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ПОРІВНЯЛЬНИЙ АНАЛІЗ БІОЛОГІЧНОЇ ЦІННОСТІ ТА ОКИСНЮВАЛЬНОЇ СТІЙКОСТІ ГОРІХОВОЇ ТА ГАРБУЗОВОЇ ОЛІЇ

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ БИОЛОГИЧЕСКОЙ ЦЕННОСТИ И ОКИСЛИТЕЛЬНОЙ СТОЙКОСТИ ОРЕХОВОГО И ТЫКВЕННОГО МАСЛА

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COMPARATIVE STUDY OF WALNUT AND PUMPKIN SEEDS OILS BIOLOGICAL VALUE AND OXIDATIVE STABILITY

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Анотація. Робота присвячена дослідженню вмісту біологічно активних компонентів та стійкості до окиснення олії із нетрадиційної сировини − волоських горіхів та насіння гарбузів. Вивчено вміст фосфоліпідів, каротиноїдів, хлорофілів, токоферолів та стеролів, а також склад жирних кислот та гомологів токоферолів, кислотність і окиснювальну стабільність у досліджуваних зразках олії. Горіхова та гарбузові олії містять значну кількість поліненасичених жирних кислот, зокрема, горіхова олія містить ліноленову кислоту і близьке до рекомендованого співвідношення ω-3:ω-6 поліненасичених жирних кислот. У жирнокислотному складі гарбузової олії переважаючими були лінолева (поліненасичена, ω-6) та олеїнова (мононенасичена) жирні кислоти, сума насичених жирних кислот була втричі вищою, ніж у горіховій. Досліджувані зразки олій майже не відрізнялись за загальним вмістом токоферолів, проте різниця в складі гомологів токоферолів була досить суттєвою, а саме − β-токоферол був основним гомологом у горіховій олії, а α-токоферол − в олії із насіння гарбузів, відповідно. Кислотність досліджених зразків олії зростала досить швидко, досягаючи значення 4 мг KOH/г протягом 63 і 70 діб для горіхової і олії із насіння гарбузів, відповідно. Окиснювальну стабільність досліджених зразків олії досліджували за зміною значення пероксидного числа протягом 98 діб зберігання. Незважаючи на високий вміст поліненасичених жирних кислот і, зокрема, ліноленової кислоти в горіховій олії, виявлено, що індукційний період окиснення олії, визначений як початок зростання пероксидного числа, становив 56 діб. Тривалість індукційного періоду окиснення олії із насіння гарбузів та її термін придатності становили 70 та 98 діб, відповідно, в той час як термін придатності горіхової олії становив 90 діб. Більш висока стійкість гарбузової олії до окиснювального псування зумовлена, перш за все, жирнокислотним складом цієї олії, а саме високим вмістом насичених та мононенасичених жирних кислот та майже вдвічі нижчим вмістом поліненасичених жирних кислот порівняно із горіховою олією. Обидві олії можна розглядати як цінне джерело поліненасичених жирних кислот, антиоксидантів та вітамінів для харчування населення.

Ключові слова: горіхова олія, гарбузова олія, токофероли, антиоксиданти, окиснювальна стійкість

