

QUALITY OF RAW MILK AND ITS FATTY ACID COMPOSITION

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**Abstract.** *The study of the influence of the season on the organoleptic and physicochemical parameters of milk is still relevant. In addition, the ratio of fatty acids in the milk of cows characterizes the level of their feeding and the study of these indicators is relevant given the prevalence of adulteration of milk and dairy products with vegetable oils. The aim was to study individual indicators of raw milk quality depending on the season and its fatty acid composition.*

*The material for the study was prefabricated chilled raw milk obtained at different times of the year on the dairy farm of NULES of Ukraine "Agronomic Research Station". The research was conducted in the accredited Ukrainian Laboratory of Quality and Safety of AIC NULES of Ukraine. Organoleptic, physicochemical, sanitary and hygienic were determined by generally accepted standard methods. The fatty acid composition of milk was investigated by gas chromatography. Extraction of lipids from milk was performed by the method of Folch. Analysis of LCD methyl esters was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector. Identification of fatty acids was performed using a standard sample Supelco 37 Component FAME Mix. Quantitative evaluation of the fatty acid spectrum of milk fat lipids was performed by the method of internal normalization with their determination in percent. The study was performed in 2 parallels. Statistical processing of experimental data was performed by conventional methods of variation statistics. The probability of the difference in indicators was assessed by Student's *t*-test. Differences between the compared indicators were considered significant at the significance level  $P < 0.05$ . Studies have shown that milk organoleptic and physicochemical parameters of raw milk vary depending on the season. In terms of microbiological indicators, the milk corresponded to the first grade, although in winter, according to other indicators, it could be attributed to the extra, or at worst, to the highest grade. In addition, in winter the content of saturated fatty acids in milk increases. The content of unsaturated fatty acids increases in summer and autumn due to the presence of green mass and corn silage in the diet of cows. The obtained results can be used as a basis for preparing a diet to obtain milk with optimal content and ratio of fatty acids, which can become competitive in the market of raw milk to produce milk and dairy products of improved chemical composition, dietary, functional products, baby food.*

**Key words:** raw milk, quality, sanitary and hygienic indicators, fatty acids

**Introduction.** Milk and dairy products always play an important role in people's diet. They are an indispensable source of energy, and it is scientifically substantiated that dairy products should make up a third of the caloric content of a person's daily diet. Importance belongs to the dietary properties of milk fat, 95% of which are triglycerides more than 40% of fatty acids (trans-isomers of unsaturated fatty acids, short chain acids with an odd number of carbon atoms and a branched chain, which are synthesized by the ruminant microflora (Magan et al., 2021).

**Analysis of recent research and publications.** The ratio of fatty acids in milk fat depends on the composition of the diet of animals and can vary widely. It is known that the highest content of unsaturated fatty acids in milk fat, as well as diene conjugates of linoleic acid is observed in the grazing period

(Yamaguchi et al., 2017; Bainbridge et al., 2017). This is since green foods contain a significant amount of their precursors - polyunsaturated fatty acids. During the stall period, the proportion of saturated fatty acids increases and diene conjugates of linoleic acid decrease (Pegolo et al., 2017).

If a high-concentration diet with a low fiber content in milk fat is used, the content of trans-isomers of acid increases by 18:1 due to incomplete biohydrogenation of linolenic and linoleic acids by the rumen microflora. If the ratio of roughage to concentrates is changed from 50:50 to 20:80, the total amount of scarring and vaccine acids in milk is halved. Also, the fatty acid composition of milk fat is influenced by the breed of cows, pregnancy, etc. (Barreiro et al., 2017) and the method of technological processing of feed (Oliveira et al., 2021; Lanza et al., 2021).

The organoleptic parameters and features of the technological properties of milk are influenced by the fatty acid composition of milk fat, because it depends on the intensity of the oxidation process. Regarding the content of biologically active compounds in milk and dairy products, which are more important for the consumer, they include: branched fatty acids and diene conjugates of linoleic acid, essential fatty acids, butyric acid. However, there are also potentially dangerous to human health fatty acids (myristic, palmitic, lauric).

