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BIOCHEMICAL FEATURES OF SEEDS IN OIL FLAX VARIETIES FOR SPECIAL PURPOSES

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Abstract. Flaxseed oil and seeds are natural sources of essential fatty acids ω-6 linoleic and ω-3 α-linolenic. However, the content of α-linolenic acid in the seeds of industrial varieties of oil flax and even in individual varieties of the food industry is several times higher than the content of fatty acids presented in the pool, including linoleic acid. Therefore, there is a need to create varieties with a ratio of ω-3:ω-6 of at least 1:5 for food and medicinal use. The article studies the biochemical parameters of seeds in oil flax varieties for different areas of use, both technical and food. The work used standard methods of biochemical analysis of plant seeds (extraction method for determining fat, gas-liquid chromatography, titration, spectrophotometric methods). Statistical analysis of research results was carried out using the programs Libre Office Calc (GNU Lesser General Public Licensev). As a result of the study of five varieties, three of which are considered for food use ('Kivika', 'Zhyvynka', 'Solnechnyj'), it was established that only the 'Solnechnyj' variety has a ω-3: ω-6 ratio in oil of 1:16.9, which corresponds to the recommended indicators. and other cultivars have high linolenic acid values and, accordingly, an altered polyunsaturated fatty acid ratio of 3:1 and 1:1.6, respectively. A comparative study of the content of fatty acids and the determination of acid, peroxide and iodine numbers showed that the iodine number and the ratio of unsaturated fatty acids are the most important criteria for identifying the quality of the oil and determine the further directions for its use. It was revealed that flax seeds of both technical and food fields are very rich in vitamin E at the level of 117–146 mg/100 g. Therefore, they are recommended to be used more widely in food as a source of this vitamin. It is proposed that when creating new varieties of flax for food purposes, also need to take into account the indicators of iodine and acid numbers, which should be low in them. These indicators are also of interest for characterizing already existing varieties in order to differentiate them. It is noted that for food use it is necessary to create varieties with a reduced content of linolenic acid to the level of 5–10%.

Keywords: oil flax, variety, biochemical characteristics of seed quality, fatty acid, oil quality, food use

Introduction. Formulation of the problem

Cultivated flax (*Linum usitatissimum* L.) has been grown for nine to ten thousand years, primarily for the manufacture of linen fabrics, canvas and ropes from stalks. Now this culture is grown all over the world as an oil and technical crop [1,2]. Flax seeds have long been valued for their medicinal properties, and the oil has been used in cooking and cosmetics [3,4,5].

In recent years, flaxseed has become popular as a healthy (functional) food product. High levels of fatty acids and minerals (Ca, Mg, Na, K, P, Cu, Fe, Mn, Zn and B) give it useful characteristics for food use [6,7,8].

In Canada, the United States and many other countries, flaxseeds are classified as natural functional foods by the Departments of Health and are considered a must for daily consumption [9].

However, to date, the level of biochemical parameters of oil and seeds of flax varieties for different uses is not clearly outlined, although this information is very important when using these products for food.

Analysis of recent research and publications

The components of the nutritional value of flax seeds are fats, proteins, vitamins, minerals, pentosans and dietary fibre. Each of these components contributes to the value of the human diet [10,11].The oil content in flax seeds is at the level of 38–49%. In addition to its high oil content, its importance as a source of essential nutrients increases the characteristic fatty acid composition. Flax seed oil contains saturated palmitic and stearic acids, monounsaturated oleic acid, doubly unsaturated linoleic acid and triple unsaturated α-linolenic acid. Flaxseed oil is the richest plant source of polyunsaturated fatty acids - α-linolenic (C18:3) and linoleic (C18:2). α-linolenic acid refers to omega-3 (ω-3) fatty acids with the location of the first double bond between the third and fourth carbon atoms. Linoleic acid is an omega-6 (ω-6) fatty acid with the first double bond located at the sixth carbon atom [12].

Linoleic and α-linolenic fatty acids are essential (essential) acids, the absence or lack of which in food inhibits the growth of a young organism, reduces reproductive functions, and lowers the tone of blood vessels. They have been proven effective in reducing the risk of chronic diseases such as heart disease, kidney disease, diabetes, high blood pressure, rheumatism, and certain types of cancer. Proven to have a positive effect on strengthening the immune system.Regular inclusion of flaxseed products has been shown to improve plasma lipids in subjects with hypercholesterolemia and lowers cholesterol levels, as well as suppresses cancer, thrombosis and allergic reactions [13,14]. Their biological role is determined by a wide range of therapeutic and prophylactic effects, in connection with which flaxseed oil can be attributed to functional vegetable oils, which ensure the entry of physiologically functional ingredients into the human body. Therefore, flaxseed oil is increasingly used in food as an additive in various food formulations such as baked goods (pastry), cereals, yogurt and bread [15-18].

