The object of this study is three lactoferrin (LF) preparations used as bioactive supplements. The problem of determining the fractional composition of proteins in lactoferrin preparations was solved.

The molecular weights of proteins in LF preparations have been determined by gel filtration on Sephadex G-25 and G-100. It was established that all three preparations contain proteins which molecular weights, including LF, are in the range from 5000 to 100000 Da. Low-molecular-weight (about 1000 Da) peptides were found in one preparation, which constitute 9 ± 3% of all protein impurities. Four different electrophoretic systems in polyacrylamide gel (PAG) were used to identify proteins in LF preparations. It was found that in order to detect whey protein fractions in preparations it is advisable to combine electrophoretic systems under native conditions with a disc electrophoresis system in the presence of sodium dodecylsulfate (SDS). Casein fractions can be detected by combining electrophoresis in the presence of urea and disc electrophoresis with SDS. Quantitative analysis of the relative content of impurity proteins was performed by the densitometry of PAG plates after disc electrophoresis in the presence of SDS. In the studied preparations (LF_1, LF_2, LF_3), in addition to LF, β-lactoglobulin (β-Lg), blood serum albumin (BSA), and α_{S1}-casein (α_{S1}-CN) were detected. The relative content of these fractions from all proteins in the preparations is as follows: in LF_1 – β-Lg (3 ± 0.4%), α_{S1}-CN (< 1%), BSA (< 1%); in LF_2 – β-Lg (3 ± 0.3%), α_{S1}-CN (1 ± 0.2%), BSA (1 ± 0.3%) and in LF_3 – β-Lg (3 ± 0.4%), α_{S1}-CN (1 ± 0.2%), BSA (5 ± 0.6%). All the studied LF preparations differ in the content and ratio of protein fractions, which may indicate the need to analyze the protein composition of each batch of the preparation

Keywords: lactoferrin preparations, gel filtration, electrophoresis, protein fractions, whey proteins, caseins

UDC 577.112.083
DOI: 10.15587/1729-4061.2025.338604

COMBINATION OF ELECTROPHORETIC SYSTEMS FOR DETERMINING OF THE FRACTIONAL COMPOSITION OF PROTEINS IN LACTOFERRIN PREPARATIONS

Volodymyr Yukalo
Doctor of Biological Sciences, Professor*
Kateryna Datsyshyn
PhD, Associate Professor*
Olha Krupa
PhD, Associate Professor*
Liudmyla Storozh
Corresponding author
PhD, Associate Professor*
E-mail: lstorozh@gmail.com
*Department of Food Biotechnology and Chemistry
Ternopil Ivan Puluj National Technical University
Ruska str., 56, Ternopil, Ukraine, 46001

Received 25.06.2025
Received in revised form 29.08.2025
Accepted date 04.09.2025
Published date 22.10.2025

How to Cite: *Yukalo, V., Datsyshyn, K., Krupa, O., Storozh, L. (2025). Combination of electrophoretic systems for determining of the fractional composition of proteins in lactoferrin preparations. Eastern-European Journal of Enterprise Technologies, 5 (11 (137)), 79–85. https://doi.org/10.15587/1729-4061.2025.338604*

1. Introduction

The bioactive milk protein lactoferrin has a wide range of positive effects on the body. These include bactericidal and antiviral activity [1], immunomodulatory and anticarcinogenic effects [2], regulation of lipid metabolism [3], and promotion of iron ion absorption [4]. Given this, lactoferrin is used in food products for children, the elderly, athletes, and for disorders of the digestive and immune systems. Multi-tonnage production of lactoferrin preparations has been established in the world. An important characteristic of such preparations is the degree of purification of lactoferrin from other milk proteins, which must be constantly monitored. Studies of lactoferrin preparations usually indicate its content, as well as the content of proteins in general. At the same time, an analysis of the fractional composition of impurity proteins is not performed. Such data may be important when using lactoferrin preparations. It is known that lactoferrin is isolated on an industrial scale from skim milk and various types of whey, which is dairy by-product in the production of acid and rennet caseins [5], as well as curds and hard cheeses [6, 7]. In this case, it is extracted from the raw material and purified by ion-exchange chromatography on cation exchangers, as well as a combination of various types of filtration. As a result are obtained the preparations that can contain from 85 to 95% lactoferrin. In addition to lactoferrin, to their composition, depending on the used raw materials and the purification technique may include other milk proteins or their fragments. First of all, this applies to proteins that are similar in properties to lactoferrin or are capable of forming complexes with it. Such proteins include lactoperoxidase (LP), α_{S1}-casein (α_{S1}-CN), α_{S2}-casein (α_{S2}-CN), β-casein (β-CN), and κ-casein (κ-CN). Most modern studies on the degree of lactoferrin purification use various modifications of the Laemmli disc electrophoresis procedure under denaturating conditions in the presence of sodium dodecylsulfate (SDS). In this case, the lactoferrin fraction in the preparation is well identified. However, the qualitative and quantitative analysis of other milk proteins in preparations can be complicated. This is due to their high heterogeneity and different properties [8]. Therefore, it may be appropriate to use other electrophoretic systems for the characterization of industrial lactoferrin preparations, which

allow for the effective identification of whey proteins and the casein complex of milk. Thus, devising and optimizing electrophoretic methods for analyzing lactoferrin preparations are critically important for ensuring their quality, efficiency, and safety in medical and food applications.