Аннотация. Работа посвящена исследованию содержания биологически активных компонентов и стойкости к окислению масел из нетрадиционного сырья − грецких орехов и семян тыквы. Исследовано содержание фосфолипидов, каротиноидов, хлорофиллов, токоферолов и стеролов, а также состав гомологов токоферолов, кислотность и антиокислительная стойкость орехового и тыквенного масла. Ореховое и тыквенное масло содержат значительное количество полиненасыщенных жирных кислот, в частности, ореховое масло содержит линоленовую кислоту и имеет близкое к рекомендованному соотношение ω-3:ω-6 полиненасыщенных жирных кислот. В жирнокислотном составе тыквенного масла превалировали линолевая (полиненасыщенная, ω-6) и олеиновая (мононенасыщенная) жирные кислоты, сумма насыщенных жирних кислот была в три раза выше, чем в ореховом. Важной особенностью орехового масла является высокое соотношение ω-3:ω-6 полиненасыщенных жирных кислот, которое составило 1:5, что практически отвечает рекомендованному диетологами для питания человека. Исследованные образцы масла почти не отличались по общему содержанию токоферолов, однако разница в составе гомологов токоферолов была довольно существенной, а именно − β-токоферол был главным гомологом орехового масла, а α-токоферол − тыквенного, соответственно. Кислотность исследованных образцов масла увеличивалась довольно быстро, достигая значения 4 мг KOH/г в течении 63 и 70 суток для орехового и тыквенного масла, соответственно. Окислительную стойкость исследованных образцов масла оценивали за изменением значения пероксидного числа в течении 98 суток хранения. Обнаружено, что индукционный период окисления орехового масла, определенный как начало увеличения пероксидного числа, составил 56 суток, несмотря на высокое содержание полиненасыщенных жирных кислот и, в частности, линоленовой кислоты в этом масле. Длительность индукционного периода окисленння тыквенного масла и его срок годности составили 70 и 98 суток, соответственно, тогда как срок годности орехового масла составил 90 суток. Более высокая стойкость тыквенного масла к окислительной порче обусловлена, в первую очередь, жирнокислотным составом этого масла, а именно высоким содержанием насыщенных и мононенасыщенных жирных кислот и более низким (почти в два раза) содержанием полиненасыщенных жирных кислот сравнительно с ореховым маслом. Оба масла можно считать ценным источником полиненасыщенных жирных кислот, антиоксидантов и витаминов для питания населения.

Ключевые слова: ореховое масло, тыквенное масло, токоферолы, антиоксиданты, окислительная стойкость

Abstract. The work is devoted to the study of the biologically active components content and the oxidation stability of oils from non-traditional raw materials such as walnuts and pumpkin seeds. The content of phospholipids, carotenoides, chlorophylls, tocopherols and sterols as well as fatty acids and tocopherol homologues composition, acidity and oxidation stability of walnut and pumpkin seeds oils were determined. The walnut and pumpkin oils contain a significant amount of polyunsaturated fatty acids, in particular, the walnut oil contains linolenic acid and has close to the recommended ratio of ω-3: ω-6 polyunsaturated fatty acids. The linoleic (polyunsaturated, ω-6) and oleic (monounsaturated) fatty acids were dominated in fatty acids composition of pumpkin oil, the sum of saturated fatty acids was three times higher than in walnut oil. The important property of walnut oil is very high ratio of ω-3:ω-6 polyunsaturated fatty acids, that was 1:5 almost the same as recommended by dietologists for human diet. The difference of total tocopherols content of two oil samples was slight, but tocopherol homologues composition was very distinctive, that is the β-tocopherol was the main in the walnut oil and α-tocopherol − in the pumpkin seeds oil, respectively. Acidity of investigated samples of oils have increased sufficiently fast, reaching the value close to 4 mg KOH/g of oil during the 63 and 70 days for walnut and pumpkin seeds oils, respectively. Oxidative stability of two oil samples were estimated as peroxide value changes during 98 days of oil storage. It was shown that the induction period of walnut oil oxidation, measured as peroxide value increase initiation, was 56 days, inspite of high content of polyunsaturated fatty acid content and particularly linolenic acid in this oil. Duration of induction period of pumpkin seeds oil oxidation and shelf life were 70 and 98 days, respectively, while shelf life of walnut oil was about 90 days. The higher resistance of pumpkin oil to oxidative damage is primarily due to the fatty acid composition of this oil, namely to the high content of saturated and monounsaturated fatty acids and almost twice lower content of polyunsaturated fatty acids compared to the walnut oil. Both oils can be recomended as a valuable source of polyunsaturated fatty acids, antioxidants and vitamins for human nutrition.

Keywords: walnut oil, pumpkin seeds oil, tocopherols, antioxidants, oxidative stability

Introduction. Formulation of the problem

Nowadays consumption of vegetable oils is rising substantially all over the world. But variety of these products in human diet is very limited in many countries. For example, soy and rape oils are the main vegetable oils in USA, Europe, China and some other regions, sunflower oil is dominative in Ukrainian diet. This so called "mono" diet of vegetable oil does not provide necessary polyunsaturated fatty acids and other bioactive compounds for human.