A significant amount of research is devoted to the characteristics of the fatty acid composition of milk fat (Silva et al., 2018; Dias et al., 2019; Musayeva et al., 2021).

Currently, several regulations on milk safety and quality have been harmonized in Ukraine. In particular, according to Order 118 of 12.03.2019 "Requirements for safety and quality of milk and dairy products" (Ministry of Agrarian Policy and Food, 2019), requirements are set for market operators that implement good practices of production, processing and marketing of milk and dairy products. , establish criteria for raw milk, among which the main ones are microbiological and somatic cell content (Yakubchak, et al. 2021), which are important for suitability for introduction into circulation of milk. Milk is also tested for mycotoxins in both Ukraine and the EU (Yakubchak et al., 2018). The maximum permissible concentrations of individual mycotoxins are regulated by the relevant regulations (Commission Regulation (EU) № 165/2010, 2010; Nakaz MON № 368, 2013).

Among the main indicators of milk quality in the current regulations there is no information on such biologically active components as fatty acids, which can fluctuate significantly at different times of the year. Given that humanity is trying to pay more attention to healthy eating, the creation of functional products (Pu et al., 2021; Prosser, 2021), it is important to study the fatty acid composition of milk obtained in small farms, which are now the main raw material base for capacity for the production of milk and dairy products in Ukraine. In particular, one of such farms is a separate subdivision of the National University of Life and Environmental Sciences of Ukraine "Agronomic Research Station" (SE NULES of Ukraine "Agronomic Research Station").

Given this, **the aim of our work** was to study individual quality indicators of raw milk depending on the season and its fatty acid composition.

**Materials and methods of research.** The milk obtained from the NUBIP of Ukraine "Agronomic Research Station" was studied. Samples were taken from whole cooling milk, respectively (DSTU ISO 707: 2002, 2002; DSTU 8553: 2015, 2015). Milk was obtained from cows of the black-spotted family with tethered keeping at different times of the year. On the farm, as a supplement - mechanical, profitable equipment of the companies "Bratslav", giving three approvals, in spring and summer; twice - in the fall. The research was conducted based on ULYABP AIC NULES of Ukraine.

Organoleptic parameters were determined: appearance, color, taste, smell, consistency. Density, mass fraction of fat, protein, and lactose were determined by ultrasound on a Master Classis LM2PI milk analyzer. The same device, in addition, determined: active acidity, dry skim milk residue, freezing point, electrical conductivity, mass fraction of minerals, mass fraction of water, freezing point.

Extraction of lipids from milk was performed by the method of Folch (Folch, 1957). The next step in sample preparation was to hydrolyze and methylate fatty acids of lipids obtained from pooled milk samples. To do this, 4 cm<sup>3</sup> of methyl sodium hydroxide solution was added to 100 mg of the obtained fat, the reflux condenser was connected to the flask containing it and boiled until the fat droplets disappeared, stirring the contents of the flask at intervals of 30-60 seconds. To the contents of the flask was added 5 cm<sup>3</sup> of methyl boron trifluoride solution, continuing to boil for up to 1 hour. 3 cm<sup>3</sup> of hexane was added to the boiling mixture through the top of the reflux condenser and removed from the heating element. To a still hot solution was added 20 cm<sup>3</sup> of saturated sodium chloride solution and stirred for 15 sec. The top (hexane) layer was selected for the study according to the current DSTU (DSTU ISO 5509-2002, 2002). Analysis of LCD methyl esters was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector. Chromatography conditions: column temperature 140-240 °C, detector temperature - 260 °C. The sample was introduced into the chromatograph using a TriPlus autosampler at a dose of 1 µl. The duration of the analysis was 65 minutes

Identification of fatty acids was performed using a standard sample Supelco 37 Component FAME Mix. Quantitative evaluation of the fatty acid spectrum of milk fat lipids was performed by the method of internal normalization, determining their content in percent. The studies were performed in 2 parallels according to the current DSTU (DSTU ISO 5508: 2001, 2001).

