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FEATURES OF THE RELATIONSHIP BETWEEN THE CHEMICAL COMPOSITION OF COW'S MILK AND ITS FREEZING POINT VALUES

This article reveals the seasonal characteristics of the formation of the most significant factors that affect the freezing point of cow's milk. The results of the generalization performed in this study on the relationship between the freezing point of milk and the content of its main components, which determine its nutritional value, can provide a significant foundation for developing organizational mechanisms to regulate milk production and implement a unified quality policy at all management levels through mathematical modeling. The chosen approach enables the ranking of basic milk quality properties, ensuring the selection of the most important ones. It has been established that an increase in the mass fraction of dry matter and lactose in milk leads to a corresponding decrease in its freezing point. Furthermore, it has been substantiated that the freezing point of milk varies throughout the year. Considering these values, we can fairly assess the degree to which milk has been adulterated with water while ensuring its high quality and safety.

Key words: *milk components, milk freezing point, season of the year.*

Meeting the needs of ordinary consumers for high-quality and safe dairy products requires comprehensive risk control throughout all stages of production, from raw materials to the finished product. Recent European regulatory documents on food safety mandate an independent and objective scientific assessment of these risks. This approach aims to enhance the competitiveness of high-quality milk production in both national and European livestock product markets [16, 20].

Typically, the nutritional value and authenticity (naturalness) of milk are primarily determined by a combination of physical, chemical, and biological parameters, the quantitative values and ranges of variation of which are influenced by the natural properties of this product. This is why the freezing point of milk is considered a key marker for assessing its quality and safety. According to DSTU 3662:2018 «Raw Cow's Milk, Technical Conditions», the freezing point of milk should not exceed — 0.520 °C for extra, higher, and first grades [6]. However, it is equally important to use this parameter to determine the probable addition of water, which may occur not only due to physical addition but also due to technical malfunctions of milking equipment [8, 13, 17].

It is widely accepted that this value is fairly constant, though it can be influenced by factors

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that significantly impact the quality and safety of milk. These factors include breed characteristics [9, 11], feeding habits [14], water supply conditions [1], seasonal changes [1, 4, 10, 18], stage of lactation and age of cows [3—5, 9, 11, 12, 14], herd size [4, 12], milking time [17], variability of individual milk components [3, 4, 11, 14, 18], and heat treatment parameters [15, 21]. According to [2], however, an increase in the mass fraction of lactose in milk can cause almost 54% of the dispersion of changes in its freezing point.

Controversial issues arise concerning the factors that determine the optimal freezing point of milk and the relationships between these factors. While we acknowledge the significant contributions of foreign scientists to assessing the quality and safety of cow's milk based on its freezing point, we must also recognize the ambiguity surrounding this issue and the lack of similar studies focusing on Ukrainian breeds of dairy cattle. This study aimed to establish the relationship between the main components in cow's milk and its freezing point values. Additionally, it aims to identify the components that influence seasonal trends in the quality and safety of milk.

MATERIALS AND METHODS

The research was conducted in the Laboratory for the Assessment of Feed and Animal Product Quality under production conditions. This laboratory is part of the Testing Center of the Livestock Farming Institute of the National Academy of Agrarian Sciences of Ukraine. It is certified for technical competence by the National Accreditation Agency of Ukraine in accordance with the requirements of DSTU ISO/IEC 17025:2006 (accreditation certificate No. 2T621) and DSTU EN ISO/IEC 17025:2018 (accreditation certificate No. 20621). The laboratory is also the base organization of the metrological service of the Ministry of Agrarian Policy and Food of Ukraine. The research focused on milk samples collected during milking from Ukrainian Black-and-White dairy cows on the base farm of the Institute.

