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FOOD IN HEALTH AND DISEASE

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

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

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Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno-tehnološki fakultet Osijek, Osijek, Hrvatska
Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia

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BOTANICAL ORIGIN AND ANTIOXIDANT CAPACITY OF BEE POLLEN FROM EASTERN CROATIA

Blanka Bilić Rajs*, Ljiljana Primorac, Milica Cvijetić Stokanović, Ana Soldić, Ilijana Vukadin, Ivana Flanjak

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek,
Franje Kuhača 20, HR-31000 Osijek, Croatia

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Summary

Bee products are considered to be a good resource of bioactive substances such as flavonoids, phenolic acids or terpenoids. Bee pollen is collected and transported by the bees as granules or pollen-loads and reserved as nutrient resource for honeycomb. Because of its nutritional value and healthful properties, bee pollen is valuable product that can increase the beekeepers' income. In this work botanical origin and antioxidant capacity of bee pollen collected in eastern Croatia in April and May 2018 were examined. Botanical origin determined by palynological analysis showed that eight out of twelve analysed samples had > 45% of the pollen grains coming from one family while in one sample *Amorpha fruticosa* pollen grains dominated by 99%. Total phenolic content, total flavonoids and antioxidant capacity determined by the ferric reducing antioxidant power (FRAP) were determined spectrophotometrically. Total phenolic content varied between 7.08 – 15.27 mg GAE/g, total flavonoids were from 1.34 to 4.25 mg QE/g while FRAP value ranged from 51.97 to 83.56 μmol Fe^{2+}/g. The highest antioxidant capacity was determined in *Amorpha fructicosa* and *Salix* spp. unifloral bee pollen samples.

Keywords: botanical origin, bee pollen, antioxidant capacity, total flavonoids, total phenolic content

Introduction

The most famous products produced by the honey bees are honey and propolis. Honey bees produce also, not less valuable products, like pollen, beebread, royal jelly, known for their positive influence on human health (Kaškoninene et al., 2015). According to Campos et al. (2010) bee pollen is defined as a food, but due to small quantities that are generally consumed, it should be classified as functional food or a food supplement.

Honey bees collect pollen grains from the anthers of the flowers and on their way to comb the grains are sticked to the thorax hairs. With addition of the bee salvia, pollen is formed in the balls which are carried to the hive. Harvesting is done by using the pollen trap and the grains must be dried before human consumption (Coe, 2007).

With certain concentration of phytochemicals such as phytosterols and phenolic compounds, pollen could be considered as beneficial for human health. It has significant antioxidant activity that mostly depends on phenolic compounds but researches showed that there are large deviations due to different botanical and geographical origin of pollen grains (Aličić et al., 2014). Beside antioxidant activity recent studies showed that bee pollen has antibacterial properties against *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica* and *Escherichia coli* (Fatrcová-Šramková et al., 2013), proteasome activation properties (Graikou et al., 2011) and ant-inflammatory activity (Campos et al., 2010). Studies showed that some types of honey have antifungal activity like heather honey (*Erica* spp.), so it is to be expected that *Erica* spp. pollen also could possess this activity (Feás and Estevinho, 2011). Palynological origin of the bee pollen is factor that affects its chemical composition and therapeutic effect. Bee pollen sample is, in most cases, composed of different botanical origin pollen species. This diversity could be seen in research of Nogueira et al. (2012) where pollen species of *Boraginaceae*, *Cytisus* spp., *Castanea* spp., *Cistus* spp. and *Trifolium* spp. were the most frequent but none of the botanical families are represented in all the samples studied. Some authors detected predominant pollen type's characteristic for some country or region like in Lithuanian pollen (Čeksteryte et al., 2013). In Croatia, studies carried out on bee pollen are scarce. Taking into account that botanical origin and composition are connected with geographical region, this research investigated bee pollen from eastern Croatia from the aspect of botanical origin and antioxidant capacity.

Materials and methods

Twelve samples of bee pollen from eastern Croatia were collected in the beekeeping season 2018 in the period of April and May. After collection, the samples were frozen at -18 °C to preserve biological and chemical properties.

*Corresponding author: blanka.bilic@ptfos.hr

Botanical origin of bee pollen

The modified method of Barth et al. (2010) was used for microscopic slide preparation. Two grams of pollen loads mixture were weighted into a 12 mL centrifuge tube, mixed with 70% ethanol just to complete 10 mL. The mixture was vortexed and placed for five minutes in ultrasonic bath for 25 minutes. After 3 minutes centrifugation at 1500 rpm, sediment was resuspended with ethanol and the procedure was repeated. Solution of water and glycerol (1:1) was added to sediment in amount of 7 mL and left for 30 minutes. Solution was mixed and dissolved two times with same mixture of water and glycerol. Sediment was stirred with Pasteur pipette and spread on microscopic slide. Microscopic slide was covered with 22×22 mm cover slide and sealed. At least 500 pollen grains were counted in each microscopic slide and identified using a 400 × magnification. CMS Celle's Melissopalynological Collection (von der Ohe, K, 2003) and Ponet Pollen databank was used for botanical origin identification.

Bee pollen extracts

Ten grams of mixed bee pollen loads were weighted in 100 mL volumetric flask, filled with methanol to the label and ultra-sonicated for 60 minutes. Suspension was filtered and 100 mL volumetric flask was filled with methanol. Each sample was made in duplicate and stored at -18 °C before analyses.

Total phenolic content

The phenolic content was evaluated by the Folin-Ciocalteu method (FC) described by Singleton et al. (1999). Water in the amount of 6 mL and 0.5 mL FC reagent were added to 100 μL of the prepared sample solution. In the period of 1-8 minutes 1.5 mL of 20% Na_2CO_3 was added and 10 mL volumetric flask was filled with water and left to incubate for two hours in dark. The absorbance was measured at 760 nm and calibration curve was constructed by testing solutions of gallic acid in concentrations from 0.02 to 0.5 mg/mL (98%).

Flavonoid content

The content of total flavonoids was determined in accordance with the method described by Pascoal et al. (2014) with quercetin as a reference standard (Kim et al., 2003). An aliquot of pollen extract was mixed with 5% $NaNO_3$, 10% $AlCl_3$ and 1 M NaOH . The absorbance of the prepared solution was measured at 510 nm. The results were calculated from the equation of the calibration line made with different concentrations of quercetin solution (0.001 – 0.5 mg/mL).

Antioxidant activity by FRAP method

The antioxidant activity of the compounds present in the prepared pollen load solution was determined according to the method described by Benzie and Strain (1999.). FRAP reagent was made by mixing acetate buffer (pH=3.6, 300 mmol/L), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) reagent (98%) and $FeCl_3 \times 6\ H_2O$. The mixture containing 3 mL FRAP reagent and 0.1 mL of pollen extract was incubated for 5 minutes at 37 °C and afterwards the absorbance was measured at 593 nm. Calibration curve was made with solutions of $FeSO_4 \times 7\ H_2O$ (0.1 – 0.8 mmol/L).

General

For bee pollen botanical origin analysis an upright research microscope (B-800 Series; Optika Microscopes, Ponteranica, Italy) and a Sigma 2-16 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) were used. Methanol (Merck KGaA, Darmstadt, Germany) was used for preparation of bee pollen extracts.
Analyses of total phenolic content were conducted using the FC reagent (Reagecon, Shannon, Ireland), Na_2CO_3 (Panreac, Barcelona, Spain) and gallic acid (98%) (Sigma-Aldrich, Switzerland). For determination of flavonoid content $NaNO_3$ (Gram-mol, Zagreb, Croatia), $AlCl_3$ (Kemika, Zagreb, Croatia), NaOH (Gram-mol, Zagreb, Croatia) and quercetin (Sigma-Aldrich, Switzerland) were used. FRAP reagent for antioxidant activity was made of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) reagent (98%) (Sigma-Aldrich, Switzerland) and $FeCl_3$ x 6 H_2O (Riedel-de Haën, Germany). For calibration curve $FeSO_4$ x 7 H_2O (Kemika, Zagreb, Croatia) was used. UV-Vis spectrophotometer UV-1800 (Shimadzu Corp., Kyoto, Japan) was used in all analyses.

Results and discussion

The botanical origin shows the presence of different plant sources attended by the bees to produce the bee pollen. This description permitted to classify them as unifloral/ bifloral/ mixed multifloral bee pollen as in the Table 1. Eight out of twelve pollen samples correspond to unifloral samples according to predominant botanical species percentage (> 45%). Samples P4 and P7 had highest percentage of predominant pollen, especially P4 sample which was almost fully unifloral *Amorpha fructicosa* (Fig. 1) sample. *Brassica* spp., which is characteristic

botanical species for area of investigation in this study, was present in just three samples. The results indicate that *Asteraceae* and *Apiaceae* pollen type, which were present in majority of samples, together with *Amorpha fructicosa* and *Salix* spp. are important source of pollen collection in this area in the collection period.

