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GAS CHROMATOGRAPHIC DETERMINATION OF FATTY AND ORGANIC ACID COMPOSITION IN SELENIUM-PROTEIN DIETARY SUPPLEMENT

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Topicality. The use of selenium-protein dietary supplements (SPDS) is relevant from a medical and economic point of view, as it can provide consumers with the required amount of selenium, and increase the value and attractiveness of food products. The diet of a modern person is very diverse in the composition of fatty ingredients. Of all natural fats, dairy is the most complex and unique one in its chemical composition. SPDS "Syvoselen Plus" contains up to 10% fat in its composition, therefore, studies of its composition are of certain scientific interest in view of the above. In many scientific studies, gas chromatography is used to determine the composition of not only the fat fraction of additives containing vegetable oils and animal fats, but also organic acids. The study of the fat fraction and the fraction of organic acids of additives by this method allows determining and predicting the composition of similar fractions in the final product from SPDS. This, in turn, allows for comprehensive control of the quality and composition of such final products. **The aim of the article** is to determine the quantitative and qualitative composition of fatty and organic acids in the SPDS "Syvoselen Plus" by the gas chromatography method, and to compare the results of the determination with the indicators of a hypothetical ideal fat. When writing the article, statistical and physico-chemical **research methods** were used, namely, the gas chromatography method, with the help of which the following problems are solved: the composition of the fat fraction of SPDS is determined quantitatively and qualitatively; the degree of correspondence of the main indicators of the fat "Syvoselen Plus" to the hypothetical ideal is established; the composition of organic acids in the additive is determined. **Results.** The fatty acid composition of milk fat, milk whey and SPDS was studied. Dietary supplement "Syvoselen Plus" contains a significant amount of palmitic (3117.71 mg/kg), stearic (1618.61 mg/kg), oleic (1397.95 mg/kg) and meristic (1296.69) mg/kg) fatty acids, which is confirmed by chromatograms of fat fractions of milk fat and SPDS. The main indicators of hypothetically ideal, milk, whey fats and DDBS were presented. As a result, it was established that the composition of fat, which is most similar to the ideal one, among the objects of the study, has SPDS "Syvoselen Plus" (the indicator of the ratio of the sum of oleic and linoleic acids to the sum of pentadecanoic and stearic acids coincides with the indicator of ideal fat, and the indicators of the ratio of linoleic to linolenic acids and linoleic to oleic acids are close to the values of the indicators of ideal fat). The study of the composition of organic acids of SPDS was carried out. Fractions of succinic, azelaic, and levulinic organic acids are predominant, which may be due to the type of dairy products from which the serum was obtained, the storage conditions of dairy products and the serum itself, the type of microorganisms that carry out the fermentation process and can produce various organic acids, and the production process of dairy products and their composition. **Conclusions and discussion.** The analysis of the fatty acid composition and the quality content of organic acids in "Syvoselen Plus" dietary supplement confirmed the feasibility of using a dietary supplement in health food technologies, since the latter contains enough polyfunctional fatty and organic acids.

Keywords: gas chromatography, selenium-protein dietary supplement, fatty acids, organic acids, selenium, whey, milk fat.

The topicality of the problem

Problem Statement. Selenium is an essential micronutrient for ensuring normal human body function. Selenium is a key component for synthesizing antioxidants, which help to protect cells from harmful free radicals and reduce the risk of developing cancer, cardiovascular, and other diseases (Roman et al., 2014).

However, most people do not get enough selenium from their diet. Therefore, adding selenium-protein supplements to food products can be an effective way to increase selenium levels in the diet. This is especially important for populations at risk of selenium deficiency, such as people with weakened immune systems, the elderly and pregnant women (Rayman, 2012).

In addition, adding selenium to food products is beneficial for producers as it can increase the value and appeal of the product to consumers. According to researchers from the American National Institutes of Health, such products will be perceived as more beneficial and healthy, which can increase their popularity and sales (National Institutes of Health, 2021). As a result, the use of selenium-protein dietary supplements (SPDS) is relevant from both medical and economic perspectives, as it can provide consumers with the necessary amount of selenium and increase the value and attractiveness of food products.

The composition of fatty ingredients in modern human nutrition is highly diverse, differing in relative content of saturated (stearic, palmitic, etc.), monounsaturated (oleic), and polyunsaturated (linoleic, linolenic, etc.) fatty acids, the physiological significance of which lies in stimulating protective functions of the body and enhancing its resistance to radiation, which is highly relevant for the population of Ukraine. Moreover, of all natural fats, milk fat is the most complex and unique in its chemical composition (Kennedy et al., 2018). Its particular feature is the presence of low-molecular-weight short-chain fatty acids.

