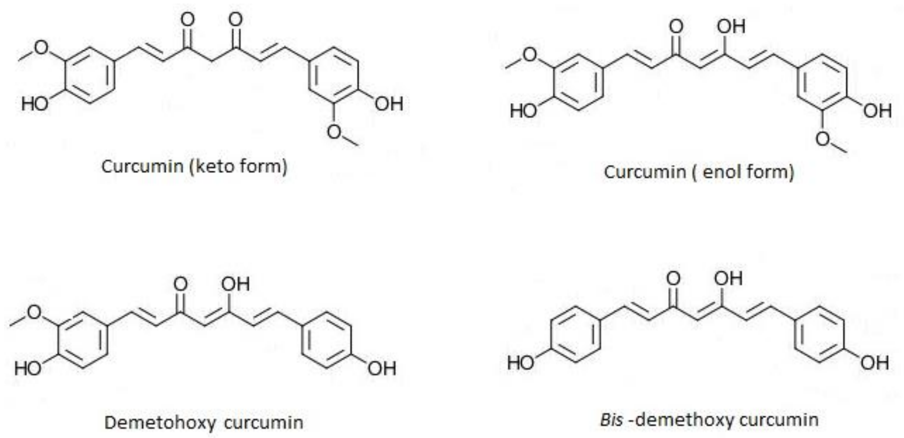

Fig. 1. Composition of curcumin

Curcumin (E,E) -1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) is extracted from the tubers of a dried and milled turmeric by using a suitable solvent. The oily resin is obtained after the extraction and removal of the solvent, and contains 25-35% of the pigment, along with the volatile oils and resin extracts. After the purification of the resin, approximately 90% of the pigment is obtained. The solvents which can be used for the extraction are: isopropanol, ethyl acetate, acetone, and ethanol. Curcumin is soluble in oils, insoluble in water at acidic and neutral pH, but soluble in alkaline conditions. The color of curcumin highly depends on the pH, at pH 2.5 to 7.0, it has an intense yellow color, and above pH 7.0 it is red.

For nutritional purposes, it is primarily used as an additive for coloring products. It is used in all sectors of the food industry, in the amount of 5-500 mg / kg, depending on the food category.

As an additive, curcumin is stable during thermal treatment and in dry food. It is relatively inert to reactions with other ingredients (may form salts with phthalates and citrates), and it is inert in the reactions with phosphates, chlorides, and bicarbonates (Stankovic, 2004).

Besides being used for food coloring, curcumin shows significant antioxidant properties and it prevents lipid peroxidation to a significant extent. The antioxidant properties are the result of the double carboxyl groups along with hydroxyl groups (Stankovic, 2004). Curcumin binds free radicals, and it becomes a short-lived, non-reactive free radical, and as such does not represent a health hazard (Majeed et al., 2000).

The absorption and metabolic pathway of curcumin

A number of previous studies have shown that curcumins exhibit poor bioavailability in the human body when administered orally. It is due to their rapid degradation and poor absorption in the gastrointestinal tract, which results in low plasma concentrations and a very lowdistribution in tissues.

Curcumin in the body is converted into dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin (HHC), and octahydrocurcumin with reductase activity, and thoseare further decomposed by the action of β-glucuronidase to dihydrocurcumin-glucuronide and tetrahydrocurcumin -glucuronide (Pan et al., 1999). Poor absorption can be seen in the example of a clinical trial in which the oral intake of 3.6 g of standardized extract per day showed that one hour after intake the plasma concentration was only 11.1 mmol / L (Sharma et al, 2004). Due to this limitation, different formulations of curcumin were tested in order to maximize its availability and activity. In addition to oral intake, subcutaneous entry was investigated and has proven effective in keeping curcumin in tissues for a longer period (Shahani et al., 2010). Intravenous intake in the form of nanoparticles has been proven effective for the treatment of tumors in animals. (Kim et al., 2011) and Wang et al., 2008, Shi et al., 2011, showed that curcumin in liposomal form is effective in enhancing the effect of chemotherapy. The use of curcumin on the skin has proven to be very effective in treating certain types of psoriasis, wound healing, candidiasis, etc. (Prasad et al., 2014). Recent research has shown that oral intake in the form of nanoparticles increases the availability of curcumin 5-6 times in comparison to the standardized extract, due to increased water solubility it dissolves better in the digestive system and itsdegradation is longer (Xie et al., 2011). Nanoparticles are also safe, non-toxic, biodegradable, do not cause allergies in the body, and can be designed to be time releasing (Sankar et al., 2016). The combination of curcumin with piperine, liposomal, and phospholipid complexes also increases bioavailability (Prasad et al., 2014). A hydrophilic carrier dispersed curcuminoid formula exhibits 46 times the bioavailability of the standard purified 95 percent curcuminoid preparation (Douglass and Clauatre, 2015).

Antioxidant properties of curcumin

Curcumin shows very significant antioxidant properties as a food additive. In the presence of curcumin, the oxidation of linoleic acid is very slow, and the antioxidant effect is about 80% (Jayaprakasha et al., 2006) when used as a dietary supplement. It works in a way that binds free radicals and it also donates a hydrogen atom (responsible for its antioxidant properties) (Wei et al., 2006). Curcumin has a property of donating electrons in order to neutralize free radicals by creating stable products, and thus breaking a chain reaction of creating free radicals in a living organism. Curcumins' ability of capturing hydrogen peroxide is higher than that of the commercial antioxidants (BHA, BHT, vitamin E) at the same concentration (20 mM) (Ak and Gülcin, 2008).

Curcumin shows a significant effect on hepatic oxidative stress in diets with high cholesterol by reducing antioxidative liver enzymes (catalase, glutathione peroxidase, and superoxide dismutase), it also reduces TNF-α (tumor necrosis factor alpha), serum IL-6 (interleukin 6), liver enzymes, and heart disease enzyme markers. Supplementation of curcumin in patients with hyperlipidemia can reduce cardiovascular problems (Hussein et al., 2016).

The study on oxidative stress and the loss of vitamins C and E during sleep in an area with little or no gravity (astronauts) showed that curcumin reduces the loss of these vitamins (Rai et al., 2010).

Curcumin prevents oxidation and lipid modification of low density lipoproteins, and as a consequence, the inhibition of prostacyclin (PGI_2), which contributes to the formation of thrombosis and arteriosclerosis, and thus contributes to the prevention of the same (Mahfouz et al., 2009).

Anti-inflammatory activity

Curcumin reduces the response of specific proteins - cytokines that occur in the processes of inflammation, such as TNF-α, interleukins (IL-1, IL-2, IL-6, IL-8, IL-12), chemokines, through the inhibitory effect on NF-κB (cellular factor kappa B), and even directly binding to TNF-α (Anthwal al., 2014). A series of studies on animals have shown that the dose of 100-200 mg per kg of body weight indicates good anti-inflammatory activity (Kohli et al., 2004). Curcumin reduces the inflammation associated with colitis by significantly reducing the activity of myeloperoxidase and TNF-α (Basnet et al., 2011). Liu et al. (2015) have shown that curcumin reduces the pathological changes in the lungs and the accumulation of inflammatory cells in the airways of asthmatic mice by downregulating the expression of pro-inflammatory cytokines with the activation of the Nrf2 / HO-1 (nuclear factor erythroid 2-related factor / heme oxygenase-1) signaling pathway. Nuclear factor erythroid 2-related factor (Nrf2) is a cytoprotective factor which regulates the expression of gene coding for anti-oxidant, anti-inflammatory, and detoxifying proteins.

Pancreatitis is another inflammatory process associated with the secretion of NF-κB cytokines. Curcumin significantly reduces the activation of this cytokine and AP-1 (activator protein 1), and reduces the mRNA induction of iNOS (nitric oxide synthase), TNF-α and IL-6 cytokines in the pancreas (Gulcubuk et al., 2013).

Studies on the effects of curcumin on allergies have shown that curcumin inhibits the NF-κB in the airway, together with the transcription factor GATA3, reduces IgE in serum, and inhibits the Notch1-GTA3 signaling pathway (Chong et al., 2014).