Milk was sampled in accordance with the requirements of DSTU ISO 707:2002 «Milk and milk products. Guidelines for sampling» [7]. The procedure for quantitatively assessing the chemical composition of milk was performed by determining the mass fractions of fat, total protein, true protein, lactose, dry matter, and dry skimmed milk residue (DSMR) using an infrared spectrometer with a Bentley 150 milk analyzer (Bentley Instruments, Inc., USA). The difference between total and «true» protein indicators relates to how these indicators are calculated. Total protein indicates the total amount of nitrogen-containing substances in milk. It is calculated by multiplying the amount of nitrogen by 6.38, which is based on the proportion of nitrogen in milk protein. However, this pa-rameter includes not only milk proteins (such as casein and whey proteins) but also non-protein nitrogen-containing compounds (NPNs), including urea, ammonia, creatine, and nucleotides. «True» protein is considered a more accurate parameter of milk's nutritional value because it only considers real protein molecules (*e.g.*, casein, β-lactoglobulin, and α-lactalbumin). At the same time, NPN components (*e.g.*, urea and ammonia) are excluded. The mass fraction of dry skimmed milk residue (DSMR) was determined by subtracting the mass fraction of fat from the total mass fraction of dry matter. «Dry matter of milk» refers to the total mass of milk excluding water and consists of proteins, fats, milk sugar (lactose), minerals, vitamins, and other organic compounds. All indices were assessed as percentages. During the study, special attention was paid to somatic cell content in milk. This was determined using fluorescence optical laser flow cytometry with a Somacount 150 somatic cell analyzer (Bentley Instruments, Inc., USA), in accordance with DSTU 3662:2018 «Raw cow's milk. Technical Conditions» [6].

To analyze the relationships between the content of the main components in milk and its freezing point values, Pearson's pair correlation coefficients were determined. The influence of the main components of milk on the quantitative values of its freezing point was established by single-factor analysis of variance using a second-degree equation. As part of the analysis of the relationship between the content of the main components in milk and its freezing point values, the total sample of the studied samples was divided into three gradations according to the criterion of normalized deviation: $<-0.580^{o}C$; within the range of -0.580 to $-0.556^{o}C$; $> -0.556^{o}C$.

Statistical analysis of the obtained data was performed using SPSS Statistics 20 software (IBM, USA). After creating a computer database, the obtained digital material was processed using methods of variational statistics to calculate arithmetic means, mean square error, and significance level. The significance of the results obtained was assessed at a level of at least 95% ($p < 0.05$) for all data.

RESULTS AND DISCUSSIONS

Laboratory studies have revealed significant seasonal variations in the chemical composition of cow's milk (Table 1).

In particular, the highest significant seasonal differences in milk quality were obtained for the mass fraction of fat in autumn compared to spring and winter ($p < 0.001$); for the mass fraction of «true» protein in winter compared to the rest of the seasons ($p < 0.001$); in the mass fraction of lactose in spring and summer compared to winter and autumn ($p < 0.001$ in both cases); in the mass fraction of dry matter in autumn compared to summer ($p < 0.01$); by mass fraction of dry skimmed milk residue in spring and winter compared to autumn and summer ($p < 0.001$ in both cases); by mass fraction of total protein in winter compared to other seasons ($p < 0.001$ in all cases of comparison) and in spring and summer compared to winter ($p < 0.001$ in both cases of comparison); in terms of freezing point in spring and summer compared to winter and autumn ($p < 0.001$ in both cases); in terms of somatic cell con-tent in spring, summer, and autumn compared to winter ($p < < 0.001$ in all cases). It is noteworthy that the somatic cell count in spring and summer was significantly higher than in autumn and winter ($p < < 0.001$). At the same time, the influence of the season on the formation of the main parameters of cow's milk was low and amounted to $\eta^2 = 0.5 \div 4.9\%$.

The slightly higher mass fraction of lactose in spring milk caused its freezing point to decrease, though it remained within the normative values of DSTU 3662:2018 [6] regardless of seasonal changes. However, as the mass fraction of «true» protein in milk increased in winter and autumn, so did its freezing point. The highest mass fraction of dry matter in winter and autumn corresponded to the lowest freezing point in the samples of manufactured products. An increase in somatic cell content in milk caused its freezing point to decrease slightly in spring and summer. During these periods, the milk's freezing point was lower than in autumn and winter, likely due to higher average outdoor temperatures, which led cows to drink more water. A similar phenomenon was described by M. Bjerg et al. [1].

Pearson's correlation coefficients were calculated to determine the relationship between the content of the main components in milk and its freezing point values (Table 2).

Data analysis revealed multidirectional correlations among the studied parameters, ranging from weak to moderate in strength and including both positive and negative directions. Specifically, the strongest correlation was observed between somatic cell count in milk and freezing point values. The coefficient for this correlation ranged from $r = = +0.413$ to $r = +0.540$ during the study. There was a significant difference between these two indicators ($p < 0.01$ in all cases of comparison). These

