

*Досліджено і уточнено склад жирних кислот рослинних олій холодного пресування. Розроблений і обґрунтований склад купажів на основі соняшникової олії з додаванням олій насіння рижію, льону та волоського горіху, що забезпечують раціональне співвідношення  $\omega$ -3: $\omega$ -6 жирних кислот з точки зору їх вмісту у харчовому раціоні. Досліджено перебіг автокаталітичного окиснення купажів при зберіганні їх за температури  $20 \pm 2$  °C за вільного доступу світла та повітря. Встановлено суттєве уповільнення швидкості накопичення пероксидів при купажуванні 45 % горіхової або 40 % рижієвої олії з відповідною кількістю соняшникової олії*

*Ключові слова: соняшникова олія, горіхова олія, рижієва олія, газова хроматографія, купажування,  $\omega$ -3 поліненасичені жирні кислоти,  $\omega$ -6 поліненасичені жирні кислоти, біологічна цінність, пероксидне число, антиокиснювальна стабільність*

*Исследован и уточнен состав жирных кислот растительных масел холодного пресования. Разработан и обоснован состав купажей на основе подсолнечного масла с добавлением масел семян рыжика, льна и грецкого ореха, которые гарантируют рациональное соотношение  $\omega$ -3: $\omega$ -6 жирных кислот с точки зрения их содержания в пищевом рационе. Исследовано автокаталитическое окисление купажей при температуре хранения ( $20 \pm 2$ ) °C со свободным доступом света и воздуха. Установлено существенное замедление скорости накопления пероксидов и свободных жирных кислот при купажировании 45 % орехового или 40 % рыжикового масла с соответствующим количеством подсолнечного масла*

*Ключевые слова: подсолнечное масло, ореховое масло, рыжиковое масло, газовая хроматография, купажирование,  $\omega$ -3 полиненасыщенные жирные кислоты,  $\omega$ -6 полиненасыщенные жирные кислоты, биологическая ценность, пероксидное число, антиокислительная стойкость*

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## NEW VEGETABLE OIL BLENDS TO ENSURE HIGH BIOLOGICAL VALUE AND OXIDATIVE STABILITY

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### 1. Introduction

Nowadays, human health and longer life expectancy are among the most important medical and social issues. A special concern in solving this problem refers to rational nutrition. In this regard, it is important to create functional products containing the necessary nutrients and not differing in taste and appearance from traditional ones [1].

Fats and oils are not only a source of energy and plastic substances but also an important pool of physiologically functional ingredients such as polyunsaturated fatty acids (PUFAs), vitamins, phospholipids, and other bioactive components. The content of the main polyunsaturated fatty acids, primarily linoleic (C18:2) and linolenic (C18:3) acids, is the most important factor in the biological value of a vegetable oil. Linoleic acid is the main representative of the

family of omega-6 ( $\omega$ -6) polyunsaturated fatty acids, and  $\alpha$ -linolenic acid represents omega-3 ( $\omega$ -3). Polyunsaturated fatty acids perform important biological functions. Primarily, they are components of the phospholipids of all cell membranes that regulate the transfer of impulses and the work of receptors. Moreover, phospholipids are precursors of the synthesis of lipid hormones (eicosanoids), which are important for the regulation of many physiological processes [2–4].

Omega-3 fatty acids stimulate the immune system as well as reduce blood coagulability, the level of triacylglycerols (TAGs) in the blood, and the risk of coronary heart disease [5–7]. It is common fact that human and animal organisms do not synthesize linoleic and linolenic acids, so they can only come from food.

The relevance of the present work is based on the need to develop blends of vegetable oils in which the ratio of polyunsaturated fatty acids ( $\omega$ -3 and  $\omega$ -6) is close to the recommendations of nutritionists. Moreover, the most important task is to create mixtures of vegetable oils that contain polyunsaturated fatty acids and have a high resistance to oxidation.