The significance of vegetable oils in modern nutrition is due to their polyunsaturated fatty acids content. Special biological function belongs to ω-3 and ω-6 polyunsaturated fatty acids and their ratio in human diet. But content of polyunsaturated fatty acids in different vegetable oils is very varying. The number of oils are abundant in linoleic acid (LA), that is ω-6 polyunsaturated fatty acid. They are sunflower, soy, corn, sesame and some other oils. Only some of vegetable oils has sufficient quantity of ω-3 polyunsaturated fatty acids and close to recommended ratio of ω-3 and ω-6

polyunsaturated fatty acids. Thus increase of vegetable oils assortment in human diet is a very actual goal for producers and consumers of vegetable oils.

Analysis of recent research and publications

The problem of modern human diet is the low content of vegetable oils with recomended ratio of ω-3 and ω-6 polyunsaturated fatty acids. According to modern understandings it has to be 1:10 in the diet of a healthy person and - from 1:3 to 1:5 in nutritional therapy [1]. It has been suggested that ω-3 fatty acids (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA ω-3), and docosahexaenoic acid (DHA)) have become less abundant in modern diets, and the average ratio of ω-6 to ω-3 fatty acids has increased from as little as 1:1 to as much as 30:1 [2].

Dietary intakes of ω-6 and ω-3 fatty acids are critical determinates of the proportions of bioactive 20- and 22-carbon ω-6 and ω-3 highly unsaturated fatty acids (HUFAs) in tissue phospholipids [3]. Tissue HUFAs, in turn, have been shown to affect multiple diseases [4-8] ranging from psychiatric [9, 10] and cardiovascular disease [11] to neurodevelopmental deficits [12].

Analysis of fatty acid composition of 15 kind of vegetable oils had shown that only some of them had ω-6 to ω-3 ratio equal to recommended by dietologists [1]. They were soy, olive and wheat germ oils. At the same time olive oil was depleted by polyunsaturated fatty acids in general and particularly by ω-3 fatty acids, and it was abounded by saturated fatty acids. On the contrary, rape, hempseed and mustard seed oil had high ω-3:ω-6 ratio. The content of ω-3 α-linolenic acid in linseed and camelina oil were higher than the linoleic and ω-3:ω-6 ratio exceeded 1 [1]. Thus vegetable oils have very different fatty acid composition and only some of them can be the source of ω-3 polyunsaturated fatty acids.

On the other hand, high content of polyunsaturated fatty acids is a cause of low oil oxidative stability. The presence of antioxidants and other substances that stabilize the oils with respect to auto-oxidation can provide the long shelf life stability. The vegetable oils contain natural antioxidants, such as tocopherols and phenolic compounds [13] and as result of this it has been shown that oxidative processes that may occur during the shelf life of cold-pressed pumpkin (Cucurbita pepo L.) seed oil does not result in an increase in oxidative stability parameters above the adopted limits [14]. But nevertheless they could cause the deterioration of oils during storage in conditions during which they are exposed to light, contacted with the air, or kept at high temperature. Deterioration occurs through rancidity resulting from oxidation which takes place at the double bond sites in the triacyleglycerol molecules. It is known that tocopherols are the most powerful antioxidants in vegetable oils. In its turn the most effective antioxidant between other homologues is α-tocopherol, it has the lowest concentration optimum of antioxidant activity: at concentrations of 10-25 mg % [15-18].

The walnut and pumpkin seeds as sources of edible oils are important because they can be used for both health benefits and they contain phytochemicals with significant antioxidative capacity [14, 19, 20]. In addition, walnut and pumpkin seeds oils have a nice taste, smell and color and could be attractive for consumers. But, still their oxidative stability and antioxidant properties are not clear.

The aim of the present study was comparative analysis of biological value and stability to accumulate primary oxidation products − peroxide and hydroperoxide. The objectives of the study were to determine the fatty acids and tocopherols homologues composition, the content of lipid fractions as well as the oxidative stability of walnut and pumpkin seeds oils. These oils are not commonly used as edible oils and assessment of their stability is very significant in order to be utilized for the food due to their health benefits.