Table 1. Botanical origin of collected bee pollen samples

Sample	Predominant pollen (>45%)		Secondary pollen (16-45%)	Important minor pollen (3-15%)	Minor pollen (<3%)	Classification
	Specie	%	Specie	Specie	Specie	
P1	*Apiaceae*	61.3	*Prunus* spp.	/	*Rubus* spp., *Papaver* spp., *Taraxacum* spp., *Castanea sativa* Mill., *Poaceae*, *Fraxinus* spp., *Juglans regia*, *Jasione montana*	Unifloral
P2	*Apiaceae*	59.5	*Prunus* spp.	*Humulus* spp.,	*Poaceae*, *Lotus* spp., *Fraxinus* spp., *Juglans regia*, *Brassica* spp.	Unifloral
P3	*Genista* spp.	51.4	/	*Rhamnaceae*, *Cornus sanguinea*, *Rubus* spp., *Prunus* spp., *Loranthus europaeus*, *Malus* spp., *Potentila* spp.	*Fraxinus* spp., *Bellis perenis*, *Campanulaceae*, *Violaceae*	Unifloral
P4	*Amorpha fructicosa*	99.0	/	/	*Cornus sanguinea*, *Juglans regia*	Unifloral
P5	*Salix* spp.	47.7	*Cerastium* spp.	*Prunus* spp., *Taraxacum* spp., *Rubus* spp., *Brassicaceae*, *Malus* spp.	*Poaceae*, *Castanea sativa* Mill., *Humulus* spp., *Pinus* spp., *Tilia* spp., *Bellis perenis*, *Trifolium* spp., *Taxus* spp., *Juglans regia*	Unifloral
P6	/	/	*Trifolium* spp., *Brassica* spp., *Apiaceae*	*Robinia pseudoacacia* L., *Prunus* spp., *Malus* spp., *Cornus sanguinea*, *Castanea sativa* Mill.	*Pinus* spp.	Multifloral
P7	*Amorpha fructicosa*	83.4	/	*Malus* spp., *Brassica* spp., *Prunus* spp.	*Trifolium* spp.	Unifloral
P8	/	/	*Asteraceae* type, *Helianthus annuus*, *Taraxacum* spp.	*Chicorium* spp.	*Zea mays*, *Carex* spp., *Iris pseudocorus*	Multifloral
P9	*Asteraceae* type	58.2	*Helianthus annuus*	*Taraxacum* spp., *Zeam mays*	*Apiaceae*, *Artemisia* spp.	Unifloral
P10	/	/	*Asteraceae t*ype, *Helianthus annuus*	*Taraxacum* spp., *Zeam mays*, *Trifolium* spp., *Cornus sanguinea*, *Artemisia* spp.	*Apiaceae*	Multifloral
P11	*Asteraceae* type	52.9		*Asteraceae* other, *Taraxacum* spp., *Bellis* spp., *Helianthus annuus*, *Trifolium* spp., *Euphorbia* spp.	*Poaceae*, *Apiaceae*, *Acacia* spp., *Cirsium* spp.	Unifloral
P12	/	/	*Tilia* spp.	*Bellis* spp., *Myrtaceae*, *Artemisia* spp., *Apiaceae*, *Amorpha fructicosa*, *Asteraceae* other, *Trifolium* spp., *Helianthus annuus*, *Prunus* spp., *Cichorium* spp.	*Cornus sanguinea*	Multifloral

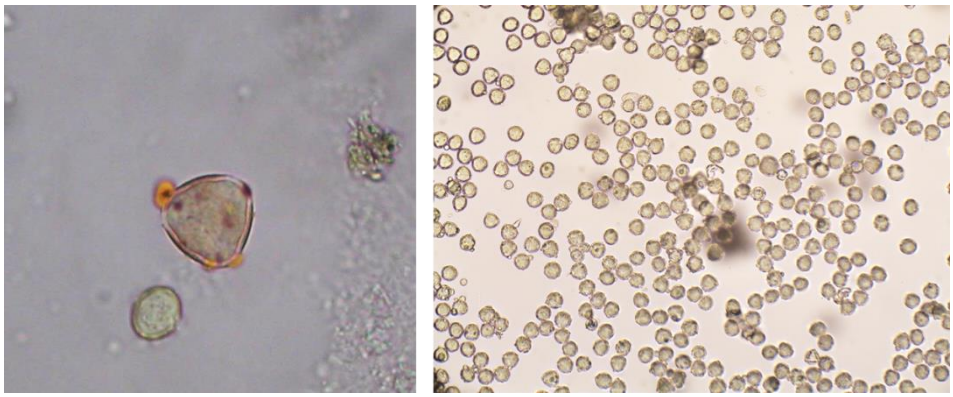

Fig. 1. Pollen grain of *Amorpha fructicosa* (800× magnification) and microscopic view of P4 sample (400× magnification)

Total phenolic content of analysed samples ranged between 7.08 – 15.27 mg GAE/g of pollen. Results were similar to those obtained by Kroyer and Hegedus (2001) and Mărghitaş et al. (2009) where values were between 7.4 – 9.7 GAE/g and 4.4 – 16.4 GAE/g were reported, respectively. In comparison to researches of Carpes et al. (2009), Araujo et al. (2017) and Velásquez et al. (2017) where results ranged between 19.28 – 48.90 GAE/g, 33.73 – 75.60 GAE/g and 6.86 – 52.99 GAE/kg, respectively, the results obtained in this study were lower. Velásquez et al. (2017) reported the highest values of total phenolic content in samples with predominant *Prunus* spp. pollen. Samples P1 and P2 with *Prunus* spp. as secondary pollen had also higher results for total phenolic content, but still significantlylower than those obtained in the above mentioned research. The highest total phenolic content had unifloral *Amorpha fructicosa* sample P7 (Table 2) while the lowest result was observed for P10 sample which was multifloral one.

Table 2. Average values and standard deviation of total phenolic content, total flavonoids and antioxidant capacity by FRAP determined in bee pollen samples

Sample	Total phenolic content [mg GAE/g]	Total flavonoids [mg QE/g]	FRAP [µmol Fe^{2+}/g]
P1	13.47	2.49	74.43
P2	12.45	2.06	60.42
P3	8.20	2.28	66.07
P4	12.62	2.30	60.18
P5	11.63	3.01	83.56
P6	12.84	1.77	67.86
P7	15.27	2.13	81.49
P8	12.35	3.58	54.67
P9	12.04	3.48	53.27
P10	7.08	3.39	51.97
P11	9.47	4.25	75.64
P12	8.79	1.34	57.57
Minimum	7.08	1.34	51.97
Maximum	15.27	4.25	83.56
Average	11.35	2.67	65.59
Standard deviation	2.43	0.86	11.03

Total flavonoid content ranged between 1.34 – 4.25 mg QE/g (Table 2). Mărghitaş et al. (2009), Araujo et al. (2017) and Carpes et al. (2009) reported higherresults than those obtained in this research (2.8 – 13.6 mg QE/g, 1.42 – 9.05 mg QE/g and 2.10 – 28.33 mg QE/g, respectively). The bee pollen of unifloral sample P11 (*Asteraceae* type) had the highest flavonoid content while the lowest value was again in mixed multifloral sample. Total flavonoid content of bee pollen was analyzed also by Feás et al. (2012) and Pascoal et al. (2014) but results were exspresed in mg catechin equivalent (CAE)/g of pollen, what enables the comparison of the results.

FRAP values ranged between 51.97 – 83.56 μmol Fe^{2+}/g (Table 2). The highest value was observed in unifloral *Salix* spp. sample (P5). Unifloral samples P7, P11 and P1 had also high FRAP values, while multifloral sample P10 had the lowest FRAP value as well as lowest total phenolic content. Other studies also showed correlation between total phenolic content and FRAP (Ulusoy and Kolayli, 2004; Borycka et al., 2016) thus the antioxidant power showed by bee pollen samples can be attributed to their phenolic content.

Conclusions

The obtained results indicate that bee pollen samples seem to be as different as honeys of different botanical origin. Due to the diversity and complexity of the bee pollen, future study should take into account more bee pollen samples for better understanding correlation between botanical origin and antioxidant properties of bee pollen. It will be interesting to broad the research with more parameters and samples from other regions of Croatia as well as from different collection periods.

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INHIBITION OF ALUMINIUM ALLOY CORROSION IN CHLORIDE SOLUTION BY CAFFEINE ISOLATED FROM BLACK TEA

Senka Gudić[1*], Marija Zlatunić[1], Ladislav Vrsalović[1], Ani Radonić[2]

[1]University of Split, Faculty of Chemistry and Technology, Department of Electrochemistry and Materials Protection, Ruđera Boškovića 35, HR-21000 Split, Croatia
[2]University of Split, Faculty of Chemistry and Technology, Department of Organic Chemistry, Ruđera Boškovića 35, HR-21000 Split, Croatia

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Summary

Caffeine (1,3,7-trimethylxanthine) was isolated from black tea and characterised using different physical methods. The corrosion inhibition performance of the caffeine isolate (in concentration from 1×10^{-5} to 1×10^{-3} mol/dm^3) on aluminium alloy corrosion in neutral 0.5 mol/dm^3 NaCl solution was investigated using potentiodynamic and linear polarization measurements at 20 °C. Corrosion potential, corrosion current and polarization resistance were determined and surface coverage of inhibitor molecules and inhibition efficiency were calculated. The obtained results show that caffeine effectively inhibited the corrosion reaction in the chloride solution with an inhibition efficiency of up to ≈ 76%. Furthermore, caffeine was found to function essentially as a mixed type with a higher influence on cathodic reaction. The adsorption behaviour of investigated inhibitor can be described by the Freundlich adsorption isotherm. The adsorption free energy closes to -10 kJ/mol indicates physical adsorption of the caffeine on the aluminium alloy surface in NaCl solution.

Keywords: aluminium alloy, corrosion, inhibition, caffeine

Introduction

The unique combinations of properties provided by aluminium and its alloys make aluminium one of the most versatile, economical, and attractive metallic materials for a broad range of uses from soft, highly ductile wrapping foil to the most demanding engineering applications. Aluminium alloys are in the second position in use as structural metals just behind the steels (Davis, 2001). The resistance of aluminium and its alloys against corrosion has been attributed to a rapidly formed surface oxide film. The major corrosion problem is the localised breakdown of the passive film in the presence of chloride ions, which leads to the initiation and growth of corrosion pits.