Statistical processing of experimental data was performed by conventional methods of variation statistics. The probability of the difference in indicators was assessed by Student's t-test. Differences between the compared indicators were considered significant at the significance level  $P < 0.05$ .

**Results of the study and their discussion.** The first stage of our work was the analysis of the fodder base on the farm of NULES of Ukraine "Agronomic Research Station" depending on the season (Table 1).

### 1. A set of feeds for the formation of the diet, depending on the season.

List of feeds	Seasons			
	winter	spring	summer	autumn
<b>Concentrated feed:</b> corn; barley; wheat; sunflower cake	+	+	+	+
<b>Vitamin and mineral supplements:</b> "Calcium" firm "Vita" (calcium, magnesium, phosphorus, sodium chloride); common salt; Molasses	+	+	+	+
Green table (alfalfa)	-	+	+	+
Corn silage. withinage (alfalfa); cino (alfalfa); witholoma (achminna)	-	+	+	+

Data Table. 1 indicate that in the diet of cows throughout the year there are concentrated feeds and vitamin and mineral supplements: "Calcium" company "Vita" (calcium, magnesium, phosphorus, sodium chloride); common salt; molasses. Green mass in the diet is from mid-April to late September. Corn silage; haylage (alfalfa); hay (alfalfa); straw (barley) is present in the diet of cows in spring, summer and autumn, and absent - in winter.

According to organoleptic parameters, milk in all seasons of the year complied with current regulations and had the following characteristics. Consistency - a homogeneous liquid without flakes of protein and sediment; taste and smell are pure, inherent in fresh milk, without extraneous tastes and smells; color - from white to light cream. From May to the end of September, raw milk has a pronounced yellowish tinge.

Physico-chemical and sanitary-hygienic parameters of milk were also compared, depending on the season and the diet of animals (Tables 2, 3).

### 2. Results of physical and chemical studies of cow's milk obtained in the conditions of THE NUBIP of Ukraine "Agronomic Research Station", $M \pm m$ , $n=16$

Name of indicators, units of measurement	Winter	Spring	Summer	Autumn	Norms according to the ND <sup>1</sup>		
					extra	higher	first
Mass fraction of solids, %	12,92 ± 0,10	12,4 ± 0,11*	12,35 ± 0,09**	12,62 ± 0,18	≥ 12,0	≥ 11,8	≥ 11,5
Mass fraction of fat, %	3,78 ± 0,29	3,41 ± 0,56	3,33 ± 0,12*	3,57 ± 0,11	≥ 3,2 %		

Density, кг/м <sup>3</sup>	1031 ± 1,10	1030 ± 1,43	10,31 ± 1,32	1031 ± 1,87	1028,0	not less than 1027,0	
Mass fraction of protein, %	3,42 ± 0,76	3,37 ± 0,11	3,32 ± 0,64	3,38 ± 0,32	≥ 2,8%		
Mass fraction of lactose, %	5,13 ± 0,28	5,05 ± 0,30	4,09 ± 0,36*	5,07 ± 0,62	is not normalized		
Mass fraction of minerals, %	0,76 ± 0,04	0,75 ± 0,06	0,75 ± 0,02	0,71 ± 0,06	is not normalized		
Mass fraction of water, %	0,00	0,00	0,00	0,00	is not normalized		
Freezing point, (minus) °C	0,592 ± 0,07	0,587 ± 0,03	0,589 ± 0,02	0,590 ± 0,06	not higher than minus 0,520		
Electroconductivity, mS/cm	5,0 ± 0,11	5,1 ± 0,32	5,1 ± 0,21	5,2 ± 0,11	is not normalized		
Active kislotn, from. pH	6,94 ± 0,11	6,90 ± 0,78	6,86 ± 0,84	6,73 ± 0,05*	from 6,6 до 6,7		from 6,55 before 6,8
Titric acidity, °T	17 ± 0,5	18 ± 0,3	19 ± 0,1**	18 ± 0,2	16–17	16–18	16–19
Milk temperature, °C, not higher than	6	6	7	6	8	8	8
Group of cleanliness, not lower than	I	I	I	I	I		

**Note:** \* P < 0,05, \*\* P < 0,01, ND<sup>1</sup> – Determination of milk quality indicators: DSTU 7057: 2009 Raw cow's milk. Ultrasonic determination of density, mass fraction of fat, protein, dry matter and lactose. DSTU 3662: 2018 Milk is raw cow. Specifications.