In our previous studies, we developed a recipe for dietary selenium-protein supplements (Prymenko & Sefikhanova, 2020) based on a detailed analysis of the structure of its raw components (Prymenko et al., 2022), studies of its chemical composition (Stepanova et al., 2022), and acute toxicity parameters (Holovko et al., 2015). We also investigated the technological parameters of the dietary supplement (Prymenko et al., 2021) and conducted medical and biological studies on it (Holovko et al., 2018). Furthermore, we modeled and developed recipes for food products using the dietary supplement and determined the quality indicators of the finished products (Helikh et al., 2020; Sefikhanova et al., 2020; Prymenko & Sefikhanova, 2022). An increase in the fat emulsifying and fat retention capacities was observed in the tested product samples (Prymenko et al., 2021). The "Syvoselen Plus" selenium-protein dietary supplement contains up to 10% fat, which is why its research is of scientific interest due to the aforementioned reasons.

State of research on the problem. Gas chromatography (GC) is an analytical method that allows for the investigation of the composition and properties of various substances. In many scientific studies, it is used to determine the composition of the lipid fraction of additives containing vegetable oils and animal fats (Coleman et al., 2018; Ivanova, 2019).

One advantage of GC is that it allows for the analysis of a product's molecular composition, particularly its fatty fraction. This provides detailed information about the composition and properties of the fats added to products. Such research can help improve the quality and safety of products, as well as create new compositions and additives. For example, a study published in the Journal of Food Science and Technology in 2015 focused on the analysis of the fatty fraction of an additive used to increase the fat content of meat products. Researchers used GC to analyze the fatty acids present in the additive. The results showed that adding this additive led to an increase in the content of saturated fatty acids in meat products (Pal et al., 2015).

Another study demonstrates the results of analyzing the fatty acid composition of additives used to increase the fat content of milk. Researchers used GC to analyze the fatty acid fraction immediately after adding the additive and storing the milk in the refrigerator. The results showed that adding such additives led to an increase in the content of saturated fatty acids in milk (Silva da Costa et al., 2022).

Using this methodology, the impact of adding flaxseed oil and vitamin E to the diet of domestic animals on the composition of SFA, MUFA, and PUFA was established (Bogolyubova & Zaitsev, 2020), as well as the effectiveness of adding different oils in the production of milk with high content of ω-3 fatty acids (Oliveira et al., 2021).

In the past five years, GC methods have become increasingly popular for analyzing the lipid fraction of dairy products. Research using this methodology includes determining the composition of fats, analyzing the impact of various factors on fat quality, as well as exploring the potential use of GC for determining the authenticity of dairy products (Zhang et al., 2022; Yu et al., 2023; Arifah & Rohman, 2021).

Since 2018, a significant amount of research has been published on the analysis of the fatty component of dairy products using GC. These studies illustrate various aspects of studying the fatty component of dairy products, such as the influence of feed type on the fatty acid composition of milk (Costa et al., 2018; Kučević et al., 2019), the importance of polyunsaturated fatty acids for improving the nutritional value of milk (Nguyen et al., 2019; Khosravi et al., 2018), differences in the fatty acid composition depending on the feeding regime of animals (Agradi et al., 2020; Barać et al., 2018; Sacchi et al., 2020), the influence of fat content on the properties of dairy products (Wang et al., 2019; Paszczyk et al., 2020; Ogrodowczyk et al., 2021), etc.

Unresolved issues. The described studies demonstrate the importance of GC in investigations of the fat fraction in additives. Therefore, GC analysis of the fat fraction in additives is a very promising direction, as it allows for the determination and prediction of the composition of the fat fraction in the final product with precision down to individual fatty acids. This, in turn, enables comprehensive control of the quality and composition of final products produced using such additives.

Investigations of the fat fraction of SPDS can be beneficial for scientists and professionals in the field of food science and technology, as well as for farmers and dairy product manufacturers who strive to improve the quality of their products and meet consumer needs.

Research aim and methods

The aims of this article are to determine the quantitative and qualitative composition of fatty and organic acids in the dietary supplement "Syvoselen Plus" using gas chromatography and to compare its results with the indicators of hypothetically ideal fat.