Anticancer activity

The transcription factor NF-κB has the main role in the creation of tumors and inflammation, and the goal of most pharmaceutical and entomological preparations is to reduce its hyper productivity. Curcumin is a natural product which reacts with various compounds in the downstream of the NF-κB pathway. Curcumin blocks IKK activation, phosphorylation, and the degradation of IκBα (Kastori et al., 2015, Fiala, 2015). A large number of curcumin analogues were investigated in order to improve its efficiency in blocking NF-κB, including the latest analogs called BAT (Katsori et al., 2011, Kasinski et al. 2008). Katsori et al. (2015) have shown that the curcumin analog BAT3 selectively inhibits NF-κB dependent gene expression by binding to chromatin DNA in the laboratory. Curcumin inhibits the growth of androgen-independent prostate cancer cells through ERK1/2- and SAPK/JNK-mediated inhibition of p65, followed by the reducing expression of MUC1-C protein. The MUC1-C is a protein that has a markedly increased expression in prostate tumors (Li et al., 2015). In combination with α-tomation, It is also effective in inhibiting PC3 prostate tumor cells by inhibiting the NF-κB and decreasing the expression of the Bcl-2 gene (Huang et al., 2015). Khosropanah et al. (2016) formulated curcumin nanoparticles to increase its bioavailability and to study the effect on breast cancer cells (MDA-MB 231), i.e. cytotoxicity on tumor cells. More than 50% of the tumor cells died within 48 hours after the administration of curcumin. The dosage of nano-formulated curcumin in this study was effective in half the dosage of the regular preparation of curcumin.

Theresearch on gastrointestinal tract tumors has shown that curcumin in combination with quercetin (enhances the absorption of curcumin) inhibits phosphorylase AKT and ERK and induces apoptosis through a mitochondrial pathway (Zhang et al., 2015). Sridhar et al. (2014) researched the effect of the nanoparticles on a breast tumor culture (MCF7) and a lungs culture (A459), and in both cases the nanoparticles composed of 1% aloe vera gel and 5% curcumin showed 15% higher cytotoxicity than nanoparticles with conventional antitumor drugs. Some of the most important anticancer activities of curcumin are summarized in Table 1.

Table 1. The anticancer activities of curcumin (Bar-Sela et al., 2010)

Immunologic modulation
Radiosensitization and radioprotection
Chemopreventive, chemotherapeutic
Inhibition of angiogenesis and metastasis
Chemosensitizing activity
Inhibition of NF-κB
Downstream of NF-κB: Inhibition of cyclin D1
Downstream of NF-κB: Inhibition of COX-2
Downstream of NF-κB: Suppression of Bcl-2 and Bcl-XL
Inhibition of cytokines inhibits the pro-survival kinase Akt
Induction of phase II enzymes
Modulation of growth factors and their signaling pathway
Inhibition of STAT3 activation

Antimutagenic, antimicrobial and gastroprotective effect

Heterocyclic amines are formed during the processing of protein rich food at high temperatures and are known for their carcinogenic effect. The study was conducted in order to explain the antimutagenic properties of curcumin, and it showed that all three curcumins are very effective in preventing S9 mediated mutagenicity (> 80%) at a concentration of 200 μg. Unsaturation in the side chain, a methoxy group on the benzene ring, and a central b-diketone moiety in the curcumin molecule are responsible for its high antimutagenic potential (Shishu and Kaur, 2008).
Curcumin shows significant antimicrobial properties. Studies carried out on G + (*B. cereus and S. aureus*) and G - *(E. coli* and *Y. enterocolitica*) bacteria have shown that curcumin and curcumin β glycoside at the concentration of 0.2-0.7 μM, have a 100% inhibitory effect on the tested G + bacteria and *Y. enterocolitica*, while they have a slightly lower effect on the *E. coli* (70%) (Parvathy et al., 2009). Wang et al. (2016) examined the effect of curcumin on the development of pneumonia in mice caused by *Staphylococcus aureus* and showed that curcumin effectively blocks the development of pneumonia by binding to α-hemolysin (virulence factor and toxin secreted by *S. aureus*).
Curcumin is highly effective in suppressing *Helicobacter pylori* in the stomach. Research has shown that curcumin inhibits the shikimate path required for the synthesis of the bacteria aromatic ring, and therefore its growth. The histological analysis in the same study showed that curcumin also restores damaged cell walls of the stomach caused by *Helicobacter pylori* (De et al., 2009).

Conclusions

In the past 30 years, numerous studies of curcumins' effect on cell tissues and animals, as well as clinical studies, have shown its multiple medical benefits (more than 10,000 published papers in the last 10 years). Despite its very poor bioavailability and absorption in the body tissues, curcumin has been recognized as an important therapeutic natural product. The highest medical value of curcumin is its strong antioxidant effect and its binding to inflammatory transcription factors as to precursors whether chronic diseases or tumors. Despite the large number of papers, a very small number of clinical trials were conducted specifically on humans in order to fully confirm and prove its effectiveness.

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POLIFENOLI I FLAVONOIDI U MEDU

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Pregledni rad

Sažetak

Biljke proizvode sve biološki aktivne sastojke hrane. Sekundarne metabolite biljaka koji su odgovorni za ljekovito djelovanje kako u samim biljkama tako i drugim organizmima mogu proizvesti samo biljke. Ljekovitost biljaka je uglavnom zasnovana na djelovanju polifenola od kojih posebno mjesto zauzimaju flavonoidi. Pčele medarice (*Appis melifera*) putem nektara, polena i medljike donose u košnicu polifenole koji su široko distribuirani u biljnom svijetu. Iako je sadržaj polifenola u medu relativno mali oni su veoma zaslužni za njegove ljekovite osobine. Pčela ih u med ugrađuje u formi u kakvoj su ih biljke proizvele, u slobodnoj formi ili formi glikozida. Njihov broj i količina koji se mogu naći u medu varira u odnosu na čitav niz faktora kao što su kvaliteta pčelinje paše, sezona sakupljanja meda, geografsko područje i sl. Znanstveno je ustanovljeno da biljke proizvode nekoliko tisuća različitih polifenola, a da im je zajednička osobina sprječavanje nastajanja bolesti kako u samim biljkama tako u sisavcima i čovjeku. Osnovno djelovanje im se zasniva na sprječavanju nastajanja veoma reaktivnih slobodnih radikala. Kemijska struktura im je zasnovana na fenilpropanskom kosturu (C_6- C_3- C_6), a njihova biološka aktivnost na broju, poziciji i vrsti supstituenata. Većina dosadašnjih istraživanja su rađena na sadržaju ukupnih polifenola u medu i sadržaja samo manjeg broja flavonoida kao što flavonoli kvercetin i kamferol ili flavonon naringenin. Neki flavonoidi su označeni kao markeri određene sorte meda. Najveći broj ispitivanja sadržaja flavonoida je rađen u aromatičnim i ljekovitim biljkama, voću, povrću i čajevima. Zbog izuzetnog značaja ovih spojeva za živi svijet za očekivati je da će se njihova naučna istraživanja dodatno intenzivirati. Med kao poseban dar prirode oduvijek važi kao hrana i lijek, međutim njegova ljekovitost nije u potpunosti istražena, stoga je za očekivati da će se znanstvena istraživanja kvalitativnog i kvantitativnog sadržaja flavonoida u medu i drugim pčelinjim proizvodima nastaviti.

Ključne riječi: flavonoidi, polifenoli, antikosidansi, biljke, med

Uvod

Više biljke proizvode ogromne varijacije sekundarnih metabolita čija je funkcija da zaštite biljku od različitih stresova kao što su UV zračenje, napadi patogena, i herbivora. Mnogi od ovih spojeva su pokazali širok raspon bioloških učinaka u prevenciji od raka, dijabetesa, kardiovaskularnih, neurodegenerativnih i drugih bolesti (Rasupuleti i sur., 2016; Rasoli, 2011). Biološki aktivni spojevi koncentrirani su u pojedinim biljnim organima (list, cvijet, sjeme, korijen, plod). Primarni metaboliti utječu na strukturnu funkciju same biljke, dok sekundarni metaboliti utječu na međustanično funkcioniranje biljke i reprodukciju unutar biljke, a nastaju kao odgovor na biotički i abiotički stres (Rasoli, 2011). Polifenoli su sekundarni metaboliti biljaka u kojima imaju višestruku ulogu kao što su: senzorska svojstva, boja, aroma ili ukus, utječu na otpornost biljke prema bolestima i mikroorganizmima, neki polifenoli indirektno utječu na rast biljke i štite osjetljive stanične dijelove od štetnog UV zračenja.

Polifenoli predstavljaju široko rasprostranjenu heterogenu grupu sekundarnih biljnih metabolita i jednu od najvažnijih klasa prirodnih antioksidanasa. Generalno, pojam „flavonoid“ (latinski naziv flavus, što znači žuto) se koristi da opiše grupu prirodnih spojeva koji u svojoj strukturi imaju C6 – C3 – C6 vezu, odnosno kemijski rečeno fenilbenzopiransku strukturu. U odnosu na poziciju aromatskog prstena na benzopiranski ciklus prirodni flavonoidi se dijele u tri grupe: flavonoidi (2 – benzopirani), izoflavonoidi (3 – benzopirani) i neoflavonoidi (4 – benzopirani) (Grotewald, 2006). Flavonoidi su podijeljeni na nekoliko podgrupa: halkoni, flavani, flavoni, flavonoli, izoflavoni, flavanoni, flavanonoli, i antocijani, čije su osnovne strukturne formule dane na Slici 1 (Brand, 2010).