One of the common methods for the corrosion protection of aluminium is the use of organic inhibitors; however, widespread application of many commercial organic inhibitors has been hindered by cost and toxicity considerations. Accordingly, several studies have focussed on identifying effective, inexpensive and nontoxic alternatives. Some of such investigations have assessed the corrosion-inhibiting properties of natural products of plant origin, which have been found to generally exhibit good inhibition efficiencies (El-Etre et al., 2005; Raja, Sethuraman, 2008; Sengeetha et al., 2011). This area of research is significant because plant products are inexpensive, readily available and renewable sources of environmentally acceptable materials. This paper focuses on aluminium alloy corrosion inhibition in NaCl using caffeine (1,3,7-trimethylxanthine) isolated from black tea.

Black tea is usually obtained from the Assamese plant (*Camellia sinensis* subsp. *assamica*) and is additionally fermented, hence more oxidized and stronger in flavour than green or white tea (Finger et al., 1992; Fernández et al., 2000). Caffeine (Fig. 1) belongs to a class of methylxanthine alkaloids present in coffee, cocoa beans, cola nuts and tea leaves (Mumin et al., 2006). Caffeine is extensively used in the production of non-alcoholic beverages and pharmaceuticals because of its stimulating and muscle relaxing properties. Accordingly, the effect of caffeine on human health and behaviour has been relatively well documented (Smith, 2002; Glade, 2010). Studies on the adsorption and protective effect of commercially available caffeine on the corrosion of various metals and alloys in different aggressive solutions have shown that this organic compound has a considerable corrosion-inhibiting potential and, thus, deserves more in-depth investigation (Fallavena et al., 2006; Trindade, Goncalves, 2009; Gudić et al., 2014). Again, in line with current efforts at promoting the utilization of biomass resources for the reasons mentioned earlier, it is also necessary to similarly assess the corrosion-inhibiting efficacy of caffeine isolated from biomass extracts.

*Corresponding author: senka@ktf-split.hr

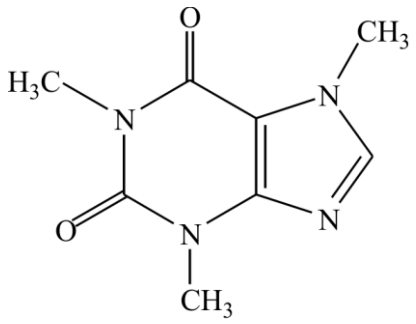

Fig. 1. Structural formula of caffeine

The present study evaluates the corrosion inhibition performance of caffeine isolated directly from black tea on AA 2017A aluminium alloy in neutral NaCl solution using potentiodynamic and linear polarization measurements.

Materials and methods

Materials preparation

The working electrode was made from AA 2017A aluminium alloy, which chemical composition was shown in Table 1. The electrical contact for the electrochemical measurements was achieved by soldering the aluminium alloy with the copper wire and then isolated them with acrylic resin leaving only one side exposed to the electrolyte.
Prior to electrochemical measurements the electrode surface (0.5 cm^2) was mechanically treated by grinding with different emery papers and polishing to a mirror finish, followed by the alkali pickling in 0.1 mol/dm^3 NaOH (T = 40 °C) for 1 min. The electrode was then rinsed in doubly distilled water. A new electrode surface was used for each run.

Table 1. Chemical composition of investigated AA 2017A aluminium alloy

element	wt. %	element	wt. %
Si	0.67709	Ni	0.00305
Fe	0.62080	Pb	0.02014
Cu	4.35373	Sn	0.00136
Mn	0.91555	Bi	0.00238
Mg	0.68498	Zr	0.12424
Zn	0.05766	Be	0.00014
Ti	0.03892	Al	92.4876
Cr	0.01238		

Isolation and characterization of caffeine

Caffeine isolation from black tea and its characterisation using different physical methods (including determination of melting point, thin layer chromatography, UV and Fourier transform infrared spectroscopy) are described in detail in previous work (Gudić et al., 2014). From the amount of 30 g of black tea (Franck, Zagreb, Croatia) 0.4332 g of caffeine was obtained.
The melting temperature was determined by differential scanning calorimetry (DSC) using a Differential Scanning Calorimeter (Mettler Toledo 823E). Thin layer chromatography (TLC) was performed on a commercial aluminium plate 20 × 20 cm coated with a 0.2-mm thin layer of silica gel and the crystalline caffeine using a 9.5:0.5, v:v mixture of chloroform and ethanol as mobile phase and visualized under a UV-lamp (λ = 254 nm). The UV-absorption spectrum of the extracted crystalline caffeine was obtained using a UV/VIS spectrophotometer (PerkinElmer Lambda EZ 201). The Fourier transform infrared (FTIR) spectrum of isolated caffeine was recorded on a Perkin Elmer FTIR spectrophotometer (Spectrum One) over a wave number range of 4000 – 650 cm^{-1} with a resolution of 4 cm^{-1} (Gudić et al., 2014).

Corrosion measurements

Electrochemical measurements were performed in a conventional three-electrode glass cell (equipped with saturated calomel electrode (SCE) as reference and platinum sheet as a counter electrode) using a potentiostat/galvanostat (PAR M273A) connected with personal computer. Measurements were performed in deaerated and stagnant 0.5 mol/dm^3 NaCl solutions at 20 ± 1 °C in which caffeine was added in concentrations from 1×10^{-5} to 1×10^{-3} mol/dm^3. Potentiodynamic current-potential curves were obtained by changing the electrode potential from –250 to +250 mV versus the open circuit potential, E_{OC}, with a scan rate of 0.5 mV/s. The polarization resistance, R_p, was determined from the slope of polarization curves obtained by measurements in the potential range ± 20 mV vs. E_{OC}, with scan rate of 0.2 mV/s.

Results and discussion

Characterization of caffeine

The analysis of the crystalline material isolated from black tea using the DSC, TLC, UV and FTIR

spectroscopy confirmed the presence of high purity caffeine.

The thermal behaviour of caffeine is presented in the DSC thermogram shown in Fig. 2 and reveals a sharp endothermic peak at 235 °C (Gudić et al., 2014).

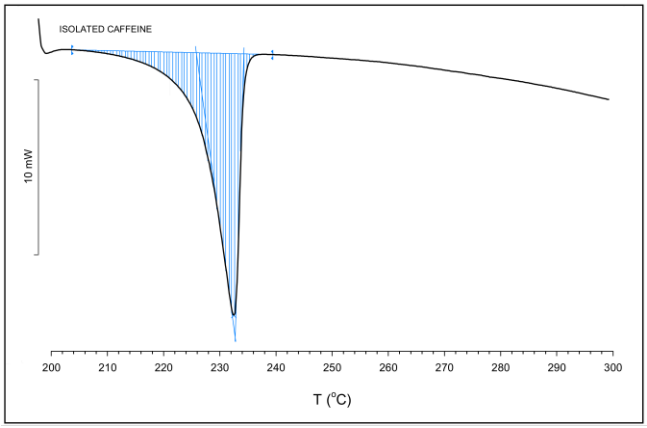

Fig. 2. DSC curve of caffeine isolated from black tea (Gudić et al., 2014)

For the TLC experiment, the plate, after evaporation of the solvent, was visualized under UV light (with λ = 254 nm) and revealed one brown spot. The R_f value of the isolated caffeine was found to be 0.63, which is consistent with the published data, and is very close to the R_f value of the reference sample of commercial caffeine (0.64) (Mumin et al., 2006). The characteristic UV absorption spectrum and absorption maximum (λ_{max}) were also employed in identification of the isolated compound. The λ_{max} was 272.9 nm for the isolate in water as solvent and 272.6 nm in ethanol as solvent, which is similar to the values reported in the literature for caffeine (Mumin et al., 2006). The λ_{max} values of the isolate also coincide with the values of the reference samples (commercial caffeine).

The FTIR spectrum of the isolated caffeine showed comparable absorption bands with that of standard caffeine (Fig. 3). The bands due to aromatic C-H stretching appear at 3112 cm^{-1} (correspond to C-H) and 2954 cm^{-1} (corresponds to C=H). The C=O stretching frequency appears at 1701 cm^{-1}. The band at 1658 cm^{-1} is due to C=C stretching. The band at 1239 cm^{-1} is attributed to C-N, while that at 1549 cm^{-1} is assigned to C=N (Gudić et al., 2014). The match between the spectra of the isolated caffeine and spectra from the base data was 97.2%. The above findings all point towards the purity of the caffeine as isolated from black tea.

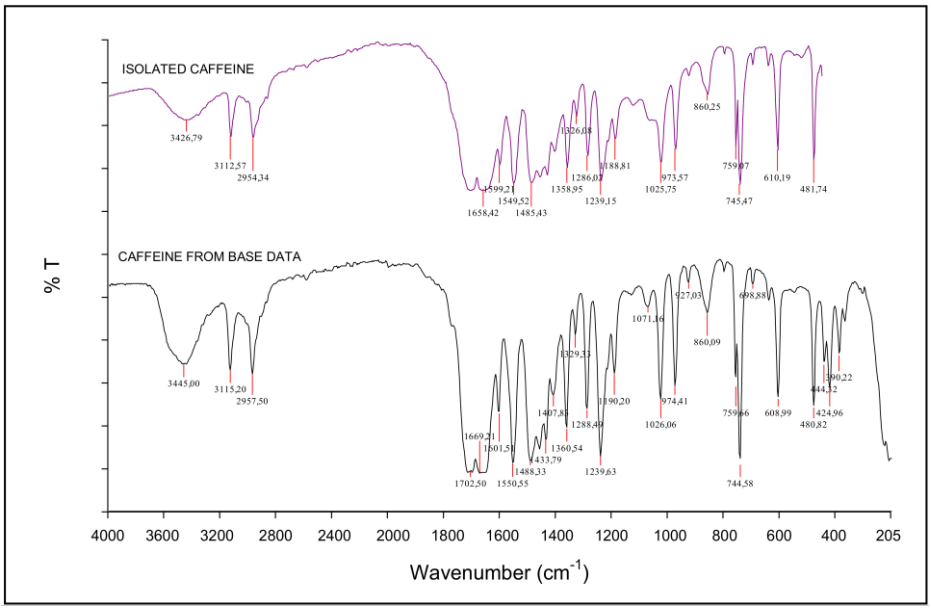

Fig. 3. FTIR spectra of caffeine isolated from black tea and caffeine from base data of instrument (Gudić et al., 2014)

Potentiodynamic polarization measurements

The nature of the corrosion inhibition process may be determined on the basis of polarization measurements. Thus, changes in the polarization curves after the addition of inhibitors typically are used as criteria for classification an inhibitor as cathodic, anodic or mixed type. Typical potentiodynamic polarization curves for AA 2017A aluminium alloy in NaCl solution in the absence and presence of the lowest and highest concentration of caffeine are shown in Fig. 4.