2. Literature review and problem statement

For analyzing industrial lactoferrin preparations, various types of modern liquid chromatography are used, as well as polyacrylamide gel electrophoresis (PAG) [9, 10]. High-performance liquid chromatography (HPLC) in combination with mass spectrometry allows for quantitative and qualitative analysis of lactoferrin preparations proteins. However, this method is very expensive and unsuitable for serial analysis of samples under production conditions. It is more often used in scientific research.

For serial studies on industrial lactoferrin preparations, electrophoresis in vertical PAG plates is more convenient and affordable, which allows the simultaneous comparison of the protein composition of about 10 preparations under absolutely identical conditions. The Committee on Milk Protein Nomenclature and Methodology of the American Dairy Science Association recommended disc electrophoresis under denaturing conditions in the presence of SDS to analyze whey proteins [11]. In practice, electrophoretic systems of disc electrophoresis in the presence of SDS, proposed by Laemmli [12], are used for analyzing lactoferrin preparations. In this system, lactoferrin itself is well identified in preparations with a high degree of purification (90–95%), as well as the main whey proteins β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). However, lactoferrin and lactoperoxidase are inefficiently separated in this system [13]. These proteins have similar molecular weights [14]. In addition, on the electrophoresis LP, which is present in the preparation in small quantities, it can be masked by a broad band of lactoferrin. As for caseins, their main fractions α_{S1}-CN and β-CN behave abnormally during disc electrophoresis in the presence of SDS. This is due to differences in SDS binding [15], which complicates their identification on electrophoresis. Also, these casein fractions can form a common band, which is explained by the closeness of their molecular mass values. Thus, determining the full fractional composition of lactoferrin preparations by disc electrophoresis alone in the presence of SDS is problematic.

There are known other electrophoretic systems that are used for the milk proteins analysis [15]. This is electrophoresis in homogeneous PAG in the presence of urea, which allows for the effective identification of all casein fractions. For analyzing whey proteins, a disc electrophoresis system can be used under native conditions [16]. These electrophoretic systems are not suitable to analyze lactoferrin itself and require some modification. However, they can be used for the analysis of impurity proteins in lactoferrin preparations.

Thus, none of the electrophoretic systems can make it possible to resolve the complete fractional composition of proteins in lactoferrin preparations. To solve this issue, combining different electrophoretic systems may be promising.

3. The aim and objectives of the study

The aim of our study is to select combinations of electrophoretic systems for determining the fractional composition of proteins in industrial lactoferrin preparations. This will allow a more objective assessment of their quality and the possible influence of protein fractions on the biological effect of lactoferrin.

To achieve the goal, the following tasks were set:

– to characterize the molecular weights of proteins in industrial lactoferrin preparations by gel filtration on columns with Sephadex G-25 and G-100;

– to obtain electrophoregrams and characterize the fractional composition of proteins in lactoferrin preparations in electrophoretic systems used for the analysis of whey proteins and casein complex;

– to obtain electrophoregrams and characterize the qualitative and quantitative composition of proteins in lactoferrin preparations by disc electrophoresis in the presence of SDS.

4. The study materials and methods

The object of our study is three lactoferrin preparations used as bioactive supplements. These preparations, conditionally designated as LF_1, LF_2, and LF_3, were obtained for research from PJSC "Ternopil Dairy Plant" (Ternopil, Ukraine).

The principal hypothesis assumes that lactoferrin preparations differ in the fractional composition of protein impurities. This may be due to the use of different types of raw materials for their production. Protein impurities may affect the biological effect of lactoferrin preparations.

Control samples of casein complex and whey proteins were isolated from skim milk obtained at PJSC "Ternopil Dairy Plant". Caseins were isolated by centrifugation (5000 rpm, 10 min) on an OPN-8 centrifuge (VAT TNK "Dastan", Kyrgyz Republic) after adjusting the pH to the isoelectric point of 1 M HCl. The resulting precipitate was dissolved in distilled water by adding 1 M NaOH, ensuring that the pH value did not exceed 7.5. The precipitation procedure was repeated twice. After the second dissolution, the casein was lyophilized. The whey preparation was obtained after isoelectric precipitation of casein.

Gel filtration of lactoferrin preparations was carried out using chromatographic columns, buffer and sample containers from the company "Reanal" (Hungary). Chromatographic fractions were collected using a Yargo fraction collector (BioMark, Ink, Ukraine). Sephadex G-25 (medium) and G-100 (super-fine) from the company "Pharmacia" (Sweden) were selected for gel filtration. The molecular weight fractionation ranges for these Sephadex species complement each other and are from 0 to 5000 Da (for Sephadex G-25) and from 4000 to 100,000 Da (for Sephadex G-100).