The methodological basis of the study is the process of determining the composition of fatty and organic acids in the dietary supplement "Syvoselen Plus" using gas chromatography with subsequent processing of the research results to establish the level of compliance with its hypothetically ideal fat.

The research methods used in this study include statistical and physicochemical approaches, specifically the gas chromatography method, which is employed to achieve the following objectives: to quantitatively and qualitatively determine the composition of the fat fraction of the selenium-protein dietary supplement (SPDS); to establish the degree of compliance between the key fat indicators of SPDS "Syvoseleen Plus" and the hypothetically ideal fat; and to determine the composition of organic acids in the supplement.

The research was conducted at the National Institute of Vine and Wine "Magarach" of the National Academy of Agrarian Sciences of Ukraine using the following methodology. 50 mg of dried plant material was placed in a vial, and an internal standard (50 mg of tridecane in hexane) was added to it in a volume of 2 ml. Then, 1.0 ml of methylating agent was added (14 % BCl_3 in methanol, Supelco 3-3033). The mixture is kept in a sealed vial for 8 hours at a temperature of 65°C. During this time, fatty oil is completely extracted from the plant material, its hydrolysis into fatty acid components and their methylation occurs. Free organic and phenolic acids are simultaneously methylated.

The reaction mixture is drained from the sediment of material and diluted with 1 ml of distilled water. To obtain methyl esters of fatty acids, add 0.2 ml of methylene chloride, gently shake several times for an hour, and then chromatograph the obtained extract of methyl esters.

The introduction of the sample (2 μl) into the chromatographic column is carried out in the splitless mode, that is, without splitting the flow, which allows to introduce the sample without loss on separation and significantly (by 10–20 times) increase the sensitivity of the chromatography method. The rate of sample introduction is 1.2 ml/min for 0.2 minutes. Agilent Technologies 6890 chromatograph with 5973 mass spectrometric detector. Chromatographic column – capillary INNOWAX with an inner diameter of 0.25 mm and a length of 30 m. Carrier gas (He) speed 1.2 ml/min. The temperature of the sample introduction heater is 250°C. The temperature of the thermostat is programmable from 50 to 250°C at a speed of 4 degrees/min.

The NIST05 and WILEY 2007 mass spectra library with a total number of over 470,000 spectra is used for component identification in conjunction with AMDIS and NIST identification programs.

The internal standard method is used for quantitative calculations. The calculation of the content of components is carried out according to the formula:

$$C = K_1 \cdot K_2 \cdot 1000, \text{ mg/kg} \qquad (1)$$

wherein: $K_1 = S_1 / S_2$ (S_1 – peak area of the substance under study, S_2 – peak area of the standard);

$K_2 = 50 / M$ (50 – weight of the internal standard (μg) introduced into the sample, M – sample weight (mg)).

The objects of the study are the fat fraction of SPDS "Syvoselen Plus", milk whey and milk fat, as well as SPDS as such.

The subject of the study is the fatty acid composition of the fatty fraction of SPDS «Syvoselen Plus», milk whey and milk fat, as well as the quantitative and qualitative composition of organic acids in the supplement.

The scientific novelty of the obtained results lies in the fact that for the first time the composition of the fat fraction of SPDS was investigated and the content of organic acids in the additive was determined, and the obtained results of gas chromatography were scientifically substantiated. The practical significance of the obtained results is revealed in their benefit to researchers and specialists in the field of food science and technology, as well as to farmers and dairy producers who seek to improve the quality of their products and meet the needs of consumers. The data on the composition of the fat fraction of DDSB and the content of organic acids in the additive and the ways of its use in food production technologies have gained further development.

Research information base – scientific articles, patents, materials of international congresses and symposia, scientific and practical conferences, regulatory and technical documentation.

Research results

In accordance with the methodology, an analysis of the fatty acid composition of SPDS «Syvoselen Plus» was carried out. In the examined samples of milk fat, fat fractions of liquid and dry milk serums and SPDS "Syvoselen Plus" identified butyric, caproic, caprylic, capric, lauric, myristic, pentadecane, palmitic, stearic, arachinic, behenic, palmitoleic, heptadecanic, oleic, linoleic, linolenic and arachidonic fatty acids (Tabl. 1). Quantification was carried out by the method of internal normalization.