Table 1. **Seasonal changes in chemical composition of cow's milk, X ± SE**

Parameter	Season			
	winter	spring	summer	autumn
Number of samples	443	702	410	911
Mass fraction, %:				
fat	4.24 ± 0.05	4.17 ± 0.05	4.34 ± 0.07 ^{ooo}	4.48 ± 0.05 $^{\#, o}$
true protein	3.29 ± 0.02 $^{*, o, \times}$	3.16 ± 0.02 $^{\times}$	2.96 ± 0.02	3.21 ± 0.01 $^{\times, ooo}$
lactose	4.83 ± 0.02	4.99 ± 0.02 $^{\#, *}$	4.95 ± 0.02 $^{\#, *}$	4.82 ± 0.01
dry matter	13.27 ± 0.06	13.26 ± 0.05	13.18 ± 0.06	13.42 ± 0.05 $^{\#\#\#, oo, \times}$
DSMR♦	9.03 ± 0.02 $^{**, \times}$	9.09 ± 0.02 $^{\#\#\#, *, \times}$	8.84 ± 0.03	8.94 ± 0.02 $^{\times}$
total protein	3.50 ± 0.02 $^{*, o, \times}$	3.39 ± 0.02 $^{\times}$	3.19 ± 0.02	3.42 ± 0.01 $^{\times}$
Freezing point, —°C	0.556 ± 0.002	0.570 ± 0.001 $^{*, \times, \#}$	0.562 ± 0.002 $^{*, \#\#\#}$	0.559 ± 0.001
Somatic cell count, thousand/cm^3	785.0 ± 44.7	1015.5 ± 43.1 $^{***, \#}$	1168.9 ± 58.1 $^{**, \#, o}$	984.9 ± 37.6 $^{\#}$

Notes: differences between indices are significant at the level of $p < 0.001$ compared to winter (#), spring (°), summer (×) and autumn (*) respectively; $p < 0.01$ compared to autumn (**) and spring (°°); and $p < 0.05$ compared to winter (###), spring (°°°) and autumn (***). ♦DSMR — dry skimmed milk residue.

results are consistent with those of P. Brzozowski and K. Zdziarski [3].

Weak, almost twice smaller, but also positive correlations were found between the mass fraction of «true» protein in milk and its freezing point values: $r = +0.187 \div r = +0.321$ ($p < 0.01$ in all cases of comparison). The closeness of the interaction between the mass fraction of total protein and the corresponding indicator remained at almost the same level and of similar strength, varying from $r = +0.137$ to $r = +0.261$ ($p < 0.01$) against the background of minimum correlation coefficients in winter ($r = +0.096$, $p < 0.05$).

As a result, the decrease in dry matter content during spring and summer led to a corresponding decline in the milk freezing point. freezing point. The correlation coefficients between these two indicators were $r = –0.350$ and $r = –0.325$, respectively, compared to $r = –0.384$ in autumn and $r = –0.459$ in winter ($p < 0.01$ in all cases).

The relationship between the mass fraction of lactose and freezing point values was negative and quite pronounced, ranging from $r = –0.922$ to $r = –0.951$ ($p < 0.01$ in all cases).

A negative correlation was also observed between the mass fraction of dry skimmed residue and the milk's freezing point, ranging from $r = –0.572$ to $r = –0.792$ ($p < 0.01$ in all comparisons).

The mass fraction of fat in cow's milk was least associated with its freezing point values in all seasons of the year ($r = –0.102$ to $–0.184$). The weakening of the correlation was mostly observed in summer ($r = –0.096$, $p < 0.01$) as opposed to the other seasons. The discrepancies in the results obtained when calculating the correlation coefficients between these indicators are probably due to differences in their respective average assessments. The relationship between the freezing point of cow's milk and its quality parameters is emphasized in works [4, 9, 11, 14, 18].

Table 2. **Correlations between fluctuations in the freezing point of milk and changes in its main components**

Parameter	Season			
	winter	spring	summer	autumn
Number of samples	443	702	410	911
Mass fraction, %:				
fat	–0.184 **	–0.102**	–0.096*	–0.113**
true protein	0.187 **	0.321**	0.303**	0.216**
lactose	–0.951 **	–0.939**	–0.922**	–0.930**
dry matter	–0.459 **	–0.350**	–0.325**	–0.384**
DSMR♦	–0.792 **	–0.580**	–0.572**	–0.687**
total protein	0.096 *	0.261**	0.244**	0.137**
Somatic cell count, thousand/cm^3	0.522 **	0.413**	0.540**	0.504**

Notes: The relationship between milk freezing point values and changes in its main components is significant at the level of $p < 0.05$ (*) and $p < 0.01$ (**).