In the human body, there must be a certain balance of linoleic and α-linolenic fatty acids. In sufficient quantities, α-linolenic acid is found only in fish oil and in flaxseed. Much lower amounts of linoleic and α-linolenic fatty acids are found in soybean, corn, camelina, hemp and sunflower oils [10]. It is believed that, for a healthy diet, an important point is to achieve the optimal ratio of omega-3:omega-6 fatty acids. There is data that human beings evolved on adiet with a ratio of omega–6 to omega–3 essential fatty acids of ∼1 whereas inWestern diets the ratio is 15:1–16.7:1, and in India ratios in urban areas rangebetween 38:1 and 50:1 whereas in rural areas the ratios range from 5:1 to 6.1:1. A lowerratioof omega-6:omega-3 fatty acids is more desirable in reducing the risk of many chronic diseases [19,20]. In flaxseed, the content of α-linolenic acid (ω-3) is more than three times higher than the content of linoleic acid (ω-6).

It has been found that the levels of fatty acids and other components of flaxseed, such as biomass and lignin content, are more dependent on genetic predisposition than on growing conditions [21]. Therefore, genetic and breeding studies are being carried out in various scientific centers in order to change the biochemical parameters of both seeds and the fatty acid composition of linseed oil. Thus, edible varieties of oil flax (*Linum humile*) with a reduced content of linolenic acid in oil have already been obtained. In varieties Y 117 up to 26% and Nilin up to 2.9%. Varieties 'Solnechnyj' and 'Linola^{TM}'(Canada) have a very low content of ω-3 acid. Variety 'Sanlin' has already been bred with a ratio of omega-3 and omega-6 fatty acids close to 1:8, and variety 'Rucheek' with a ratio of 1:4.3.Kiralanwith co-authors (2010) propose to carry out targeted breeding programs, using the selection of variability in fatty acid composition and mineral content (Ca, Mg, Na, K, P, Cu, Fe, Mn, Zn, B) [22].

In Ukraine, breeding and genetic work is being carried out to create special-purpose varieties, both food and technical [21]. The result of this work is the already created varieties of oilseed flax for the food industry 'Kivika' and 'Zhyvynka'. They are listed in the State Register of Plant Varieties of Ukraine [23].

In this regard, **the purpose** of our research was to compare the biochemical parameters of seeds in flax varieties from different areas of use.

Objectives of the study

1. To compare the fatty acid composition of flax seed oil of different uses.
2. Investigate oil quality indicators of the researched varieties.
3. To determine the content of vitamin E in the seeds of flax varieties of different uses.

Research materials and methods

The experiments were conducted during 2019–2021. Material used from the collection of the Department of Genetics and Plant Resources of the Zaporizhzhia National University – 'Pivdenna Nich', variety of technical use (year of registration 2000, Institute of Oilseeds, Zaporizhzhia, Ukraine), 'Kivika', variety of food use (2007, Institute of Oilseeds, Zaporizhzhia, Ukraine), Vodohrayi, variety of technical use (2010, Institute of Oilseeds, Zaporizhzhia, Ukraine), Zhyvynka, variety of food use (2018, Institute of Oilseeds, Zaporizhzhia, Ukraine) (State register of plant varieties, 2021), Solnechnyj, variety of food use (foreign breeding).

The oil content was determined according to [24].

The fatty acid composition was determined by gas-liquid chromatography in the form of methyl esters using a gas chromatograph GC-16A "Shimadzu" (Japan) with a flame ionization detector, a thermostat with a temperature not lower than 200°C, an evaporator with a temperature not lower than 300°C, software "GC solution", column THERMO TR-FAME 30m x 0.25mm with filler (70% Cyanopropyl (equiv) Polysiphenylene-siloxane), carrier gas with nitrogen not lower than 99.99% purity. The content of fatty acids was expressed as % of the total fatty acid. The acid value was calculated according to [24]. The free fatty acid content was calculated as oleic acid in which case 1 mL of 0.1 MKOH is equivalent to 0.0256 g of oleic acid.

The peroxide value was determined according to [24].

The iodine value was determined using the ASTM D2078 analytical procedure [24].

