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ELECTROPHORETIC SYSTEMS FOR THE FRACTIONAL COMPOSITION ANALYSIS OF PROTEINS IN MILK OF COWS OF DIFFERENT BREEDS

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Abstract A comparative analysis of the fractional composition of the main milk proteins of the casein complex and whey from Holstein and Jersey cows from the farms in the Ternopil region of Ukraine was carried out in the work. The milk of these breeds differs in the qualitative and quantitative composition of protein fractions, which may be important for the production of certain types of products. Analysis of literature data showed that genetic variants of the main milk proteins primarily differ in charges and the ability to aggregate in solutions. Therefore, two electrophoretic systems were used to analyze protein fractions, which allow separating caseins and whey proteins according to their charges. These are polyacrylamide gel electrophoresis in the presence of urea for the analysis of caseins and express electrophoresis in native conditions for the analysis of whey proteins. Studies of skim milk samples from Holstein and Jersey breeds using two electrophoresis systems allowed the identification of only three main fractions: α_{S1}-casein, β-casein and β-lactoglobulin (β-Lg). Identification of other fractions and quantitative assessment of all fractions is complicated by the overlapping of protein bands on electrophoregrams. Better results were obtained with separate analysis of caseins and whey proteins from milk of each breed. Analysis of caseins by electrophoresis in the presence of urea allowed to identify and establish the relative content of the following fractions in Holstein milk: α_{S1}-CN B-8P (35,4±2,1%), α_{S2}-CN A-XP (15,2±1,1%), β-CN A^2-5P (36,2±1,6%), and in Jersey milk: α_{S1}-CN C-8P (36,6±1,8%), α_{S2}-CN A-XP (16,2±1,2%) and β-CN A^2-5P (32,8±1,9%). Analysis of whey samples by express electrophoresis in native conditions showed the presence of two genetic variants of β-Lg, but in different ratios – in milk of Holstein cows: β-Lg A (16,7±1,3%) and β-Lg B (23,7±2,2%), and in milk of Jersey cows: β-Lg A (14,9±1,5%) and β-Lg B (27,4±2,3%). The results of express electrophoresis also showed that the same variant α-La – α-La B is present in both milk samples.

Key words: electrophoresis, caseins, milk whey proteins, genetic variants.

Introduction. Formulation of the problem

In Ukraine, the use of purebred cow milk for the production of dairy products is increasing. Milk obtained from individual breeds may differ in the qualitative and quantitative composition of protein fractions. First of all, this concerns to casein complex proteins. Such differences may affect the technological properties of milk and, accordingly, its use for the production of certain types of dairy products. Currently, a common and accessible method for analyzing the fractional composition of milk proteins is polyacrylamide gel electrophoresis (PAG) in the presence of sodium dodecyl sulfate (SDS), which allows separating protein molecules by the difference in their molecular mass values. However, differences in the molecular masses of proteins of different breeds or genetic variants of proteins within the same breed may be insignificant and relate more to the charge of the protein molecule. This complicates the interpretation of the analysis results. Therefore, for fractional analysis of proteins and identification of the relationship between their composition and certain technological properties, it may be advisable to combine electrophoresis in the presence of SDS with a native electrophoresis system for whey proteins and an electrophoretic system in the presence of urea for identification of casein fractions

Analysis of recent research and publications

Before choosing electrophoretic systems in PAG for the analysis of genetic variants of caseins and whey proteins of cow's milk, it is necessary to consider the

features of their structure and properties. It is known that the genes of the casein complex proteins are connected and located on the sixth chromosome [1]. The genes of the three fractions of calcium-sensitive caseins (α_{S1}-CN, α_{S2}-CN, β-CN) are similar in structure and originate from a single ancestor gene. There is a hypothesis that this gene was related to the development of teeth [2]. The gene of the fourth fraction resistant to calcium ions κ-CN is evolutionarily close to the fibrinogen genes [3]. Many years of research on milk proteins using electrophoretic systems, isoelectric focusing, chromatography, and modern mass spectrometry have allowed us to establish many genetic variants of the casein fractions of milk of various species of the genus *Bos*. This primarily concerns the milk of various breeds of cows of the *Bos taurus* species, and also those close to them *Bos indicus* (zebu), *Bos grunniens* (yak) and *Bos javanicus* (banteng) [4, 5]. Table 1 shows the genetic variants of the main casein fractions – α_{S1}-casein.

The most characteristic mutations for α_{S1}-CN are those that phenotypically manifest themselves in the replacement of one or more amino acid residues in the primary structure. In most cases, this leads to a change in the charge of the molecule. Only in two cases (variants A and H) deletions occur and, together with the change in charge, the molecular weight decreases. The comparison is made with the most common variant B.