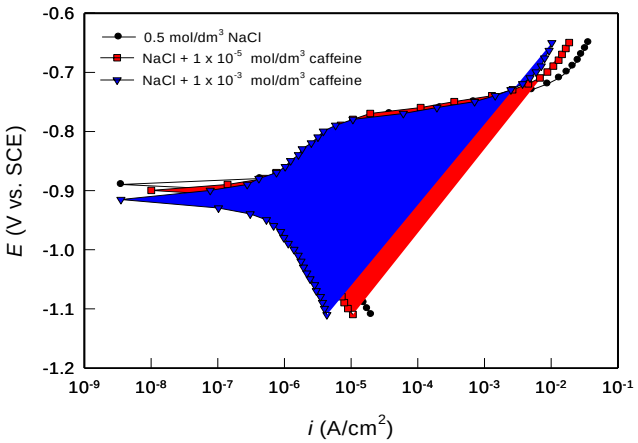

Fig. 4. Potentiodynamic polarization curves for AA 2017A alloy in NaCl solution in the absence and presence of lowest and highest concentration of caffeine

On the polarization curve for Al alloy in NaCl solution, a tight passive area is observed just above the corrosion potential in the potential range from ≈ -0.98 V to ≈ -0.80 V. In this potential area growth of the oxide layer takes place on the alloy surface. Anodic current slowly rises until a so-called breakdown potential is reached (≈ -0.77 V). Above breakdown potential a passive layer breakdown takes place due to the pitting corrosion and anodic current rises sharply. As the solution was deaerated by purging with argon, a cathodic part of the polarization curves probably represent hydrogen evolution reaction.

The presence of caffeine causes significant changes in the polarization behaviour of the Al alloy. The reduction of the anodic and cathodic current density along with the slight changes the values of corrosion potential in the cathodic side can be observed. Decreasing the cathodic current density along with the changes of corrosion potential in cathodic direction are characteristic of cathodic corrosion inhibitors, while decreasing the anodic current density along with the changes of corrosion potential in positive direction is characteristic of anodic corrosion inhibitors. Mixed inhibitors act by reducing both the cathodic and anodic current densities without any significant changes of corrosion potential values. Generally, an inhibitor can be classified as cathodic or anodic type if the shift of corrosion potential in the presence of the inhibitor is more than 85 mV with respect to corrosion potential in the absence of the inhibitor (Tao et al., 2009).

According to the observed changes in polarization behaviour of AA 2017A alloy, it could be said that caffeine belongs to a mixed type of inhibitor, with increased influence on cathodic reaction. Table 2 shows the electrochemical polarization parameters for AA 2017A alloy in NaCl solution in the presence and absence of coffeine. The parameters include the corrosion potential (E_{corr}), corrosion current density (i_{corr}), as well as inhibition efficiency (η) which were determined using the relation (1):

$$\eta = \frac{i_{corr} - (i_{corr})_{inh}}{i_{corr}} \times 100 \qquad (1)$$

where i_{corr} and $(i_{corr})_{inh}$ represent corrosion current in the absence and presence of inhibitor.

Inhibitor action can be explained by simple adsorption on the electrode surface and by blocking active sites on the surface, which leads to a reduction of corrosion. In general, the adsorption processes in the metal/solution interface resulting in separation of the solute in solution (inhibitor), and its concentration on the metal surface. This process takes place until the establishment of the dynamic equilibrium between the concentration of the residual solute in the solution and its concentration on the metal surface. Thus, in the adsorption process metal surface is covering with inhibitor which slows down the corrosion of metal. According to the Table 2 it can be seen that the inhibition efficiency increased with caffeine concentration.

Table 2. Corrosion parameters for AA 2017A aluminium alloy in 0.5 mol/dm^3 NaCl solution in the absence and presence of caffeine

$c_{caffeine}$ (mol/dm^3)	E_{corr} (mV)	i_{corr} (μA/cm^2)	η (%)
0	-889.0	1.44	
1×10^{-5}	-899.5	0.64	55.90
1×10^{-3}	-915.0	0.35	75.98

Linear polarization method measurements

By linear polarization method (polarization resistance determination method), the influence of caffeine on the inhibition of corrosion of AA 2017A alloys in NaCl solution was investigated in detail. Fig. 5 shows the linear parts of the polarization curves for the alloy in all tested solutions. The addition of caffeine changes the slope of the linear part of polarization curves, i.e. lead to a rise of curve slopes. The polarization resistance (R_p) can be defined by equation:

$$R_p = \frac{\Delta E}{\Delta i} \qquad (2)$$

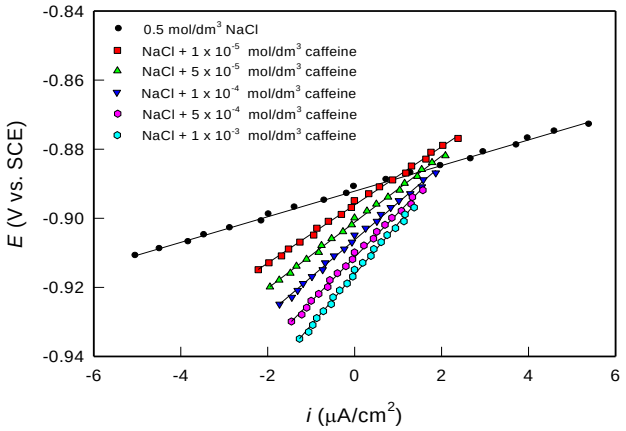

Fig. 5. Linear polarization curves for polarization resistance determination of AA 2017A alloy in NaCl solution in the absence and presence different concentration of caffeine

Thus, increasing the slope of the linear part of polarization curve indicates an increase in the value of polarization resistance. Since the electrochemical theory assumes that $1/R_p$ is directly proportional to the corrosion rate, the surface coverage (θ) and inhibition efficiency (η) was calculated according to equation 3:

$$\eta = \theta \times 100 = \left(\frac{(R_p)_{inh} - R_p}{(R_p)_{inh}} \right) \times 100 \quad (3)$$

where R_p and $(R_p)_{inh}$ represent the values of polarization resistance in the absence and presence of inhibitor respectively. The values of polarization resistance, surface coverage and the inhibition efficiency for AA 2017A alloy in investigated caffeine solution were shown in Table 3. Polarization resistance and corrosion inhibition efficiency increase with increasing caffeine concentrations.

Table 3. Corrosion parameters for AA 2017A in 0.5 mol/dm^3 NaCl solution in the presence of different concentration of caffeine, determined by linear polarization method

$c_{caffeine}$ (mol/dm^3)	R_p (kΩ cm^2)	Θ	η (%)
0	3.83		
1×10^{-5}	8.70	0.5597	55.97
5×10^{-5}	9.99	0.6166	61.66
1×10^{-4}	10.89	0.6484	64.84
5×10^{-4}	12.94	0.7040	70.40
1×10^{-3}	14.85	0.7420	74.20

Using the linear polarization method, the influence of the stabilization time (up to 4 hours) on the inhibition of AA 2017A alloy corrosion with the highest concentration of caffeine (1×10^{-3} mol/dm^3) was investigated and the results obtained were shown in Fig. 6. As can be seen, the polarization resistance increases with the stabilization time of alloy on E_{OC}, which means that the protective properties of the surface film are improved.

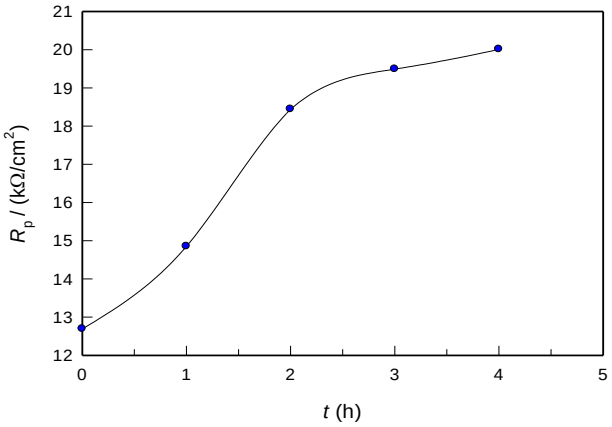

Fig 6. Change of polarization resistance of AA 2017A alloy with stabilization time in 0.5 mol/dm^3 NaCl + 1×10^{-3} mol/dm^3 caffeine solution

According to the obtained results the mechanism of caffeine action on AA 2017A alloy corrosion can be explained by the formation of the 3D surface layer consisting of inhibitor and products of corrosion. The formed surface layer acts as a physical barrier and prevents direct contact between metal and aggressive medium, and thus metal corrosion. Protective properties of the surface film are better if the inhibitor concentration is higher and the exposure time is longer.