Research Materials and Methods

The samples of cold-pressed walnut and pumpkin seeds oils were selected from the local market (GOLDEN KINGS of UKRAINE).

Quality parameters of pressed oils evaluation

Peroxide value (PV), acid value and sterol fraction content were determined according to procedures given by IUPAC (2.501 and 2.201, respectively) [21]. Total phosphorus content was determined by a spectrophotometric method [22] measuring absorbence of yellow molybdenumvanadiyphosphoric acid at λ= 400 nm using dry ashing and magnesium oxide as an ashing aid.

Determination of oils fatty acid composition

Fatty acid composition was determined by gas-liquid chromatography of fatty acid methyl esters. They were prepared by IUPAC standard method 2.301 [21] and analyzed on Hewlett Packard gas chromatograph model HP 6890 with capillary column HP-88 (88%-cyanopropyl aryl-polysiloxane, 100 m x 0.25 mm, 0.25 γm film thickness (Agilent Technologies). The temperature of injector was 280 ℃, and pf detector was 290 ℃. The column temperature program of heating rate was from 60 to 230 ℃. The rate of carrier gas was 1.2 ml/min. Identification of the fatty acids was performed by comparison of the retention times with standards mixture of fatty acid methyl esters (37 Component FAME Mix, Supelco).

Total carotins content determination

Total carotenes content was determined by a spectrophotometric method. Solution of oil in hexane (1:9) was used for absorbance measurement at λ=451 nm. Total carotines content (g/100 ml) was calculated using the following equation:

$C=10A/10 \cdot 256$,

where A correspond to the absorbance of oil solution at 451 nm and cuvette thickness equal to 10 mm and 256 is the specific absorption coefficient of β carotene at 451 nm [23].

Total tocopherols content determination.

Total tocopherols content was determined by a spectrophotometric method after saponification of oil and reaction unsaponfiable matters with o-phenantrolin**.** For this purpose 0.5 g of oil was saponificated in ethanol solution of potassium hydroxide during 30 min. Unsaponifiable matters were extracted thrice by diethyl ether. Extract was properly washed by disstiled water and dryed by sodium sulphate during 30 min. Ether was evaporated on rotor evaporater at 40…50˚C and residual was diluted in 5 ml of methanol. 1 ml of solution was used for reaction with 0.1 % solution of o-phenantrolin in methanol in the presence of $FeCl_3$ (0.25 % solution in methanol). Absorbance was measured at 490 nm and tocopherol concentrations were calculated using standard tocopherol solution with concentration from 1 to 100 mg/ml.

Determination of the tocopherols homolougue composition by HPLC using a reverse phase column [21].

Saponification of oil sample (5 g) was carried out in a water bath at a temperature of 85-90 °C for 30 minutes in a presence of 15 ml of methyl alcohol, 10 sm^3 of an 10 % aqueous solution of ascorbic acid and 4 ml of a 50 % aqueous potassium hydroxide solution. The non-saponifiable matters were extracted similar to that of a spectrophotometric determination and the dry residue of non-saponifiable matters was immediately dissolved in methyl alcohol and transferred quantitatively to a volumetric flask of 10 ml. The volume of the solution was adjusted by methanol to the label, closed and stirred. The extract was used for chromatographic determination of tocopherol homolougues.

Hewlett-Packard HP-1100 Liquid chromatograph with fluorescent (excitation wavelength of 295 nm, an absorption of 330 nm) and diode-matrix detectors, a Hypersil MOS reverse-phase column (2.1 mm diameter, 200 mm length) were used. Chromatography conditions: mobile phase acetonitrile:water (70:80), flow rate 0.4 ml/min, thermostat temperature 40 °C. Identification of the tocopherols isomers was performed by comparison of the retention times with standards mixture of tocopherol homolougues (Supelco).

Statistical analysis.

Samples were analyzed in triplicate. Statistical analysis was performed using Microsoft Excel 2007 (Microsoft, City of Redmond, USA). The results were reported as mean±SD. Differences were considered to be significant at validity of α=0.95.