According to the complex of physicochemical parameters (Table 2): mass fraction of dry matter, density, titrated acidity, purity group, freezing point, temperature of milk obtained in the conditions of NULES of Ukraine "Agronomic Research Station" corresponded to the first grade. All studied indicators corresponded to the current standard. However, it should be noted that the mass fraction of dry matter, density, purity group, freezing point, temperature, milk corresponded to the extra grade. However, the worst indicator was the titratable acidity index, according to which the first grade was exhibited. Titrated acidity of milk is a sanitary and hygienic indicator of its production, which indicates non-compliance with hygienic requirements for obtaining milk of proper quality on this farm.

Other indicators that are normalized but do not affect the grade also met the requirements of current regulations (DSTU 3662: 2018, 2018). The mass fraction of fat and protein was lowest in summer and highest in winter, which can be explained by the predominance in the diet of green cows in summer and concentrated and dry feed in winter.

Although the mass fraction of lactose in Ukraine is not regulated, it is an important indicator of milk quality and regulated by European legislation, it is especially important to produce dairy products for baby food. This figure was lowest in summer, and at other times of the year almost at the same level, and in general was quite high. Mass fraction of minerals, electrical conductivity and active acidity did not differ statistically, depending on the season.

### 3. Results of the study of sanitary and hygienic indicators of milk, M±m, n=16

Name of indicators, units of measurement	Winter	Spring	Summer	Autumn	Norms according to the ND*		
					extra	higher	first

Number of mesophilic aerobic and optional anaerobic microorganisms* (KMAFANM for temperatures 30 <sup>C</sup> ), ted. KUO/cm <sup>3</sup>	476,31 ± 1,06	489,12 ± 3,7*	331,27 ± 1,13**	427,02 ± 2,21**	≤ 100	≤ 300	≤ 500
Number of somatic cells*, thousand/cm <sup>3</sup>	486,31 ± 28,02	463,13 ± 21,01	378,41 ± 23,03*	425,14 ± 18,02	≤ 400	≤ 400	≤ 500
In the appearance of bacteria of the group of intestinal sticks, the presence of 1 cm <sup>3</sup>	< 3	< 3	< 4	< 4	< 3	< 3	< 3
In the appearance of pathogenic microorganisms, including bacteria of the genus <i>Salmonella</i> , 25 cm <sup>3</sup>	–	–	–	–	не дозволено	не дозволено	не дозволено
Detection of coagulase-positive <i>Staphylococcus aureus</i> , in 1 cm <sup>3</sup>	–	–	–	–	не дозволено	не дозволено	не дозволено

**Note:** \*P < 0.05, \*\*P < 0.001. Indicators were determined by the variable geometric mean of the relevant monthly analyzes for a certain period: the content of microorganisms - for a two-month period, on samples that are taken at least twice a month; somatic cell content - for a three-month period, at least one sample per month.

According to the hygienic indicators of KMAFANM and the number of somatic cells, the milk corresponded to the first grade, except for the number of somatic cells and the summer period, mother's milk according to this indicator could be attributed to the highest grade. For other microbiological parameters that do not affect the grade of milk, bacteria of the *Escherichia coli* group in 1 cm<sup>3</sup> were higher than normal in summer and autumn. Pathogenic microorganisms, including bacteria of the genus *Salmonella*, in 25 cm<sup>3</sup> and coagulase-positive *Staphylococcus aureus*, in 1 cm<sup>3</sup> was not found to meet the requirements of current regulations.

Milk of different species is characterized by a specific fatty acid composition. It is known that cow's milk is also characterized by a certain content of fatty acids. The species identification of milk can be determined by the fatty acid composition, which can fluctuate slightly, but is relatively stable. Significant changes in the fatty acid content may indicate milk from mastitis in cows or its adulteration (replacement of milk fat with non-dairy) (Paredes et al, 2018).