Electrophoresis of lactoferrin preparations under native conditions was performed in an analytical disc electrophoresis system in PAG for neutral and acidic proteins as described in [16], as well as in an express electrophoresis system in homogeneous PAG [17]. Electrophoresis of lactoferrin preparations in homogeneous PAG in the presence of urea was performed according to the method proposed earlier [16]. The listed electrophoretic studies were performed on a Stadier-type apparatus manufactured in the laboratory at the Department of Food Biotechnology and Chemistry of the Ivan Puluj Ternopil National Technical University (TNTU, Ukraine). The characteristics of the apparatus are described in [16].

Disc electrophoresis in the presence of SDS of lactoferrin preparations was performed in the scientific laboratory at the Department of Dairy Products and Quality Management, the University of Warmia and Mazury (Olsztyn, Poland), according to the method reported in [18]. For the analysis, the Mini-PROTEAN® 3 Cell apparatus from Bio-Rad (Italy), ready-made 12% PAG plates, and a set of ten marker proteins with molecular weights from 10,000 to 250,000 Da from Bio-Rad Laboratories (USA) were used. All lactoferrin preparations were analyzed in quadruplicate on two PAG plates for quantitative evaluation. Quantitative processing of PAG plates was performed using the *imread* graphic image reading function in the MATLAB package (MathWorks, USA).

The concentration of proteins in the samples was determined spectrophotometrically in the ultraviolet region of the spectrum on an Agilent Cary 60 spectrophotometer (Agilent Technology, USA). The following absorption coefficients at a wavelength of $\lambda = 280$ nm were used to calculate the concentration: 12.3 – for whey proteins; 8.2 for total casein; and 9.91 for lactoferrin [8].

Mathematical and statistical processing of the results was carried out using the Microsoft Office Excel 2007 software package (Microsoft Corporation, USA). Electrophoretic analysis of lactoferrin preparations was carried out with four repetitions to increase the reliability of the results. The data obtained during quantitative evaluation were entered into spreadsheets in the Microsoft Office Excel 2007 program. For each set of results, the average value of the value obtained from four repetitions and the standard deviation were calculated.

5. Results of investigating the fractional composition of proteins in lactoferrin preparations in different electrophoretic systems

5. 1. Characterization of molecular weights of proteins in industrial lactoferrin preparations

To estimate the molecular weights of possible impurities of protein and peptide nature in lactoferrin preparations, gel filtration was used on two types of Sephadex – G-25 and G-100. The results of gel filtration of three lactoferrin preparations on Sephadex G-25 are shown in Fig. 1. Preparations LF_1 and LF_2 on chromatograms (Fig. 1, *a, b*) are represented by one symmetrical peak, the elution volume of which from the column is equal to the free volume. The molecular weights of proteins and polypeptides (except lactoferrin) included in this peak are ≥ 5000 Da. Impurities with $M < 5000$ Da are almost absent. In the LF_3 preparation (Fig. 1, *c*), in addition to the main peak, there is also a small amount of peptides (M about 1000 Da), which elute with a volume close to the full column volume. Their amount of all impurities is 9 ± 3%.

The results of lactoferrin preparation gel filtration on a Sephadex G-100 column are shown in Fig. 2.

The shape of the chromatographic profiles of all three preparations is similar and includes one main peak, which consists mainly of lactoferrin molecules, as well as impurities with molecular weights of about 100,000 Da and above. A significant number of polypeptide impurities have molecular weights between 4000 and 100,000 Da and are represented in the chromatogram of all preparations by two small peaks. This range includes the proteins of the casein complex of milk and most of the major whey proteins [11].