Table 3. **Regression equations and coefficients of determination of the relationship between the content of the main components in milk (*x*) and its freezing point values (*y*)**

Components of milk	Regression equation	Coefficient of determination (r^2)	Significance, (p)
Mass fraction, %:	$y = –0.529–0.013\,x+0.001\,x^2$ (1)	0.016	0.001
fat	$y = –0.561–0.018\,x+0.006\,x^2$ (2)	0.076	0.001
true protein	$y = –0.107–0.120\,x+0.005\,x^2$ (3)	0.876	0.001
lactose	$y = –0.234–0.109\,x+0.004\,x^2$ (4)	0.196	0.001
dry matter	$y = 0.702–0.243\,x+0.011\,x^2$ (5)	0.438	0.001
DSMR♦	$y = –0.522–0.037\,x+0.007\,x^2$ (6)	0.046	0.001
total protein			
Somatic cell count, thousand/cm^3	$y = –0.580+2.498\times10^{-5}\,x–2.833\times10^{-9}\,x^2$ (7)	0.233	0.001

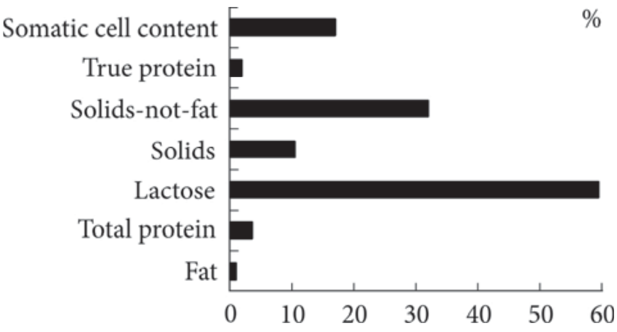

Fig. 1. Influence of the content of the main components of milk on its freezing point

Second-degree equations were used in a regression analysis to determine the relationship between milk's main components and its freezing point.

The results of the calculations established that all relationships between the indicators are nonlinear (Table 3).

It was confirmed that the regression equations are statistically significant and adequately represent the empirical data according to the variance analysis. However, it is important to note that among the equations given in the table, the highest coefficient of determination is characteristic of the dependence of milk freezing point values on lactose content ($r^2 = +0.876$).

Using a step-by-step procedure for introducing variables into the calculation of the multiple regression equation, we obtained expression 8:

$$y = -0.104 - 0.069\,x_1 - 0.009\,x_2, \qquad (8)$$

where x_1 is the lactose content; x_2 is the dry matter content; –0.104, 0.069, and 0.009 are the calculated indices of the equation.

The coefficient of determination ($r^2 = 0.994$) proved to be quite significant when calculating regression parameters, indicating the high statistical significance of the obtained results ($p < 0.001$).

However, when the remaining indicators were included in the calculation and second-degree equations were used, this coefficient did not increase.

In general, the analysis of the equation proves that the higher the mass fraction of dry matter and lactose in milk, the lower its freezing point. Under these conditions, the coefficient of determination for the mass fraction of lactose is more significant than that for the mass fraction of dry matter. Similar conclusions regarding the established features have been recorded in studies [2, 3, 11].

Table 4. **Relationship between milk freezing point values and content of its main components, x ± SE**

Milk component	Grouping of samples by milk freezing point, °C	*n*, samples	The values of the main components of milk	Significance according to the F-criterion
Mass fraction of fat, %	< –0.580	826	4.18 ± 0.05	4.12×10^{-06}
	–0.580 — –0.556	857	4.30 ± 0.05	
	> –0.556	783	4.50 ± 0.05 **, #	
Mass fraction of true protein, %	< –0.580	826	3.27 ± 0.02 ×, #	1.66×10^{-20}
	–0.580 — –0.556	857	3.18 ± 0.01 ×	
	> –0.556	783	3.05 ± 0.01	
Mass fraction of lactose, %	< –0.580	826	4.43 ± 0.01	1.51×10^{-215}
	–0.580 — –0.556	857	4.98 ± 0.01 *	
	> –0.556	783	5.28 ± 0.01 *, #	
Mass fraction of dry matter, %	< –0.580	826	12.76 ± 0.05	7.487×10^{-60}
	–0.580 — –0.556	857	13.39 ± 0.04 *	
	> –0.556	783	13.80 ± 0.04 *, #	
Mass fraction of dry skimmed residue, %	< –0.580	826	8.58 ± 0.02	2.57×10^{-207}
	–0.580 — –0.556	857	9.09 ± 0.01 *	
	> –0.556	783	9.29 ± 0.01 *, #	
Mass fraction of total protein, %	< –0.580	826	3.45 ± 0.02 ×	8.382×10^{-11}
	–0.580 — –0.556	857	3.41 ± 0.01 ×	
	> –0.556	783	3.31 ± 0.01	
Somatic cell count, thousand/cm^3	< –0.580	826	1619 ± 46.0 ×, #	$1.76 \times 10^{-10}0$
	–0.580 — –0.556	857	796 ± 31.0 ×	
	> –0.556	783	533 ± 27.0	