Adsorption of caffeine

Adsorption of caffeine on the AA 2017A alloy surface was further characterised by fitting the experimental data to several adsorption isotherms. The Freundlich's isotherm isotherm (Eq. 4) was found to most suitably describe the adsorption behaviour of caffeine on AA 2017A alloy:

$$Kc^n = \theta; \qquad 0 < n < 1 \qquad (4)$$

were K is the equilibrium adsorption constant. The relation between the equilibrium adsorption constant, K, and free energy of adsorption ΔG°_{ads}, is given by:

$$K = \frac{1}{c_{solvent}} \exp\left(\frac{-\Delta G^\circ_{ads}}{RT} \right) \qquad (5)$$

where $c_{solvent}$ represents the molar concentration of the solvent, which in the case of water is 55.5 mol/dm^3, R is the universal gas constant, and T is the absolute temperature. The Freundlich's isotherm can also be written in the form of:

$$\ln\theta = \ln K + n\ln c \qquad (6)$$

Accordingly, a linear relationship can be obtained when ln θ is plotted as a function of ln c, where a ordinate intercept represents ln K (Fig. 7).

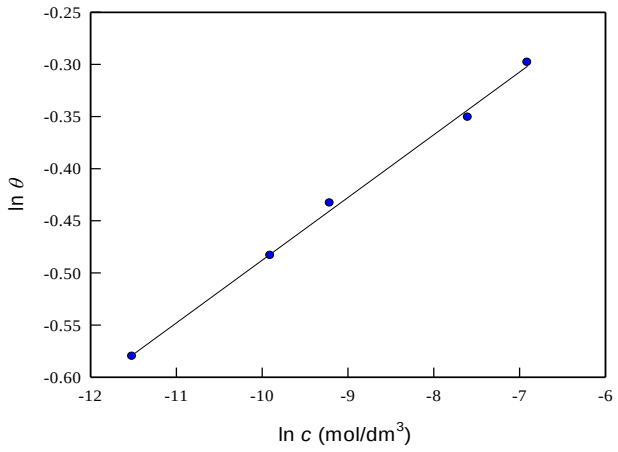

Fig. 7. The Freundlich adsorption isotherms of caffeine adsorption onto AA 2017A alloy surface in 0.5 mol/dm^3 NaCl solution determined by linear polarization measurements

The free energy of adsorption was calculated from experimental data and found to be equal to -9.93 kJ/mol. It is well known that values of ΔG°_{ads} in the order of -20 kJ/mol or lower indicate a physisorption, while those of order of -40 kJ/mol or higher involve charge sharing or charge transfer from the inhibitor molecules to the metal surface to form a coordinate type of bond (chemisorption) (Donahue, Nobe, 1965). The adsorption free energy closes to -10 kJ/mol indicates physical adsorption of the caffeine on the AA 2017A alloy surface in NaCl solution.

Conclusions

The presence of caffeine causes significant changes in the polarization behaviour of the AA 2017A aluminium alloy. The reduction of the anodic and cathodic current density along with the slight changes the values of corrosion potential in the cathodic side can be observed. It can be said that the caffeine belongs to a mixed type of inhibitor, with higher influence on cathodic reaction.
Inhibition efficiency increased with caffeine concentration and the highest value of ≈ 76% was obtained with 10^{-3} mol/dm^3 caffeine.
The adsorption behaviour of investigated inhibitor can be described by the Freundlich adsorption isotherm. The adsorption free energy closes to -10 kJ/mol indicates physical adsorption of the caffeine on the AA 2017A aluminium alloy surface in NaCl solution.
The mechanism of caffeine action on AA 2017A alloy corrosion can be explained by the formation of the 3D surface layer consisting of inhibitor and products of corrosion. The formed surface layer acts as a physical barrier and prevents direct contact between metal and aggressive medium, and thus metal corrosion. Protective properties of the surface film are better if the inhibitor concentration is higher and the exposure time is longer.

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THE IMPACT OF EDUCATION ABOUT SPECIFIC COOKING METHODS ON SERUM POTASSIUM LEVELS IN PATIENTS ON HEMODIALYSIS

Ivica Vrdoljak[1], Anja Vukomanović[2], Martina Bituh[2], Ivana Rumora Samarin[2], Ines Panjkota Krbavčić[2*]

[1]Clinical Hospital Center Rijeka, Krešimirova 42, HR-51000 Rijeka, Croatia
[2]University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Food Quality Control, Pierottijeva 6, HR-10000 Zagreb, Croatia

original scientific paper

Summary

Progression of chronic kidney disease often results with developing hyperkalemia; the increased serum level of potassium, which causes cardiac, neuromuscular and gastrointestinal complications. Hyperkalemia is generally associated with cardiac arhythmias and higher risk of mortality in patients on hemodialysis. The aim was to determine the impact of education on potassium control among patients on hemodialysis, while basing additional education on potassium-reducing techniques during food preparation and applying diet prepared accordingly to learned techniques. Participants were 47 patients on hemodialysis divided in control (n=22) and intervention (n=25) groups. All participants were educated by trained dietitian and received materials about proper nutrition at the beginning of the 1-year longitudinal study. The intervention group was educated additionally on potassium-reducing food preparation techniques. While both groups received two hospital meals per day during hemodialysis, meals for the intervention group were prepared accordingly to suggested food preparation techniques. Biochemical parameters were monitored during the study according to standard methods. The results showed that there was significant change in reduction of serum levels of potassium in intervention group compared to control group after one year of the study ($p=0.037$). Also, monthly serum levels of potassium were significantly reduced ($p<0.05$), compared to baseline of the study, during first 8 months in the control group and during all 12 months in the intervention group. Education about food preparation, proper diet alterations and its implementation can be useful in decreasing serum potassium levels and preventing hyperkalemia in patients on hemodialysis.

Keywords: hemodialysis, hyperkalemia, potassium, cooking methods

Introduction

Chronic kidney disease is characterized by imbalance of electrolyte levels, which can cause many health complications. One of the progression results of chronic kidney disease is hyperkalemia, or increased serum level of potassium, with high prevalence among dialysis patients (Einhorn et al., 2009; Kutlugun et al., 2017). It is estimated that there is approximatelly 50 mmol/kg of total body potassium, of which 2% is extracelullar, so the smallest changes in its serum level can cause homeostasis disorder (Putcha and Allon, 2007). Fluctuations in serum potassium level lead to serious health problems, such as neuromuscular (Kes, 2001; Montague et al., 2008) and gastrointestinal complications (Kes, 2001). Also, hyperkalemia is closely associated with cardiac arhythmias (Kes, 2001; Bleyer et al., 2006; Montague et al., 2008) and consequently higher risk of mortality in patients on hemodialysis (Noori et al., 2010; Pani et al., 2014; Yusuf et al., 2016). Reference range for an adequate serum potassium level is 3.9 - 5.1 mmol/L, while concentrations over the higher range value are considered increased (Flegar - Meštrić et al., 2000). This emphasizes the importance of potassium regulation, which depends on renal secretion, intracellular and extracellular distribution, and dietary intake (Brown, 1986). Dietary intake of potassium is the factor that can be controlled by dietitians and patients themselves and correlates with higher 5-year mortality (Noori et al., 2010).

Control over fluid and electrolyte levels in patients on hemodialysis is one of the main goals in maintaining patients health status. Recommendations for daily dietary intake of potassium for patients on hemodialysis are 1500 - 2700 mg (Fouque, 2003).

For managing disease, patients with chronic kidney disease require effort of medical stuff and education about adjusting their eating habits according to appropriate recommendations and hemodialysis treatments frequency (Jahanpeyma et al., 2017). Education of patients on hemodialysis is usually based on limitations, in this case on limiting foodstuffs naturally high in potassium or in which high concentration of potassium is a result of industrial process. Published studies proved that potassium content in food can be reduced by certain techniques during food preparation (Yaseen, 1993; Burrowes and Ramer, 2008; Bethke and Jansky, 2008; Cubadda et al., 2009). For example, it can be reduced by double boiling, (boiling, changing water and boiling food again) (Burrowes and Ramer, 2008). The potassium content in pasta can be

*Corresponding author: ipanjkota@pbf.hr

decreased by boiling up to 32%-39% of the baseline value (Yaseen, 1993) or even up to 70% (Cubadda et al., 2009). In potatoes, reduction can be achieved by boiling thin potato slices instead of dices (Bethke and Jansky, 2008). Therefore, these cooking techniques could be applied while preparing meals for patients on hemodialysis with the presumption that it may result by reduction of serum potassium level.

The aim of this study was to determine the impact of education, potassium-reducing food preparation techniques and application of accordingly prepared diet on potassium control among patients on hemodialysis.

Materials and methods

Participants and education

The one-year study was conducted at General Hospital „Dr. Josip Benčević" in Slavonski brod during 2016. The study included 47 participants, of which 28 men and 19 women, aged 61 to 67 years. All participants were patients with chronic kidney disease who underwent hemodialysis treatment three times per week in time period from 3 to 9 years. Duration of hemodialysis was 4-5 hours and it was performed by using bicarbonate solution on high- and low-permeability polysulfonate dialyzers with standard flow rates of blood and dialysate. The dialysate contained 0.5 mmol/L magnesium, 2.0 mmol/L potassium and 1.25 - 1.50 mmol/L calcium.

Participants were randomly divided in two groups, control group (n=22) and intervention group (n=25). At the beginning of the study, all participants finished an educational program for hemodialysis patients, which lasted for three days and was held by trained dietitian. Participants got information about recommended nutrition for patients on hemodialysis and were advised about their serum potassium level control, primarily by avoiding foods naturally rich in potassium. Written educational materials with menu samples and recommendations on nutrition and potassium intake were provided for all participants.