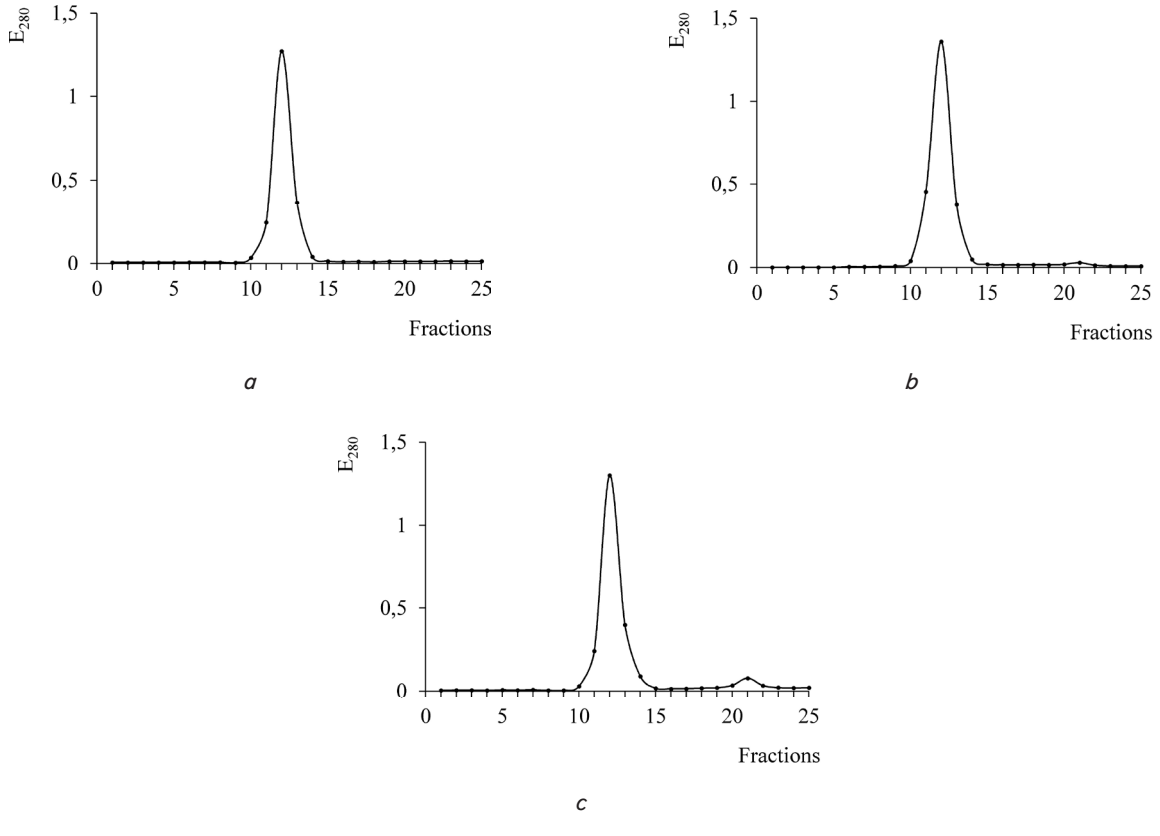

Fig. 1. Chromatograms of lactoferrin preparations obtained by gel filtration on a Sephadex G-25 column: *a* – LF_1; *b* – LF_2; *c* – LF_3

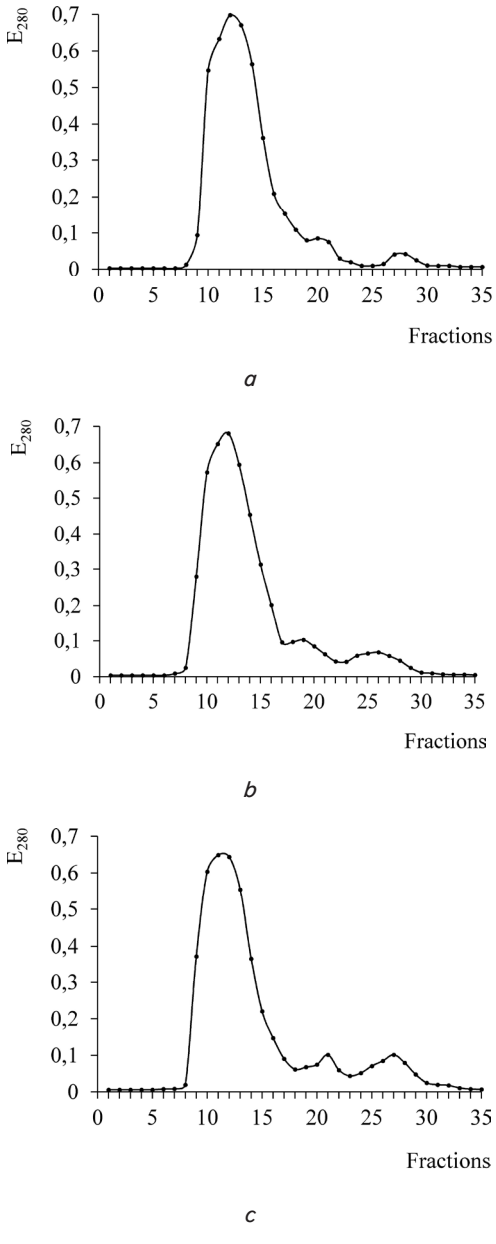

Fig. 2. Chromatograms of lactoferrin preparations obtained by gel filtration on a Sephadex G-100 column: *a* – LF_1; *b* – LF_2; *c* – LF_3

5. 2. Studies of lactoferrin preparations in electrophoretic systems used for the analysis of caseins and whey proteins

Considering the methods of isolation and purification of lactoferrin from different types of dairy raw materials, it can be predicted that the main impurity proteins will be, first of all, whey proteins. For serial analyses of such proteins, the analytical system of disc electrophoresis under native conditions is effective [16]. The results of disc electrophoresis of the control whey sample, three lactoferrin samples, and total casein are shown in Fig. 3, *a*. The electrophoregram of the control whey and casein samples (lanes 1 and 5) clearly shows all the main whey fractions (β-Lg A and B, α-La, BSA, Ig), as well as two casein fractions (α_{S1}-CN, β-CN) and a mixture of α_{S2}-CN fractions.

To improve the efficiency of protein separation of lactoferrin preparations in the following analyses, an express system of electrophoresis in homogeneous PAG under native conditions with an increased pH value of PAG and electrode buffer to 9.0 was used. The results of electrophoresis of a control whey sample and three lactoferrin preparations in such a system are shown in Fig. 3, *b*.