Note: differences between groups are significant at the level of $p < 0.05$ compared to the freezing point of milk –0.580 °C (*), –0.580 — –0.556°C (#) and –0.556 °C (×), respectively; $p < 0.01$ compared to –0.580 °C (**).

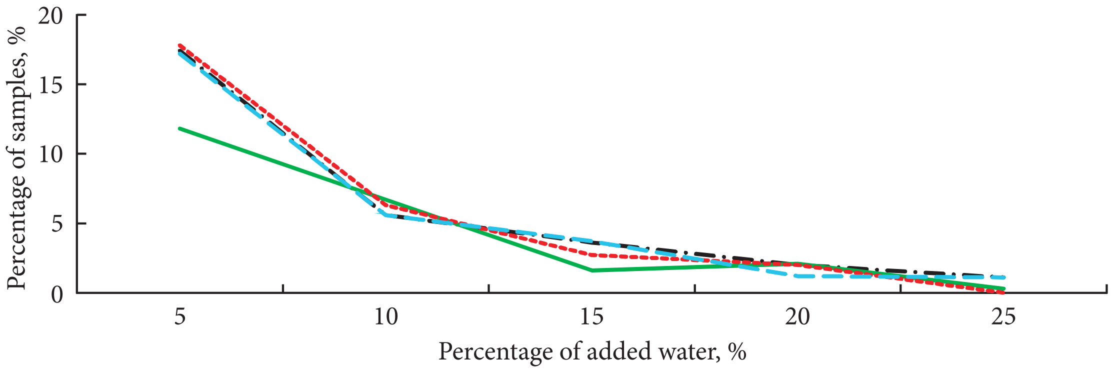

Fig. 2. The proportion of milk samples with probable water addition, determined based on their freezing point: winter — black line; spring — green line; summer — red line; autumn — blue line

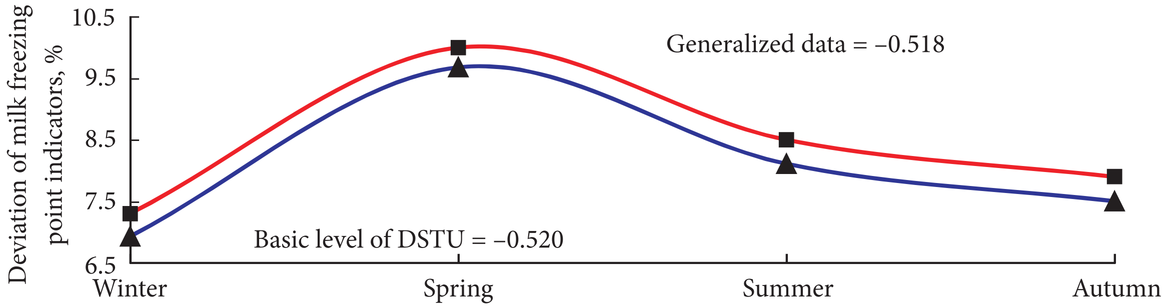

Fig. 3. Deviations in the freezing point of natural milk in different seasons of the year, %: ■ — red line deviation from the national standard; ▲ — blue line deviation from the generalized data

Analysis of regression coefficients calculated for different seasons of the year made it possible to prove differences in the mass fraction of lactose, which turned out to be slightly higher for the winter-autumn period than for the spring-summer period, and are represented by equations 9—12:

Winter: $y = -0.1095-0.0705\ x^1-0.0080\ x^2$; $r^2 = 0.996$ (9)

Spring: $y = -0.1016-0.0691\ x^1-0.0093\ x^2$; $r^2 = 0.997$ (10)

Summer: $y = -0.1072-0.0679\ x^1-0.0090\ x^2$; $r^2 = 0.989$ (11)

Autumn: $y = -0.1027-0.0696\ x^1-0.0090x^2$; $r^2 = 0.998$ (12)

Thus, the proposed regression equations quite accurately ($r^2 = 0.989 \div 0.997$) and statistically significantly ($p < 0.001$) describe the main patterns of seasonal formation of the mass fraction of lactose. In addition, the results obtained (Fig. 1, Table 4) indicate that with an increase in the average values of the mass fractions of fat, lactose, dry matter, and dry skimmed residue in milk, the values of its freezing point also increased. The strength of the influence of the content of the main components of milk on the values of its freezing point varied from $\eta^2 = 3.6$ to $\eta^2 = 59.5\%$.