Tabl. 1. Fatty acid composition of milk fat, milk whey and SPDS

No	Fatty acid	Concentration in samples, % by mass			
		Natural milk fat	Milk whey		SPDS «Syvoselen Plus»
			liquid	dry	
	Saturated				
1.	Butyric C4:0	2.0–5.0	6.45	6.32	–
2.	Caproic C6:0	1.0–3.5	–	1.05	2.73
3.	Caprylic C8:0	0.4–2.0	–	1.05	1.23
4.	Capric C10:0	0.8–6.5	3.23	2.11	2.63
5.	Lauric C12:0	0.8–4.0	–	–	4.07
6.	Myristic C14:0	7.6–14.6	12.90	10.53	14.18
7.	Pentadecanoic C15:0	–	–	–	0.09
8.	Palmitic C16:0	20.0–38.0	35.48	34.74	34.10
9.	Stearic C18:0	5.5–13.7	9.68	10.53	17.70
10.	Arachinic C20:0	0.3–1.3	–	–	0.39
11.	Behenic C22:0	до 0.1	–	–	0.14
	Monounsaturated				
11.	Palmitoleic C16:1	1.5–4.0	3.23	3.16	2.49
12.	Heptadecanoic C17:1	–	–	–	2.07
13.	Oleic C18:1	16.7–37.6	25.81	26.32	15.29
	Polyunsaturated				
14.	Linoleic C18:2	1.0–5.2	3.23	3.16	2.37
15.	Linolenic C18:3	0.1–2.1	–	1.05	0.52
17.	Arachidonic C20:4	0.2–1.7	–	–	–
	Total:	**100.0**	**100.0**	**100.0**	**100.0**

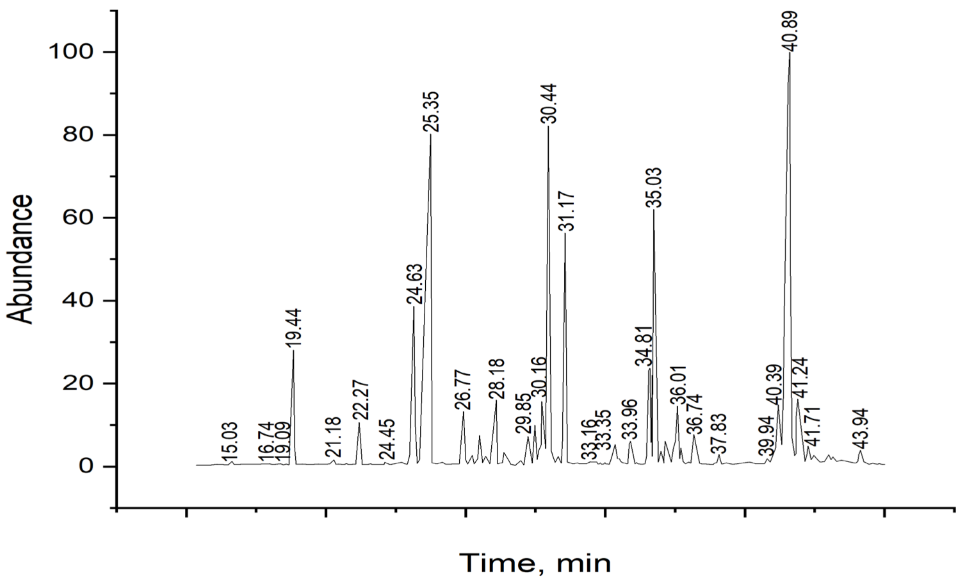
Source: own elaboration

The results of the study of the fatty acid composition of milk fat (control) and SPDS are shown on the chromatograms (Pic. 1. a, b).