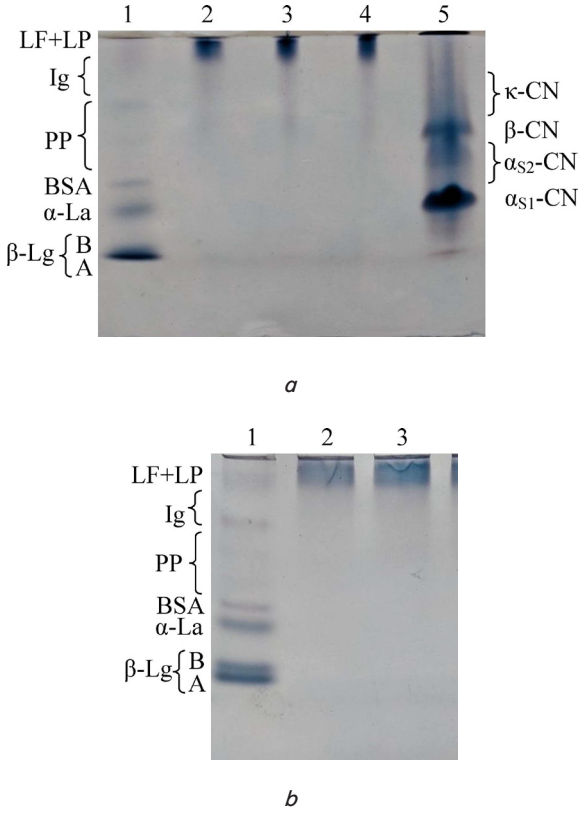

Fig. 3. Electrophoregrams: *a* – control sample of milk whey (1), three preparations of lactoferrin LF_1 (2), LF_2 (3), LF_3 (4), and control sample of total casein (5), obtained by disc electrophoresis under native conditions; *b* – control sample of milk whey (1), three preparations of lactoferrin LF_1 (2), LF_2 (3), and LF_3 (4), obtained by express electrophoresis with increased pH PAG

To identify caseins, which according to literature data are present in lactoferrin preparations [13], an electrophoretic system of homogeneous PAG with urea was used [16]. This system allows us to detect all casein fractions. The results of electrophoresis in the presence of urea of the control total casein, the LF_3 preparation, and milk whey are shown in Fig. 4.

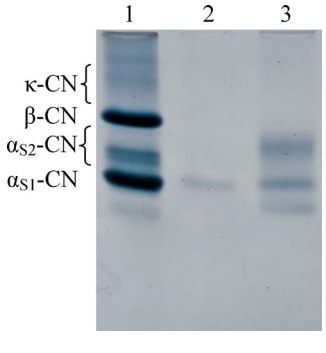

Fig. 4. Electrophoregram obtained by electrophoresis in homogeneous PAG in the presence of urea: 1 – control casein sample; 2 – lactoferrin LF_3 preparation; 3 – control whey sample

The electrophoregram of total casein shows all casein fractions (Fig. 4, 1). Whey proteins in this system form blurred bands (Fig. 4, 3) due to the denaturing effect of urea. Among the three lactoferrin samples, only in the LF_3 preparation there was a small amount of the α_{S1}-CN fraction detected (Fig. 4, 2). In the other preparations, caseins in the free state are absent.

5. 3. Analyzing the qualitative and quantitative composition of lactoferrin preparations by disc electrophoresis in the presence of sodium dodecylsulfate

To study the protein composition of lactoferrin preparations by molecular weights, a variant of the Laemmli disc electrophoresis system in the presence of SDS was used [18]. The results of the qualitative analysis of lactoferrin preparations by disc electrophoresis in the presence of SDS are shown in the electrophoregram (Fig. 5). In parallel, to identify the protein fractions of the preparations, a separation of a set of marker proteins with known molecular weights was carried out (Fig. 5, 1). The electrophoregram shows that the composition of the preparations, in addition to lactoferrin, includes three distinct protein fractions, which are marked on the electrophoregram as X_1, X_2, and X_3. Given their location relative to the marker proteins, they can be identified in the PAG plate as β-lg (X_1), α_{S1}-CN (X_2), and BSA (X_3). In addition to the three fractions mentioned, all preparations contain minor fractions in trace amounts (<1%).

Based on densitometry of all lanes of lactoferrin preparations, the relative content of lactoferrin and fractions designated as X_1, X_2, and X_3 were calculated. The results are given in Table 1.