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## 2. Literature review and problem statement

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Despite the fact that most scientists are of the opinion that the biological effectiveness of lipids depends on the content of omega-3 fatty acids, the question of the optimal ratio of individual classes of lipids in the human diet remains controversial. According to the recommendations of the Institute of Nutrition of the Russian Academy of Medical Sciences, Russia, the ratio of  $\omega$ -3: $\omega$ -6 polyunsaturated fatty acids in the diet of a healthy person should be 1:10, and in the medical diet, it should be range from 1:3 to 1:5 [8]. Based on the majority of clinical and experimental studies, the ratio of  $\omega$ -3 and  $\omega$ -6 in the human diet is recommended to be from 1:4 to 1:2 [3, 4, 9]. The British Nutrition Foundation maintains that the ideal ratio between the families of  $\omega$ -3 and  $\omega$ -6 PUFAs should be 1:6 [4]. To achieve this ratio, the UK population is advised to increase the consumption of fatty fish containing a significant amount of PUFAs of the  $\omega$ -3 family. According to the author of study [10], the consumption of fatty fish twice a week reduces the risk of angina and atrial fibrillation by 50 % in patients with cardiovascular diseases.

The  $\omega$ -6 fatty acid family includes linoleic (C18:2),  $\gamma$ -linolenic (C18:3) and arachidonic (C20:4) acids. Linoleic acid can be elongated in vivo to arachidonic acid, and the latter is a precursor to the formation of eicosanoids. In most traditional vegetable oils, linoleic acid predominates. The exception is olive oil, in which oleic acid ( $\omega$ -9) predominates, contributing to a decrease in the level of cholesterol in the plasma and being necessary for the balance of polyunsaturated fatty acids in the body.

The three essential fatty acids (eicosapentaenoic (C20:5), docosahexaenoic (C22:6), and  $\alpha$ -linolenic (C18:3)) belong to the family of  $\omega$ -3 polyunsaturated fatty acids. Elongation and desaturation of  $\alpha$ -linolenic acid convert it into eicosapentaenoic acid, a precursor of the synthesis of eicosanoids. Docosahexaenoic acid is an important structural component of cell membrane phospholipids.

Eicosanoids, synthesized from  $\omega$ -3 and  $\omega$ -6 polyunsaturated fatty acids, have different structures and biological effects [10]. Eicosanoids that are formed from  $\omega$ -3 fatty acids (prostaglandins PGE3, PGI3, thromboxane TXA3, as well as leukotrienes LTB5 and LTC5-LTE5) intensify blood cir-

ulation, dilate blood vessels, and have anti-inflammatory, antiallergic and thrombolytic effects.

Conversely, eicosanoids that are synthesized from  $\omega$ -6 arachidonic acid (prostaglandins PGE2 and PGI2, thromboxane TXA2 and leukotrienes LTB4 and LTC4-LTE4) contribute to the development of inflammation and allergy, platelet aggregation, clot formation, and narrowing of blood vessels. The exception is prostaglandin E1, which is derived from  $\gamma$ -linolenic acid ( $\omega$ -6); it has an anti-inflammatory effect and lowers histamine synthesis, reducing allergic reactions. Clinical studies have shown that shortage of the main polyunsaturated fatty acids (especially  $\omega$ -3) in cells creates a high potential for inflammations [11]. It has also been found that the content of  $\omega$ -3 below 4 % in the fatty acid diet is associated with the greatest risk of death from an ischemic heart disease [12].

It has been suggested that for normal life the content of arachidonic acid in the diets should be 2 g, and an excess of this acid can lead to a number of undesirable changes in metabolism [12]. Therefore, for effective metabolism of  $\omega$ -3 PUFAs, there is a need to block the source of arachidonic acid synthesis [13].

Thus, the published works on the enrichment of daily nutrition with  $\omega$ -3 PUFAs disclose studying the effect of fatty fish consumption on the prevention of common diseases. Much less attention is paid, in research by technologists and nutritionists, to the use of vegetable oils with a high content of  $\omega$ -3 PUFAs to optimize daily nutrition. One of the reasons of this may be the low antioxidant resistance of such vegetable oils. A possible solution to this problem is the creation of blends of vegetable oils with optimal PUFA content and higher antioxidant resistance. This is especially essential for people with low consumption of fish products.

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## 3. The aim and objectives of the study

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The aim of the work is to develop blends of vegetable oils with the optimal ratio of  $\omega$ -6 and  $\omega$ -3 PUFAs in terms of healthy nutrition and high antioxidant resistance.