Table 2 shows data on 12 genetic variants of the second largest casein fraction - β-casein. In all genetic variants of β-casein, amino acid residues are replaced. In this case, with the exception of one variant (I), the overall charge of the molecule changes compared to the most common variant A^2.

The least genetic variants were found in the α_{S2}-casein fraction. In addition to the main one (A), three more are known: B, C and D. Variant C is characteristic only for the *Bos grunniens* species, variant B is found in Asian breeds of cows, as well as in *Bos grunniens* and *Bos indicus*. Genetic variant D, in which the primary structure is shortened by 9 amino acid residues and the negative charge of the molecule is reduced from -6 to -1 due to deletion, is not characteristic for α_{S2}-caseins of Holstein and Jersey milk, which are analyzed in this work [6].

Table 1 – Differences in the primary structure of genetic variants of α_{S1}-casein [5, 6]

Genetic variant α_{S1}-casein*	Amino acid residue number						
	14–26	51–58	53	59	66	84	192
A (-14)	Deletion						
B (-15)			Ala	Gln	SerP	Glu	Glu
C (-14)							Gly
D (-16)			ThrP				
E (-13)				Lys			Gly
F (-14)					Leu		
H (-13)		Deletion					
I (-15)						Asp	

Note: The value of the excess negative charge of the α_{S1}-casein genetic variant molecule is given in parentheses.

Table 2 – Differences in the primary structure of genetic variants of β-casein [6]

Genetic variant β-casein*	Amino acid residue number													
	18	25	35	36	37	67	72	88	93	106	122	137/ 138	152	?
A^1 (-7)						His								
A^2 (-8)	SerP	Arg	SerP	Glu	Glu	Pro	Gln	Leu	Met	His	Ser	Leu/ Pro	Pro	Gln
A^3 (-9)										Gln				
B (-6)						His					Arg			
C^1 (-4)			Ser		Lys	His								
D (-6)	Lys													
E (-6)				Lys										
F (-7)						His							Leu	
G (-7)						His						Leu		
H^1 (-9)		Cys						Ile						
H^2 (-10)							Glu		Leu					Glu
I (-8)									Leu					

Note: The value of the excess negative charge of the β-casein genetic variant molecule is given in parentheses.

The heterogeneity of κ-caseins is formed due to numerous genetic variants (about 14), as well as different degrees of glycosylation. All this gives numerous variants that differ in charges and molecular weights [7]. For their identification, modern mass spectrometry must be used. Therefore, we do not consider their characteristics in this work.

The main whey proteins are coded by genes located in chromosome 11 (β-lactoglobulin) and 5 (α-lactalbumin). β-lactoglobulin (β-Lg) belongs to the family of transport glycoproteins – lipocalins. The structure of the α-lactalbumin (α-La) gene is similar to the lysozyme gene, with which they have a common origin [1]. Changes in the structure and excess negative charge of twelve genetic variants of β-Lg compared to the most common variant B are presented in Table 3. Except of two variants (J and W), all of them relate to the charge of the molecule. The differences in molecular masses, at the same time, are not significant. For the evolutionarily conserved α-La, apart from the main genetic variant B, were found only two variants: A and C [6]. Variant C is characteristic only for the species *Bos javanicus*, and variant A is mainly found in the species *Bos indicus* and rarely in *Bos taurus* cows. Moreover, the molecule of variant A does not differ from variant B in charge and has a very close molecular weight.

Taking into account the above data, it can be concluded that the use of the most common electrophoresis system, namely disc electrophoresis in the presence of SDS, has limited possibilities for identification of genetic variants of such main fractions of milk proteins as α_{S1}-casein, β-casein, β-lactoglobulin, α-lactalbumin. So, in all these mentioned fractions, from 31 genetic variant, only two have significantly different molecular weights, and charge changes are characteristic for 26 compared to the main variants. In addition, the main fractions of caseins have similar molecular weights and during electrophoresis with SDS can partially overlap in PAG plates [8]. In this regard, for the electrophoretic analysis of genetic variants of the main milk proteins, it may be advisable to use electrophoretic systems, where separation occurs according to the charge value of their molecules. Such systems include electrophoresis in the presence of urea for the analysis of caseins, as well as express electrophoresis in native conditions for whey proteins [9, 10].

The aim of the work is to select a combination of electrophoretic systems to detect differences in the fractional composition of milk proteins of Jersey and Holstein breeds of cows.