Results and Discussion

The content of main fatty acids in cold-pressed walnut and pumpkin seeds oils are shown in Table 1. The content of saturated fatty acids (SFA) was three time lower, monounsaturated fatty acids (MUFA) − about two time lower in walnut oil comparing with pumpkin seeds oils. Accordingly, polyunsaturated fatty acid (PUFA) content in this oil was almost twice higher than in pumpkin seeds oils. The important property of walnut oil is very high ratio of ω-3:ω-6 polyunsaturated fatty acids, it is exactly the same as recommended by dietologists for human diet. The pumpkin seeds oil contains only minor conttent of α-linolenic acid (ω-3) and respectivly this oil cannot be the source of ω-3 polyunsaturated fatty acids.

Table 1 The content of main fatty acids in walnut and pumpkin seeds oils

Fatty acid	Fatty acid content, % of total content*	
	Walnut oil	Pumpkin seeds oil
Palmitic (C16:0)	7.46±0.15	11.52±0.14
Stearic (C18:0)	0.68±0.13	12.76±0.20
Oleic (C18:1)	16.92±0.17	32.35±0.13
Linoleic (C18:2)	60.2±0.20	37.79±0.21
Linolenic (C18:3)	12.24±0.19	0.48±0.14
∑SFA	8.14±0.14	24.28±0.17
∑MUFA	16.92±0.17	32,35±0.13
∑PUFA	72.44±0.20	38.27±0.18
ω-3 :ω-6 ratio	1:5	1:79

*Fatty acid contents are given as % peak area, For both oils: some fatty acids which have peak areas below 1% are not shown in the table. Each value is the mean ± SD of triplicate determinations.

The content of other lipid fractions in two vegetable oils is shown in Table 2. Obtained data have demonstrated that content of phospholipids, total tocopherols and sterols was higher in pumpkin seeds oil, while chlorophylls were not detected in this oil. At the same time carotenoids content of pumpkin seeds oil was more than 10 time higher comparing with walnut oil.

Table 2 The content of lipid fractions in walnut and pumpkin seeds oils*

Lipid fractions	Walnut oil	Pumpkin seeds oil
Phospholipids (calculated on srearic oleic lecitin), mg/100 g of oil	0.8 ±0.05	1.0 ±0.03
Carotenoids, mg/100 g of oil	0.06 ±0.01	0.78 ±0.06
Tocopherols, mg/100 g of oil	55.8 ±0.07	62.1 ±0.09
Chlorophylls, mg/100 g of oil	0.19 ±0.02	undetected
Sterols, %	0.28 ±0.03	0.32 ±0.02

*Each value is the mean ± SD of triplicate determinations.

The tocopherol concentration is an important factor that influences tocopherol antioxidant activity in vegetable oils. It was shown, that antioxidant activity is greatest at lower concentrations and decreases or may become prooxidant at higher concentrations [24-27]. For example, α-tocopherol exhibits optimal antioxidant activity at concentrations between 10 and 25 mg/100 g of oil [25, 26]. The optimal concentration for γ-tocopherol is between 25 and 50 mg/100 g of oil, and the optimal concentration for δ-tocopherol is between 50 and 100 mg/100 g of oil [25, 26]. The optimal concentration for the mixture of tocopherol homologues present in soybean oil is between 50 and 75 mg/100 g of oil [27] and according to [18] − between 34 and 66 mg/100 g of oil.

From this point of view total tocopherols content in both investigated oil samples was near the optimal concentration for the mixture of tocopherol homologues. Curiously that the antioxidant activity of the tocopherols diminished when the tocopherol levels exceeded their optimal concentrations. Above their optimal concentrations, the individual tocopherols and the tocopherol mixture exhibited prooxidation behavior [25].

In spite of slight difference of tocopherols content in walnut and pumpkin seeds oils the homologues composition of this lipid fraction was very distinctive, that is the β-tocopherol was the main in the walnut oil and α tocopherol − in pumpkin seeds oil, respectively (table 3). As it was mentioned above, the α-tocopherol has the highest biological activity and the lowest concentration optimum of antioxidant activity [14-17]. A comparison of the antioxidant activity of the individual tocopherols at their optimal concentrations revealed that α-tocopherol (~100 ppm) was 3–5 times more potent than γ-tocopherol (~300 ppm) and 16–32 times more potent than δ-tocopherol (~1900 ppm) [25]. Thus even low concentration of α-tocopherol in walnut oil may provide oxidative stability of this oil. At the same time high concentration of β-tocopherol in this oil may also facilitate walnut oil oxidative stability.