To achieve this aim, the following objectives were set:

- to study the fatty acid composition of common vegetable oils;
- to develop blends of vegetable oils with the optimal ratio of  $\omega$ -6 and  $\omega$ -3 PUFAs;
- to study the antioxidant stability of the blended vegetable oils with the optimal ratio of  $\omega$ -6 and  $\omega$ -3 PUFAs.

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## 4. Materials and methods for determining the fatty acid composition and the oxidative stability of the vegetable oil blends

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### 4.1. Materials

The present research concerns vegetable oils of cold pressing, presented on the consumer market. Namely, it deals with oils such as sunflower, soybean, rapeseed, flaxseed, mustard, camelina, hempseed, amaranth, sesame, cedar, pumpkin, walnut, wheat germ, grapeseed, and extra virgin olive.

### 4.2. Determination of the content of fatty acids in the vegetable oils

For the preparation of methyl esters of fatty acids, 100 mg of an oil was dissolved in 2 ml of a solution (0.5 g/l) of hydroxytoluene (butylated hydroxytoluene, BHT) in heptane. Then there was addition of 100  $\mu$ l of sodium in a meth-

anol solution (46 mg/ml); the mixture was stirred for 2 minutes and exposed for 15 minutes. It was followed by adding 1 to 2 g of sodium hydrogen sulphate. The samples were filtered through anhydrous sodium sulphate, and then a heptane BHT solution was added in the amount of 2 ml. The resulting solution was filtered once again through a 0.45  $\mu\text{m}$  membrane cellulose filter, which was washed with 1 ml of the same solvent. The two filtrates were combined and used for analysis.

The composition of the methyl esters of the fatty acids was determined by gas-liquid chromatography. The analysis was carried out on the HP 6890 gas chromatograph Hewlett Packard with a HP-88 capillary column (88 % of cyano-propylaryl-polysiloxane, 100 m  $\times$  0.25 mm, film thickness 0.25  $\mu\text{m}$  (Agilent Technologies)). The temperature of the injector was 280  $^{\circ}\text{C}$ , and the temperature of the detector was 290  $^{\circ}\text{C}$ . The temperature program of the rate of heating from 60 to 260  $^{\circ}\text{C}$  was as follows:

- holding at 60  $^{\circ}\text{C}$  for 4 minutes;
- heating from 60 to 150  $^{\circ}\text{C}$  at 4  $^{\circ}\text{C}/\text{min}$  and holding at 150  $^{\circ}\text{C}$  for 10 minutes;
- heating from 150 to 180  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$  and holding at 180  $^{\circ}\text{C}$  for 5 minutes;
- heating from 180 to 190  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$  and holding at 190  $^{\circ}\text{C}$  for 2 minutes;
- heating from 190 to 230  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$  and holding at 230  $^{\circ}\text{C}$  for 2 minutes;
- heating from 230 to 260  $^{\circ}\text{C}$  at 4  $^{\circ}\text{C}/\text{min}$  and holding at 260  $^{\circ}\text{C}$  for 2 minutes.

The gas carrier velocity was 1.2 ml/min, and the sample volume was 1.0  $\mu\text{l}$ . The fatty acids were identified by comparing the retention time with the standard mixture of methyl esters of fatty acids (37 components of the FAME Mix, SUPELCO) [14, 15].

#### 4.3. Determination of the oxidation stability of the oil mixtures

Tests of the oxidative stability of the mixtures were carried out during the storage of the samples at a temperature of (20 $\pm$ 2)  $^{\circ}\text{C}$  with free exposure to light and air. The blends of oils were stored in glass cups. The peroxide values of the oil samples were determined every 7 days according to [16]. The peroxide values of the mixtures stopped being measured when the value had reached 10 mmol  $\frac{1}{2}\text{O}/\text{kg}$ .

Sunflower oil was used as reference.