In addition to standard education, intervention group received instructions for preparing and thermaly processing foods at home on its own by techniques which significantly reduce potassium food content. Additional education was held in one-on-one sessions with trained dietitian for 15-20 minutes. It was provided once per week during the first two months of the study and later on participants requests during the one-year period of the study.

Table 1. Example of a standard hospital menu for patients on hemodialysis and an optimized menu according to changes in nutritive content considering thermal processing

	Menu – fresh ingredients	Menu – standard processing	Menu - optimized
Breakfast	Bread, white (100 g)		
	Fruit tea		
	Honey (20 g)		
	Butter (20 g) Marmelade (20g)		
	Cottage cheese (60g)		
	Apple (100 g)		
Lunch	Vegetable soup (200 mL)		
	Fresh beef round (100 g)	Cooked beef round (100 g)	Stewed beef round (meat soaked in water 1 h before thermal processing) (100 g)
	Sunflower oil (10 g)		
	Salt (1 g)		
	Fresh potato (200 g)	Steamed potatoes (160g)	Potatoes boiled in water (160 g) (potatoes soaked in water 1 h before thermal processing)
	Olive oil (10 g)		
	Salt (1 g)		
	Fresh carrot (100 g)	Fresh carrot boiled in water (100 g)	Stewed fresh carrot (100 g)
	Red onion (10 g) White onion (1 g)		
	Olive oil (5 g) Salt (1 g)		
	Orange (100g)		
Dinner	Turkey fillet (60 g)	Roasted turkey fillet (60 g)	Stewed turkey fillet (meat soaked in water 1 h before thermal processing) (100 g)
	Sunflower oil (10 g)		
	Salt (1g)		
	Rice (60 g)	Cooked rice (60 g)	Cooked rice (60g)
	Red onion (10 g)		
	Sunflower oil (10 g), Salt (1g)		
	Freezed broccoli (100 g)	Frozen broccoli boiled in water (100 g)	Stewed frozen broccoli (100 g)
	Oil (10 g)		
	Salt (1g)		
	Yogurt with probiotics (100 g)		

Table 1. Cont.

	Nutritional value of fresh foodstuffs from standard nutritional tables	Nutritional values based on standard thermal processing	Nutritional values based on optimized thermal processing
Energy (kcal)	1832	1826	1828
Protein (g)	66.9	65.3	65.8
Change in protein content during thermal processing of foodstuffs (%)	/	-2.4	-1.64
Potassium (mg)	3012	2635	2068
Change in potassium content during thermal processing of foodstuffs (%)	/	-12.5	-31.3

Participants in both groups received two hospital meals per day during hemodialysis. The control group received meals prepared by following standard recommendations for hemodialysis patients, while the intervention group received meals prepared accordingly to suggested techniques for lowering potassium food concentration.

Computer software (Prehrana, Infosistem d.d., Zagreb, Croatia) was used for designing standard hospital menu for patients on hemodialysis and optimized menu, with adjusted cooking methods for increasing potassium loses during foodstuff preparation. All standard menus were generated according to standard processing methods and optimized menus according to potassium-reducing techniques during food preparation (example Table 1). Literature references were used to modify food composition while designing menus and calculating food potassium loses: concentration of potassium in peas could be reduced 44% and in beans 60% (Rickman et al., 2007) by boiling; boiling diced potatoes could reduce its potassium content by 50% (Bethke and Jansky, 2008), while steaming potatoes reduces it by 10% (Vrdoljak, 2010).

The study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the ethics committee. All participants signed a written informed consent before the start of the study.

Biochemical methods

Blood samples from each participant were collected at the baseline of the study and once a month during the one-year period of the study. Analysis of the samples were carried out at the Biochemical laboratory of the General Hospital „Dr. Josip Benčević" in Slavonski Brod. Standard biochemical method for assessing blood levels of potassium was used (Flegar-Meštrić et al., 2000).

Statistical analysis

Descriptive statistical methods were used in describing data. Normally distributed variables were expressed as average values and standard deviations, while not normally distributed ones were expressed as median (interquantile range). Intergroup differences were estimated by using the χ^2 test and Fischer's exact test. Intragroup differences were estimated by using Friedman's or Wilcoxon test. For data analysis *Excel 2013* (Microsoft, Seattle, WA, USA) and *SPSS* statistical package (version 17.0, SPSS Inc., Chicago, IL, USA) were used. Differences were considered significant when $p<0.05$.

Results and discussion

There were 47 participants included in this study, of which 28 (59.6%) men and 21 (44.7%) women. Participants were randomly divided in control group (n=22; 46.8%) and intervention group (n=25; 53.2%). There was no significant difference in age between these two groups (p=0.084) (Table 2). The most participants (44.7%) were aged 65-75 years.

Median of duration of hemodialysis for all participants was 5 years, without significant difference (p=0.441) between control and intervention groups (Table 2).

During one year period, standard menus were applied on the control group and optimised menus on the intervention group. Considering thermal processing of foodstuffs in both menues, it was showed that potassium content in the standard menu could be reduced by 12.5% and by 31% in the optimized menu (example Table 1). Also, it was showed that potassium concentration in food prepared for patients on hemodialysis can be decreased with minimum food protein changes (example Table 1). Preserving the protein intake is an important factor in nutrition of

hemodialysis patients, since studies proved connection between low protein intake and increased risk of mortalily in this population (Shinaberger et al., 2006). Also, the ability to incorporate foodstuffs, which patients on hemodialysis usually avoid, brings variety and enriches their diet.

Table 2. Characteristics of participants according to groups, age and duration of hemodialysis

	Median (interquantile range)			p*
	Control group (n=22)	Intervention group (n=25)	All participants	
Age (years)	68 (60 – 73)	73 (62 – 77)	69 (61 – 76)	0.084
Duration of hemodialysis (years)	4 (2.5 – 8)	5 (3 – 10)	5 (3 – 9)	0.441

*Mann Whitney test

At the baseline of the study, there was no significant difference observed in serum potassium levels between control (5.9 mmol/L [5.4 - 6.5]) and intervention (6.2 mmol/L [5.6 - 6.7]) groups, but significant intergroup differences were found at 1, 3, 4, 10 and 11 months ($p<0.05$), where serum potassium levels in the intervention group were lower than in the control group (Table 3). During the one-year period, serum potassium levels decreased in both groups; mean reduction of serum potassium levels was -0.3 mmol/L [-0.8 do 0] in the control group and -0.7 mmol/L [-1.1 do -0.2] in the intervention group. The average change in serum potassium levels between groups was significant ($p=0.037$). Taking into consideration the reference range for serum potassium level, median of monthly values for both groups were showing that patients were in chronic hyperkalemia, except for months 3, 4 and 5 for patients in the intervention group. Despite of decrease, the control group stayed in chronic hyperkalemia with median values of serum potassium levels do not descending below 5.2 mmol/L (Table 3). Another study (Kovesdy et al., 2007) also showed frequency of hyperkalemia among hemodialysis patients. Three-year cohort study conducted on 81,013 hemodialysis patients associated serum potassium levels between 4.6-5.3 mmol/L with greatest survival among patients on hemodialysis, while increased mortality was associated with serum potassium levels <4.0 and ≥5.3 mmol/L. The same study conducted additional biochemical measurments on 74,219 patients on hemodialysis, which showed that 12.5% of them had an average serum potassium level 5.5 mmol/L or higher measured during three months (Kovesdy et al., 2007).

Table 3. Average serum potassium levels at the baseline and during the study compared between grups

Potassium*	Median (interquantile range)			p**
	Control group (n=22)	Intervention group (n=25)	All participants	
Baseline	5.9 (5.4 - 6.5)	6.2 (5.6 - 6.7)	6.1 (5.4 - 6.6)	0.529
Month 1	5.7 (5.3 - 6.2)	5.2 (4.6 - 5.5)	5.4 (4.8 - 5.8)	**0.005**
Month 2	5.5 (5.1 - 5.8)	5.2 (4.8 - 5.7)	5.3 (4.9 - 5.7)	0.253
Month 3	5.5 (4.9 - 5.9)	5.0 (4.7 - 5.3)	5.2 (4.7 - 5.7)	**0.041**
Month 4	5.6 (5.0 - 5.8)	5.0 (4.6 - 5.7)	5.3 (4.9 - 5.7)	**0.049**
Month 5	5.4 (5.0 - 5.8)	5.0 (4.7 - 5.8)	5.3 (4.8 - 5.8)	0.109
Month 6	5.4 (5.0 - 5.9)	5.3 (4.7 - 5.9)	5.4 (4.8 - 5.9)	0.509
Month 7	5.2 (4.9 - 5.9)	5.5 (5.0 - 5.9)	5.3 (4.9 - 5.8)	0.369
Month 8	5.4 (5.0 - 6.1)	5.5 (4.9 - 5.8)	5.4 (5.0 - 5.9)	0.991
Month 9	5.8 (5.4 - 6.4)	5.8 (5.2 - 6.4)	5.8 (5.3 - 6.4)	0.593
Month 10	6.0 (5.5 - 6.6)	5.5 (5.0 - 5.9)	5.7 (5.2 - 6.2)	**0.018**
Month 11	6.0 (5.5 - 6.4)	5.4 (4.8 - 6.1)	5.8 (5.3 - 6.3)	**0.044**
Month 12	5.8 (5.3 - 6.4)	5.5 (5.0 - 6.1)	5.6 (5.1 - 6.1)	0.138

*reference range is 3.9-5.1 mmol/L for both men and women
**Mann Whitney test (significance at $p<0.05$); significant results are bolded

Monthly serum levels of potassium for control and intervention groups were compared with data from the baseline of the study, provided in Table 3. Data analysis showed that serum potassium levels were significantly

reduced ($p<0.05$) in the control group in the first 8 months and in all 12 months of the study in the intervention group (Table 4). Nutritional education had the impact on decreasing dietary potassium intake in both groups. Greater changes in potassium intake were seen from results of the intervention group compared to the control, that can be explained by providing additional education on potassium-reducing techniques during food preparation.