The opposite pattern was observed for the mass fractions of «true» protein, total protein, and somatic cells in milk. As these values decreased, the freezing point of the milk increased with moderate influence ($\eta^2 = 1.9 \div 17.0\%$). The mass fraction of lactose in cow›s milk had the strongest effect on its freezing point ($\eta^2 = 59.5\%$).

Subsequently, the degree to which milk was adulterated with water, which directly affects its freezing point, was determined (Fig. 2).

It was proven that the season of the year had no significant effect on the milk's freezing point, which averaged between –0.556 °C and –0.580 °C. However, samples exceeding –0.555 °C were recorded within each season, indicating the addition or penetration of water into the milk.

The obtained results align with the published reports [8, 17], which emphasize the key role of the freezing point of cow's milk in detecting water adulteration. Figure 3 illustrates the graphical representation of deviations in the freezing point of natural milk, as determined by monitoring studies, from standard values according to DSTU 3662:2018 [6] and from generalized data presented in the publication [19].

By comparing the actual freezing point values obtained for cow's milk with generalized data, we can conclude that none of the samples were falsified. However, the values given for this indicator (–0.520 to –0.518 °C) are underestimated. The baseline should be the freezing point of milk, which was determined through laboratory analysis of approximately 75 000 samples of cow's milk to be –0.555 °C. This value is considered the reference value for the eastern region of Ukraine. Therefore, the obtained data underscore the importance of determining the freezing point of natural milk to assess its adulteration with water.

CONCLUSIONS

1. The results confirm a correlation between the content of milk's main components and its freezing point values. These values are represented by positive and negative coefficients of weak and moderate strength. In the context of this comprehensive assessment of the quality and safety of cow's milk, a group of parameters has been identified, as well as the most important ones that characterize the multifaceted nature of the object under study from the perspective of food use.

2. The calculated equations, which consider the primary patterns of lactose mass fraction formation over the course of a year, allow us to evaluate the impact of increased dry matter and lactose content on milk's freezing point.

3. This mechanism explains seasonal variations in freezing point values and must be considered to ensure reliable milk quality control, objective falsification detection, and authenticity confirmation in the safety assurance system.

The research was conducted as part of the fundamental task of the state thematic research plan, «Scientific Foundations of Quality and Safety Management of Livestock and Crop Products Based on Adequate Measurement Competence» (state registration number 0116U002307), of the LFI NAAS.
There is no potential conflict of interest.

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ОСОБЛИВОСТІ ЗВ'ЯЗКУ МІЖ ХІМІЧНИМ СКЛАДОМ МОЛОКА КОРІВ ТА ВЕЛИЧИНАМИ ТОЧКИ ЙОГО ЗАМЕРЗАННЯ

У матеріалах статті розкрито сезонні особливості формування найбільш вагомих чинників, що впливають на величини точки замерзання молока корів. Мета досліджень — установити зв'язки між вмістом основних компонентів у молоці корів і величинами точки його замерзання та виділити ті з них, які детермінують їх в сезонному тренді змін якості та безпечності. Методологічними засадами виконаної роботи є комплекс загальноприйнятих методів: узагальнення та систематизації; зоотехнічних; лабораторних; графічних; популяційно-статистичних. Результати проведеного в роботі узагальнення про залежність між величинами точки замерзання молока та вмістом основних його компонентів, які визначають його харчову цінність, можуть стати істотним підґрунтям для розробки організаційних механізмів регулювання виробництва молока і реалізації єдиної політики в сфері його якості на всіх рівнях господарювання за рахунок математичного моделювання. Обраний підхід дає змогу ранжувати базові властивості якості молока, що, перш за все, забезпечує виділення тих із них, які є найважливішими. Установлено, що чим більше міститься в молоці масових часток сухої речовини й лактози, тим нижча точка його замерзання. Обґрунтовано, що точка замерзання молока упродовж року варіює. Зважаючи на її величини об'єктивно оцінено ступінь фальсифікації молока водою на тлі забезпечення високої його якості та безпечності.

Ключові слова: компоненти молока, точка замерзання молока, сезон року.