COMPARATIVE STUDY OF THE BIOLOGICAL VALUE AND OXIDATIVE STABILITY OF WALNUT AND PUMPKIN-SEED OILS

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Abstract. The work is devoted to the study of the biologically active components and the oxidation stability of oils made from non-traditional raw materials such as walnuts and pumpkin seeds. The characteristics that have been determined are the content of phospholipids, carotenoids, chlorophylls, tocopherols, and sterols, the composition of fatty acids and tocopherol homologues, the acidity and oxidation stability of walnut and pumpkin-seed oils. Walnut and pumpkin-seed oils contain a significant amount of polysaturated fatty acids, in particular, walnut oil contains linoleic acid and has the ratio \( \omega-3: \omega-6 \) of polysaturated fatty acids, which is close to the recommended ratio. The linoleic (polysaturated, \( \omega-6 \)) and oleic (monounsaturated) fatty acids dominated in the fatty acid composition of pumpkin-seed oil, and the sum of saturated fatty acids was three times as high as that in walnut oil. An important property of walnut oil is a very high ratio of \( \omega-3: \omega-6 \) polysaturated fatty acids, 1.5, which is almost what is recommended by dietitians for the human diet. The difference in the total tocopherol content of the two oil samples was slight, but the composition of tocopherol homologues was very distinctive, i.e. \( \beta \)-tocopherol was the main homologue in the walnut oil and \( \alpha \)-tocopherol in the pumpkin-seed oil, respectively. The acidity of the oil samples increased quite rapidly, reaching the value close to 4 mg KOH/g in 63 days for walnut oil, and in 70 days for pumpkin-seed oil. The oxidative stability of the two oil samples was estimated by changes of the peroxide value during 98 days of oil storage. It has been shown that the induction period of walnut oil oxidation, defined as the start of an increase of the peroxide index, was 56 days, in spite of a high content of polysaturated fatty acids, particularly, linolenic acid. The duration of the induction period of pumpkin-seed oil oxidation and the shelf life of this oil were 70 and 98 days, respectively, while the shelf life of walnut oil was about 90 days. The higher resistance of pumpkin-seed oil to oxidative damage is primarily due to the fatty acid composition of this oil, namely to a high content of saturated and monounsaturated fatty acids and almost twice as low a content of polysaturated fatty acids compared to that of walnut oil. Both oils can be recommended as a valuable source of polysaturated fatty acids, antioxidants, and vitamins for human nutrition.

Keywords: walnut oil, pumpkin-seed oil, tocopherols, antioxidants, oxidative stability.

ПОРІВНЯЛЬНИЙ АНАЛІЗ БІОЛОГІЧНОЇ ЦІННОСТІ ТА ОКИСНЮВАЛЬНОЇ СТИЖКОСТІ ГОРІХОВОЇ ТА ГАРБУЗОВОЇ ОЛІЇ

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Анотація. Роботу присвячено дослідженню вмісту біологічно активних компонентів та стійкості до окиснення олій із нетрадиційної сировини – вовчих горіхів та насіння гарбузів. Вивчено вміст фосфоліпідів, каротиноїдів, хлорофілів, токоферолів та стеролів, а також склад жирних кислот та гомологій токоферолів, кислотність та окиснену стабільність у досліджуваних зразках олій. Горіхова та гарбузова олії містять значну кількість поліненасичених жирних кислот, а головними вони є \( \omega-3 \) та \( \omega-6 \) поліненасичені кислоти, а особливо \( \omega-6 \) поліненасичені кислоти, хлорофілів та токоферолів. Прийнято, що кислотність досліджених зразків олій зростала досить швидко, досягнувши значення 4 мг KOH/g у 63 діб для гарбузової олії та у 70 діб для гаріхової олії.

Keywords: walnut oil, pumpkin-seed oil, tocopherols, antioxidants, oxidative stability.
Introduction. Formulation of the problem

Nowadays consumption of vegetable oils is rising substantially all over the world. But the range 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 dominant in Ukrainian diet. This, so to say, monodiet of only one vegetable oil does not provide a person with necessary polyunsaturated fatty acids and other bioactive compounds.