Raznovrsnost flavonoida kontroliraju geni biljke, ali i zrelost biljke, klima i način uzgoja. U prirodi se flavonoidi nalaze uglavnom u obliku glikozida, tj. povezani su s različitim molekulama šećera. Osim šećera, supstitucijske grupe koje se nalaze na osnovnoj jezgri su hidroksilna i metoksi grupa što pridonosi velikoj raznolikosti i velikom broju tih spojeva. Bogati izvori flavonoida su: voće i povrće, zeleni i crni čaj, čokolada, crna vina i bobičasto voće, međutim značajne količine se nalaze u cvijeću, čajevima, sjemenkama, medu i propolisu (Grotewald, 2006; Harborne i sur., 1999). Oksidativne procese u ljudskom organizmu

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pokreću slobodni radikali koji nastaju djelovanjem UV zračenja, metabolizma, radijacije, pušenja, polutanata i životne sredine, upalnih procesa u organizmu i drugo (Tapas i sur., 2008). Loš način prehrane može biti glavni uzročnik kroničnih bolesti kao što su srčani udar i rak. Međutim, teško je kazati točno koji su to "zdravi" sastojci hrane koje bi trebalo konzumirati kako bi se imala zdravstvena korist. Ipak, s velikom sigurnošću je utvrđeno da unos adekvatnih količina voća i povrća doprinosi prevenciji od nastajanja bolesti. Veliki broj istraživanja je potvrdio da flavonoidi imaju zaštitnu ulogu zdravlja kod sisavaca posebno djelujući antikancerogeno i antiteratogeno, zaštitu DNK, lipoproteina niske gustoće (LDL), nastajanje upala, inhibiciju nastajanja trombocita, estrogeni efekt i očuvanje receptora. S aspekta prehrane međutim do danas nije s potpunom sigurnošću utvrđena potpuna uloga flavonoida unatoč mnogim istraživanjima koja su do sada urađena (Rice-Evans i Packer, 2003).

Uloga i značaj polifenola

Polifenoli uključuju više tisuća spojeva različite kemijske strukture, od jednostavnih hidroksimetilnih kiselina i antocijana (biljni pigmenti) do složenijih flavonoida i tanina s jednim ili više hidroksiliranih benzenskih prstenova u svojim strukturama. S obzirom na veliku raznovrsnost njihova klasifikacija je veoma kompleksna, uglavnom je zasnovana na kemijskoj strukturi i biosintetskom porijeklu (Herken i sur., 2009; The national Honey Board, 2007; Kazazić, 2004). Pregled strukturnih klasa fenola od prostih do najkompleksnijih dan je u Tablici 1. Flavonoidi, uključujući flavone, izoflavone i antocijanidine nastaju kondenzacijom fenilpropanoida pri čemu prvo nastaju halkoni, a zatim dolazi do sinteze raznih strukturnih klasa. Proces sinteze flavonoida kataliziraju čitav niz biljnih enzima (Grotewold, 2006).

Ove supstance se mogu naći u slobodnom obliku ili češće u obliku glikozida ili u obliku kompleksa s drugim spojevima. Kao sastavne komponente hrane biljnog porijekla mnogi od njih do sada poznatih različitih flavonoida su dio redovne prehrane (Ferreira i sur., 2009; McKibben i sur., 2002). Flavonoidi su uključeni u fotosenzitizaciju, tranfer energije, djelovanje na hormone i regulatore rasta kod biljaka, kontrolu respiracije i fotosinteze, mofrologiju i spol biljaka (Falcone i sur., 2012). Oni su sastavni dio i ljudske i životinjske prehrane, ali se ne mogu sintetizirati u ljudima niti u životinjama (Keler, 2009). U posljednjih nekoliko godina znanstvenici su proveli opsežna istraživanja flavonoida i ustanovili njihove biološke učinke, kao što su antibakterijski, antifungalni, antivirusni, antitumorski i drugi (Tapas i sur., 2008).

Flavonoli i flavoni su od posebne važnosti jer je utvrđeno da posjeduju antioksidacijske i sposobnosti čišćenja slobodnih radikala. Flavonoidima se pripisuje svojstvo utjecaja na boju i ukus hrane (Ferreira i sur., 2009; McKibben i sur., 2002). Jedna od važnih karakteristika ovih spojeva je antioksidantna aktivnost, koja je prvenstveno rezultat njihove sposobnosti da budu donori vodikovih atoma i da kao takvi uklanjaju slobodne radikale, uz formiranje fenoksil radikala, koji je stabiliziran rezonancijom te postaje manje reaktivan (Kukrić i sur., 2013). Flavonoidi kao donori protona sprječavaju redukciju dehidroaskorbinske kiseline ili inhibiraju određene enzime i tako usporavaju proces razlaganja elastina (Gokce i Haznedaroglu, 2008).

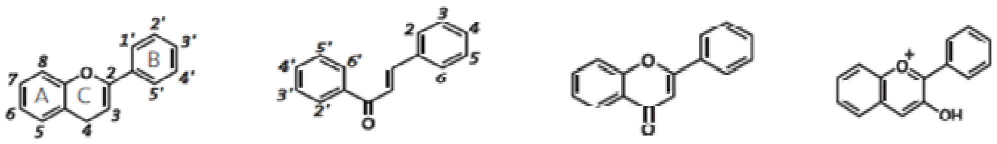
Tablica 1. Pregled strukturnih klasa fenola

Redni broj	Broj C atoma	C - skelet	Klase biljnih fenola
1.	6	C6	jednostavni fenoli
2.	7	C6-C1	hidroksibenzoati
3.	8	C6-C2	acetofenoni i fenilacetati
4.	9	C6-C3	hidroksicinamati, fenilpropeni,
5.	10	C6-C4	kumarini i hromoni
6.	13	C6-C1-C6	naftokinoni
7.	14	C6-C2-C6	ksantoni
8.	15	C6-C3-C6	stilbeni i antrahinoni
9.	18	(C6-C3)2	flavonoidi
10.	30	(C6-C3-C6)2	lignani
11.	n	(C6)n	bioflavonoidi i katehol melanini
12.	n	(C6-C3)n	lignini
13.	n	(C6-C3-C6)n	kondenzirani tanini

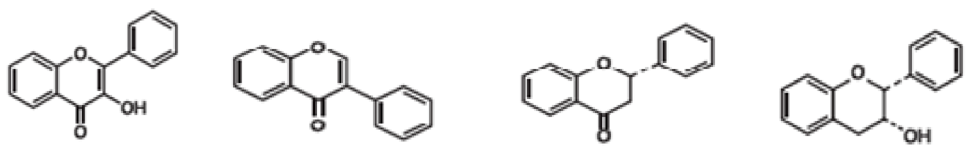
Fenolne kiseline i njihova aktivnost su već godinama predmet brojnih bioloških, kemijskih i medicinskih studija. Posjeduju snažnu antioksidativnu, antiinflamatornu, antikancerogenu i antimikrobnu aktivnost, a također su pokazale i pozitivan učinak u liječenju mnogih kardiovaskulranih bolesti i sprječavanju ateroskleroze (Hwang i sur., 2006; Mandal i sur, 2010). Za biološku aktivnost je zaslužan isključivo aglikonski dio molekula. Fenolni spojevi u biljkama nisu ravnomjerno raspoređeni na nivou tkiva, staničnoj i podstaničnoj razini. Netopljivi fenoli su sastavni dio stanične stijenke, dok se topljivi fenoli nalaze u staničnim vakuolama. Na nivou tkiva, površinski slojevi sadrže veći nivo fenola od onih koji se nalaze u njihovim središnjim dijelovima. Fenoli staničnog zida, kao što su lignini i hidroksicimetna kiselina, povezani su različitim staničnim komponentama. Ovi spojevi doprinose mehaničkoj otpornosti stanične stijenke, imaju regulatornu ulogu u rastu i morfogenezi biljke, kao i u reakciji na stres i patogene (Naczk, 2004).
Akumulacija polifenolnih spojeva varira i u zavisnosti od fiziološkog stanja biljke, kao rezultat ravnoteže između biosinteze i daljeg metabolizma (Harborne, 1994). U biljkama flavonoidi vrše redukciju reaktivnih spojeva kisika čime potpomažu redoks procese u stanicama. Međutim, flavonoidi također sudjeluju i u procesima prijenosa bola.
Kod biljaka vrše značajnu ulogu u procesu oplodnje kao npr., neke biljke su klijale usprkos nedostatku flavonoida, rasle i davale sjeme, međutim većini su bili potrebni flavonoidi za oplodnju i normalan razvoj polena. Utvrđeno je također da flavonoidi podešavaju transport auxina (Andersen i Markham, 2006).