Table 4. Significance (p-values) between the baseline and serum potassium levels during the study

PB* vs. month	p**	
	Control group (n=22)	Intervention group (n=25)
PB vs. month 1	**0.044**	**<0.001**
PB vs. month 2	**0.002**	**<0.001**
PB vs. month 3	**0.001**	**<0.001**
PB vs. month 4	**0.003**	**<0.001**
PB vs. month 5	**0.012**	**<0.001**
PB vs. month 6	**<0.001**	**<0.001**
PB vs. month 7	**<0.001**	**0.002**
PB vs. month 8	**0.023**	**0.002**
PB vs. month 9	0.455	**0.013**
PB vs. month 10	0.897	**<0.001**
PB vs. month 11	0.638	**0.008**
PB vs. month 12	0.432	**0.002**

*PB - potassium baseline

**Wilcoxon test (significance at $p<0.05$); significant results are bolded

Study conducted on 23 patients on hemodialysis showed that six-month implementation program has positive impact on changing dietary habits, resulting with significant decrease in phosphorous, potassium and sodium intake and improving nutritional status of patients (Yang et al., 2003). Another study conducted on 30 hemodialysis patients also showed positive results after short education, resulting with signifficant serum potassium level decrease (Jahanpeyma et al., 2017). Also, study showed that education on food preparation methods resulted in decreasing serum phosphate levels in patients on hemodialysis (Vrdoljak et al., 2017).

Despite the fact that dialytic therapies make the foundation in treating hemodialysis patients, dietary strategies must not be ignored because recommended and individually adjusted nutrition can help to mantain chronic kidney disease and avoid hyperkalemia (Kovesdy et al., 2007; Pani et al., 2014).

Despite given instructions for potassium reducing techniques in food preparation at home, one of the limitations of this study was the inability to monitor adherence of its use. Although participants confirmed adherence during consultations with dietitian and monthly blood control showed reduction in potassium levels in the intervention group, its hard to distinguish to what extent preparation at home contributed to its reduction. Therefore, further studies could be focused on exploring this limitation.

Conclusions

Patients with chronic kidney disease are specific population with demanding dietary recommendations. Hyperkalemia is a common disorder in patients on hemodialysis, which can be influenced by proper diet. Additional education about specific cooking methods proved beneficial for hemodialysis population, as the change of serum potassium levels in the intervention group was significant. Moreover, the role of trained dietitian mantaining patients with chronic kidney disease in hospitals should be unquestionable. Education about food preparation, proper diet alterations and its implementation can be useful in decreasing serum potassium levels and preventing hyperkalemia in patients on hemodialysis.

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FORMULATION OF SUNFLOWER AND FLAXSEED OIL BLENDS RICH IN OMEGA 3 FATTY ACIDS

Tanja Lužaić*, Ranko Romanić, Snežana Kravić, Bojana Radić

University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

professional paper

Summary

The recommendations of the World Health Organization (WHO) are aimed at increasing the consumption of foods rich in omega 3 fatty acids. The recommended ratio of omega 6 and omega 3 fatty acids in diet is 4-10 : 1, which allows muscle building, hormone production, reduces risk of the cardiovascular diseases, reduces blood pressure, triglyceride concentrations, improves brain functions, mood, intelligence etc. However, just some foods (eg. flaxseed oil, fish oils etc.) are rich in these compounds. The aim of this study was to examine the possibility of enriching refined sunflower oil (RSO) with omega 3 fatty acids by adding cold pressed flaxseed oil (CPFO). Samples: refined sunflower oil, cold pressed flaxseed oil, their blends in the mass ratio 70:30, 50:50 and 30:70 were analised, and the obtained results were compared with the one comertial vegetable oil blend present on the market of R. Serbia. The content of saturated fatty acids in analysed samples ranged from 9.63 to 10.3 2%, monounsaturated from 17.15 to 30.44 % and polyunsaturated from 59.78 to 73.15 %. The ratio of omega 6 : omega 3 fatty acids found in the samples was between 853 : 1 and 0.33 : 1.

Keywords: oil blends, flaxseed oil, omega 6 : omega 3 ratio

Introduction

A large number of health problems are closely related to inadequate composition of fatty acids in the diet. Essential fatty acids are polyunsaturated fatty acids with 18, 20 and 22 carbon atoms and containing 2 to 6 double bonds. All double bonds have *cis* konfiguration. Humans can not synthesize fatty acids which in alkyl chain have double bonds after 10th carbon atom. Deficit of essential fatty acids in human body is clinicaly exposed as disturbance of growth and skin changes. Brain is particulaty sensitive on a lack of essential fatty acids (Dimic, 2005).

Omega 3 fatty acids protect from cardovascular diseases through the modification of cell membrans' phospolipids and helpig the creation of eicosanoids (Nair et al., 1997). Having a diet rich in omega 3 prevents from inflammatory reactions (Serraino and Thompson, 1991), atherosclerosis and high blood pressure (Rotondo, 1995; Harris, 1997), also strengthens immunity, even helps with autoimmune diseases (Parbtani and Clark, 1995).

From the nutritional point of view, the ratio of essential omega 6 and omega 3 fatty acids is very important, and recommended healty ratio ranges from 4 : 1 to 10 : 1 (Lepsanovic and Lepsanovic, 2000). The most represented oil in the Serbian market is sunflower oil because of the availibility of the raw material and its price. Sunflower oil is source of omega 6 fatty acids and it doesn't contain omega 3 fatty acids. On the other hand, flaxseed oil is rich in omega 3 fatty acids and with affordable price. Flaxseed oil differs from other commercial oils due to the very high contribution of ALA (alpha - linolenic fatty acid), which is usually in concetration above 50% (Przybylski, 2005). Due to the high content of this "unique" fatty acid, flaxseed oil is often used as a dietary supplement, in cases where it is necessary to enrich diet with omega-3 fatty acids such as alpha-linoleic fatty acid. Flaxseed oil contains small amounts of saturated fatty acids compared to soy and sunflower oil (Przybylski, 2005; Shukla et al., 2002).

The way to get to the recommended healty ratio of omega 3 and 6 fatty acids is to make blends of oils rich in those acids. Mostafa et al. (2013) investigated seven blends formulated from flaxseed, olive and canola oil. Blends were different in content of omega 3 to 6 and omega 9 to 6 to 3 fatty acids ratio. The aim of this study is to examine the possibility of enriching refined sunflower oil with flaxseed oil. The mathematical model of the dependence of the share of flaxseed oil in the blend and the ratio of omega 6 and omega 3 fatty acids will be formed. In this way, it will be possible to calculate which is the share of flaxseed oil most preferred from the aspect of the ratio of omega 3 and omega 6 fatty acids.

Materials and methods

Materials: For the purposes of this study, six samples were used: two initial sampls: refined sunflower oil (RSO), cold pressed flaxseed oil (CPFO), three blends from this two oils and one commercially available blend from the market, according to the list shown in Table 1. Blended vegetable oils were obtained by blending refined sunflower seed oil (S) and cold

*Corresponding author: tanja.luzaic@tf.uns.ac.rs

pressed flaxseed oil (F), in appropriate proportions, bought in the local store. The required mass of the oil is carefully measured into a 1000-ml glass beaker, using a glass stick. The oil which was present in the blend in lower proportion was added into the beaker first, following the oil present in the blend in higher proportion. The oils were blended with a magnetic stirrer and immediately transferred to PET bottles of 500 ml volume which were completely filled with oil (without empty space), sealed with an original seal, and the oil was stored at 4 °C before testing.
The obtained results are compared with the results obtained by the analysis of the sample 6 which represents a commercially available blend of three refined vegetable oils: rapeseed, sunflower and corn oils, in decreasing order. The ratio of these oils in the blend is not known. This oil is the only avaible blended oil rich in omega 3 fatty acids in the Serbian market.

Table 1. Labels and identification of samples

Sample	RSO (%)	CPFO (%)
1	100	0
2	70	30
3	50	50
4	30	70
5	0	100
6	-	-

Methods: The fatty acids composition in oils was determined by the application of gas chromatography - Mass Spectrometry (GC-MS) (according to SRPS EN ISO 12966-2: 2015 and SRPS EN ISO 12966-2: 2017) using the HP 5890 gas chromatograph with HP 5971A mass detector ("Hewlett Packard", USA). All determinations were done in three repetitions. Based on the fatty acids composition, the content of saturated, monounsaturated and polyunsaturated fatty acids and their ratio, as well as the ratio between omega 6: and omega 3 fatty acids and ratio between omega 3 ratio: omega 6: omega 9, were calculated. The results are presented as the mean ± standard deviation, and the differences between the results were tested using the Student t-test with a level of significance of 95%. Statistical analyse of the obtained results and graphical representations were made using Microsoft Excel 2010 (Microsoft, Washington, USA) and Statistica 13.0 (StatSoft, Tulsa, USA).