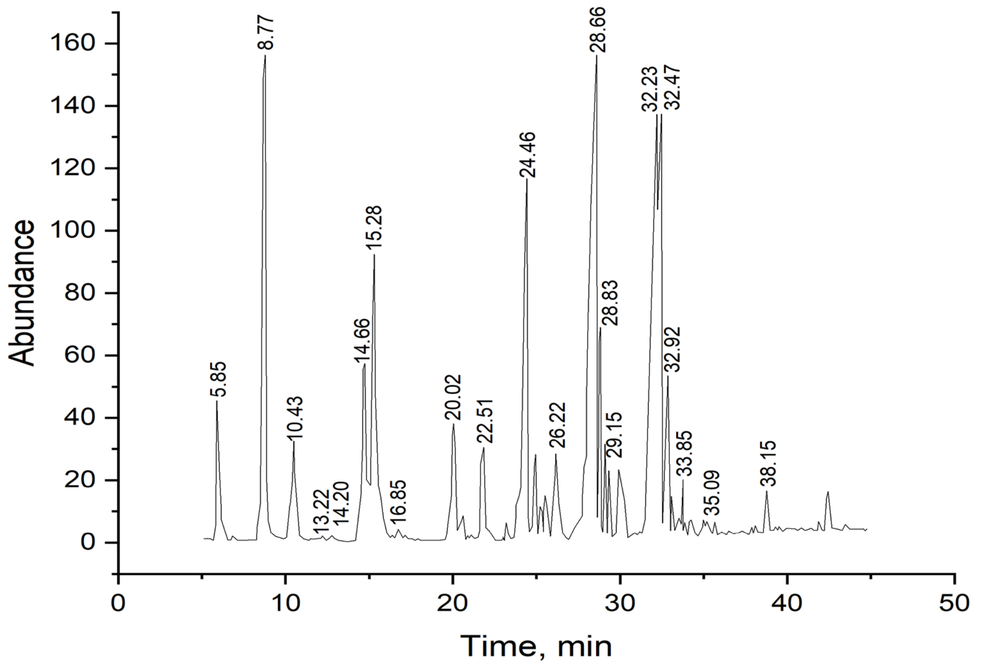
A quantitative expression of the biological effectiveness of SPDS is the correspondence of its fatty acid composition to the formula of a hypothetical ideal fat (Tabl. 2). Thus, the tested milk fat and dry and liquid serum fat do not meet the ideal criteria in terms of their fatty acid composition.

Among the objects of the study, the composition of fat most closely related to the ideal one has the "Syvoselen Plus" dietary supplement (the ratio of the sum of oleic and linoleic to the sum of pentadecanoic and stearic acids coincides with the ideal fat, and the ratio of linoleic to linolenic acids and linoleic to oleic acids close to the values of ideal fat indicators).

An analysis of the composition of organic acids in the supplement "Syvoselen Plus" was also carried out (Tabl. 3).



a



b

Pic. 1. Chromatograms of the fat fraction of: *a* – milk fat; *b* – SPDS
Source: own elaboration

Tabl. 2. The main indicators of hypothetically ideal fat and SPDS

No	Ratio of fatty acid content	Studied fat				
		Ideal fat	Milk fat	Milk whey		SPDS «Syvoselen Plus»
				liquid	dry	
1.	Unsaturated to saturated	0.6–0.9	0.56	0.48	0.51	0.29
2.	Linoleic to linolenic	7.0–40.0	2.82	–	3.0	4.55
3.	Linoleic to oleic	0.25–4.0	0.11	0.125	0.12	0.15
4.	The sums of oleic and linoleic to the sum of pentadecane and stearic	0.9–1.4	3.15	3.0	2.8	0.99

Source: own elaboration

Tabl. 3. Composition of organic acids of SPDS "Syvoselen Plus"

No	Spectrum length, μm	Name of the defined organic acid	Content, mg/kg
1.	10.801	Oxalic acid	6.81
2.	13.216	Malonic acid	19.63
3.	14.197	Fumaric acid	0.56
4.	14.660	Levulinic acid	325.34
5.	15.279	Succinic acid	624.35
6.	26.216	Azelaic acid	352.64

Source: own elaboration

Fractions of succinic, azelaic and levulinic organic acids are predominant, which may be due to the following factors. First, the type of dairy product from which the whey was obtained. Thus, milk for whey production was obtained from farms, and it was characterized by high fat content (over 5.0%), which could contain a predominant amount of levulinic and azelaic acids (Ribeiro et al., 2022). Secondly, the storage conditions of dairy products and the whey itself. Thus, storage at low temperatures can cause an increase for succinic acid in whey (Q. Nie & S. Nie, 2019). Third, the type of microorganisms that carry out the fermentation process and can produce various organic acids (Coorevits et al., 2008).

The technological process of dairy production and its composition can also influence the composition of organic acids in SPDS. Thus, the content of individual amino acids can affect the production of succinic acid in milk whey (Xiong et al., 2020), and heat treatment can affect the composition of whey and its organic acids (Bulat & Topcu, 2021).

Conclusions and discussion of results

Therefore, research using GC is ongoing, and it can be expected that in the future GC methods will be even more widely used for the analysis of the fat component of dairy products. This makes it possible to detect the influence of various factors on the quality of dairy products, as well as to identify possible problems with their authenticity.