Determination of vitamin E content was carried out according to methodological principles [25].

The experiments were carried out in 3-fold repetition.

Statistical analysis of the research results was carried out using the program Libre Office Calc (GNU Lesser General Public Licensev 3).

Results of the research and their discussion

As can be seen from the data obtained, the studied flax varieties had significant differences in the fatty acid composition of the oil (table 1). In the standard variety PivdennaNich, the ratio of fatty acids is the most typical for most varieties of flax, the so-called typical "linum-type", where linolenic acid is predominant with a content of 50–55%. Saturated fatty acids palmitic $C_{15}H_{31}COOH$ and stearic $C_{17}H_{35}COOH$ in total in linseed oil are 5–10%.

During genetic breeding work on the creation of genotypes with a modified content of fatty acids in the composition of linseed oil, forms with a high content of individual unsaturated acids are selected. So, the variety of the technical direction 'Vodohrai' has an increased content of up to 70.0–73.0% of linolenic acid $C_{17}H_{29}COOH$ due to a decrease in linoleic $C_{17}H_{31}COOH$ and oleic $C_{17}H_{33}COOH$ acids.

In the opposite direction, food varieties are being created, and they are characterized by a reduced content of linolenic acid. At the same time, the 'Kivika' variety has the highest content of oleic acid among them, and the 'Zhyvynka' and 'Solnechnyj' varieties have linoleic acid. It is important to note that the 'Solnechnyj' variety is distinguished by an extremely low content of linolenic acid, which greatly distinguishes it from the other studied oil flax genotypes.

When considering the ratio of the amount of saturated acids to the amount of unsaturated acids, a decrease in the content of saturated acids in the high linolenic variety is clearly seen (Figure 1).

So, if in a "typical" variety 'PivdennaNich' this ratio is 1:10, in food varieties 'Zhyvynka'– 1:9.6, 'Solnechnyj'– 1:12, 'Kivika' – 1:14, then in the technical variety 'Vodohrai' with a content of linolenic acid more 70% it was 1:29 (Figure 1).

To characterize flax varieties for food purposes, it is also important to take into account the ratio of polyunsaturated fatty acids ω-3:ω-6. In the studied flax varieties, this ratio was: for the variety 'Pivdenna Nich'3.3:1, for the technical variety 'Vodohrai' 7.7:1, for food varieties 'Solnechnyj' 1:16.9, 'Zhyvynka'– 1:1.6, 'Kivika' – 3:1 (Figure 2).

The balance of the two types of polyunsaturated fatty acids is important for homeostasis and normal development of the human body. Introducing flax seeds or flax seed oil into the diet can bring this ratio closer to life-saving. To obtain a lipid complex of designed products with the recommended optimal ratio of polyunsaturated fatty acids ω-3:ω-6, it is desirable to use lipid blending from seeds of various flax varieties.

Table 1–**Indicators of the fatty acid composition of the oil of edible flax varieties in comparison with the technical variety and the standard varieties (% of the total fatty acids)**

Variety	Fatty acid %				
	palmitic $C_{15}H_{31}COOH$	stearic $C_{17}H_{35}COOH$	oleic $C_{17}H_{33}COOH$	linoleic $C_{17}H_{31}COOH$	linolenic $C_{17}H_{29}COOH$
'PivdennaNich' variety-standard	4.8 ± 1.11	4.2 ± 0.96	20.8 ± 1.36	16.3 ± 0.78	53.9 ± 1.92
'Vodohrai'	1.2 ± 0.41	2.1 ± 0.57	16.2 ± 1.24	9.3 ± 0.65	71.2 ± 1.87
'Solnechnyj'	5.2 ± 1.07	2.6 ± 0.61	18.8 ± 1.77	69.3 ± 1.93	4.1 ± 0.97
'Zhyvynka'	5.4 ± 0.75	4.0 ± 0.56	19.1 ± 1.09	44.2 ± 1.68	27.3 ± 1.31
'Kivika'	2.9 ± 0.48	3.8 ± 0.65	35.6 ± 1.86	13.9 ± 1.51	43.8 ± 1.94
max	5.4	4.2	35.6	64.3	71.2
min	1.2	2.1	16.2	9.3	4.1
$\bar{x}$	3.9±0.8	3.2±0.4	22.0±3.5	29.6±10.6	40.1±11.5
CV,%	46.3	28.7	35.1	80.17	64.0

In the results, presented in Table 2, it can be seen that varieties with a high content of linolenic acid, especially 'Vodohrai' 194.2 g/100 g, have higher iodine values, which reflect the concentration of mono- and polyunsaturated fatty acids in the total pool of fatty acids. And the 'Solnechnyj' variety with the lowest content of linolenic acid also has the lowest iodine value of 150.1 g/100 g. A comparative study of the content of fatty acids and the determination of the iodine number showed that these parameters serve as important criteria for identifying the quality of the oil and determine further directions for its use.