The significance of vegetable oils in modern nutrition is due to their polyunsaturated fatty acids content. A special biological role is played by ω-3 and ω-6 polyunsaturated fatty acids and their ratio in human diet. But the content of polyunsaturated fatty acids in different vegetable oils varies greatly. A number of oils are abundant in linoleic acid (LA), that is ω-6 polyunsaturated fatty acid. They are sunflower, soy, maize, sesame, and some other oils. Only a few vegetable oils have enough of ω-3 polyunsaturated fatty acids and the ratio of ω-3 and ω-6 polyunsaturated fatty acids close to recommended. Thus increasing of the vegetable oil assortment in human diet is a topical goal for producers and consumers of vegetable oils.

Analysis of recent research and publications

The problem of modern human diet is a low content of vegetable oils with the recommended ratio of ω-3 and ω-6 polyunsaturated fatty acids. According to the modern view, it should be 1:10 in a healthy person’s diet, and 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 determine the proportion of bioactive ω-6 and ω-3 highly unsaturated fatty acids (HUFAs) in tissue phospholipids [3]. Tissue HUFAs, in its turn, have been shown to cause multiple diseases [4-8] ranging from psychiatric [9,10] and cardiovascular disease [11] to neurodevelopmental deficits [12].

Analysis of fatty acid composition of 15 kinds of vegetable oils has shown that only some of them have the ω-6 to ω-3 ratio equal to that recommended by dietitians [1]. They are soy, olive, and wheat germ oils. However, olive oil contains almost no polyunsaturated fatty acids in general, and particularly, no ω-3 fatty acids, but it abounds with saturated fatty acids. On the contrary, rape, hempseed, and mustard seed oil have a high ω-3:ω-6 ratio. The content of ω-3 α-linolenic acid in linseed and camelina oil was higher than that of linoleic acid, and the ω-3:ω-6 ratio exceeded 1 [1]. Thus, vegetable oils have very different fatty acid compositions, 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 in oils is a cause of low oxidative stability. The presence of antioxidants and other substances that stabilize oils against auto-oxidation can provide a long shelf life. Vegetable oils contain natural antioxidants, such as tocopherols and phenolic compounds [13]. As a result of this, it has been shown that oxidative processes that can occur during storage of cold-pressed pumpkin seed oil (Cucurbita pepo L.) do not result in an increase in oxidative stability above the permissible limits [14]. But nevertheless they could cause the deterioration of oils during storage in conditions when they are exposed to light, contact with the air, or kept at high temperature. Deterioration occurs through rancidity resulting from oxidation that takes place at the dibond sites in triacylglycerol molecules. It is known that tocopherols are the most powerful antioxidants in vegetable oils. In its turn, the most effective antioxidant among other homologues is α-tocopherol, it has the lowest concentration optimum of antioxidant activity: 10–25 mg % [15-18].

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

The aim of this study was comparative analysis of the oils as for their biological value and resistance to accumulating primary oxidation products – peroxide and hydroperoxide. The objectives of the study were to determine the composition of fatty acids and tocopherol homologues, the content of lipid fractions, and the oxidative stability of walnut and pumpkin-seed oils. These oils are not commonly used as edible oils, and assessment of their stability is very significant as they can be used as food due to their healthiness.
### Research materials and methods

The samples of cold-pressed walnut and pumpkin-seed oils were purchased in the local market (the manufacturer is GOLDEN KINGS of UKRAINE).

**Quality parameters of cold-pressed oils.** The peroxide value (PV), acid value, and sterol fraction content were determined according to the procedures given by IUPAC (2.501 and 2.201, respectively) [21]. The total phosphorus content was determined by the spectrophotometric method [22] measuring absorbance of yellow molybdenumvanadiyphosphoric acid at λ=400 nm using dry ashing and magnesium oxide as an ashing aid.