Results and discussion

Flaxseed oil is rich in alpha linolenic fatty acid and is the source of omega 3 fatty acids, while sunflower oil is rich in linoleic acid and is the source of omega 6 fatty acids. Orsavova et al. (2015) determinated the next fatty acid content in sunflower oil: SFA 9.4%, MUFA 28.3% and PUFA 62.4%. 62.2% of total fatty acid content made omega 6 and 0.16 % made omega 3 fatty acids. Omega 6 and 3 ratio was 311:1. Guimaraes et al. (2013) investigated the nutritional value of flaxseed and sesame oil. The content of SFA, MUFA and PUFA in flaxseed oil was 9.97%, 18.0%, 52.24%, respectively. Founded amount of omega 6 fatty acids was 12.34% while amount of omega 3 fatty acid was 39.90%. Omega 6 and 3 ratio was 0.31:1.
The results of the fatty acids analysis of sunflower and flaxseed oil in this study were in agreement with those stated in literatures, as shown in Tables 2 and 3. Results of analysis obtained of their blends are between the results of individual oils (sample 1 and 5). Among the tested blends the highest content of saturated fatty acids was found in the sample 2, while the smallest content was found in the sample 4. Sunflower oil is rich in monounsaturated linoleic acid, so the highest content of monounsaturated fatty acids was found in the sample 1 and it was in amount of 30.44 ± 0.07 %. With the reduction of RSO content in blends, the content of monounsaturated fatty acids was reduced to 17.15 ± 0.02 % found in initial cold pressed flaxseed oil. The highest content of polyunsaturated fatty acids was found in the sample 5 and in amount of 73.15 ± 0.09 %. Flaxseed oil is rich in polyunsaturated alpha linolenic acid, so with the reduction in the content of flaxseed oil in the blends, the content of polyunsaturated fatty acids was reduced to 59.78 ± 0.02 %, found in initial refined sunflower oil.The content of saturated and monounsaturated fatty acids in the sample 6 was significantly lower compared to the tested blends, with only 6.41 ± 0.04 % (SFA) and 28.48 ± 0.02 % (PUFA), while the content of monounsaturated fatty acids was considerably higher, 65.10 ± 0.02 %.

Table 2. The content of saturated, monounsaturated and polyunsaturated fatty acids in the tested blends and their ratio

Sample	SFA (%)	MUFA (%)	PUFA (%)	SFA:MUFA:PUFA
1	9.78 ± 0.05	30.44± 0.07	59.78 ± 0.02	1 : 3.11 : 6.16
2	10.32 ± 0.04	26.86 ± 0.06	62.82 ± 0.02	1 : 2.60 : 6.09
3	9.88 ± 0.01	23.97 ± 0.02	66.15 ± 0.01	1 : 2.43 : 6.70
4	9.63 ± 0.00	21.31 ± 0.05	69.06 ± 0.05	1 : 2.21 : 7.17
5	9.70 ± 0.07	17.15 ± 0.02	73.15 ± 0.09	1 : 1.77 : 7.54
6	6.41 ± 0.04	65.10 ± 0.02	28.48 ± 0.02	1 : 10.15 : 4.44

Table 3. The content of omega 3, 6 and 9 fatty acids as well as their ratio in the tested blends

Sample	n-3 (%)	n-6 (%)	n-9 (%)	n-3 : n-6 : n-9	n-6 : n-3	n-3 : n-6
1	0.07 ± 0.00	59.71 ± 0.02	30.36 ± 0.07	1 : 853 : 433.71	853 : 1	0.00 : 1
2	14.48 ± 0.03	48.34 ± 0.01	26.75 ± 0.06	1 : 3.34 : 1.85	3.34 : 1	0.30 : 1
3	26.02 ± 0.00	40.13 ± 0.00	23.92 ± 0.02	1.09 : 1.68 : 1	1.54 : 1	0.65 : 1
4	36.93 ± 0.07	32.13 ± 0.02	21.26 ± 0.05	1.74 : 1.51 : 1	0.87 : 1	1.15 : 1
5	54.84 ± 0.12	18.31 ± 0.03	17.11 ± 0.02	3.20 : 1.07 : 1	0.33 : 1	2.99 : 1
6	4.78 ± 0.00	23.70 ± 0.02	65.00 ± 0.02	1 : 4.96 : 13.6085	4.96 : 1	0.20 : 1

The content of omega 3, 6 and 9 fatty acids as well as the ratio of these acids in the blends is shown in Table 3.

The determined content of omega 3 fatty acids was the highest in the initial sample of flaxseed oil and was 54.84 ± 0.12 %, while the expected lowest content was found in the initial sunflower oil as it was expected and was only 0.07 ± 0.00 %. With omega 6 fatty acid content, the situation was reversed. The highest content was found in the sample 1 and was 59.71 ± 0.02 %, while the lowest value was determined in the sample 5 and was 18.31 ± 0.03 %. The highest content of omega 9 fatty acids was found in the sample 1 and amounted to 30.36 ± 0.07 %, while the lowest amount was found in the sample 5 and was 17.11 ± 0.02 %. Blended vegetable oil 6 is significantly different in the content of omega 3, 6 and 9 fatty acids compared to the tested blends and the content of omega 3 and omega 6 fatty acids was significantly lower and amounts 4.78 ± 0.00 % and 23.70 ± 0.02 %, respectively. The content of omega 9 fatty acids in this oil is higher than the content of these acid in the tested blends and amounts to 65.00 ± 0.02 %. The ratio of omega 6 and omega 3 fatty acids is 4.96, which is in line with recommendations (Lepsanovic and Lepsanovic, 2000).

Based on the results determinated with t-test with 95% probability it is concluded that there is no statistically significant difference in the total amount of saturated fatty acids between samples 1 to 5 ($p>0.05$). Also has been established that there is a statistically significant difference in the total amount of monounsaturated fatty acids ($p<0.05$), except between samples 2 and 3 where no statistically significant difference was found ($p=0.012$). Samples 4 and 5 are not significant differnet in content of polyunsaturated fatty acids ($p=0.015$) while all the other samples have significant differece. In the n-3, n-6 and n-9 content was found a statistically significant difference between all samples, except between samples 2 and 3 where in n-9 content wasn't found statistically significant difference ($p=0.013$).

The dependence of flaxseed oil content in blends ($\omega_{\%CPFO}$) and the ratio of omega 6 and omega 3 fatty acids ($\omega_{n\text{-}6/n\text{-}3}$) is described by the logarithmic function:

Eq. 1. Logarithmic dependence of flaxseed oil content in blends ($\omega_{\%CPFO}$) and the ratio of omega 6 and omega 3 fatty acids ($\omega_{n\text{-}6/n\text{-}3}$)

$$\omega_{\%CPFO} = -10.9 \ln(\omega_{n\text{-}6/n\text{-}3}) + 65.689 \quad (R^2 = 0.792) \qquad (1)$$

Based on the recommended ratio of omega 6 and omega 3 fatty acids in the diet of 4-10:1, using the obtained dependence, it has been determined the most optimal ratio of vegetable oils in the blend. The most favorable content of cold pressed flaxseed oil in the blend is from 40.59 to 50.58 %, ie. the share of refined sunflower oil from 59.41 to 49.42 %.

The results of cluster analysis of samples of blended vegetable oils obtained from the calculated values shown in Tables 2 and 3 are shown using the dendrograms in Fig. 1. The results of clustering were obtained by the minimum variance method by the Ward's method, and the clustering is based on Euclidean distances. On the dendrogram two separate subclasses are allocated. In the first subclass, the difference between samples (5, 4 and 3), expressed as Euclidean distance, ranges from 14.4 to 38.1, while the difference between the samples in the second subclass (sample 1 and 2) is 19.3. It is concluded that sample 3 and 4 are the most similar according to data shown in Table 2 and 3, value of Euclidean distance between sample 3 and 4 is the lowest (14.4). Similar to this two samples is sample 5, but values of Euclidean distances between sample 5 and samples 3 and 4 are higher, 38.1 and 23.8, respectively.

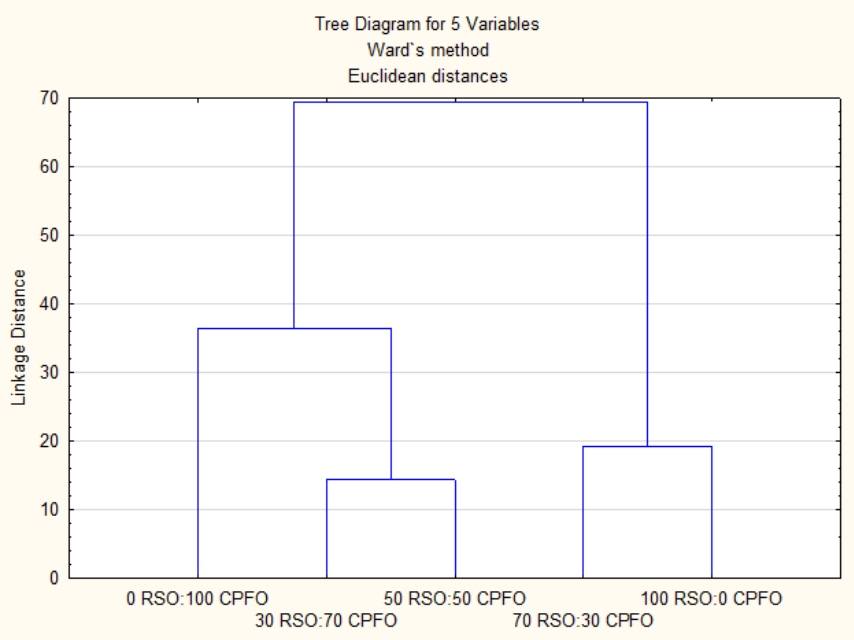

Fig. 1. Dendrogram of the hierarchical cluster analysis of the tested samples based on content of SFA, MUFA, PUFA, n-3, n-6, n-9 and n-3 : n-6 ratio

Conclusions

Blending vegetable oils with certain properties directly affects the improvement of certain oil characteristics. With the addition of cold pressed flaxseed oil in refined sunflower oil, the optimal ratio of omega 6 and omega 3 fatty acids can be achieved. Using the logarithmic function of the dependence between the ratio of omega 6 and omega 3 fatty acids and the share of cold pressed flaxseed oil in blends, it is determined that the preferred blending ratio of these two vegetable oils is one in which the proportion of cold pressed flaxseed oil is between 40.59 and 50.58%.

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