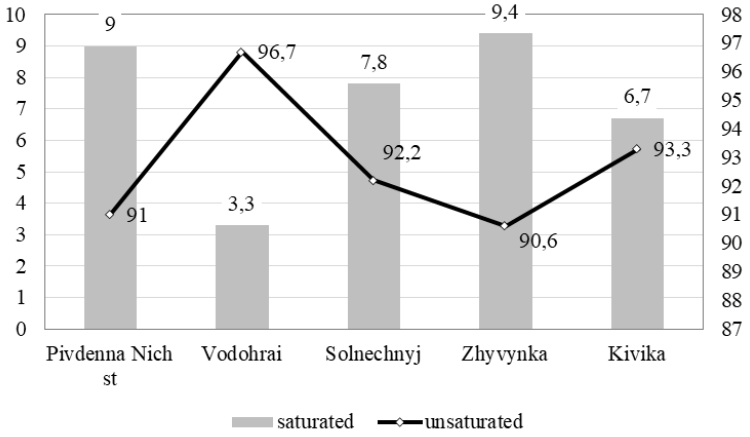

Fig.1 The ratio of the sum of saturated acids to the sum of unsaturated acids in the studied varieties of oil flax

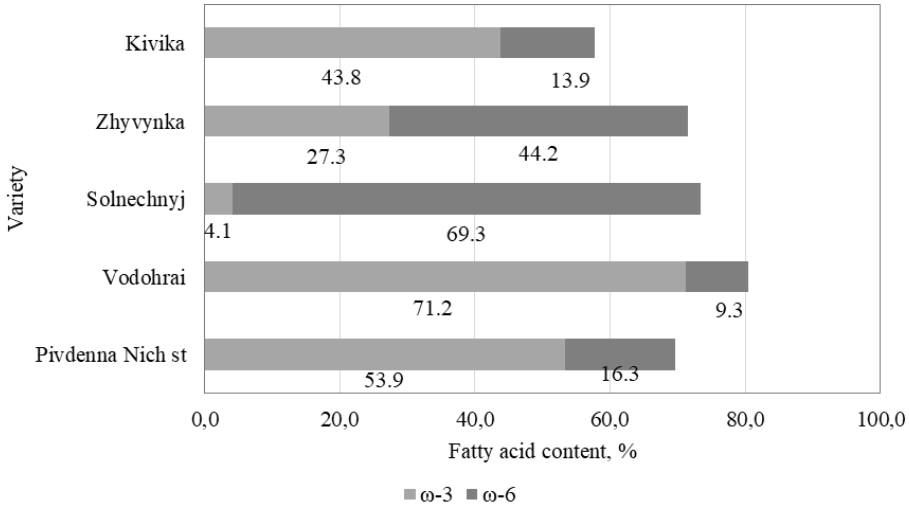

Fig. 2. The ratio of polyunsaturated fatty acids ω-3:ω-6 in the studied varieties of oil flax

Table 2 – Oil quality indicators of edible flax varieties in comparison with the technical grade and the standard grade

Variety	Acid valueor acidity		Peroxide number		Yodine number
	mg KOH	% free fatty acids from seed weight	% I_2	mM/kg ½ O	g/100 g
'PivdennaNich' variety-standard	1.77±0.01	0.89±0.006	0.117±0.001	9.130±0.06	168.9±0.6
'Vodohrai'	1.72±0.009	0.86±0.003	0.047±0.001	3.660±0.120	194.2±1.3
'Solnechnyj'	1.17±0.007	0.58±0.003	0.323±0.005	25.194±0.40	150.1±1.9
'Zhyvynka'	1.79±0.02	0.90±0.010	0.332±0.0003	25.922±0.026	192.6±4.1
'Kivika'	1.87±0.05	0.94±0.003	0.029±0.003	2.335±0.263	164.6±1.6
max	1.87	0.94	0.332	25.922	194.2
min	1.17	0.58	0.029	2.335	150.1
$\bar{x}$	1.66±0.12	0.83±0.06	0.17±0.07	13.25±5.10	174.1±8.5
CV,%	16.91	17.37	87.19	86.99	10.90