**Determining the fatty acid composition of oils.** The fatty acid composition was determined by gas-liquid chromatography of methyl esters of fatty acids. They were prepared by the standard method (IUPAC, 2.301 [21]) and analysed on a Hewlett Packard gas chromatograph model HP 6890 with the capillary column HP-88 (88%-cyanopropyl aryl-polysiloxane, 100 m x 0.25 mm, 0.25 γm film thickness – Agilent Technologies). The temperature of the injector was 280 °C, and of the detector 290 °C. The column heating program was 60 to 230°C. The rate of the carrier gas was 1.2 ml/min. Identification of the fatty acids was performed by comparison of the retention time, using a standard mixture of fatty acid methyl esters (Supelco).  

**Determination of the total carotenes content.** The total carotenoids content was determined by the spectrophotometric method. A solution of oil in hexane (1:9) was used for absorbance measurement at λ=451 nm. The total carotenoids content (g/100 ml) was calculated by the following equation: C=10A/10-256, where A corresponds to absorbance of oil solution at 451 nm, with the cuvette thickness 10 mm, and 256 is the specific absorption coefficient of β carotene at 451 nm [23].

**Determining the total tocopherols content.** The total tocopherols content was determined by the spectrophotometric method after saponification of oil and a reaction of unsaponifiable substances with o-phenantrrol. To this end, 0.5 g of oil was saponified in the ethanol solution of potassium hydroxide for 30 min. Unsaponifiable substances were extracted thrice by diethyl ether. The extract was properly washed with distilled water and dried with sodium sulphate for 30 min. The ether was evaporated on a rotor evaporator at 40–50°C, and the residual was dissolved in 5 ml of methanol. 1 ml of the solution was used for the reaction with the 0.1% solution of o-phenantrrol in methanol in the presence of FeCl₃ (a 0.25% solution in methanol). The absorbance was measured at 490 nm, and tocopherol concentrations were calculated using the standard tocopherol solution with the concentration 1 to 100 mg/ml.

**Determining the composition of tocopherol homologues by HPLC using a reverse phase column** [21]. An oil sample (5 g) was saponified in a bain-marie at a temperature of 85–90°C for 30 minutes in the presence of 15 ml of methyl alcohol, 10 ml of a 10% aqueous solution of acetic acid, and 4 ml of a 50% aqueous potassium hydroxide solution. The non-saponifiable matters were extracted in a similar way as during the spectrophotometric determination, the dry residue of non-saponifiable matters was immediately dissolved in methyl alcohol and transferred quantitatively into a volumetric flask of 10 ml. Then the volume of the solution was filled up to the measuring mark, closed and stirred. The extract was used for chromatographic determination of tocopherol homologues.

A Hewlett-Packard HP-1100 Liquid chromatograph with fluorescent (excitation wavelength 295 nm, absorption 330 nm) and diode-matrix detectors, and a Hypersil MOS reverse-phase column (2.1 mm diameter, 200 mm length) were used. The chromotography conditions: mobile phase acetonitrile/water (70:30), flow rate 0.4 ml/min, thermostat temperature 40°C. The tocopherol isomers were identified by comparing the retention time with a standard mixture of tocopherol homologues (Supelco).

**Statistical analysis.** The samples were analysed three times. 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 the validity ε=0.05.

### Results of the research and their discussion

The content of the main fatty acids in cold-pressed walnut and pumpkin-seed oils is shown in Table 1. The content of saturated fatty acids (SFA) was three times lower, and that of monounsaturated fatty acids (MUFA) about two times lower in walnut oil compared with pumpkin-seed oil. Accordingly, the polyunsaturated fatty acid (PUFA) content in this oil was almost twice as high as in pumpkin-seed oil. An important property of walnut oil is a very high ratio of ω-6:ω-3, that is why this oil cannot be the source of ω-3 polyunsaturated fatty acids, it is exactly the same as recommended by dietitians for human diet. The pumpkin-seed oil contains but a minor amount of α-linolenic acid (ω-3), that is why this oil cannot be the source of ω-3 polyunsaturated fatty acids. The content of other lipid fractions in the two vegetable oils is shown in Table 2. The obtained data have demonstrated that the content of phospholipids, total tocopherols, and sterols was higher in pumpkin-seed oil, while chlorophylls were not detected in this oil. At the same time, the content of carotenoids of pumpkin-seed oil was more than 10 times higher than that of walnut oil.