To achieve this goal, it is necessary to solve the following tasks:

– obtain samples of casein complex proteins and whey proteins from milk of Jersey and Holstein breeds of cows for analysis of the fractional composition;

– characterize the composition of the main protein fractions of milk of both breeds by electrophoresis in the presence of urea and express electrophoresis in native conditions;

– compare the results of the analysis of caseins from milk of both breeds, obtained by electrophoresis in the presence of urea;

– establish the fractional composition of whey proteins obtained from milk of Jersey and Holstein breeds by express electrophoresis under native conditions.

Materials and methods.

The work used whole milk of Holstein and Jersey cows from PJSC «Ternopil Dairy Plant». The total composition of milk and its physicochemical parameters were determined on the «MilkoScan FT2» analyzer. Preparation of samples of skim milk, total casein and whey for electrophoretic analysis was carried out as shown in the diagram (Fig. 1).

Table 3 – Differences in the primary structure of genetic variants of β-Lg [1, 6]

Genetic variant β-Lg*	Amino acid residue number												
	28	45	50	56	59	64	70	78	108	118	126	129	158
A (-7)						Asp				Val			
B (-6)	Asp	Glu	Pro	Ile	Gln	Gly	Lys	Ile	Glu	Ala	Pro	Asp	Glu
C (-5)					His								
D (-5)		Gln											
Dr (-5)	Asn												
E (-5)													Gly
F (-4)			Ser									Tyr	Gly
G (-5)								Met					Gly
H (-8)						Asp	Asn			Val			
I (-5)									Gly				
J (-6)											Leu		
W (-6)				Leu									

Note: The value of the excess negative charge of the β-lactoglobulin genetic variant molecule is given in parentheses.

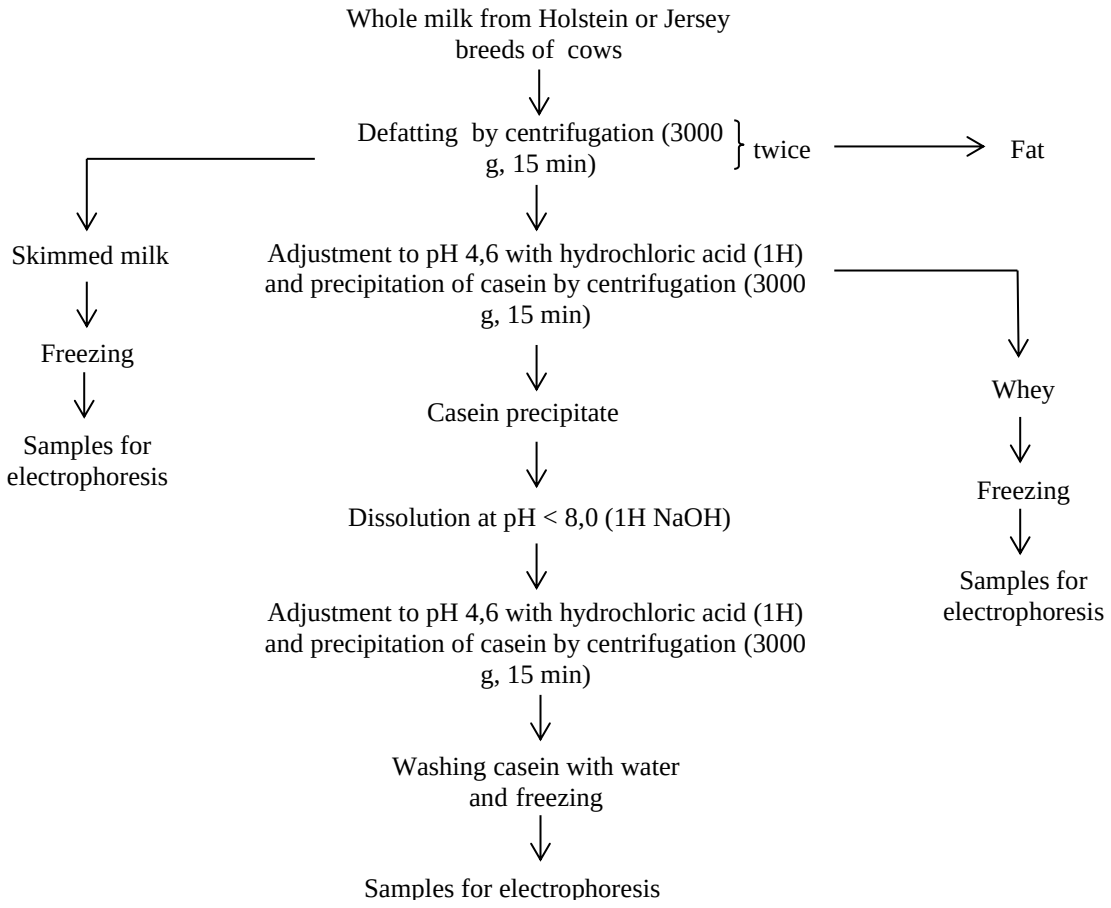

Fig. 1. Scheme of preparation of samples of skim milk, total casein and whey for electrophoretic analyses

In order to reduce the influence of denaturing factors on proteins, the sample preparation scheme was simplified as much as possible. In particular, gel filtration was not used to separate low-molecular-weight whey compounds, which have little effect on the results of electrophoresis. When purifying caseins from skim milk, only two precipitations of it at the isoelectric point were used.