Kemijska struktura

Osnovna kemijska struktura flavonoida se sastoji od 15 atoma ugljika koji su povezani u jezgru od tri fenolna prstena: A, B i C (Tapas i sur., 2008). Benzenski prsten A kondenziran je s tročlanim alifatskim nizom koji zajedno s kisikom tvori šesteročlani prsten C, a na poziciji 2 prstena C nalazi se benzenski prsten B (Slike 1 i 2). Za flavonoide koji imaju vezanu karbonilnu grupu na C-4 atomu prstena C često se koristi izraz 4-okso-flavonoidi (Slika 2b). Raznovrsnost klase flavonoida je bazirana na oksidaciji C – prstena. Samo određeni broj flavonoida daje boju biljkama zbog apsorpcije u vidljivom dijelu spektra (Davies, 2004).

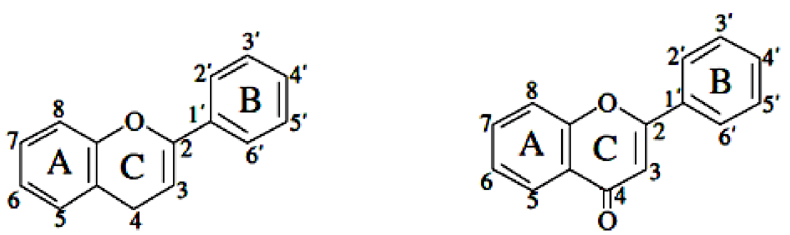


flavonoid halkon flavon antocijan



flavonol izoflavon flavanon flavanol

Slika 1. Osnovne strukture flavonoida i osnovne kemijske strukture različitih subklasa



(a) (b)

Slika 2. Osnovna struktura flavanoida: (a)-flavan jezgra, (b)-okso-flavonoid jezgra

S povećanjem broja hidroksilnih grupa povećava se antioksidantna aktivnost flavonoida (Marinova i Yanishlieva, 1992). Flavanoni i flavoni često se nalaze zajedno, npr. u južnom voću i povezani su sa specifičnim enzimima za razliku od flavona i flavonola koji se međusobno isključuju u mnogim biljkama.
Razlike između pojedinih flavonoidnih podgrupa proizlaze iz varijacija u broju i rasporedu hidroksilnih grupa, kao i iz prirode i stupnja njihove alkilacije i/ili glikozidacije. Najčešće se javljaju flavoni i flavonoli s hidroksilnim grupama u položajima 3'- i 4'- u prstenu B, a rjeđe oni s hidroksilnom grupom samo u položaju 4'-. Glikozidacija kod flavonoida događa se najčešće u položaju 3-, a manje u položaju 7-. Šećer koji se najčešće javlja je glukoza, no javljaju se i galaktoza, ramnoza i ksiloza. Flavonoidi koji su zastupljeni u hrani razlikuju se po položaju hidroksilnih, metoksi i glikozidnih grupa i u konjugaciji između prstena A i B. Flavonoidi u biljkama uglavnom su u obliku 3-O-glikozida ili polimera. Do polimerizacije flavanola u tanin i ostale složene spojeve dolazi npr. prilikom fermentacije enzimskom oksidacijom lišća zelenog u crni čaj. Tanini ili galotanini su esteri 3,4,5-trihidroksibenzojeve kiseline. Galoilna skupina tih tanina i monomernih katehina u zelenom čaju djelomično je odgovorna za svojstva helatiranja i hvatanja slobodnih radikala. Kondenzirani tanini ili proantocijanidini sastoje se od flavanolskih jedinica kojih može biti i do 17 u jednoj molekuli. Za ljudsku prehranu najvažniji od tih spojeva su procijanidini koji se sastoje od monomera (+)-katehina i (–)-epikatehina. (–)-Katehini i (+)-epikatehini su fitotoksični i neke ih biljke sintetiziraju u korijenu da bi spriječile naseljavanje drugih biljaka na tom teritoriju (Bais i sur., 2003). Flavonoidi daju uobičajene fenolne reakcije kao što su stvaranje intenzivno plavo-crnog kompleksa na tretman sa željezo (III) solima. Imaju izražena posebna svojstva kao što su sposobnost da taložе alkaloide i proteine. Zdravstveni učinak polifenola ovisi o konzumiranoj količini i njihovoj biodostupnosti (Pichichero, 2009). Danas su u značajnoj mjeri uspostavljene metode izolacije, identifikacije i kvantifikacije polifenola i drugih fitokemikalija (Ximing i Horward, 2012).

Zastupljenost pojedinih flavonoida u prehrani

Tokom redovne prehrane unos polifenola u ljudski organizam može biti i do nekoliko stotina miligrama na dan (Manach i sur., 2005). Polifenoli su vezani za procese fotosinteze u biljkama pa su prisutni u hrani biljnog porijekla, voću, povrću, sjemenkama, cvijeću, čaju, kavi, vinu, medu i propolisu (Lachman i sur., 2010; Grotewold, 2006). Flavonoidi su glavne obojene komponente cvijetova biljaka i obojene komponente u hrani (Keler, 2009). Brojna istraživanja potvrđuju da je koncentracija polifenolnih spojeva manja u zrelom plodu, osim kod crvenih plodova kod kojih se flavonoidi i antocijani akumuliraju na kraju sazrijevanja (Macheix i sur, 1990). U hrani se pojavljuju kao glikozidi ili esteri s drugim spojevima kao što su steroli, alkoholi, glukozidi i masne kiseline (Mandal i Chakraborty, 2010). Flavonoidi u hrani su uglavnom odgovorni za boju, okus, sprječavanje oksidacije masti i zaštitu vitamina i enzima (Yao i sur., 2004). U voću su najzastupljeniji katehini, flavonoli i proantocijanidini. Flavonoli su najobilniji flavonoidi u hrani od kojih su najzastupljeniji kvercetin, kaempferol i miricetin. Flavanoni su uglavnom nađeni u citrusnom voću, a flavoni u celeru. Katehini su prisutni u velikim količinama u zelenom i crnom čaju, i u crvenom vinu, dok se antocijanini mogu naći u jagodama i ostalom bobičastom voću. Izoflavoni se gotovo isključivo nalaze u hrani na bazi soje. Flavonske kiseline su uglavnom prisutne u cerealijama (pšenici, ječmu, raži), a posebno ferulna kiselina, esterifikovana s polisaharidima koji su sastavni dio stanične stijenke biljke (Cai i sur., 2001). U citrusima kao što su naranče i limun najzastupljeniji su flavoni i flavononi. Citrusni flavanoni nađeni su u narančama, grejpfrutu i limunu, među kojima naringenin u najvećoj količini. U nekom voću (npr. jabuke) flavonoli su primarno zastupljeni u kori pa se guljenjem njihova količina značajno smanjuje, međutim katehini se u najvećoj količini nalaze u svježem voću. Crni grejp je voće najbogatije sa katehinima, a zatim slijede jabuke. U manje značajnim količinama katehini se nalaze u stolnom voću, a u formi estera u manjim količinama se nalaze u bobičastom voću. Najveća količina kvercetina se nalazi u bazgi i brusnici. Bobičasto voće i ribizle sadrže najveće količine keamferola i mirecitina. Ova dva flavonoida su također detektirana i drugim vrstama voća, ali u veoma malim količinama. Veće količine su nađene u svježem voću. Utvrđeno je da masline sadrže značajne količine luteolina i apigenina (Andersen i Markham, 2006). Od povrća lukovi, kupusnjače, lisnato povrće i rajčica su najveći izvori flavonoida pogotovo kvercetina i keampferola. Flavonoidi su nađeni u celeru, slatkoj paprici i salati. Rajčica je jedino povrće koje može sadržavati flavanone naringenin i hesperetin. U crvenom luku je nađena najveća količina kvercetina od 95 mg/100 g. Pića uglavnom sadrže katehine, a njihov broj, vrsta i količina zavise od sirovine od koje su napravljeni.