Therefore, the next stage of our work was the study of the fatty acid composition of milk. By gas chromatography, 17 fatty acids were identified in milk-raw samples (Table 4).

Depending on the time of year (the effect of the diet), the content of individual fatty acids changed.

#### 4. Fatty acid composition of cow's milk, obtained in the conditions of the NUBIP of Ukraine "Agronomic Research Station", M ± m, n = 16

Name and code of fatty acid	Winter	Spring	Autumn	Summer	*Norm by ND
Butyric acid (C4:0)	3,65 ± 0,16	2,84 ± 0,13**	3,84 ± 0,18	2,90 ± 0,23*	2.0-4.5
Capronic acid (C6:0)	2,78 ± 0,10	2,17 ± 0,11**	2,57 ± 0,22	1,87 ± 0,09***	1.5-3.0
Caprylic acid (C8:0)	1,70 ± 0,08	1,21 ± 0,06**	1,61 ± 0,04	1,54 ± 0,14	1.0-2.0
Capric acid (C10:0)	3,41 ± 0,21	2,77 ± 0,08*	3,17 ± 0,07	2,54 ± 0,02**	2.0-3.5
Lauric acid (C12:0)	3,54 ± 0,12	3,12 ± 0,11*	3,32 ± 0,06	3,18 ± 0,14	2.0-4.0
Miristinova acid (C14:0)	11,60 ± 1,84	10,74 ± 0,56	12,74 ± 0,64	10,03 ± 0,60	8.0-13.0

Miristinoleinova acid(C14:1)	0,79 ± 0,06	1,18 ± 0,09**	1,20 ± 0,08*	1,23 ± 0,11**	0.6-1.5
Pentadecanic acid (C15:0)	1,96 ± 0,11	1,23 ± 0,06***	1,87 ± 0,32	1,80 ± 0,05	3.0-4.5
Palmitic acid (C16:0)	28,83 ± 2,65	28,17 ± 0,95	22,17 ± 1,1*	26,17 ± 1,08	22.0-33.0
Palmatinoleic acid (C16:1)	1,87 ± 0,09	1,90 ± 0,06	1,87 ± 0,07	1,93 ± 0,13	1.5-2.0
Heptadecanic acid (C17:0)	0,87±0,04	0,85 ± 0,05	0,80 ± 0,11	0,80 ± 0,10	2.0.-4.0
Stearic acidC18:0	12,11±0,87	11,90 ± 1,43	10,62 ± 1,12	10,11 ± 1,07	9.0-13.0
Oleic acid (C18:1ω9c)	22,62 ± 1,98	26,94 ± 1.12	28,74 ± 2,78	31,05 ± 2,35*	22.0-32.0
Lynolev acid (C18:2ω6c)	3,76 ± 0,32	4,48 ± 0,11	4,86 ± 0,16	4,22 ± 0,2	3.0-5.5
Arachic acid (C20:0)	0,16 ± 0,06	0,12 ± 0,04	0,15 ± 0,03	0,13 ± 0,02	до 0.3
Tinolenic acid (C18:3ω3)	0,27 ± 0,09	0,30 ± 0,07	0,41 ± 0,05	0,44 ± 0,03	до 1.5
Begenic acid (C22:0)	0,08 ± 0,02	0,08 ± 0,02	0,06 ± 0,02	0,06 ± 0,02	до 0.1