The concentration of casein complex proteins and whey proteins was determined spectrophotometrically by absorption at a wavelength of λ=280 nm on an SF-46 spectrophotometer. The following absorption coefficients ($D_{1cm}^{1\%}$) were used to calculate the protein concentration: 8,2 for total casein and 12,3 for whey proteins [11].

The fractional composition of caseins in samples of skim milk and total casein was analyzed by electrophoresis in homogeneous PAG in the presence of urea [9]. The fractional composition of whey proteins was analyzed by express electrophoresis in native conditions [10]. Densitograms for quantitative analysis were constructed using the *imread* graphic image reading functions in the Matlab system. Mathematical and statistical processing of the results was performed using Microsoft Office Excel 2007 software packages.

Results of the research and their discussion

It is known that the milk of Holstein and Jersey breeds can differ significantly in fat content, as well as in the content and fractional composition of proteins. Therefore, first of all, for samples of chilled milk of both breeds, the total composition was determined using the «MilkoScan FT2» analyzer. The analysis results are shown in Table. 4. The obtained data indicate a significantly higher fat and protein content in Jersey milk, which is consistent with the results described in the literature [12].

Cow's milk is characterized by high heterogeneity of casein and whey protein composition. Therefore, two electrophoresis systems were selected for electrophoretic analysis. This is an anodic system in homogeneous PAG in the presence of urea, which effectively separates the proteins of the casein complex of milk, and an express electrophoresis system in native conditions for the separation of whey proteins [9, 10].

In the first series of experiments, samples of skim milk from Holstein and Jersey breeds were analyzed using both electrophoretic systems. Moreover, in each case, the samples were analyzed in parallel in one gel plate in absolutely identical conditions. The results of the analysis of skim milk samples by electrophoresis in the presence of urea are shown in Fig. 2.

Table 4 – Composition of whole milk samples of Holstein and Jersey cows

Cows breeds	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of casein, %	Mass fraction of lactose, %	Dry substances, %	Acidity, °T
Holstein	3,82	3,37	2,49	4,88	12,87	17
Jersey	5,05	4,12	3,2	4,75	14,84	18

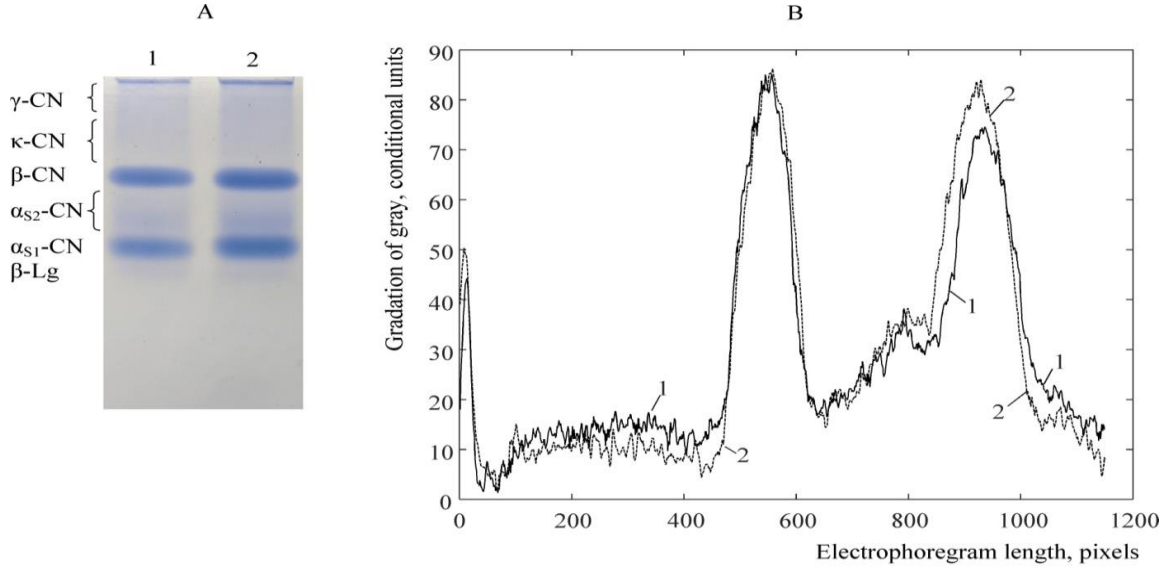

Fig. 2. Electrophoregrams (A) and densitograms (B) of skim milk from Holstein cows (A1, B1) and Jersey cows (A2, B2) obtained by electrophoresis in homogeneous PAG in the presence of urea