Table 3 The content of tocopherol homologues in walnut and pumpkin seeds oils

Homologues	The content of tocopherol homologues, % of ещефд tocopherol content	
	Walnut oil	Pumpkin seeds oil
α	12.1 ±0.2	73.7 ±0.5
β	70.5 ±0.4	26.3 ±0.2
γ+δ	12.1 ±0.3	undetected

*Each value is the mean ± SD of triplicate determinations.

Indeed the induction period of walnut oil oxidation, measured as peroxide value increase, was sufficient (56 days, Fig. 1) taking into account high content of polyunsaturated fatty acid content and particularly linolenic acid in this oil. Duration of induction period of pumpkin seeds oil oxidation and shelf life were 70 and 98 days, respectively, while shelf life of walnut oil was about 90 days. We have estimated the shelf life of oils as period of peroxide value reaching 10 mequiv O/kg oil, so far as Codex Alimentarius Commission (1982) stipulated a permitted maximum peroxide level of not more than 10 mequiv O/kg oil [23].

Acidity of investigated samples of oils have increased sufficiently fast (Fig. 2) and with about the same speed, reaching the value close to 4 mg KOH/g of oil during the 63 and 70 days for walnut and pumpkin seeds, respectively.

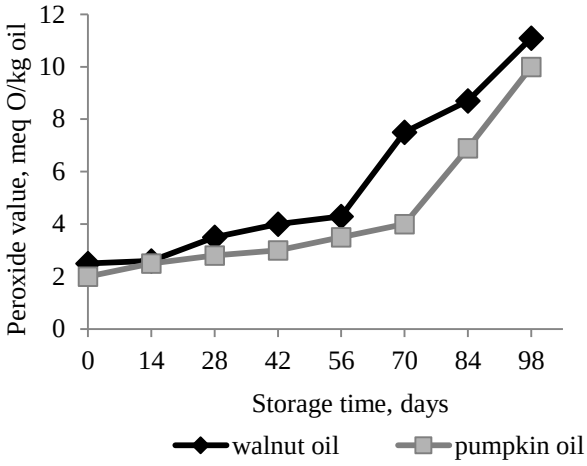

Fig. 1. Peroxide values of the oil samples versus the time of their storage.

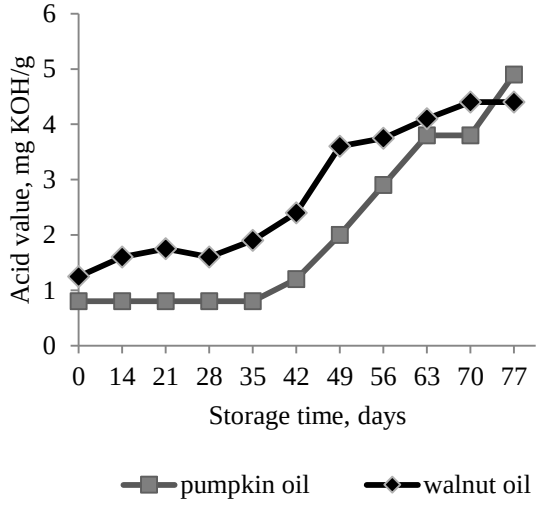

Fig. 2. Acid values of the oil samples versus the time of their storage.

Conclusion

Cold-pressed walnut and pumpkin seeds oils contain significant levels of polyunsaturated fatty acids which are important to health. Particularly, walnut oil has a high level of linolenic acid and closed to recommended ω-3:ω-6 ratio of polyunsaturated fatty acids. This oil contains significant level of total tocopherols and β-tocopherol was dominated between other homologues. The high content of this antioxidants have provided high oxidation stability and long shelf life of walnut oil. Pumpkin seeds oil contains high level of α-tocopherol, which has demonstrated the highest biological activity between others homologues. Both of these oils can be an important source of polyunsaturated fatty acids, antioxidants and vitamins for human diet. To investigate the other chemical compounds of these oils and their potential biological activities, further research is required.

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