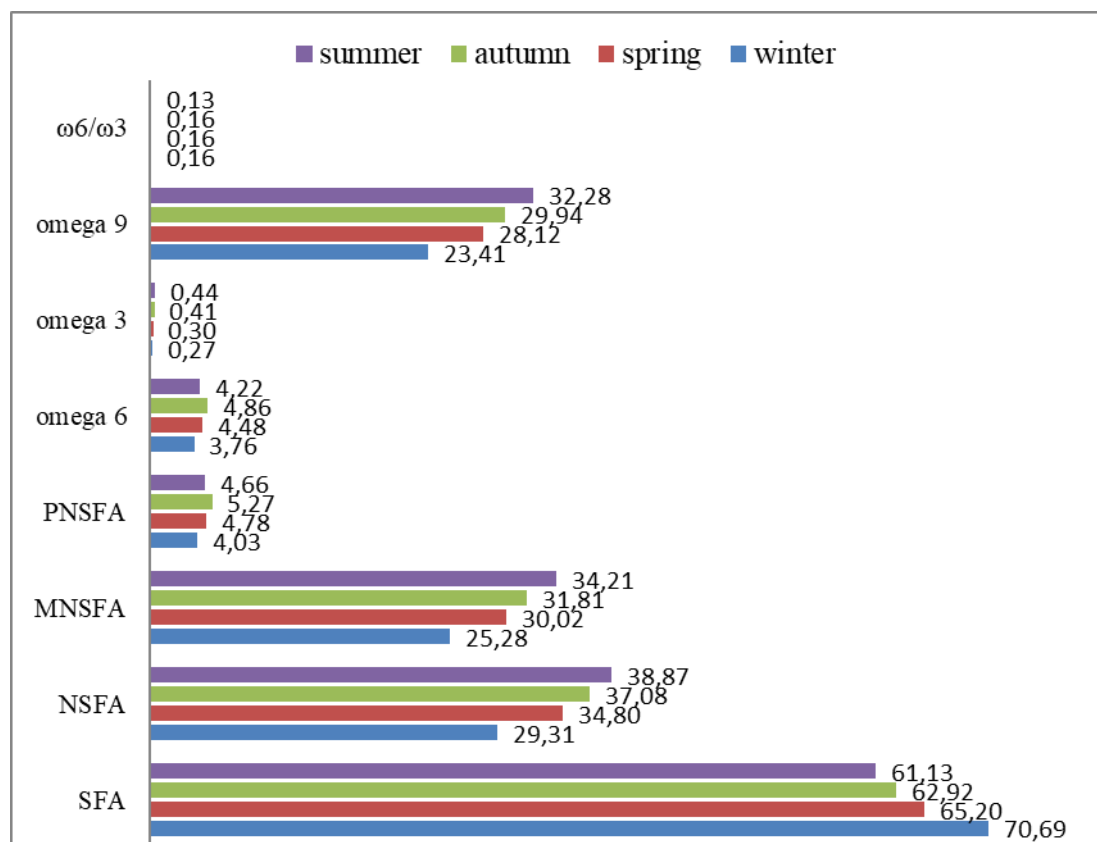
**Note:** \*P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001. Interstate standard GOST 52253-2004 Butter and butter paste from cow's milk. General technical conditions (with change №1) (Butter and butter paste from cow's milk. General technical conditions (with changes №1)). Valid, but does not operate in Ukraine. Interstate standard GOST 52253-2004 Butter and butter paste from cow's milk. General technical conditions (with change №1) (Butter and butter paste from cow's milk. General technical conditions (with changes №1)).

As can be seen from the data presented in table. 3, milk fat differs in the ratio of fatty acids depending on the season and feeding of cows.

The content of saturated fatty acids in the experimental milk was mostly normal. In winter and autumn, a higher content of butyric, kapron, caprylic, capric, lauric, myristic, pentadecanoic and other saturated fatty acids was noted. Milk of all seasons showed a slightly lower content compared to the normative indicators of pentadecanoic (C15: 0) and heptadecanoic (C17: 0) fatty acids.

Unsaturated fatty acids myristic and palmitic in milk were also within normal limits and their number varied according to the seasons. The content of myristic acid was higher in summer and palmitic was stable throughout the year, oleic acid ω 9 predominated in autumn and summer milk, linoleic acid ω 6 predominated in autumn and spring milk, linolenic ω 3 - in autumn and summer when present in the diet. feed and silage.

It should be noted that the amount of saturated fatty acids (SFA) in the studied raw milk in the winter increases by 9.56% compared to summer milk. Monounsaturated fatty acids are contained in summer milk by 8.93% more than in winter. Polyunsaturated fatty acids, including ω 3, are most abundant in summer and autumn milk (Fig. 1).