#### 5. The results of evaluating the biological values of various vegetable oils and the oxidative stability of the vegetable oil blends

Analysis of the fatty acid composition of 15 vegetable oil types has shown that only in some of them the ratio of  $\omega$ -3: $\omega$ -6 corresponds to the one recommended by dietitians (1:10, Table 1). This refers to soybean, olive and wheat germ oils. At the same time, olive oil contains a small amount of polyunsaturated fatty acids and, in particular,  $\omega$ -3 fatty acids. In contrast, rapeseed, hempseed and mustard oils have high ratios of  $\omega$ -3: $\omega$ -6. The content of  $\omega$ -3  $\alpha$ -linolenic acid in flaxseed and camelina oils is higher than the content of linoleic acid, and the ratio of  $\omega$ -3: $\omega$ -6 exceeds 1. Thus, it is obvious that natural vegetable oils are not balanced

in the content of fatty acid fractions, as recommended by nutritionists.

Table 1

The content of the main fractions of fatty acids in the tested vegetable oils

Vegetable oils	Fatty acids, %				The ratios of $\omega$ -3: $\omega$ -6
	Polyunsaturated		Mono-unsaturated	Saturated	
	$\omega$ -3 ( $\alpha$ -linolenic)	$\omega$ -6 (linoleic)	$\omega$ -9 (oleic)		
Sunflower	0.09	62.58	24.61	11.34	1:695
Soybean	5.73	55.60	21.36	15.64	1:10
Rapeseed	9.13	18.68	58.99	6.86	1:2
Corn	0.65	44.90	43.1	11.31	1:69
Olive	0.59	7.12	72.06	15.53	1:12
Flaxseed	57.26	14.31	17.30	10.24	1:0.25
Camelina	33.85	19.26	15.99	9.96	1:0.6
Hempseed	15.32	55.40	13.53	10.74	1:3.6
Mustard	11.25	10.96	33.53	4.87	1:1
Amaranth	1.31	53.75	23.97	17.83	1:41
Sesame	0.34	40.71	38.0	11.31	1:130
Pumpkin	0.14	58.38	21.47	19.71	1:417
Walnut	13.58	61.35	16.56	8.21	1:4.5
Wheat germ	6.69	57.03	14.86	18.24	1:8.5
Grapeseed	0.45	68.15	19.6	11.51	1:151

For the development of mixtures of vegetable oils with a balanced composition of essential fatty acids, sunflower oil was used as a traditional edible vegetable oil, along with camelina, flaxseed and walnut oils. Camelina and flaxseed oils are sources of  $\alpha$ -linolenic acid, and walnut oil is used as the most balanced oil according to the recommended ratio of  $\omega$ -3: $\omega$ -6. The composition of fatty acids of the oils, which was determined by gas chromatography, is shown in Table 2. Samples of sunflower, camelina, flaxseed and walnut oils were analyzed three times. The statistical processing of the results was performed using Microsoft Excel 2007 (Microsoft, Redmond, USA). The results were presented as a mean $\pm$ standard deviation. The deviations were calculated at a significance level of  $\alpha$ =0.95 (Table 2).

According to the data presented in Table 2, sunflower oil does not practically contain linolenic acid, whereas in the other oils tested this  $\omega$ -3 acid is contained in a rather high amount (13.6–57.3 %). Therefore, the study suggests developing mixtures of sunflower oil and one of the so-called  $\omega$ -3 oils – camelina, flaxseed or walnut oil. The composition of the blends has been calculated mathematically, and the calculated ratios of  $\omega$ -3: $\omega$ -6 fatty acids are presented in Table 3. The fatty acid ratios presented in Table 3 were calculated based on the data of Table 2. The values of these ratios for 30 blends of sunflower oil with one of the  $\omega$ -3 oils depended on the type and content of the  $\omega$ -3 oil, and they ranged from 1:82 to 1:1.3. Based on the values of the  $\omega$ -3: $\omega$ -6 ratios of the PUFAs, three researched mixtures were selected, namely: 55 % of sunflower oil plus 45 % of walnut oil (1), 75 % of sunflower oil plus 25 % of flaxseed oil (2), and 60 % of sunflower oil plus 40 % of camelina oil (3). The ratios of  $\omega$ -3: $\omega$ -6 were 1:10 for blend 1, 1: 3.5 for blend 2, and 1: 3.3 for blend 3.