As can be seen from the electrophoregram, the qualitative composition of the protein fractions of both samples is identical. The main fractions such as α_{S1}-CN and β-CN are clearly visible. All other fractions are difficult to identify, since it is known that in the presence of urea β-Lg, α-La and BSA do not give clear bands and overlap with α_{S1}- and α_{S2}-caseins. Also, the components of the proteose-peptone fraction coincide on the PAG plate with κ-caseins. All this makes it impossible to identify proteins and quantitatively process electrophoregrams.

Fig. 3 shows the results of the analysis of skim milk samples in the express electrophoresis system in native conditions. The qualitative composition of the protein fractions in both samples is very similar. β-Lg, α_{S1}-CN and β-CN can be identified. In addition, the densitograms indicate a significant disordered background, which is formed from minor fractions of whey proteins and aggregates of casein fractions. It is known that caseins in native conditions can form aggregates of different sizes and compositions [11]. All this significantly complicates the identification of even the main protein fractions, as well as the quantitative processing of electrophoregrams.

However, in both electrophoregrams (Fig. 2 and 3) it is possible to note the difference in the electrophoretic mobility of α_{S1}-casein fractions. This casein moves faster to the anode in a sample of skimmed milk from Holstein cows.

Further, separate samples of caseins and whey proteins isolated from milk of each breed were used for electrophoretic analysis as shown in the diagram (Fig. 1). In this case, electrophoresis of casein was performed in a homogeneous PAG system in the presence of urea, and whey proteins were analyzed by express electrophoresis in native conditions. The results of electrophoresis of casein complex proteins from milk of Holstein and Jersey cows are shown in Fig. 4. Both electrophoregrams (A1 and A2) show all groups of caseins: α_{S1}-, α_{S2}-, β-, κ- and γ-caseins. The main difference between the casein fractions of both samples concerns α_{S1}-casein. Its mobility is higher in the casein sample from Holstein milk. The densitograms of β-caseins and, to a large extent, κ-caseins completely coincide. The densitograms of α_{S2}- and γ-caseins differ quantitatively. These fractions are more abundant in Jersey milk. Quantitative calculations based on densitograms of five casein samples from each breed showed the following relative contents of α_{S1}-, α_{S2}- and β-caseins: 35,4±2,1%, 15,2±1,1%, 36,2±1,6% for Holstein and 36,6±1,8%, 16,2±1,2%, 32,8±1,9% for Jersey.

Express electrophoresis of whey proteins (Fig. 5) showed quantitative differences in the composition of the main fractions (β-Lg, α-La and IG). The content of these fractions is higher in Jersey milk. It is also necessary to note the differences in the relative content of the genetic variants β-Lg A and β-Lg B. The relative content of β-Lg A and β-Lg B in the milk of Holstein cows is 16,7±1,3% and 23,7±2,2%, and in the milk of Jersey cows – 14,9±1,5% and 27,4±2,3%, respectively.