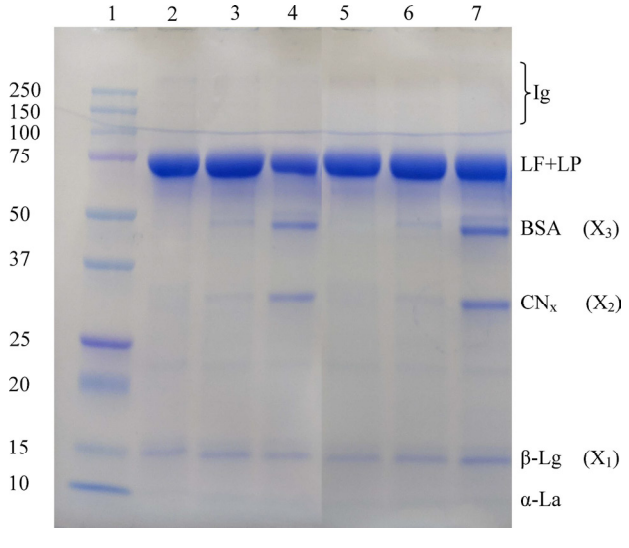

Fig. 5. Electrophoregram of a set of marker proteins (×1000 Da) — lane 1 and lactoferrin preparations: LF_1 — lanes 2 and 5; LF_2 — lanes 3 and 6; LF_3 — lanes 4 and 7, obtained by disc electrophoresis in PAG in the presence of SDS

Table 1

Relative content of protein fractions in lactoferrin preparations (M ± m, n = 4)

Lactoferrin preparations	Relative content of protein fractions, %				
	β-lactoglobulin	α_{S1}-casein	whey albumin	lactoferrin	other proteins
Preparation LF_1	3 ± 0.4	Traces*	Traces*	93 ± 2.5	4 ± 0.6
Preparation LF_2	3 ± 0.3	1 ± 0.2	1 ± 0.3	90 ± 2.3	5 ± 0.7
Preparation LF_3	3 ± 0.4	3 ± 0.5	5 ± 0.6	84 ± 1.9	5 ± 0.6

Note: Traces – content less than 1%.

6. Discussion of results based on the analysis of the fractional composition of proteins in lactoferrin preparations in different electrophoretic systems

Gel filtration of lactoferrin preparations on two types of Sephadex (G-25 and G-100) with different fractionation ranges allowed us to preliminarily estimate the content and molecular weights of protein impurities in them. Only in one preparation (LF_3) there was a small amount of peptides with molecular weights of about 1000 Da (Fig. 1). The remaining proteins and polypeptides have a molecular weight exceeding 5000 Da. This is evidenced by the chromatographic profiles of lactoferrin preparations obtained by gel filtration on Sephadex G-100 (Fig. 2). Comparing the gel filtration data and the known values for the proportion of lactoferrin in the total protein of its preparations, we can conclude that the molecular weights of the main part of the proteins and polypeptides of the impurities are in the range from 5000 to 100,000 Da. At the same time, the upper limit may have a lower value since the first peak in the chromatograms, which includes lactoferrin (M = 76,110 Da), does not start with protrusions and elutes from the column with a volume exceeding the free volume (Fig. 2). This excludes the presence of proteins with significantly higher molecular weights in lactoferrin preparations. Among the impurity proteins with molecular weights from 5,000 to 100,000 Da, there may be the main whey proteins (β-Lg, α-La, and BSA), all casein fractions (α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN), fragments of β-casein [8]. In lactoferrin preparations obtained from cheese whey, there may be various peptides, in particular glycomacropeptide (GMP), which are formed under the action of milk-clotting enzymes on caseins [19, 20].

Gel filtration methods cannot establish the fractional composition of impurity proteins in lactoferrin preparations. This is not possible because of their low resolution [13]. However, the information obtained on the molecular mass distribution of protein fractions allows for adequate selection of electrophoretic analysis conditions. This can be achieved by combining electrophoretic systems used for the analysis of milk proteins [16, 18]. Given the wide variety of proteins and polypeptides that may be part of lactoferrin preparations, it should be noted that none of the known electrophoretic systems can equally effectively separate all of these proteins [15]. Thus, to identify the most probable whey proteins in lactoferrin preparations, the analytical disc electrophoresis system can be successfully used under native conditions. In this case, it is possible to reliably identify all the main whey proteins in the undenatured state, including the genetic variants A and B of β-lactoglobulin [16]. Comparison of the results of our analysis of lactoferrin preparations in this system with the results of electrophoresis in the presence of SDS can provide valuable information about the temperature conditions for the isolation and purification of lactoferrin. It is known that some types of biological action of lactoferrin are very sensitive to heating [10]. When using the original methodology of the analytical system of disc electrophoresis under native conditions, it is not possible to visualize the band of lactoferrin itself, which remains near the starting line (Fig. 3, *a*, 2–4). In addition, this band may include a lactoperoxidase fraction with similar properties [13].

To improve the visualization of the lactoferrin fraction, another variant of electrophoresis under native conditions was proposed – express

electrophoresis in homogeneous PAG [17] with an increased pH value of the electrode buffer and gel. As can be seen in the electrophoregram (Fig. 3, *b*, 1), the express system allows one to detect all the main fractions of whey proteins. Traces of these proteins are visible on the tracks of lactoferrin preparations (Fig. 3, *b*, 2–4). First of all, this applies to the BSA and β-Lg fractions. Lactoferrin under such conditions forms a clear band in the gel plate. The blurring of protein bands on electrophoregram may be associated with partial thermal denaturation of whey proteins during the isolation and purification of lactoferrin. Casein fractions were not detected in all three preparations. The use of a homogeneous gel in a native system significantly simplifies analysis without reducing the efficiency of whey protein separation. This is due to the preservation of the effect of concentration of sample proteins after their entry into the gel. Casein fractions are not detected in the analysis of lactoferrin preparations in native systems (Fig. 3). They may form complexes with lactoferrin [11] or aggregates of different sizes under native conditions and do not appear as clear bands [8]. To identify caseins, an anodic electrophoretic system is used in a homogeneous PAG in the presence of urea [16]. Only in one (LF_3) of the three preparations was it possible to detect a fraction that is similar in electrophoretic mobility to α_{S1}-CN (Fig. 4).