Table 2

The content of basic fatty acids in the tested vegetable oils

Fatty acids	Vegetable oils			
	flaxseed	camelina	walnut	sunflower
Palmitic (C16:0)	4.7±0.01	5.8±0.02	6.1±0.02	6.7±0.02
Palmitoleic (C16:1)	0.1±0.0003	0.1±0.0003	0.1±0.0003	0.1±0.0003
Stearic (C18:0)	5.2±0.02	2.4±0.007	2.1±0.006	3.5±0.01
Oleic (C18:1 ω-9)	17.3±0.05	15.9±0.05	16.6±0.05	24.6±0.07
(C18:1n11)	0.6±0.002	0.9±0.003	–	1.1±0.003
Linoleic (C18:2 ω-6)	14.3±0.04	19.3±0.06	61.3±0.2	62.6±0.2
α-linolenic (C18:3 ω-3)	57.3±0.2	33.8±0.1	13.6±0.04	0.09±0.0003
Arachic (C20:0)	0.2±0.001	1.3±0.004	0.1±0.0003	0.2±0.001
Gondoic (C20:1 ω-9)	–	14.1±0.04	0.2±0.001	0.2±0.001
Behenic (C22:0)	0.1±0.0003	0.3±0.001	0.03±0.0001	0.6±0.002
Erucic (C22:1)	–	2.7±0.01	–	–
Saturated (SFA)	10.2±0.03	9.96±0.03	8.2±0.03	11.3±0.03
Unsaturated (USFA), including: polyunsaturated (PUSFA)	89.5±0.3	86.9±0.3	91.5±0.3	88.7±0.3
monounsaturated (MUSFA)	71.6±0.2	53.1±0.2	74.9±0.2	62.7±0.2
PUSFA:MUSFA:SFA	17.9±0.05	33.8±0.1	16.6±0.05	25.7±0.08
The ratios of ω-3:ω-6	72:18:10	53:34:10	75:17:8	63:26:11
	1:0.25	1:0.6	1:4.5	1:695

Table 3

The calculated ratios of ω-3:ω-6 PUFAs in the blends of vegetable oils

The blend number	The content of ω-3 oil in the blends (the mixtures of sunflower oil plus an ω-3 oil)	ω-3 oil		
		walnut	flaxseed	camelina
1	50	1:9	1:1.3	1:2.4
2	45	1:10	1:1.6	1:2.7
3	40	1:11	1:1.9	1:3.3
4	35	1:13	1:2.3	1:3.8
5	30	1:15	1:2.8	1:4.7
6	25	1:18	1:3.5	1:5.9
7	20	1:22	1:4.6	1:7.6
8	15	1:29	1:6.4	1:10.5
9	10	1:50	1:9.9	1:16.2
10	5	1:82	1:20.4	1:34.1

The presence of ω-3 PUFAs in fats increases their biological value, but at the same time, it increases the oxidation rate: the higher the content of ω-3 PUFAs, the higher the oxidation rate of the oil. It follows from Fig. 1 that the rate of increase in the peroxide value of the researched mixtures with free access of air and light was different. The highest rate of accumulation of peroxides was observed in blend 2 (ω-3:ω-6=1:3.3) when the peroxide value had reached 10 mmol ½O per kg in 21 days (Fig. 1). The same peroxide value of sunflower oil was detected after 22 days of storage despite the absence of linolenic acid.

The longest period (27.5 days) of oxidation as to the peroxide value of 10 mmol ½ O per kg was observed for the blend containing 45 % of walnut oil (1), which is due to the content of antioxidants in walnut oils, mainly tocopherols. A similar oxidation period was recorded for sample 3, amounting to 25.7 days. It is obvious that the increase in the

oxidative stability of this mixture was due to the presence of antioxidants in camelina oil.

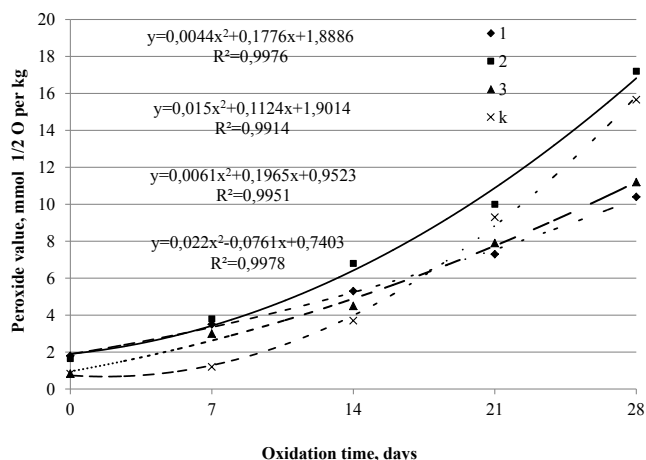


Fig. 1. Changes in the peroxide value of sunflower oil and the oil blends based on it, with K being sunflower oil, sample 1 containing 55 % of sunflower oil plus 45 % of walnut oil, sample 2 containing 75 % of sunflower oil plus 25 % of flaxseed oil, and sample 3 containing 60 % of sunflower oil plus 40 % of camelina oil