Flavonoli kvercetin, keampferol i mirecitin su nađeni u čajevima. Proizvodi na bazi bilja, voća i povrća sadrže značajne količine flavonoida. Med sadrži male količine flavonola i flavanone, a od flavanone nađen je naringenin. Sadržaj flavonoida u aromatičnim biljkama nije dovoljno istražen. Sadržaj apigenina u peršinu je nađen u visokom iznosu (217.9 mg/100 g), a u kadulji i majčinoj dušici nađen je luteolin (39.5 mg/100 g) (Andersen i Markham, 2006). Biodostupnost raznih klasa polifenola i flavonoida je danas predmet mnogih istraživanja i većina od njih ukazuje na njihovu relativno slabu apsorpciju što zavisi od mnogih faktora kao što su molekulske težine, stupanj glikolizacije i hidrolizacije, metabolizma organizma i sl. (Surangi i Vasantha, 2013). U nekim istraživanjima kvercetin glukozid iz luka i jabuke su pokazali najbolju biodostupnost (Hollman i sur., 1997). Pokazalo se da stupanj apsorpcije flavonoida u probavnom sustavu zavisi i od aglikonskog dijela molekule. Tako je u slučaju kvercetina utvrđeno da njegov šećerni dio molekule igra veoma važnu ulogu u apsorpciji (Surangi i Vasantha, 2013).

Flavonoidi i fenolne kiseline u medu

Glavne grupe flavonoida nađenih u medu su flavoni, flavonoli i flavononi (Lachman i sur., 2010). Osim flavonoida med sadrži i druge fenole od kojih se najviše fenolne kiseline poput galne, kumarinske, kofeinske, elaginske i ferulične te njihovi esteri (Rasupuleti, i sur., 2016). Osim estera navedenih kiselina prisutni su i neki drugi kao npr. metil-vanilat, metil-siringat i metil-4-hidroksibenzoat koji su pronađeni u različitim vrstama meda poput bagremovog, kestenovog, jelinog, narančinog, suncokretovog i meda od uljane repice (Jaganathan i sar., 2010; Herken, 2009). Flavonoidi koji se najčešće nalaze u medu su pinocembrin, apigenin, kamferol, kvercetin, galangin, krisin, pinobanksin, luteolin i hesperitin (Turkmen i sur., 2006). Prisustvo navedenih flavonoida u medu prvenstveno ovisi o botaničkom podrijetlu, pa se pojedini flavonoidi označavaju i kao markeri botaničkog porijekla meda (Lachman i sur., 2010; Kaškoniene i sur., 2009). U desetine vrsta ispitivanih uzoraka meda utvrđeno je prisustvo flavonoida pinocembrina (Bogdanov, 1989). U nekim studijama, flavanon hesperetin je služio kao marker za tzv. citrusni med, flavonol kempferol za med ružmarina i kvercetin za med suncokreta. Ferreres i suradnici su HPLC analizom flavonoidnog profila 14 vrsta uniflornih i multiflornih medova odredili hesperetin kao marker za identifikaciju meda citrusa budući da je i identificiran samo u toj vrsti meda (Ferreres i sur., 1994). Isti autori su flavonoid miricetin predložili kao marker za med vrijeska te kempferol za med ružmarina (Tomas-Barberan i sur., 2001). Treba imati u vidu da se određene flavonoidne komponente koje ulaze u sastav propolisa (npr. pinobanksin, galangin, krisin, pinocembrin) ne mogu koristiti kao pokazatelji botaničkog porijekla jer njihova koncentracija u medu zavisi od kontaminacije meda propolisom (Tomas-Barberan i sur., 2001). U ispitivanjima flavonoida u medu često su identificirane i fenolne kiseline. Tako je elaginska kiselina identificirana kao mogući marker za med vrijeska (*Erica* sp.), dok je za med majčine dušice karakteristična prisutnost ružmarinske kiseline (Andrade i sur., 1997). S druge strane, apscisinska kiselina, koja se smatrala mogućim markerom meda od vrijeska, identificirana je i u medovima od repice, limete i bagrema. Također, moguću povezanost s botaničkim porijeklom meda pokazuju i derivati fenolnih kiselina prisutni u medu, ali to svakako treba dodatno ispitati (Tomas-Barberan i sur., 2001). Neke fenolne kiseline su označene kao markeri meda lijeske, hidroksicimetne (kofeinske, p-kumarinska i ferula kiselina kao markeri kestenovog meda (Jaganathan i sur., 2010; Herken, 2009). Ispitivanja sadržaja fenolnih spojeva i flavonoida u medu su pokazala da postoji korelacija s cvjetnim i geografskim porijeklom s jedne strane i antimikrobnim djelovanjem s druge strane (Pasupuleti i sur., 2016; Bertoncelj, 2008). Količine flavonoida u medu su veoma različite, ali mogu iznositi i do 6000 μg/kg, dok je njihov udio puno veći u polenu (0,5 %) i u propolisu (10 %) (Reshma, i sur., 2016; Youngsu, i sur., 2015; Anklam, 1998). Neki znanstvenici su ispitivali ukupni udio fenola (ne uključujući flavonoide) u 27 uzoraka različitih vrsta meda. Pri tom se udio fenola u ispitanim medovima kretao između 32,59 i 114,75 mg/100 g uz prosječnu vrijednost od 74,38 mg/100 g. Također se pokazalo da medljikovci imaju veći udio fenola nego cvjetni medovi (Meda i sur., 2005). Utvrđeno je da antioksidativni kapacitet medova je u značajnoj mjeri povezan s flavonoidima, ali da svakako ukupna antioksidacijska aktivnost meda zavisi kako od ukupnog sadržaja polifenola (Reshma i sur., 2016; Youngsu i sur., 2015) tako i svih drugih antioksidanasa (Mohamed i sur., 2010). Do danas su flavonoidi analizirani različitim kromatografskim tehnikama kao što su tankoslojna (TLC), gasna (GC) i tečna (HPLC), kapilarna elektro-foreza (CE), gasna kromatografija-spektrometrija masa (GCMS) i drugim (Pengpeng i sur., 2010). U današnje vrijeme HPLC - DAD je našla najveću primjenu u određivanju aktivnih sastojaka u biljkama, a samim tim i flavonoida (Pengpeng i sur., 2010; Makawi-

Alabedeen, 2009). Za flavonoide su karakteristične dvije UV apsorpcijske trake; traka 2, s maksimumom u intervalu od 240-285 nm, vjeruje se da nastaje iz (A) prstena, dok traka 1 s maksimumom u intervalu od 300-550 nm, vjerojatno nastaje iz (B) prstena. Kvantifikacija flavonoida je još jedna prednost HPLC metode sa UV detektorom. Procjena količine flavonoida u medu vrši se usporedbom rezultata kalibracija standarda flavonoida s rezultatima u uzorcima meda (Kaskoniene i Venskutonis, 2010). Neka kromatografska istraživanja su pokazala bitnu razliku u količini i vrsti flavonoida između pojedinih vrsta meda kao što su količine flavonoida kvercetina, naringenina i hesperetina (Kurtagić i sur., 2013; Čeksteryte i sur., 2006).

Zaključci

Imajući u vidu ogromnu rasprostranjenost polifenola i flavonoida u biljnom carstvu s pravom se može očekivati da se mnogi od njih mogu naći u medu. Kao što dosadašnja istraživanja pokazuju neki flavonoidi se već mogu koristiti kao markeri određenih vrsta meda, međutim jasno je da se radi samo o malom broju flavonoida. S obzirom na raznolikost uslova sakupljanja meda, pasmine pčela, sezone, meteorološke uslove i sl., može se pretpostaviti da od nekoliko tisuća flavonoida koje proizvode biljke i veliki broj vrsta pčelinjeg meda može se očekivati prisutnost polifenola i flavonoida u medu u različitim kvalitativno-kvantitativnim omjerima. Iz dostupne znanstvene literature može se zaključiti da je ova tema jako popularna na svim meridijanima svijeta međutim ni blizu nije dovoljno istražena.

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POLYPHENOLS AND FLAVONOIDS IN HONEY

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Review paper

Summary

The plant produces all biologically active ingredients of the food. Secondary metabolites of plants that are responsible for the beneficial effects in plants and other organisms but it can produce only plants. Therapeutic properties of plants is mainly based on the activity of polyphenol, of which special place occupy the flavonoids. Honeybees (*Appis melifera*) colecting nectar, pollen and honeydew brings in the hive poliphenols that are widely distributed in the plant world. Although the content of polyphenols in honey are relatively small, but they are very meritorious for its medicinal qualities. Bee embeds them in honey in the form of what they plant produced, in free form or the form of glycosides. Their number, and the amount of which can be found in honey varies in relation to the whole range of factors such as the quality of bee season, season of collecting honey, geographic area, etc. Science has found that plants produce several thousand different polyphenols and their common characteristics are preventing the emergence of a disease while in plants that in mammals and human. Their basic action is based on preventing the emergence of highly reactive free radicals. Their chemical structure is based on the phenylpropanoic structure (C_6-C_3-C_6) and their biological activity on the number, position and type of the substituents. Most of the results of the research were made on the content of total polyphenols in honey and the contents of only a small number of flavonoids like flavonols quercetin and kamferol or flavonon naringenin. Some flavonoids are marked as markers of a certain varieties of honey. The largest number of studies of the content of flavonoids was made in the aromatic and medicinal plants, fruits, vegetables and teas. Due to the exceptional importance of these compounds for the live it should be expected that their scientific research to further intensify. The honey as a special gift of nature has always been named as a food and medicine, however, his healing powers is not fully explored, it is therefore to be expected that there will be scientific research qualitative and the quantitative content of flavonoids in honey and other bee products will continue.