A similar trend can be traced in terms of acid number. The acid number is one of the main quality indicators characterizing the degree of freshness of fat and is regulated by standards for all types of edible fats. In the case of improper storage of seeds or oil, the amount of free fatty acids increases and their further oxidation leads to the appearance of defects in taste and smell, and in the case of deeper processes, the unsuitability of fat for food purposes. The amount of free fatty acids in fat is not constant and depends on the amount of fatty raw materials, the method of obtaining fats, the duration and storage conditions, and other factors. Their accumulation is due to the hydrolytic breakdown of glycerides into diglycerides, monoglycerides, glycerol and fatty acids. Partially free fatty acids are also formed as a result of oxidative transformations of fat in the later stages of its oxidation. In the studied varieties, the lowest value of the acid number (1.17 mg KOH) was found in the Solnechnyj variety, the highest – in the 'Kivika' variety (1.87 mg KOH) (Table 2).

According to the indicators of peroxide number, which reflects the degree of oxidation of fat (oil), due to the accumulation of peroxide compounds (peroxides and hydroperoxides) during the oxidation of fat (oil) during storage, which is especially active in the light, another dependence was revealed. The highest values of the peroxide value are typical for the 'Solnechnyj' and 'Zhyvynka' varieties with a high content of linoleic acid of more than 25 mM/kg ½ O. Moreover, the excess over other studied varieties was very significant (Table 2).

The use of whole flax seeds in the bakery and confectionery industry is increasing every year. At the same time, flax seeds are used regardless of the variety, although there are already varieties of food.

Table 3 shows the seed weight and oil content of the food varieties 'Kivika', 'Zhyvynka' and 'Solnechnyj' in comparison with the technical variety 'Vodohrayi' and the standard variety 'Pivdenna Nich'. As can be seen, the varieties of the food direction have a significantly smaller seed mass. When comparing the oil content with the standard variety, the 'Kivika' and 'Solnechnyj' varieties showed a significant decrease in fat content. And the variety of the food direction 'Zhyvynka' exceeds 'Pivdenna Nich', although it is inferior to the variety of the technical direction Vodohrayi.

As can be seen from the data presented in Table 3, both industrial and food flax seeds are very rich in vitamin E, which is a key antioxidant and plays an important role in overall human health. Our results indicate broad prospects for the use of flax seeds as a source of vitamin E in food. After all, its content in flax seeds is 117–146 mg / 100 g.

Table 3 – **Indicators of seeds of edible flax varieties in comparison with the technical variety and the standard variety**

Variety	Weight of 1000 seeds, g	Oil content, %	Vitamin E, mg/100 g
'PivdennaNich' variety-standard	8.0	45.4	146.47±2.99
'Vodohrai'	8.0	50.2	138.48±4.39
'Solnechnyj'	5.3	42.7	139.44±2.23
'Zhyvynka'	6.9	47.0	117.22±2.85
'Kivika'	6.0	43.5	134.79±2.65
Max		50.2	146.47
Min		42.7	117.22
$\bar{x}$		45.45±1.68	135.28±4.89
CV,%		7.4	8.09
LSD	0.25	1.13	

Taking into account that the daily requirement for this vitamin is 4–10 mg per day, daily consumption products with whole flax seeds (yogurts, cookies, bakery products) can be recommended as dietary and prophylactic to ensure its required amount.

Vitamin E in foods is maximally preserved in raw foods. The main natural source of this vitamin is natural unrefined cold-pressed vegetable oils. Most vitamin E is found in sunflower seed oils 56 mg/100 g, grape seed oils 32 mg/100 g, wheat germ 20–25 mg/100 g, soybeans 15–20 mg/100 g [9,10].

Thus, the considered biochemical criteria can be recommended for the breeding of flax varieties for food purposes, and in the future the number of indicators will need to be expanded.

The variety of fields of application of flax seeds requires breeders to develop varieties with different indicators corresponding to the purpose of the final product. According to the FAO, linseed oil can be divided into four groups according to the content of linolenic acid: 1) high – more than 50% – the oil is suitable mainly for use for technical purposes; 2) average – 36–49% – the oil is suitable for technical purposes, in medicine and perfumery; 3) low – 10–35% – suitable mainly for food purposes; 4) very low – less than 10% – only for food purposes [21].