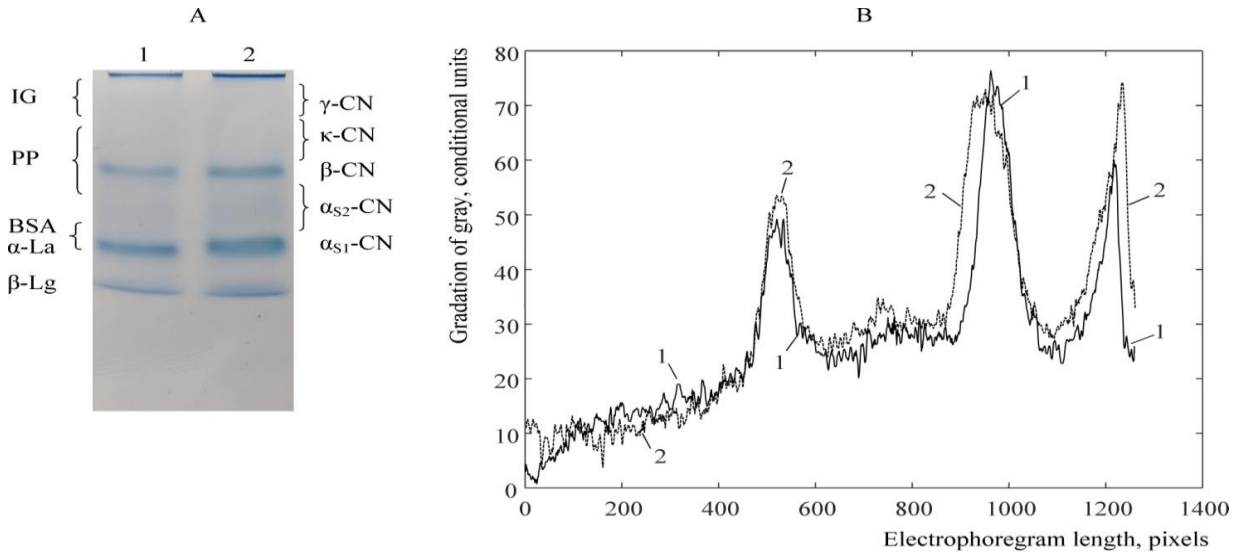

Fig. 3. Electrophoregrams (A) and densitograms (B) of skim milk from Holstein cows (A1, B1) and Jersey cows (A2, B2) obtained by express electrophoresis in native conditions

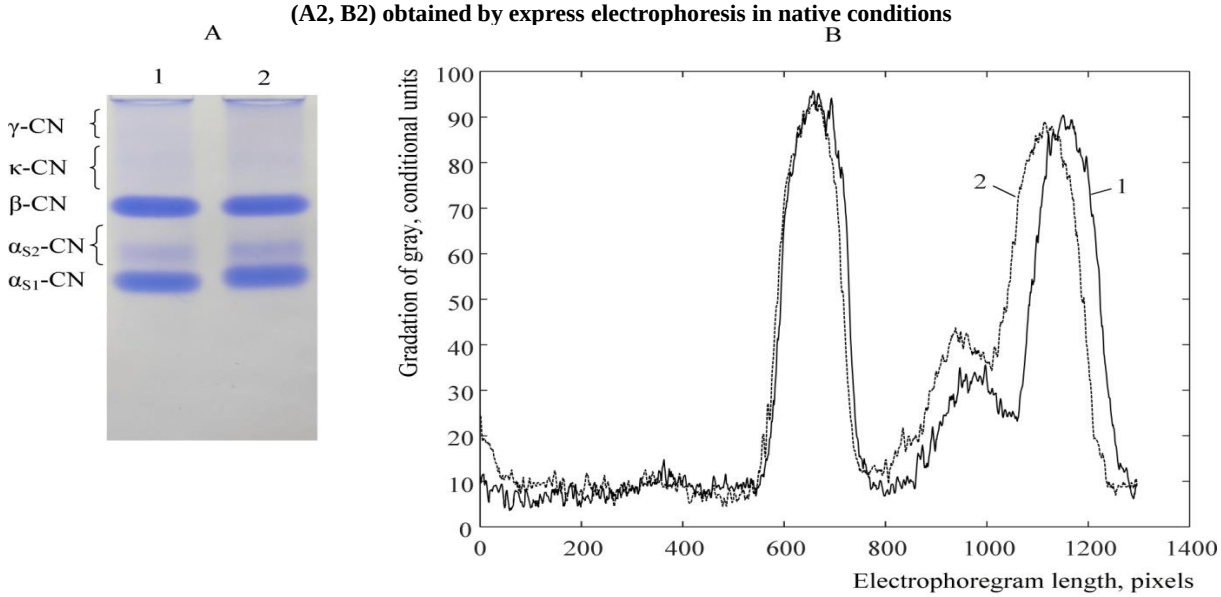

Fig. 4. Electrophoregrams (A) and densitograms (B) of caseins isolated from milk of Holstein cows (A1, B1) and Jersey cows (A2, B2) obtained by electrophoresis in homogeneous PAG in the presence of urea

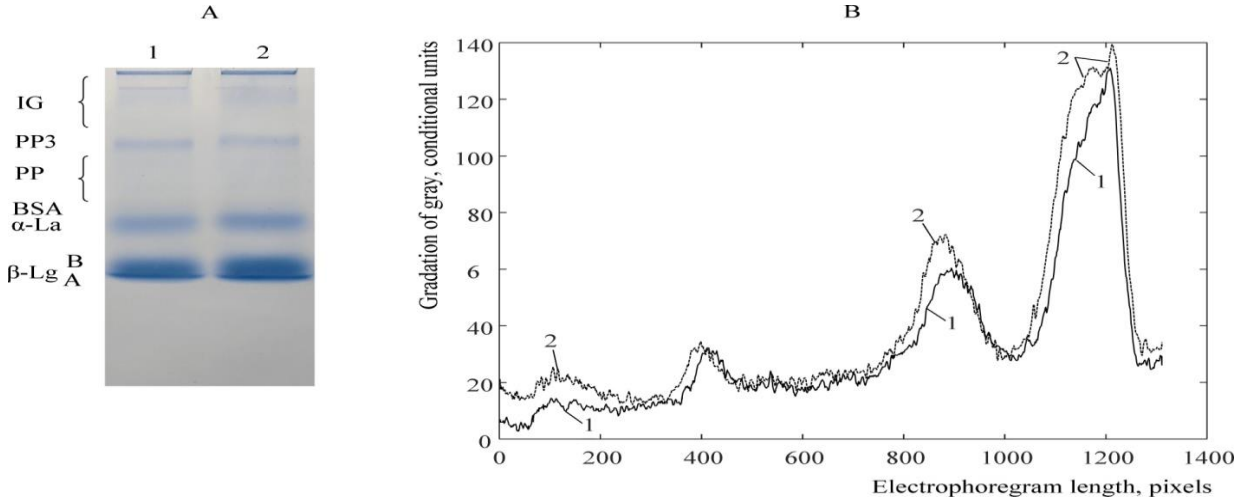

Fig. 5. Electrophoregrams (A) and densitograms (B) of whey proteins isolated from milk of Holstein cows (A1, B1) and Jersey cows (A2, B2) obtained by express electrophoresis in native conditions

The obtained results of electrophoresis of caseins from milk of both breeds can be used to establish genetic variants of the three main fractions – α_{S1}-, β- and α_{S2}-caseins. For Holstein milk, the most characteristic genetic variant of α_{S1}-casein is α_{S1}-CN B-8P. Its prevalence is about 97% [13]. Therefore, it can be affirmed with high probability that this variant is present in the pooled milk of Holstein cows used for analysis. In the studied milk of Jersey cows, α_{S1}-casein has a lower electrophoretic mobility. This is clearly visible on the electrophoregram of caseins (Fig. 4). Among the known genetic variants of α_{S1}-casein (Table 1), only five have a lower negative charge and, accordingly, a lower electrophoretic mobility in the anodic system than variant B. These are variants A, C, E, F and H [5, 6]. Variant E is found only in milk of the *Bos grunniens* species. Variants F and H are less common, variant A is more common, but all of them are not characteristic for Jersey milk. Therefore, it is most likely that the α_{S1}-CN C-8P variant is present in the samples of studied Jersey milk. This genetic variant is often found in Jersey milk [6].

The placement of β-caseins on the electrophoregrams and, accordingly, densitograms of both casein samples completely coincides. It is obvious that this is the most common variant of β-casein in both breeds – β-CN A^2-5P [1]. As for α_{S2}-casein, of its four genetic variants, only the α_{S2}-CN A-XP variant is characteristic for the Holstein and Jersey breeds [6]. In general, the densitograms of the κ-casein sections of the electrophoretic plots of caseins from the milk of both breeds are similar. It is difficult to establish the genetic variants of κ-caseins. The electrophoretic properties of κ-caseins are affected by the degree of glycosylation, in addition to changes in the primary structure. It is obvious that to identify the genetic variants of κ-caseins, it is necessary to conduct comparative electrophoresis of purified κ-casein preparations or use more sensitive methods.

Two genetic variants of β-Lg were predicted to be found in the composition of the milk of both breeds: β-Lg A and β-Lg B. These variants are the most common and characteristic for the milk of Holstein and Jersey breeds. The α-La fraction is similar in electrophoretic characteristics in both samples and is the genetic variant α-La B. Two other known variants are characteristic for the *Bos indicus* (A) and *Bos javanicus* (C) species [6].

The composition of genetic variants of caseins and whey proteins can affect the enzymatic and acid coagulation of milk. Thus, for Holstein and Jersey cows, it was found that the B variant of β-casein and β-Lg is the best for the production of rennet cheeses [14, 15]. The presence of the β-CN A^2 genetic variant in milk reduces its ability to rennet coagulation [16, 17]. The α_{S1}-CN C genetic variant, which is characteristic of Jersey cows, promotes rennet coagulation and the formation of a good clot [18, 19]. The decrease of coagulation properties of milk may be associated with a higher content of α_{S2}-CN [20]. Genetic variants of β-casein may affect the properties of the gel obtained by acid coagulation. For example, milk with β-CN A^1 forms a stronger gel than with the genetic variant β-CN A^2 [21].

Approbation of research results. The obtained results were used in the laboratory of milk biochemistry of Ivan Puluj TNTU in the isolation of proteins-precursors of bioactive peptides, as well as at PJSC «Ternopil Dairy Plant» in the selection of milk for the production of curds.

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Conclusion

As a result of the conducted studies, the following conclusions can be made:

– the combination of electrophoretic systems in PAG in the presence of urea and express electrophoresis allows us to establish certain differences in the composition of the main protein fractions when analyzing skim milk from Holstein and Jersey cows. This concerns to α_{S1}-casein, β-casein and β-Lg. However, the quantitative assessment of all fractions and the identification of other protein fractions is very complicated due to their overlap on electrophoregrams. It is necessary to conduct a separate analysis of caseins and whey proteins.

– the analysis of casein samples from Holstein and Jersey milk allowed us to identify qualitative and quantitative differences (relative content) in the composition of their fractions. Taking into account the results of electrophoresis in the presence of urea and literature data, it was found that the genetic variants α_{S1}-CN B-8P (35,4±2,1%), α_{S2}-CN A-XP (15,2±1,1%), β-CN A2-5P (36,2±1,6%) were present in the studied milk of the Holstein breed, and in the milk of the Jersey breed –α_{S1}-CN C-8P (36,6±1,8%), α_{S2}-CN A-XP (16,2±1,2%) and β-CN A2-5P (32,8±1,9%).

– express electrophoresis in native conditions of whey samples revealed two genetic variants of β-Lg, but in different ratios, in milk of Holstein cows – β-Lg A (16,7±1,3%) and β-Lg B (23,7±2,2%), and in milk of Jersey cows – β-Lg A (14,9±1,5%) and β-Lg B (27,4±2,3%). According to the results of express electrophoresis, it was also found that the same variant α-La – α-La B is present in both milk samples.

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ЕЛЕКТРОФОРЕТИЧНІ СИСТЕМИ ДЛЯ АНАЛІЗУ ФРАКЦІЙНОГО СКЛАДУ БІЛКІВ МОЛОКА КОРІВ РІЗНИХ ПОРІД

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Анотація. У роботі проведено порівняльний аналіз фракційного складу основних білків казеїнового комплексу і сироватки молока корів голштинської і джерсейської порід з фермерських господарств Тернопільської області України. Молоко цих порід відрізняється якісним і кількісним складом білкових фракцій, що може бути важливим для виготовлення певних видів продуктів. Аналіз літературних даних показав, що генетичні варіанти основних молочних

білків насамперед відрізняються зарядами і здатністю до агрегації в розчинах. Тому для аналізу білкових фракцій були використані дві електрофоретичні системи, які дозволяють розділяти казеїни і білки сироватки за їх зарядами. Це електрофорез в поліакриламідному гелі в присутності сечовини для аналізу казеїнів і експрес-електрофорез в нативних умовах для аналізу білків сироватки молока. Дослідження взірців знежиреного молока голштинської і джерсейської порід двома системами електрофорезу дозволили ідентифікувати лише три основні фракції: α_{S1}-казеїн, β-казеїн і β-лактоглобулін (β-Lg). Ідентифікація інших фракцій і кількісна оцінка всіх фракцій ускладнена накладенням білкових смуг на електрофореграмах. Кращі результати були отримані при окремому аналізі казеїнів і білків сироватки з молока кожної породи. Аналіз казеїнів електрофорезом в присутності сечовини дозволив ідентифікувати і встановити відносний вміст таких фракцій в молоці голштинської породи: α_{S1}-CN B-8P (35,4±2,1%), α_{S2}-CN A-XP (15,2±1,1%), β-CN A^2-5P (36,2±1,6%), а в молоці джерсейської породи: α_{S1}-CN C-8P (36,6±1,8%), α_{S2}-CN A-XP (16,2±1,2%) і β-CN A^2-5P (32,8±1,9%). Аналіз взірців сироватки експрес-електрофорезом у нативних умовах показав наявність двох генетичних варіантів β-Lg, але в різних співвідношеннях – у молоці корів голштинської породи: β-Lg A (16,7±1,3%) і β-Lg B (23,7±2,2%), а у молоці джерсейської породи: β-Lg A (14,9±1,5%) і β-Lg B (27,4±2,3%). За результатами експрес-електрофорезу також встановлено, що в обох взірцях молока присутній один і той же варіант α-La – α-La B.

Ключові слова: електрофорез, казеїни, білки сироватки молока, генетичні варіанти.