For visualization of native and denatured whey proteins in lactoferrin preparations, disc electrophoresis under denaturing conditions in the presence of SDS is well suited [15]. This system allows for effective quantitative and qualitative analysis of the main whey proteins (Fig. 5). The results of quantitative analysis based on densitometry reveal that the majority of the impurity proteins are whey proteins (Table 1). The separation of caseins in this system is complicated by the closeness of their molecular masses. However, in combination with electrophoresis in the presence of urea, casein fractions can be identified. In this case, this is confirmation of the presence of the α_{S1}-casein fraction in the LF_3 preparation. When comparing the results of electrophoresis with SDS (Fig. 5) and under native conditions (Fig. 3), it can be concluded that in these preparations in the denatured state there is a β-Lg fraction, and in the LF_3 preparation there is denatured BSA and traces of α-La. To obtain a complete picture of the fractional composition of protein impurities in lactoferrin preparations, it is advisable to use a combination of three electrophoretic systems: express electrophoresis under native conditions, electrophoresis in the presence of urea, and disc electrophoresis under denaturating conditions in the presence of SDS. Such a combination of electrophoresis can be practically applied for the analysis of lactoferrin preparations at enterprises where it is produced or used. All of the above methodologies can be implemented in a Stadier-type apparatus, which was adapted for the analysis of milk proteins [16].

The disadvantage of our study when using the proposed combination of electrophoresis is the inability to detect most minor proteins. In addition, the set of standard marker proteins used in the work does not allow for the unambiguous identification of individual protein fractions. First of all, this drawback concerns casein fractions. Further development of this study may involve the use of special markers from purified milk proteins.

7. Conclusions

1. Gel filtration of three lactoferrin preparations (LF_1, LF_2, LF_3) using a combination of Sephadex G-25 and G-100 showed that about 90% of protein impurities have molecular weights in the range from 5000 to 100,000 Da. Low-molecular peptides (about 1000 Da) in significant quantities were found only in one preparation (LF_3) and constitute 9 ± 3% of all protein impurities.

2. For the identification of whey proteins, it is advisable to use express electrophoresis under native conditions in combination with disc electrophoresis in the presence of SDS. Such a combination also allows one to establish their denatured state, in contrast to studies that were conducted earlier and used only disc electrophoresis with SDS. Electrophoresis with SDS separates proteins exclusively in the denatured state. Casein identification can only be effectively performed by a combination of urea electrophoresis and disc electrophoresis with SDS. This is due to the abnormal binding of casein fractions to SDS. These combinations allow reliable detection of the following protein fractions in lactoferrin preparations: β-Lg, α-La, BSA, α_{S1}-CN, α_{S2}-CN, and β-CN.

3. For quantitative analysis, PAG plates obtained by disc electrophoresis of lactoferrin preparations in the presence of SDS are better suited. β-Lg, BSA, and α_{S1}-CN were detected in the studied lactoferrin preparations. The relative content (%) of these fractions from all proteins of the preparations is: in LF_1 – β-Lg (3 ± 0.4%), α_{S1}-CN (< 1%), BSA (< 1%); in LF_2 – β-Lg (3 ± 0.3%), α_{S1}-CN (1 ± 0.2%), BSA (1 ± 0.3%); and in LF_3 – β-Lg (3 ± 0.4%), α_{S1}-CN (1 ± 0.2%), BSA (5 ± 0.6%).

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

Funding

The study was conducted without financial support.

Data availability

The data will be provided upon reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Acknowledgments

The authors express their gratitude to the Chairman of the Board at PJSC "Ternopil Dairy Plant" Vitalii Kovalchuk and the Main Technologist Borys Lukiianchuk for the provided lactoferrin preparations, milk samples, as well as assistance in conducting the research.

The authors are also sincerely grateful to the Head of the Department of Dairy Products and Quality Management at the University of Warmia and Mazury in Olsztyn (Poland), Professor Justyna Zhulevska, postgraduate student Justyna

Tarapata, and engineer Hanna Novak for the opportunity and assistance in conducting disc electrophoresis in the presence of SDS of lactoferrin preparations at the scientific laboratory of the Department.