The tocopherol concentration is an important factor that influences tocopherol antioxidant activity in vegetable oils. It has been shown that antioxidant activity is the greatest at lower concentrations and decreases or can become prooxidant at higher concentrations [24-27]. For example, α-tocopherol exhibits the optimum antioxidant activity at concentrations between 10 and
25 mg/100 g of oil [25,26]. The optimum concentration of γ-tocopherol is between 25 and 50 mg/100 g of oil, and the optimum concentration of δ-tocopherol is between 50 and 100 mg/100 g of oil [25,26]. The optimum concentration of 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.

### Table 1 – The content of the main fatty acids in walnut and pumpkin-seed oils

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fatty acid content, % of total content*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Walnut oil</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>7.46±0.15</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>0.68±0.13</td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>16.92±0.17</td>
</tr>
<tr>
<td>Linoleic (C18:2)</td>
<td>60.2±0.20</td>
</tr>
<tr>
<td>Linolenic (C18:3)</td>
<td>12.24±0.19</td>
</tr>
<tr>
<td>∑ SFA</td>
<td>8.14±0.14</td>
</tr>
<tr>
<td>∑ MUFA</td>
<td>16.92±0.17</td>
</tr>
<tr>
<td>∑ PUFA</td>
<td>72.44±0.20</td>
</tr>
<tr>
<td>ω-3:ω-6 ratio</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*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 triple determination.

### Table 2 – The content of lipid fractions in walnut and pumpkin-seed oils*

<table>
<thead>
<tr>
<th>Lipid fractions</th>
<th>Walnut oil</th>
<th>Pumpkin-seed oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids (calculated as stearic oleic lecitin), mg/100 g of oil</td>
<td>0.8±0.05</td>
<td>1.0±0.03</td>
</tr>
<tr>
<td>Carotenoids, mg/100 g of oil</td>
<td>0.06±0.01</td>
<td>0.78±0.06</td>
</tr>
<tr>
<td>Tocopherols, mg/100 g of oil</td>
<td>55.84±0.07</td>
<td>62.14±0.09</td>
</tr>
<tr>
<td>Chlorophylls, mg/100 g of oil</td>
<td>0.19±0.02</td>
<td>undetected</td>
</tr>
<tr>
<td>Sterols, %</td>
<td>0.28±0.03</td>
<td>0.32±0.02</td>
</tr>
</tbody>
</table>

*Each value is the mean ± SD of triple determination.

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

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

### Table 3 – The content of tocopherol homologues in walnut and pumpkin-seed oils

<table>
<thead>
<tr>
<th>Homologues</th>
<th>The content of tocopherol homologues, % of the total tocopherol content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Walnut oil</td>
</tr>
<tr>
<td>α</td>
<td>12.1±0.2</td>
</tr>
<tr>
<td>β</td>
<td>70.5±0.4</td>
</tr>
<tr>
<td>γ+δ</td>
<td>12.1±0.3</td>
</tr>
</tbody>
</table>

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

Indeed, the induction period of walnut oil oxidation, measured as a peroxide value increase, was sufficient (56 days, Fig. 1), taking into account a high content of polyunsaturated fatty acid content, and particularly, linoleic acid in this oil. The duration of the induction period of pumpkin-seed oil oxidation and the shelf life were 70 and 98 days, respectively, while the shelf life of walnut oil was about 90 days. We have estimated the shelf life of the oils as a period of peroxide value reaching 10 mequiv O/kg oil, so far as the Codex Alimentarius Commission (1982) stipulated the maximum permitted peroxide level of not more than 10 mequiv O/kg oil [23].
The acidity of the studied samples of oils has increased quite rapidly (Fig. 2) and with about the same speed, reaching the value close to 4 mg KOH/g of oil during 63 and 70 days for walnut and pumpkin seeds, respectively.

Conclusion

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

List of References:

References:


