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PECULIARITIES OF PROTEINS FRACTIONATION AT ELECTROACTIVATION OF WHEY

Irina Paladii [1*], ORCID: 0000-0002-5683-5248,
Elvira Vrabie [1], ORCID: 0000-0001-8607-8981,
Mircea Bologa [1], ORCID: 0000-0002-5262-9666,
Valeria Vrabie [2], ORCID: 0000-0002-2651-7150,
Tatiana Stepurina [1], ORCID: 0009-0002-4589-8443,
Albert Policarpov [1], ORCID: 0009-0008-4762-1775,
Catalina Sprincean [1], ORCID: 0009-0001-1540-007X

[1] *Institute of Applied Physics, Moldova State University, 5 Academiei St., Chisinau, Republic of Moldova*
[2] *Moldova State University, 60 Alexei Mateevici St., Chisinau, Republic of Moldova*
* Corresponding author: Irina Paladii, *irina.paladii@ifa.usm.md*

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Abstract. Food processing, which generates both by-products and waste, requires a revision of modern processes in the framework of the development of non-residual, environmentally friendly processes. Dairy by-products require the development of complex zero-waste technologies. Electroactivation is an emerging process to overcome those challenges, which allows for non-residual processing of milk by-products. Electroactivation, managing both the technological regimes and the geometric/technical parameters of the electrolyzers used to process different types of whey, allows both the electrofractionation of the whey proteins recovered in protein mineral concentrates enriched, under certain process conditions, with a certain protein content, but also the simultaneous isomerization of lactose into lactulose following two mechanisms, and creating a closed process cycle. The geometry of the electrolyzers and the content of the secondary liquid (anodic liquid) influence both the extraction and the formation of protein compounds enriched with alpha-lactalbumin, as well as the formation of a complex between calcium and isomerized lactulose.

Keywords: *Alpha-lactalbumin, electrofractionation, electrolyzers, isomerization, lactose, lactulose, secondary dairy products.*

Rezumat. Procesarea alimentelor, care generează atât subproduse, cât și deșeuri, necesită o revizuire a proceselor moderne în contextul dezvoltării unor procese non-reziduale, ecologice. Subprodusele lactate necesită dezvoltarea tehnologiilor complexe - zero deșeuri. Electroactivarea este o metodă emergentă de soluționare a acestor provocări, și permite prelucrarea non-reziduală a subproduselor lactate. Gestionând atât regimurile tehnologice, cât și parametrii geometrici/tehnici a electrolizoarelor utilizate la procesarea diferitor tipuri de zer, procedeul dat permite atât electrofracționarea proteinelor serice ale zerului

recuperate în concentrate proteice minerale ce le înnobilează, în anumite condiții de tratare, cu anumit conținut proteic, dar și crearea unui ciclu închis de tratare însoțit de izomerizarea lactozei în lactuloză după două mecanisme concomitent. Geometria electrolizoarelor și conținutul lichidului secundar (lichidului anodic) influențează atât recuperarea și formarea compușilor proteici înnobilați cu alfa-lactalbumine, cât și formarea unui complex între calciu și lactuloza izomerizată.

Cuvinte-cheie: *Alpha-lactalbumina, electrofracționare, electrolizoare, izomerizare, lactoză, lactuloză, produse lactate secundare.*

1. Introduction

Non-waste technology development issues and approaches to their implementation are widespread in several areas. One of them, of great importance for mankind, is the food industry, primarily food processing, which often generates environmental problems. In the manufacturing sector, there is currently an intensive process of reviewing the environmental requirements for waste. These include dairy production, mainly by-products. Developing zero-waste technologies and closed-loop whey processing is one of the main global challenges [1, 2].

Milk sugar represented by lactose, protein fractions, and an impressive calcium content, extremely important for cell signaling mechanisms, and also beneficial for human metabolism and skeletal system, all those and other milk ingredients provide a vital biologically active base for the human body and represent the biological value of milk [3]. Those vital substances for humans, in the most part, are found in dairy by-products, obtained after primary processing of milk [4].

Skimmed milk, ultrafiltrates, caseinates, and different types of whey are the main dairy by-products to be used efficiently and entirely [5-7].

There are two types of whey produced during the primary processing of milk: sweet (pH 5.5-6.0, produced during the manufacture of many types of hard cheese) and acid (pH 4.5-5.1, produced during the manufacture of such products as cottage cheese or curd products) [6].

Solids content of whey is 6-8%, which is about 50-70% of that of whole milk [8]. However, in the respective literature, the solid content of whey was found to be different and depended on the primary processing of milk [9, 10].

Lactose is a disaccharide found only in milk. During the primary processing of milk, a large part of the valuable milk solids passes into whey, constituting 70-80% of its solids content varying according to the primary processing method [11].

The whey proteins are structurally similar to blood proteins, and one of their important functions is immune activity. Amino acids, including essential amino acids, are a significant part of the biologically active substances in food. The whey lipids are more dispersed than milk lipids and are digestive biochemicals [12]. The mineral composition of whey is very high and is optimally balanced and biologically varied. The whey contains both water-soluble and fat-soluble milk vitamins [12, 13].

Various techniques and technologies are available for processing dairy by-products to obtain protein concentrates which are used in various beneficial (dietary) supplements and pharmaceuticals as biologically active substances. The use of recovered whey proteins is of significant interest. Several studies have shown that breast milk contains 60% whey protein and about 40% casein. However, the use of whey proteins for the above-mentioned purposes

requires working under certain specific strict conditions - specific technological regimes to ensure a high degree of purity and maintenance of natural qualities [14].

The environmentally-friendly technologies for dairy by-product processing are: new high-tech and efficient methods, including electroactivation; establishing parameters for process operation; and further development of energy-saving techniques for the process [15].

Investigation of the technological processes related to the application of electroactivation involves the optimization of chemical-free processing at low temperature and low energy consumption.

Protein recovery from whey and obtaining high-value protein-mineral concentrates (PMC) under the action of an electric current, avoiding the direct use of chemicals, is an advantageous process based on modern principles, which ensures non-residual processing of whey with simultaneous isomerization of lactose into lactulose, which is subsequently separated from deproteinized whey (DW) [16].

Whey protein concentrates are globally valuable. They can be used as biologically active additives, food supplements and dietary products [12].

The whey proteins can be fractionated by electroactivation and the recovered PMCs enriched with different protein fractions depending on the processing regime [17].

The recovery of whey protein fractions to prepare different compositions for infant diets is a particular focus for researchers. The review of whey processing leads to the conclusion that the combination of different methods allows a more efficient recovery of whey components [18].

Lactose is the main carbohydrate of milk, following the manufacture of primary dairy products it passes into whey and makes up about 70% of its solid content. The use of lactose via extraction, crystallization, and isomerization - remains a major issue in the world and is the subject of much research and many discussions [19, 20].

Lactulose is a carbohydrate, falling into the class of oligosaccharides, subclass disaccharides, and it consists of one galactose and one fructose molecules [21, 22].

Isomerization of lactose into lactulose is one of the most effective ways to use lactose.

The use of whey as a raw material for this transformation presents a new direction for both lactose extraction and lactose isomerization [20, 23].

Lactulose is considered to be an effective prebiotic, which has the properties of activating the growth of protective intestinal microflora (bifido- and lactobacteria), it is used as a therapeutic and prophylactic remedy for several diseases, namely for dysbiotic symptoms [24, 25].

There are two known mechanisms of lactose isomerisation into lactulose.

The first refers to the reaction of conversion of aldose to ketoses - the Lobry de Bruyn-Alberda van Ekenstein reaction, called L-A- transformation, conditioned by the formation in alkaline solutions of the enolic intermediate form of lactose and epilactose and subsequent conversion to lactulose. Alkaline reagents are used as catalysts. Electroactivation meets the conditions for isomerization of lactose to lactulose when processing whey in the cathode cell [26].

The second mechanism of isomerization is the Amadory rearrangement, which occurs through the formation and hydrolysis of lactulozamine. Under the action of catalysts (bases and acids) lactose reacts with amines to form lactulozylamylamine, which then leads to the rearrangement [27].

Amadori regrouping/rearrangement is a reaction between proteins and carbohydrates, it is the incipient phase of the Maillard reaction, which has the property of being reversible,

where upon electroactivation of whey in the case under investigation, lactose reacts with amines, due to the presence in whey of amino acids from proteins, and amine groups from nitrogenous substances of non-protein origin (creatinine, carbamide, uric acid) which ensure the transformation of lactose into lactulose. The reversible reaction of Amadori rearrangement is the Heyns reaction or Amadori retro–rearrangement (Figures 1 and 2) [28-30].

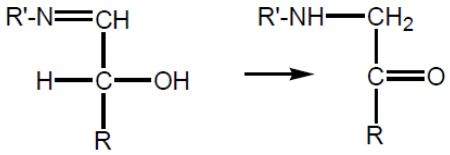

Figure 1. Amadori rearrangement [30].

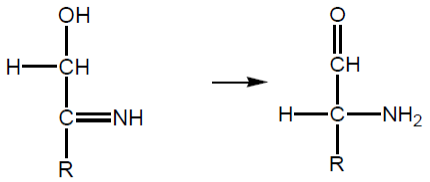

Figure 2. Heyns rearrangement [30].

Lactose is an extremely important component of milk responsible for maintaining 50% of the osmotic pressure, being, respectively, an indirect property of α-Lactalbumin (α-La) - a small protein with acidic properties (pH 4-5), with a high affinity towards Ca^{2+} ions and other bivalent ions. Calcium binding to α-La participates in the formation of disulfide bridges during protein folding [31].

The aim of this work was to determine the influence of the geometrical and technical parameters of electrolyzers on whey protein fractionation and their recovery in PMCs with simultaneous lactose isomerization into lactulose via electroactivation.

2. Materials and methods

The electroactivation of whey allows the obtaining of two major fractions: PMCs with different protein and mineral content and DW containing isomerized lactulose, both simultaneously obtained by electrochemical activation.

Whey electroactivation was carried out in diaphragm electrolyzers with different geometrical casings of different types of whey with low and medium protein content (supplied by the Joint Stock Company "JLC", Chisinau, Republic of Moldova):

1. whey with low protein content - whey obtained after the manufacture of "cheese product", 18% fat content, whey protein content in the initial whey 17-19 mg/mL;

2. whey with medium protein content - whey obtained after the production of cottage cheese, 5% fat content, whey protein content in the initial whey 23-25 mg/mL.

In order to understand the processes that are involved both in the electroactivation of whey with the extraction of PMCs and in the simultaneous isomerization of lactose into lactose, here the research on the electroactivation of 4% lactose solution was carried out.

The studies were performed in two diaphragm electrolyzers, conventionally referred to as: EDP-2 with parallelepiped casing and EDC-pilot with semi-cylindrical casing. The experimental layout and the technical realization of the diaphragm electrolyzers were developed by the authors at the Institute of Applied Physics and are shown in Figures 3 and 4 [31-33].

The electrolyzer EDP-2 has the ratio between the volume of the processed whey (*V*, mL) and the electrode surface (*S*, cm^2) *V/S*- 1.4 mL/cm^2, and the electrolyzer EDC-pilot - 0.75 mL/cm^2.

The electroactivation of whey was carried out at different densities of the electric current: *j*=10 and 20 mA/cm^2, keeping them constant during the processing, in a stationary

flow of the working liquid (different types of whey: that passing into the cathode cell (CC)) and that of the secondary liquid (2% $CaCl_2$ solution, passing into the anode cell (AC)). The PMCs were collected at certain treatment periods (5-10 min) [31, 32].

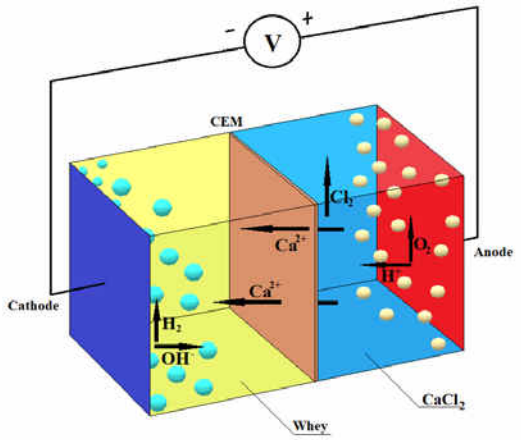

Figure 3. Layout of experimental electrolyzer EDP-2.

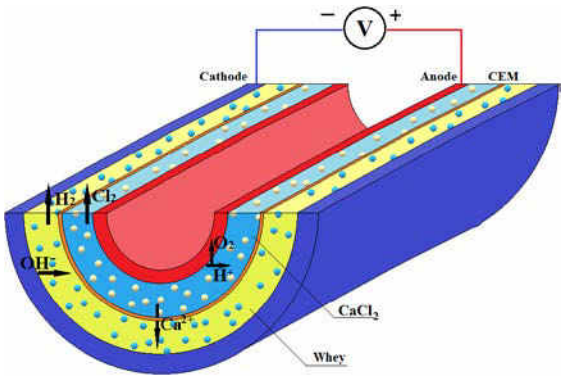

Figure 4. Layout of experimental electrolyzer EDC-pilot.

The amount of calcium ions *v*(Ca^{2+}), mol of the anodic liquid (sol. 2%, $CaCl_2$) for EDP-2 was 0.0144 and for EDC-pilot - 0.054.

The content of protein fractions (*Qp*, %) extracted from the PMCs with the buffer solution 0.05M Tris-HCl 0.5M NaCl, 0.5mM EDTA, pH 8.0, were analyzed by the SDS-PAGE 15% electrophoresis [34]. The obtained gels were scanned by an HP Scanget 3800 and analyzed with a Phoretix 1D Advans to determine the amount of protein fractions in the PMCs.

The method allowed the identification of the major fractions extracted from the PMCs, which were distributed in four groups: high-weight proteins **(HWP),** with molecular mass (MW) of 54-249 kDa; caseins (**CSN**), in which 2-3 fractions were identified–α-CSN, β CSN and κ-CSN with respective MW of 37, 33 and 46 kDa; β-lactoglobulins (**β-Lg**), MW 18.3; α-lactalbumins (**α-La**) with MW 14.2 kDa.

Optical rotation (angle of rotation) of deproteinized whey was determined with a Kruss P3000 polarimeter. Tube length - 200 and 100mm.

The pH values were registered at pH meter 767, Knick, Germany.

3. Results and Discussion

The research was carried out on different types of whey in two different electrolyzers, where the maximum extraction of α-La was registered in PMCs.

The extraction of α-La at the electroactivation of whey differs from the extraction of β-Lactoglobulin (β-Lg) in PMCs.

The processing of whey obtained after the manufacture of the "cheese product", 18% fat content, analyzed with SDS-PAGE 15%, upon electroactivation in EDP-2, stationary regime: j=20 mA·cm^{-2}, allows the extraction of a significant amount of α-La of about 70% at the end of electroactivation (after 20 min of treatment).

This type of whey has an initially higher content of calcium ions due to the primary processing of the milk with calcium chloride, and further supplementation with calcium ions from the anode cell, which leads to the formation of PMCs with increased α-La content due to their high affinity for Ca^{2+} ions, Figures 5-8.

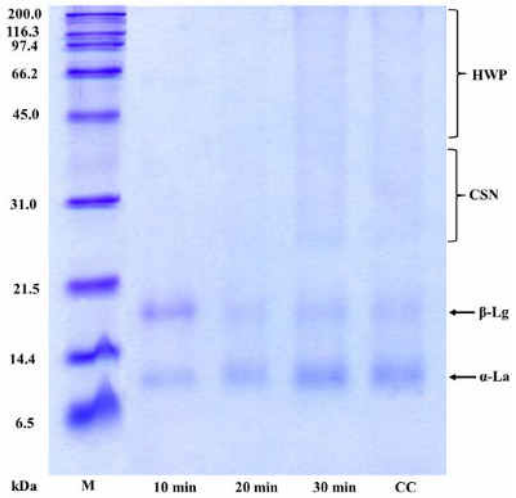

Figure 5. SDS-PAGE 15%, of soluble proteins extracted with 0.05M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from PMCs obtained at the electroactivation of whey with low protein content, in EDP-2, stationary regime, j=10 mA/cm^2 (M - marker, 10-30 min - processing time, CC - cathode cell content).

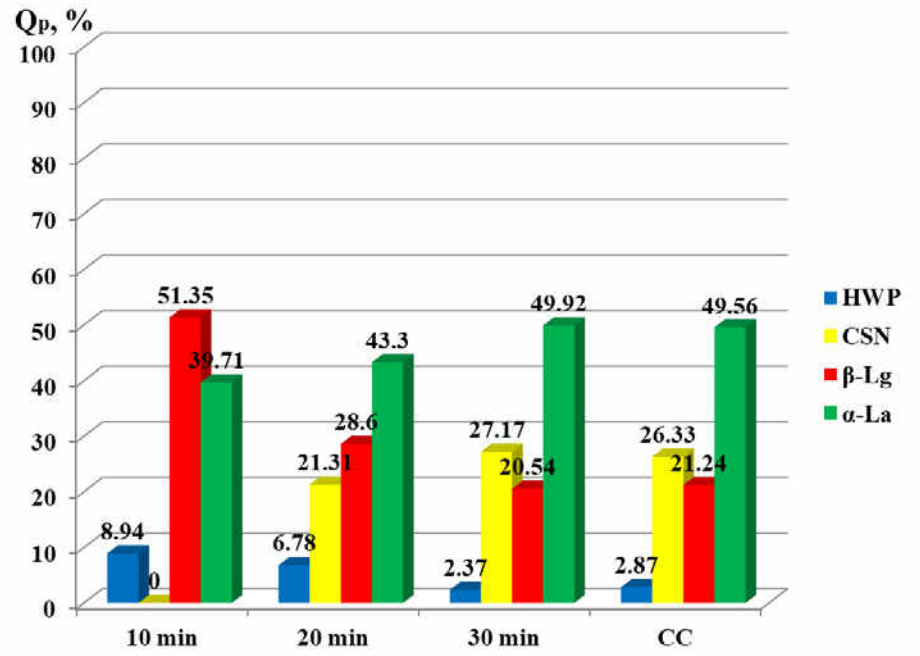

Figure 6. Variations of the content (Q_p, %) of major soluble protein fractions extracted with 0.05 M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from PMCs obtained at the electroactivation of whey with low protein content, in EDP-2, stationary regime, j= 10 mA/cm^2 (10-30 min - processing time, CC - cathode cell content).

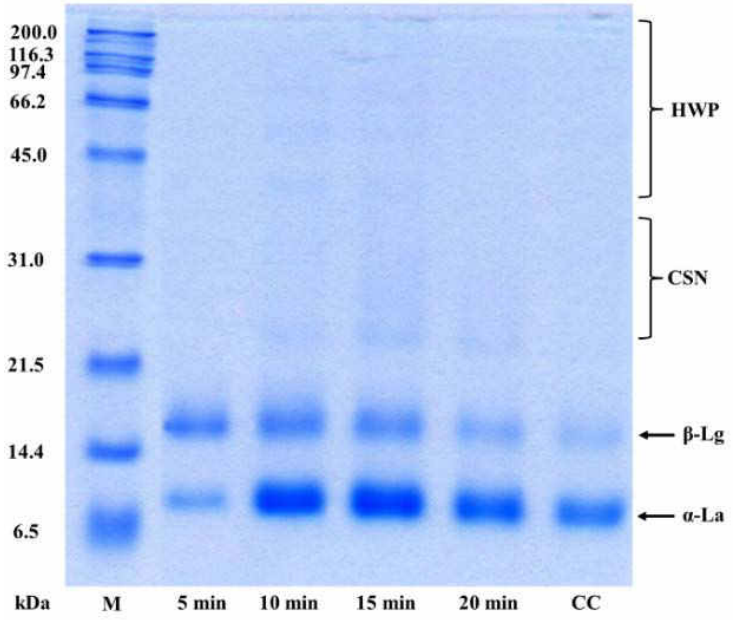

Figure 7. SDS-PAGE 15% of soluble proteins extracted with 0.05 M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from the PMCs obtained at the electroactivation of whey with low protein content, in EDP-2, stationary regime, j=20 mA/cm^2 (M - marker, 5-20 min - processing time, CC - cathode cell content).

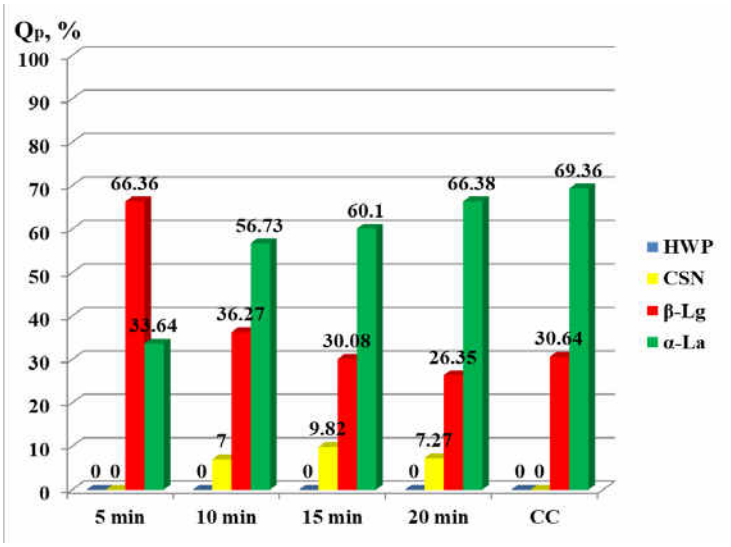

Figure 8. Variations of the content (Q_p, %) of major soluble protein fractions extracted with 0.05 M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from the PMCs obtained at the electroactivation of whey with low protein content, in EDP-2, stationary regime, j=20 mA/cm^2 (5-20 min - processing time, CC - cathode cell content).

The polarimetric method makes it possible to determine the anomeric composition of lactose. The decrease of the polarization angle - $\alpha°$ during electroactivation indicates the isomerization of lactose into lactulose, reaching negative values towards the end of processing. It is known that for lactose $\alpha°$ has positive values and for lactulose $\alpha°$ has negative values.

The polarimetric analysis of DW after the electroactivation of the whey with low protein content, at j= 10 and 20mA/·cm^2, indicates negative values of the polarization angle $\alpha°$ towards the end of processing.

The polarization angle $\alpha°$ of DW after the electroactivation of the whey with low protein content, in EDP-2, stationary regime, at j=10 mA/cm^2 is (-0.08) at 30 min of treatment and (-0.1) for the CC content, while the $\alpha°$ value of the initial whey is (4.3), tube length l=200 mm.

The polarization angle $\alpha°$ for the same type of whey at j=20 mA/cm^2 is (-1.0) for 20 min of treatment and (-1.4) for CC (Figures 9, 10) [31].

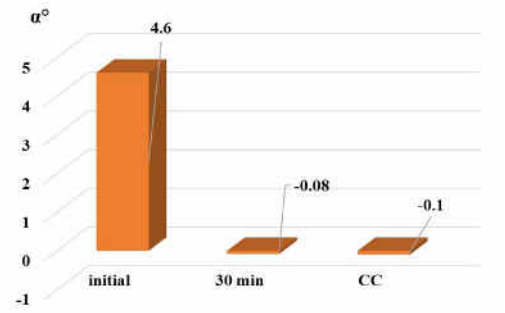

Figure 9. Polarization angle $\alpha°$ of DW after the electroactivation of whey with low protein content in EDP-2, j= 10 mA/cm^2 (3 h 12 min time of relaxation).

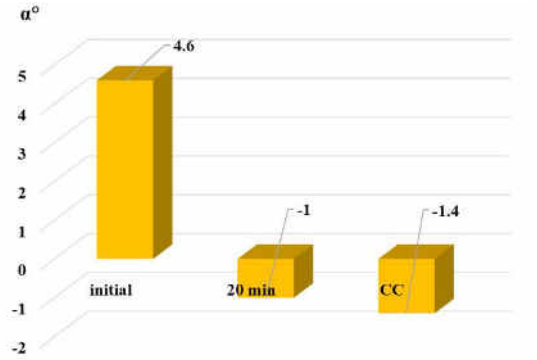

Figure 10. Polarization angle $\alpha°$ of DW after the electroactivation of whey with low protein content in EDP-2, j=20 mA/cm^2 (7 h 9 min time of relaxation).

Electroactivation of whey with medium protein content, in the stationary regime, at j=10 mA/cm^2, in the electrolyser EDC-pilot, which has optimized technical parameters in order to increase the surface of activation and has V/S=0.75 mL/cm^2, allowed an intense extraction of α-La even from the first minutes of processing, both in percentage (Q_p, %) and quantitative (Q_p, mg/g PMC) determination.