References

1. Kell, D. B., Heyden, E. L., Pretorius, E. (2020). The Biology of Lactoferrin, an Iron-Binding Protein That Can Help Defend Against Viruses and Bacteria. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.01221
2. Cutone, A., Rosa, L., Ianiro, G., Lepanto, M. S., Bonaccorsi di Patti, M. C., Valenti, P., Musci, G. (2020). Lactoferrin's Anti-Cancer Properties: Safety, Selectivity, and Wide Range of Action. Biomolecules, 10 (3), 456. https://doi.org/10.3390/biom10030456
3. Min, Q.-Q., Qin, L.-Q., Sun, Z.-Z., Zuo, W.-T., Zhao, L., Xu, J.-Y. (2018). Effects of Metformin Combined with Lactoferrin on Lipid Accumulation and Metabolism in Mice Fed with High-Fat Diet. Nutrients, 10 (11), 1628. https://doi.org/10.3390/nu10111628
4. Superti, F. (2020). Lactoferrin from Bovine Milk: A Protective Companion for Life. Nutrients, 12 (9), 2562. https://doi.org/10.3390/nu12092562
5. Duran, A., Kahve, H. I. (2017). The use of lactoferrin in food industry. Academic Journal of Science, 07 (02), 89–94. Available at: https://universitypublications.net/ajs/0702/html/DE6C406.xml
6. Slyvka, I., Tsisaryk, O., Musii, L., Kushnir, I., Koziorowski, M., Koziorowska, A. (2022). Identification and Investigation of properties of strains Enterococcus spp. Isolated from artisanal Carpathian cheese. Biocatalysis and Agricultural Biotechnology, 39, 102259. https://doi.org/10.1016/j.bcab.2021.102259
7. Tsizh, B., Skulska, I., Tsisaryk, O., Slyvka, N., Musiy, L., Slyvka, I. (2024). Characteristics of the course of proteolytic processes in brynza with reduced common salt content. Zywnosc Nauka Technologia Jakosc/Food Science Technology Quality, 31 (4), 23–44. https://doi.org/10.15193/zntj/2024/141/519
8. Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., O'Mahony, J. A. (2015). Dairy Chemistry and Biochemistry. Springer International Publishing. https://doi.org/10.1007/978-3-319-14892-2
9. Krolitzki, E., Schwaminger, S. P., Pagel, M., Ostertag, F., Hinrichs, J., Berensmeier, S. (2022). Current practices with commercial scale bovine lactoferrin production and alternative approaches. International Dairy Journal, 126, 105263. https://doi.org/10.1016/j.idairyj.2021.105263
10. Wakabayashi, H., Yamauchi, K., Abe, F. (2018). Quality control of commercial bovine lactoferrin. BioMetals, 31 (3), 313–319. https://doi.org/10.1007/s10534-018-0098-2
11. Yukalo, V. H. (2021). Biolohichna aktyvnist proteiniv i peptydiv moloka. Ternopil: Vyd-vo TNTU imeni Ivana Puliuia, 372. Available at: https://elartu.tntu.edu.ua/handle/lib/36801
12. Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature, 227 (5259), 680–685. https://doi.org/10.1038/227680a0
13. Perraudin, J.-P., Valck, L. D. (2020). Lactoferrin Production from Bovine Milk or Cheese Whey. Journal of Engineering and Applied Sciences Technology, 2 (1), 1–9. https://doi.org/10.47363/jeast/2020(2)103
14. Deeth, H. C., Bansal, N. (Eds.) (2019). Whey Proteins: From Milk to Medicine. Academic Press. https://doi.org/10.1016/c2016-0-02581-0
15. Sharma, N., Sharma, R., Rajput, Y. S., Mann, B., Singh, R., Gandhi, K. (2021). Separation methods for milk proteins on polyacrylamide gel electrophoresis: Critical analysis and options for better resolution. International Dairy Journal, 114, 104920. https://doi.org/10.1016/j.idairyj.2020.104920
16. Yukalo, V., Datsyshyn, K., Krupa, O., Storozh, L. (2024). Adaptation of Stadier's apparatus for electrophoresis of main milk proteins. Eastern-European Journal of Enterprise Technologies, 1 (11 (127)), 73–80. https://doi.org/10.15587/1729-4061.2024.296753
17. Yukalo, V., Datsyshyn, K., Storozh, L. (2019). Electrophoretic system for express analysis of whey protein fractions. Eastern-European Journal of Enterprise Technologies, 2 (11 (98)), 37–44. https://doi.org/10.15587/1729-4061.2019.160186
18. Tarapata, J., Maciejczyk, M., Zulewska, J. (2022). Microfiltration of buttermilk: Partitioning of proteins and modelling using a resistance-in-series model. International Dairy Journal, 134, 105445. https://doi.org/10.1016/j.idairyj.2022.105445
19. Kukhtyn, M., Kremenchuk, I., Horiuk, Y., Salata, V., Kochetova, H., Kladnytska, L. et al. (2025). Development and evaluation of technology for preserving hard cheese with staphylococcal bacteriophage. Scifood, 19, 208–223. https://doi.org/10.5219/scifood.16
20. Kukhtyn, M., Arutiunian, D., Pokotylo, O., Kravcheniuk, K., Salata, V., Horiuk, Y. et al. (2024). Microbiological characteristics of hard cheese with flax seeds. Potravinarstvo Slovak Journal of Food Sciences, 18, 281–296. https://doi.org/10.5219/1956