Keywords: flavonoids, polyphenols, antioxidants, plants, honey

PEDAGOŠKI ASPEKTI PRAVILNE PREHRANE I ZDRAVIH STILOVA ŽIVOTA U OSNOVNOŠKOLSKOM ODGOJU I OBRAZOVANJU

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Stručni rad

Sažetak

Uvod: Pedagoški rad, odnosno edukacija, u ustanovama odgoja i obrazovanja posebno u periodu ranog djetinjstva predstavlja temeljno djelovanje za razvoj pozitivnih navika i zdravih stilova života. Ranija pedagoška, a posebno longitudinalna istraživanja pokazala su kako predškolski odgoj, utjecaj obitelji i škole značajno doprinose razvoju higijenskih, zdravstvenih, ekoloških, kulturnih i emocionalnih navika kod djece. Smatra se da su predškolska i školska dob ujedno razdoblja koja stručnjaci definiraju kao vrijeme i pedagoško-odgojnu, obiteljsku i društvenu obavezu za prikladno započinjanje prevencije kroničnih bolesti odrasle dobi, u prvom redu kardiovaskularnih bolesti. Kroz pravilnu prehranu djeca zadovoljavaju svoje energetske potrebe, ali i unos vitamina i minerala. To znači da periodu njihovog razvoja treba prilagoditi prehranu, pa zato u vrtiće i škole treba uvesti promjene.
Cilj rada: Istražiti pedagoške aspekte utjecaja pravilne prehrane na razvoj pozitivnih navika kod djece u školama kroz zastupljenost takvih sadržaja u nastavnim programima osnovnog obrazovanja Tuzlanskog kantona.
Materijal i metode: Ovo presječno istraživanje provedeno je tijekom rujna 2015. godine na osnovu podataka dobivenih iz nastavnih planova i programa za osnovno obrazovanje na Tuzlanskom kantonu. Korištena je analitičko deskriptivna metoda i postupak analize sadržaja četiri nastavna predmeta: „Moja okolina", „Biologija", „Kemija" i „Kultura življenja". Ovi predmeti se uče na nivou druge i treće trijade osnovnog obrazovanja.
Zaključak: Rezultati istraživanja ukazuju da je u devetogodišnjem osnovnom obrazovanju obuhvaćeno jedanaest predmeta koji obrađuju ili u sebi imaju zastupljene sadržaje koji tretiraju prehranu i zdrave stilove života djece u osnovnom obrazovanju. Važno je osvijestiti da je potrebna veća zastupljenost ovakvih sadržaja kroz nastavne predmete, kao i kontinuirana edukacija odgajatelja i nastavnika u području nutricionizma i ekologije te kulture o zdravom životu i razvoju mladih. Kao mjeru prevencije, predlaže se da nadležne stručne pedagoške službe i ministarstva hitno učine promjene u pogledu sadržaja nastavnih programa osnovnog obrazovanja kroz model izborne nastave koja će predvidjeti prehranu i zdrave stilove života kao važnu pedagošku djelatnost za razvoj mladih.

Ključne riječi: pedagoški utjecaj, odgoj i obrazovanje, ekološke navike, curiculum, zdrav stil života

Uvod

Odgojno obrazovni rad u školi zauzima značajno mjesto u kognitivnom razvoju djeteta, a posebno u razvoju njegove ekološke svijesti u susretu s prirodom. Za normalan psihofizički, kognitivni i konativni razvoj djetetove osobnosti, neophodni su adekvatni faktori socijalizacije unutar obitelji, škole i sredine u kojoj dijete odrasta i provodi svoje slobodno vrijeme (Omerović i Čirić, 2014). Sredina u kojoj dijete boravi i s kojom se identificira u kulturološkim i drugim vrijednosnim obrascima, ispunjena je riziko faktorima koji se negativno odražavaju na sveukupni razvoj djetetove osobnosti. Utjecaj medija i novina direktno podstiče određene oblike ponašanja, koje dijete prihvaća kao adekvatne zbog nemogućnosti ispravnog rasuđivanja, a prateći razne emisije i sadržaje na elektronskim i printanim medijima. Nastavnim planom i programom u osnovnoj školi zastupljeni su sadržaji vezani za prehranu, odnosno namirnice koje čovjek treba da konzumira u procesu življenja. Nastavnim programima u osnovnoj školi utvrđeni su sadržaji prehrane i zdravog stila življenja. Anlizirajući nastavne planove i programe odnosno curriculum-e pojedinih nastavnih predmeta dolazi se do saznanja da su u određenoj mjeri zastupljeni sadržaji koji usmjeravaju djecu na važnost korištenja raznovrsne hrane. Za uspješan proces formiranja ličnosti važan je pedagoški utjecaj koji se odvija u školi, ali i obitelji. Važno mjesto u razvoju djeteta ima pedagoško djelovanje u školskoj zajednici. Zbog toga se mora posvetiti pažnja pedagogiji kao nauci koja proučava, istražuje i unapređuje odgoj i obrazovanje. "Suvremena pedagogija je znanost o suvremenom odgoju" (Vukasović, 1999).
Uloga nastavnika u pedagoškom procesu je veoma značajna, jer osim prenošenja činjenica nastavnik treba aktivirati i učenikovu kognitivnu i emotivnu stranu. Uključivanjem učenika u različite aktivnosti usmjerava ih se na samostalan rad s krajnjim ciljem osiguravanja bolje budućnosti. Proces ekološkog odgoja je dugoročan, on se mora provoditi od predškolskog do sveučilišnog obrazovanja. Posebno značajnu ulogu i

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utjecaj na zdravlje tokom cijelog života svakog pojedinca ima prehrana. Pojedini hranjivi sastojci su potrebni i važni ljudskom tijelu ukoliko ga želimo održati u zdravom stanju. Prehrana djece školske dobi mora biti blagovremena, pravilna i uravnotežena. Ukupna hrana koja se unosi u organizam mora biti u ravnoteži s tjelesnim potrebama za raznovrsnim hranjivim tvarima te sadržavati dovoljnu količinu kalorija, bjelančevina, ugljikohidrata, minerala, masti i vitamina (Paklarčić i sur., 2013).

Cilj rada

Cilj ovog rada jeste istražiti pedagoške aspekte utjecaja na razvoj pozitivnih navika kod djece u školama u pogledu zdravih stilova prehrane kroz zastupljenost takvih sadržaja u nastavnim programima osnovnog obrazovanja Tuzlanskog kantona. Polazna hipoteza bila je da se u nastavnom planu i programu osnovnog obrazovanja na području Tuzlanskog kantona u dovoljnoj mjeri posvećuje pažnja pitanjima prehrane, zdravlja, ekoloških navika i zdravih stilova života kroz programske sadržaje pojedinih nastavnih predmeta.

Metode rada

U ovom preglednom istraživanju je korištena analitičko-deskriptivna metoda. Osnovna pretraga obavljena je pretraživanjem nastavnog plana i programa za osnovno obrazovanje na području Tuzlanskog kantona, a temeljila se na sljedećim ključnim riječima u postavljenim sadržajima nastavnih predmeta: zdravi životni stilovi, prehrana, ekološke navike, ishrana čovjeka, higijenske navike, njega i bolesti. Pretraživanje je ograničeno isključivo na nastavni plan i program na području Tuzlanskog kantona. Za separaciju i razvrstavnje nastavnih predmeta i nastavnih programa korišten je postupak analize sadržaja pedagoške dokumentacije. Sadržajni kriterij za razvrstavanje radova, bila je kompatibilnost predmeta istraživanja s temama koje obuhvaćaju nastavni programi predmeta koji tretiraju ovu problematiku. Za potrebe pisanja rada, s aspekta osnovnog obrazovanja obuhvaćeno je ukupno 4 nastavna predmeta na nivou osnovnog obrazovanja koji su zadovoljili sadržajni kriterij s temom istraživanja:

- „Moja okolina“ (Livnjak, 2012; Mujakić i sur., 2009; Bijedić i Livnjak, 2014; Livnjak, 2014), koji se uči na nivou razredne nastave u prvoj trijadi osnovnog obrazovanja,
- „Biologija“ (Hadžihalilović i sur., 2010; Numić i Vidović, 2008; Begić i Hadžihalilović, 2012),
- „Kemija“ (Miličević i Music, 2012) i
- „Kultura življenja“ (Neidhart i Numić, 2015; Mujakić i sur., 2013) koji se uče na nivou druge i treće trijade osnovnog obrazovanja.