Thus, the mixtures of sunflower oil with walnut or camelina oil had the longest oxidation period and, therefore, the longest shelf life.

## 6. Discussion of the effects of the chemical composition of the oils on their oxidative stability

The study of the autocatalytic oxidation of the oil mixtures when stored at (20±2) °C with free access of light and air has shown that the mixture of sunflower oil with flaxseed

oil has the lowest resistance to oxidation. Despite this, such a mixture can be recommended for correction of lipid metabolism in patients with type II diabetes and cardiovascular diseases. Oil blends with flaxseed oil cannot be recommended for long-term storage. However, their shelf life can be increased under conditions that prevent their oxidation (low temperature and exclusion of oxygen access).

Moreover, there is evidence that even during 6 months of storage the peroxide value of flaxseed oil increases insignificantly [17].

The mixtures of sunflower oil with walnut oil (1) or camelina oil (3) were more resistant to oxidation than the mixture of sample 2 or sunflower oil itself. Earlier it had been shown [18] that camelina oil stored for 28 days at 22 °C had a sufficiently low peroxide value at the end of storing. A significant increase in the accumulation of peroxides was observed after 14 days at a storage temperature of 42 °C.

The obtained data indicate that adding camelina oil improves the antioxidant capacity of the test mixture. This is probably due to the presence of phenolic compounds in camelina oil, such as sinapinic acid and its derivatives. It was shown in [19, 20] that these compounds possess high antioxidant properties.

Among the derivatives of sinapinic acid, cannolol (4-vinyl-2,6-dimethoxyphenol) is of particular importance, as it demonstrates higher antioxidant abilities as well as more anticancer and antimutagenic properties than  $\alpha$ -tocopherol and flavonoids.

The high oxidative stability of the mixture of sunflower and walnut oils can be the result of a low (13.6 %) content of  $\alpha$ -linolenic acid and a high content of  $\alpha$ -tocopherol in the walnut oil.

The obtained data have proved that the oxidative stability of fats depends not only on the composition of fatty acids but also on the chemical composition in general and on the presence of natural antioxidants in vegetable oils.

The developed sunflower blends with camelina oil or walnut oil have a higher biological value and a close to optimal ratio of  $\omega$ -3: $\omega$ -6 polyunsaturated fatty acids. These blends are recommended for direct consumption with food as well as for the preparation of salad sauces. This research can be continued to create new blends of vegetable oils with the necessary ratio of essential fatty acids.

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## 7. Conclusion

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1. It has been proven that natural vegetable oils are not balanced by the content of fatty acid fractions in terms of modern requirements for rational nutrition. The study of the fatty acid composition of 15 vegetable oil types has shown that only soybean, olive and wheat germ oils have an  $\omega$ -3: $\omega$ -6 PUFA ratio close to the one recommended by dietitians (1:10).

2. A blend of 55 % of sunflower oil plus 45 % of walnut oil has a ratio of  $\omega$ -3: $\omega$ -6 close to the one recommended for healthy nutrition, which is 1:10. For recreational nutrition, blends with higher ratios of  $\omega$ -3 and  $\omega$ -6 PUFAs are recommended: 75 % of sunflower oil plus 25 % of flaxseed oil ( $\omega$ -3: $\omega$ -6=1:3.5) and 60 % of sunflower oil plus 40 % of camelina oil ( $\omega$ -3: $\omega$ -6=1:3.3).

3. Two of the suggested mixtures of vegetable oils – 55 % of sunflower oil plus 45 % of walnut oil and 60 % of sunflower oil plus 40 % of camelina oil – have proved to have the highest resistance to oxidation.

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