The analysis of the fatty acid composition and the quality content of organic acids in "Syvoselen Plus" dietary supplement confirmed the feasibility of using a dietary supplement in health food technology, since the latter contains enough polyfunctional fatty and organic acids.

The practical significance of the obtained results is revealed in their benefit to researchers and specialists in the field of food science and technology, as well as to farmers and dairy producers who seek to improve the quality of their products and meet the needs of consumers.

The data on the composition of the fat fraction of SPDS and the content of organic acids in the additive and the ways of its use in food production technologies have gained further development.

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ГАЗОХРОМАТОГРАФІЧНЕ ВИЗНАЧЕННЯ СКЛАДУ ЖИРНИХ І ОРГАНІЧНИХ КИСЛОТ У ДОБАВЦІ ДІЄТИЧНІЙ СЕЛЕН-БІЛКОВІЙ

Актуальність. Використання добавок дієтичних селен-білкових (ДДСБ) є актуальним із медичного та економічного погляду, оскільки може забезпечити споживачів необхідною кількістю селену та підвищити цінність і привабливість харчових продуктів. Харчування сучасної людини дуже різноманітне за складом жирових інгредієнтів. З усіх природних жирів молочний за своїм хімічним складом є найбільш складним та унікальним. ДДСБ «Сивоселен Плюс» містить у своєму складі до 10 % жиру, тому його дослідження викликають певний науковий інтерес з огляду на вищезначене. В багатьох наукових дослідженнях газова хроматографія використовується для визначення складу не тільки жирової фракції добавок, що містять рослинні олії та тваринні жири, а й органічних кислот. Дослідження жирової фракції та фракції органічних кислот добавок цим методом дозволяє визначати і прогнозувати склад аналогічних фракцій у кінцевому продукті з ДДСБ. Це, зі свого боку, дозволяє контролювати комплексно якість та склад таких кінцевих продуктів. **Метою дослідження** є визначення кількісного та якісного складу жирних і органічних кислот у добавці дієтичній селен-білковій «Сивоселен Плюс» методом газової хроматографії та порівняння результатів визначення з показниками гіпотетично ідеального жиру. При написанні статті використовувались статистичний і фізико-хімічний **методи дослідження,** а саме метод газової хроматографії, за допомогою яких вирішуються наступні завдання: кількісно та якісно визначається склад жирової фракції ДДСБ; встановлюється ступінь відповідності основних показників жиру ДДСБ «Сивоселен Плюс» гіпотетично ідеальному; визначається склад органічних кислот у добавці. **Результати.** Досліджений жирнокислотний склад молочного жиру, молочної сироватки та ДДСБ. ДДСБ «Сивоселен Плюс» містить значну, у порівнянні із молочним жиром, кількість пальмітинової (3117,71 мг/кг), стеаринової (1618,61 мг/кг), олеїнової (1397,95 мг/кг) та міристинової (1296,69 мг/кг) жирних кислот, що підтверджується хроматограмами жирових фракцій молочного жиру і ДДСБ. Наведені основні показники гіпотетично ідеального, молочного, сироваткового жирів та ДДСБ, в результаті чого встановлено, що складом жиру, який найбільш споріднений з ідеальним, серед об'єктів дослідження володіє ДДСБ «Сивоселен Плюс» (показник співвідношення суми олеїнової та лінолевої до суми пентадеканової і стеаринової кислот збігається із показником ідеального жиру, а показники співвідношення лінолевої до ліноленової кислот та лінолевої до олеїнової наближені до значень показників ідеального жиру). Проведене дослідження складу органічних кислот ДДСБ «Сивоселен Плюс». Переважними є фракції бурштинової, азелаїнової та левулінової органічних кислот, що може бути обумовлено видом молочної продукції, з якої була отримана сироватка, умовами зберігання молочних продуктів та самої сироватки, видом мікроорганізмів, які здійснюють процес ферментації і можуть виробляти різні органічні кислоти, технологічним процесом виробництва молочної продукції та її складом. **Висновки та обговорення.** Аналіз жирнокислотного складу та якісного вмісту органічних кислот у ДДСБ «Сивоселен Плюс» підтвердив доцільність застосування дієтичної добавки у технологіях харчових продуктів оздоровчого призначення, оскільки остання містить достатньо поліфункціональних жирних та органічних кислот.

Ключові слова: газова хроматографія, добавка дієтична селен-білкова, жирні кислоти, органічні кислоти, селен, сироватка молочна, молочний жир.