Korištena je i relevantna udžbenička literatura za elaboraciju navedenog problema istraživanja.

Rezultati istraživanja

Analizirajući programske i nastavne sadržaje prema Okvirnom nastavnom planu i programu za devetogodišnju osnovnu školu u Federaciji Bosne i Hercegovine (MONKSTK, 2016) za odabrane predmete, primjećuje se različitost u sadržaju i tjednom fondu sati te raspodjeli po razredima. Analizirano je koliko je akcenat na ekološki te odgojno kulturološki odnos prema prehrani i prehrambenim navikama odgajitelja unutar samih sadržaja posmatranih predmeta.
Analizom sadržaja predmeta koje se bave istraživanom problematikom vidljivo je u Tablici 1 da je u devetogodišnjem osnovnom obrazovanju obuhvaćeno jedanaest predmeta. Primjećuje se da je kroz svaku godinu školovanja zastupljen po najmanje jedan predmet koji uči u svom sadržaju ekološki odgoj, prehrambene navike, upoznavanje s biljnim svijetom i zaštitom životne sredine, prevenciju bolesti i kulturu zdravih navika življenja. Samom analizom sadržaja nastavnih jedinica vidljivo je da nije napravljen dobar balans u tematskim cjelinama. Važno je istaknuti veliku ulogu nastavnika (pogotovo u prvoj trijadi obrazovanja djeteta) da pravi korelaciju nastavnih sadržaja s ostalim predmetima koji se uče, te samoj nastavnikovoj kreativnosti, edukatorskoj sposobnosti i empatiji prema pozivu. Ukoliko nastavnik sam radi na osvještavanju zdravog života i sadržaj nastavne jedinice napravi na kreativan, interaktivan način i pretvori u poticanje istraživačkih sposobnosti onda se može reći da bi ovi nastavni sadržaji bili zadovoljavajući za odgajatelje.
U ovom istraživanju na osnovu prikazanih rezultata može se zaključiti da nastavni program za osnovno obrazovanje s nastavnim predmetima koje obuhvaća se vrlo malo bavi problematikom prehrane i zdravih stilova života. Tek četiri predmeta pokrivaju djelomično sadržaje koji su od vitalnog značaja za razvoj djece i mladih. Postavlja se pitanje da li ovakvo obrazovanje ima za cilj zdrav psihofizički razvoj osobnosti, s obzirom da ono što nudi kroz programske sadržaje vrlo malo posvećuje pažnju ovoj problematici.

Tablica 1. Sistematski pregled nastavnih predmeta po razredima, sadržaju i broju sati

Nastavni predmet	Razred	Sadržaj nastavnih predmeta	Tjedni fond sati po predmetu
Moja okolina	1	Svijest o mom tijelu, Higijena, Sredstva za higijenu Prehrana	2
Moja okolina	2	Higijena u školi Čuvanje zdravlja Svijest o mom tijelu Higijena Sredstva za higijenu Prehrana Značaj boravka u prirodi za zdravlje	3
Moja okolina	3	Značenje biljaka za ljudsku prehranu Osobna čistoća Čistoća i kultura stanovanja Zdravstvene ustanove Prehrana Duhan, alkohol i droga Zdrav okoliš	3
Moja okolina	4	Odmor i rekreacija kao higijenska potreba Uzročnici bolesti-neprijatelji zdravlja Kultura življenja	3
Priroda	5	Značaj biljaka za čovjeka Ekologija; očuvanje životne sredine	2
Kultura življenja	5	Bolesti uzrokovane nehigijenom (bolesti prljavih ruku i druge bolesti suvremenog svijeta) Hranjivi sastojci hrane – neophodni za rast, razvoj, rad i očuvanje zdravlja, pravilan raspored dnevnih obroka i njihova kalorijska vrijednost Prehrambene namirnice – vrste namirnica, njihova upotrebna i hranjiva vrijednost, higijena i pravilno čuvanje namirnica Kulinarski postupci pripreme hrane i kontrola kvaliteta hrane, ekološka proizvodnja hrane Bolesti uzrokovane nedostatkom hranjivih sastojaka u hrani, nedostatkom hrane, nepravilnom, prekomjernom i nehigijenskom prehranom	1
Biologija	6	Građa i funkcija biljaka Biosistematika biljaka Virusi Bakterije Značaj biljaka	1
Biologija	7	Značaj životinja za čovjeka Jestive životinje vodene i zračne sredine Korištenje u prehrani u izvanrednim uvjetima	2
Biologija	8	Ekologija i svakodnevni život čovjeka Značaj šume za čovjeka Osobine vode kao životne sredine Utjecaj čovjeka na prirodu Zagađivanje/onečišćenje i zaštita zraka, vode i zemljišta/tla	1
Kemija	9	Ugljikohidrati Alkoholi Masti i ulja Sapuni i deterdženti Aminokiseline i bjelančevine Enzimi	2
Biologija	9	Njega i bolesti organa za probavu Njega i bolesti organa za disanje Njega i bolesti organa za krvotok Njega i bolesti povrede kože Njega i bolesti živčanog sustava	2

Rasprava

Čovjek je dio prirode i kroz čitavu svoju povijest u snažnoj je vezi s prirodom. Značajan period formiranja i usvajanja ispravnih prehrambenih navika koje trebaju biti osnova dobrog zdravlja predstavlja predškolski period, osnovna i srednja škola. U školskom sustavu potrebna je permanentna edukacija nastavnika o potrebi realizacije sadržaja kulture življenja i prehrane djece. Važnu ulogu imaju nastavnici na satovima razredne nastave i na satovima izvannastavnih aktivnosti (sekcije). Nepotpuna ili nepravilna prehrana može nepovoljno utjecati na rast i razvoj djeteta. S druge strane, prekomjerno uzimanje hrane te nepravilan omjer prehrambenih tvari mogu utjecati na pojavu niza kroničnih bolesti u kasnijoj životnoj dobi. Pravilan odabir namirnica i pravilna prehrana mogu prevenirati mnoge zdravstvene probleme (Pandžić, 2015).

Djeca u predškolskoj dobi pokazuju veće ili manje sklonosti pojedinim jelima i načinima pripreme hrane. Prehrana predškolskog djeteta ne smije biti stihijska. Veoma je važno što i koliko djeca jedu? Pravilne prehrambene navike izgrađuju se u najranijem djetinjstvu (Jusufbegović, 2013). Obaveza je odraslih da planiraju i kontroliraju dječju prehranu i da je prilagode njihovim potrebama. Ukoliko se ustanove nepravilnosti u prehrani moraju se pažljivo i uporno otklanjati bez svađe i nasilja. Dječja prehrana treba osigurati dovoljno energije, hranjivih i zaštitnih tvari. Djeca također svakodnevno trebaju voće (Matek Saric i Knežević, 2016). Preporuka je da se bazira na žitaricama jer su one bogate složenim ugljikohidratima, koji ne dovode do naglog podizanja glukoze u krvi, već daju organizmu energiju za duži period. Uz tri glavna obroka predlažu se i dva ili tri manja međuobroka.Veoma je važno dan započeti doručkom, koji djeci osigura početnu dnevnu energiju jer doručak doprinosi raspoloženju, jačem imunološkom sustavu i boljem obavljanju dnevnih zadataka. Međuobrok bi se trebao sastojati od voća, povrća, proizvoda od cjelovitog zrna žitarica ili mliječnih proizvoda, a ne grickalica (smoki, čips, keksi), koje daju osjećaj sitosti, bogate su energijom, siromašne hranjivim tvarima, a mogu uzrokovati karijes i debljinu (Pavičić Žeželj, 2016).

Osnovni postulati pravilne prehrane uče se u petom razredu kroz predmet Kultura življenja s 1 satom tjedno. Kako se navodi u Usporednoj analizi nastavnog plana osnovnog obrazovanja i odgajanja u Crnoj Gori, zemljama regije i zemljama Europske Unije, predmet „Kultura življenja“ se ne nalazi u planu i programu zemalja u okruženju: Hrvatska, Srbija i Crna Gora. Tako se slični sadržaji npr. u Crnoj Gori, uče kroz predmet „Zdravi stilovi života“, u 8. i 9. razredu, kroz 2 sata tjedno, što je duplo više nego što je to u BiH (VCG, 2013).

U istoj analizi navodi se da je u Srbiji, Hrvatskoj i Sloveniji zadržan predmet „Domaćinstvo“ u okviru kojeg učenici stječu znanja vezana za pravilnu prehranu (VCG, 2013).