**Fig. 1. The ratio of individual groups of fatty acids in raw milk depending on the season**

As can be seen from Fig. 1. in summer the highest content of milk was NSFA, MNSFA and omega 9, in autumn the content of omega 6 was slightly higher, in spring all indicators had an average level, in winter - the content of SFA was the highest in milk.

**Conclusions.** Organoleptic, physicochemical, sanitary and hygienic indicators (except for bacteria of the *Escherichia coli* group in summer and autumn) meet the current requirements and change depending on the season. So the color of milk from May to September has a pronounced yellowish tinge. The mass fraction of fat and protein is lowest in summer and highest in winter, which probably depends on the presence of green mass in the diet of cows and concentrated and dry feed.

The fatty acid composition of milk corresponds to the fatty acid composition of milk fat. The amount of saturated fatty acids in winter increases by 9.56% compared to summer. The presence of green mass and corn silage in the diet of cows in milk increases the content of unsaturated fatty acids omega 3.

**Prospects for further research and the practical significance of the results is that the results** can be used as a basis for formulating a diet to obtain milk with optimal content and ratio of fatty acids, which can become competitive in the market of raw milk for milk and dairy products improved chemical composition, dietary, functional products, baby food.

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**ЯКІСТЬ МОЛОКА-СИРОВИНИ ТА ЙОГО ЖИРНОКИСЛОТНИЙ СКЛАД**  
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***Анотація.** Вивчення впливу пори року на органолептичні та фізико-хімічні показники молока і досі залишається актуальним. Крім того, співвідношення жирних кислот у молоці корів характеризує рівень їх годівлі та дослідження цих показників є актуальним з огляду на поширення фальсифікації молока і молочних продуктів рослинними оліями. Метою було дослідження окремих показників якості молока-сировини залежно від пори року та його жирнокислотного складу. Матеріали і методи. Матеріалом для дослідження слугувало збірне охолоджене молоко-сировина, отримане в різні пори року на молочно-товарній фермі ВП НУБіП України "Агрономічна дослідна станція". Дослідження проводили в акредитованій Українській лабораторії якості і безпеки АПК НУБіП України. Органолептичні, фізико-хімічні, санітарно-гігієнічні визначали загальноприйнятими стандартними методами. Жирнокислотний склад молока досліджували методом газової хроматографії. Екстракцію ліпідів з молока проводили за методом Фолча. Аналіз метилових ефірів ЖК проводили на газовому хроматографі Trace GC Ultra (США) з полум'яно-іонізаційним детектором. Ідентифікування жирних кислот проводили за допомогою стандартного зразка Supelco 37 Cotropept FAME Mix. Кількісну оцінку спектру жирних кислот ліпідів молочного жиру здійснювали методом внутрішньої нормалізації з визначенням їх у відсотках. Дослідження проводили у 2-х паралелях. Статистичну обробку експериментальних даних проводили загальноприйнятими методами варіаційної статистики. Вірогідність різниці показників оцінювали за t-критерієм Ст'юдента. Відмінності між показниками, що порівнювались, вважали вірогідними за рівня значимості  $P < 0,05$ . Результати і обговорення. Проведені дослідження засвідчили, що молоко органолептичні та фізико-хімічні показники молока-сировини змінюються залежно від пори року. Щодо мікробіологічних показників, то молоко відповідало першому гатунку, хоча зимою за іншими показниками його можна було віднести до екстра, чи в гіршому випадку, до вищого татунку. Крім того, вміст насичених жирних кислот у молоці зростає. Влітку та восени підвищується вміст ненасичених жирних кислот, що пов'язано з наявністю у раціоні корів зеленої маси та кукурудзяного силосу. Отримані результати можна використовувати як основу для складання раціону з метою отримання молока з оптимальним вмістом і співвідношенням жирних кислот, що може стати конкурентним на ринку молока-сировини з метою виробництва молока і молочних продуктів поліпшеного хімічного складу, дієтичних, функціональних продуктів, дитячого харчування.*

***Ключові слова:** молоко-сировина, якість, санітарно-гігієнічні показники, жирні кислоти*