Therefore, for food use, it is necessary to create varieties with a reduced content of linolenic acid to a level of 5–10%. At this stage, neither the "typical" and technical varieties, which contain too much linolenic acid, nor the already established food grade varieties 'Zhyvynka' and 'Kivika', which synthesize significantly less linolenic acid, until they give the optimal ratio of ω-3: ω-6 fatty acids.

In our opinion, one of the best ways to obtain the necessary initial breeding material of a special direction is the method of experimental mutagenesis, which makes it possible to create mutant lines with various biochemical characteristics in a relatively short period

of time. In our earlier works, we successfully used mutation breeding to obtain valuable mutant forms of oil flax with new valuable traits, including those with modified fatty acid composition of the oil [26] and to create flax varieties based on them [23]. Other authors also obtained mutants with a changed fatty acid composition of the oil and an improved ratio of ω-3:ω-6 as a result of chemical mutagenesis of different genotypes of oil flax among 27 types of mutations. The authors proposed to use the resulting mutant samples as initial forms for conducting breeding work on flax in various directions [27].

Conclusion

In the group of studied varieties of oil flax, only the variety 'Solnechnyj' revealed a ratio close to the recommended ratio ω-3:ω-6 – 1:16.9. The remaining varieties have high values of linolenic acid, which changes this ratio and is characteristic of the fatty acid composition of a typical linseed oil.

Varieties of flax for food purposes with the required ratio of ω-3:ω-6 within 1:5 should also be characterized by such biochemical characteristics as reduced iodine and acid numbers. It should be remembered that fiber flax seeds do not have the required ratio of such polyunsaturated fatty acids, because this crop is not selected for oil quality.

To create varieties of oil flax for special purposes, it is necessary to create breeding material using the method of experimental mutagenesis, which makes it possible to obtain forms with a modified composition of individual fatty acids in flax oil and their ratio.

In the course of research, it was found that flax seeds, both technical and food, are very rich in vitamin E 117–146 mg / 100 g. This indicates broad prospects for the use of flax seeds as food as a source of this vitamin.

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БІОХІМІЧНІ ОСОБЛИВОСТІ НАСІННЯ СОРТІВ ЛЬОНУ ОЛІЙНОГО СПЕЦІАЛЬНОГО ПРИЗНАЧЕННЯ

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Анотація. Лляна олія та насіння льону є природніми джерелами есенціальних жирних кислот ω-6 лінолевої та ω-3 α-ліноленової. Однак, в насінні технічних сортів льону олійного й навіть у окремих сортах харчового напряму вміст α-ліноленової кислоти в декілька разів перевищує вміст жирних кислот, що представлені в пулі, включаючи лінолеву. Тому існує необхідність створення сортів із співвідношенням ω-3:ω-6 не менш 1:5 для харчового та лікувального застосування. У статті проведено дослідження біохімічних показників насіння сортів льону олійного різних напрямів використання як технічного, так і харчового. У роботі використовували стандартні методи біохімічного аналізу насіння рослин (екстракційний метод визначення жиру, газорідинна хроматографія, титрування, спектрофотометричні методи). Статистичну обробку результатів досліджень проведено з використанням програм Libre Office Calc (GNU Lesser General Public Licensev). У результаті дослідження п'яти сортів, три з яких вважаються харчового використання ('Kivika', 'Zhyvynka', 'Solnechnyj'), встановлено, що тільки сорт 'Solnechnyj' має співвідношення ω-3:ω-6 в олії 1:16.9, що відповідає рекомендованим показникам, а інші сорти мають високі значення ліноленової кислоти та, відповідно, змінене співвідношення поліненасичених жирних кислот на рівні 3:1 та 1:1.6, відповідно. Порівняльне вивчення вмісту жирних кислот й визначення кислотного, перекисного та йодного чисел показало, що показники йодного числа і співвідношення ненасичених жирних кислот слугують найбільш важливими критеріями для ідентифікації якості олії й визначають подальший напрямок її використання. Виявлено, що насіння льону як технічного, так і харчового напряму дуже багате на вітамін Е на рівні 117–146 мг/100 г. Тому його рекомендується ширше використовувати в їжу, як джерело цього вітаміну. Запропоновано при створенні нових сортів льону харчового напряму також вказувати показники йодного й кислотного чисел, які у них мають бути низькими. Дані показники представляють інтерес і для характеристики вже існуючих сортів з метою їх диференціації. Зазначається, що для харчового використання необхідно створювати сорти зі зменшеним вмістом ліноленової кислоти до рівня 5–10%.

Ключові слова: льон олійний, сорт, біохімічні показники якості насіння, жирнокислотний склад, якість олії, харчове використання