S druge strane, ekološki problemi svakim danom sve su više evidentni (Omerović i sur., 2015). Ekološki obrazovani ljudi trebaju razumjeti prirodni poredak, ekološki sustav, ali i da se obrazuju o ekonomiji, politici, zdravlju, poljoprivredi i kako rješavati probleme koji su sve više izraženi u suvremenom svijetu. Ciljevi ekološkog obrazovanja uključuju sve ono čime bi se poboljšao odnos unutar ekologije, odnos ljudi prema prirodi i odnos ljudi jednih prema drugima. On podrazumijeva promoviranje pozitivnih promjena u kvaliteti okoliša u zajednici, odnosno učenje školske djece, omladine i svih građana o značaju ekološkog djelovanja s obzirom na potrebe društva.

U osnovnim školama Tuzlanskog kantona ekologija se uči kroz osnovne predmete kao što su „Biologija“ i „Kemija“ te „Priroda“ i „Moja okolina“. Svi ovi predmeti su zastupljeni u jednakom omjeru kao i u zemljama u okruženju (VCG, 2013).

Kako bi se postigla potpuna kvaliteta i svrha u procesu učenja, nastavnik mora ulagati napore u kreiranje pozitivne pedagoške i socioemocionalne klime na nastavnom satu, sekcijama i drugim oblicima odgojno obrazovnih djelatnosti u školi. Danas škola nije ustanova gdje se kroz nastavni sadržaj samo prenosi znanje, već je to mjesto gdje se znanje stječe, proizvodi, zarađuje kroz aktivan zajednički rad nastavnika i učenika. Da bi postigli adekvatno elementarno obrazovanje, ono se mora provoditi kako u institucijama tako i izvan njih, a ti načini obrazovanja trebaju biti povezani, koordinirani i integralni, kako bi stečeno znanje obuhvaćalo što veći krug pojedinaca iz svakog socijalnog okruženja.

Obrazovanje o zdravim stilovima života prevenstveno treba započeti u okvirima obitelji, zatim preko medija (televizija, knjige, novine i sl.) ali i vršnjaka, a proširiti institucionalno počevši od predškolskog do sveučilišnog obrazovanja, a svoju ekspanziju nastaviti putem procesa cjeloživotnog obrazovanja, odnosno permanentnim učenjem kroz različite oblike aktivnog učešća u neformalnom i informalnom obrazovanju. Svako pedagoško nastojanje u odgojno obrazovnom radu utemeljeno je prema onome koga podučavamo, prema onome čemu podučavamo, kao i prema onome kako podučavamo (Slatina, 1998). Osjeti koja čovjek donosi rođenjem

uobličavaju se odgojem i obrazovanjem, koji omogućavaju da se čovjekove individualne snage dijelom potvrđuju, a dijelom razvijaju. Odgojem i obrazovanjem razvijaju se i duhovna osjetila (moralnost, volja, ljubav, zainteresiranost, mašta, itd.) (Slatina, 1998).
S aspekta ekološke pedagogije, treba skrenuti pažnju da se stalnom degradacijom životne sredine, ekspanzijom stanovništva i naseljenosti, te prekomjernim nagomilavanjem otpada koji se taloži oko nas, uključujući naš prostor življenja, ali i oceane, dovodi u pitanje naš vlastiti opstanak, kao i opstanak drugih živih bića oko nas. Ekološko obrazovanje propisano je u mnogim međunarodnim aktima, u vidu preporuka i obaveza koje proizilaze kao odgovorno ponašanje građana i države (Banović i Karadžin, 2012). Krajem 20.-og stoljeća čovječanstvo je poduzelo određene aktivnosti na zaštiti planeta Zemlje, što je rezultiralo donošenjem Programa o zemlji (Agenda 21) na konferenciji o okolišu i razvoju održanoj u Rio de Jeneiru 1992. godine. Ovim programom izražena je potreba za promjenom čovjekove svijesti o okolišu i planeti Zemlji i porastom pozitivne ekološke svijesti.
Odgojno obrazovni sustav zauzima značajno mjesto u formiranju ekološke svijesti, ekološke kulture i ekološke pismenosti kod djece, mladih, ali i odraslih. Zanimljivo je da u osnovnim školama Tuzlanskog kantona nema obveznog predmeta koji u svom središtu njeguje ekološke principe. Kada su u pitanju zemlje okruženja, ekologija se posebno nalazi u planu i programu u Crnoj Gori i Kosovu (VCG, 2013).
Kako bi čovjek opstao na planetu, a s obzirom na evidentan i sve prisutniji nemaran odnos, on brzo mora promijeniti svoje ponašanje i navike u suglasju s prirodom u čemu nastavnička uloga ima prvobitan značaj. Formiranjem ekološke kulture postiže se ekološka održivost u svim sferama društvenog razvoja čovječanstva. Ekološka kultura određuje odnos čovjeka i prirode kao pojedinca i kao zajednice, osigurava siguran i brižan način življenja u zdravom okruženju.

Zaključak

Rezultati ovog istraživanja ukazuju da u nastavnom programu za osnovni odgoj i obrazovanje koji je u primjeni na području Tuzlanskog kantona postoje zastupljeni sadržaji koji tretiraju prehranu i zdrave stilove života. Radi se uglavnom o sadržajima u okviru nastavnih predmeta „Moja okolina“, „Biologija“, „Kemija“ i „Kultura življenja“. Iako navedeni predmeti svojim sadržajima pokrivaju određene dijelove koji su bili predmet ovog istraživanja, na osnovu svega se može zaključiti da su cjelokupni sadržaji o prehrani, higijeni, ekološkim navikama i zdravim stilovima života vrlo skromni. Činjenica je da se u školama priča s djecom o pravilnoj prehrani, ali glavni problem je u tome što edukacija o pravilnoj prehrani nema svoj periodični niz i kontinuitet. Sigurno je da kultura prehrane polazi od obiteljskog stola, pa roditelji igraju jako važnu ulogu u formiranju prehrambenih navika koje škola ostvaruje i kasnije podstiče kao mjesto socijalizacije djece. Potrebno je da stručne službe Pedagoškog zavoda, katedre fakulteta i lokalna zajednica unaprijede sadržaje zdravog stila življenja. Rezultati istraživanja ukazuju na potrebu veće zastupljenosti sadržaja prehrane djece u NPP-u osnovnog obrazovanja, kao i kontinuirana edukacija odgajatelja i nastavnika u području nutrcionizma i ekologije, te kulture o zdravom životu i razvoju mladih.
Kao mjeru prevencije, predlaže se da nadležne stručne pedagoške službe i ministarstva hitno učine promjene u pogledu sadržaja nastavnih programa osnovnog obrazovanja kroz model izborne nastave koja će predvidjeti prehranu i zdrave stilove života kao važnu pedagošku djelatnost za razvoj mladih.

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PEDAGOGICAL ASPECTS OF HEALTHY EATING AND HEALTHY LIFESTYLES IN ELEMENTARY EDUACTION

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Professional paper

Summary

Introduction: Pedagogical work in educational and fostering institutions, especially in early childhood, is a basis for development of positive attitude and habits, as well as healthy lifestyle. Earlier pedagogical, and longitudinal research has shown that pre-school up-bringing, and influence of family and school environment significantly contribute to development of hygienic, health, ecological, cultural and emotional habits of pre-school aged children. It is defined by the experts that pre-school and elementary/middle school age would be the most appropriate timing, as well as educational and social obligation, for development of prevention of chronic diseases, such as cardiovascular disease. Through the appropriate nutritional habits, children will ensure intake of appropriate level of vitamines and mineral. This means that nutrition in kindergardens and schools should be adjusted to their needs, what is leading to necessary changes in nutrition plans.
Goal: To explore pedagogical aspects of an influence of appropriate nutrition on development of positive habits of children in school, through existance of such educational materials in educational school programs in Tuzla Canton.
Materials and methods: This research has been initiated in September 2015 using information from educational plans and programs for pre, elementary and middle schools in Tuzla Canton, by using analitical-descriptive method and analysis of four subjects: „My surroundings“, „Biology“, „Chemistry“, and „Culture of Living“. These subjects are learned in the second and third trimester of elementary education.
Conclusion: Research results are showing, that in 9-year elementary educaiton, there are eleven subjects that are includinng, or in some form contain elements that are relevant to proper nutrition and healthy lifestyles of young children. It is important to promote higher representation of such material through different subjects, as well as continuous education of teachers in fields of nutrition and ecology, healthy life and development of young population. As a preventive measure, it is suggested that an authorized professional pedagogical services and relevant ministries immediatelly implement necessary changes of educational material through the model of extracurricular activities that will include healthy nutritional habits and lifestyles, as an important pedagogical approach in fostering young population.

Keywords: pedagogical influence, fostering and education, ecological habits, curriculum, healthy lifestyle

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