β-Lg, being the major whey fraction, has a tendency towards an intense extraction from the first minutes at the electroactivation in EDP-2 [35].

An increase of the surface of activation provides the conditions for rapid inter- and intramolecular transformations, which favours both the hydrolysis of whey proteins and the isomerization of lactose into lactulose, which allowed a maximum extraction of α-La at 20 min of treatment of this type of whey under the mentioned conditions (Figures 11-13).

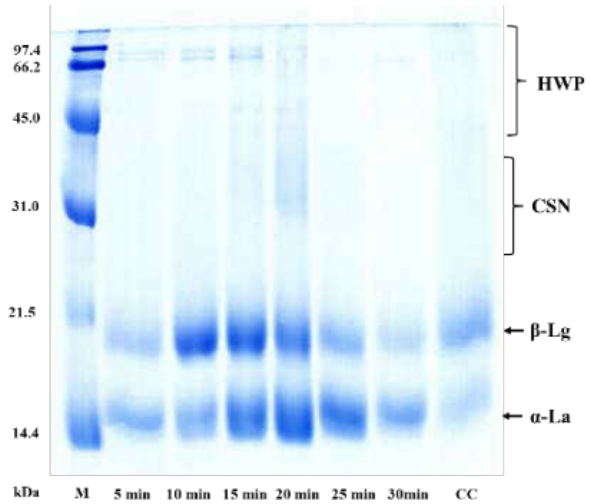

Figure 11. SDS-PAGE 15% of soluble proteins extracted with 0.05M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from the PMCs obtained at the electroactivation of whey with medium protein content, in EDC-pilot, stationary regime, j= 10 mA/cm^2 (M - marker, 5-30 min - processing time, CC - cathode cell content).

α-La is a regulatory protein of the lactose synthase enzyme complex. The extraction of α-La is directly influenced by the isomerization of lactose into lactulose. The isomerization of lactose into lactulose via the electroactivation by the Amadori rearrangement mechanism "releases" α-La from the lactose synthase complex and allows its "capturing" into PMCs towards the end of processing [31, 36].

Hypothetically, this explains the extraction of α-La in the PMCs at the end of the processing, which corresponds to a more intense isomerization of lactose into lactulose.

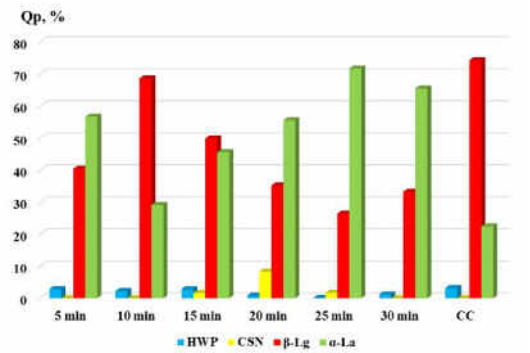

Figure 12. Variations of the content (Q_p, %) of major soluble protein fractions extracted with 0.05 M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from the PMCs obtained at the electroactivation of whey with medium protein content, in EDC-pilot, stationary regime, j=10 mA/cm^2 (5-30 min - processing time, CC - cathode cell content).

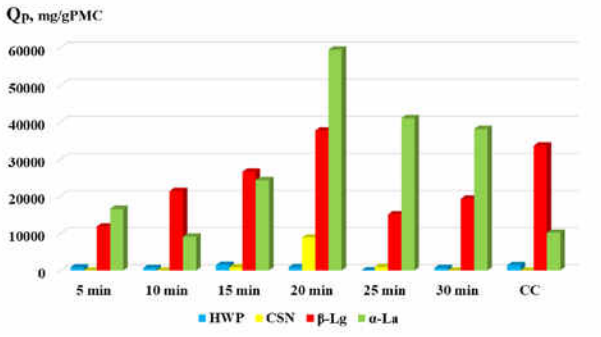

Figure 13. Variations of the content (Qp, mg·gPMC-1) of major soluble protein fractions extracted with 0.05 M Tris-HCl, 0.5 M NaCl, 0.5 mM EDTA, pH 8.0, from the PMCs obtained at the electroactivation of whey with medium protein content, in EDC-pilot, stationary regime, j=10 mA/cm^2 (5-30 min - processing time, CC - cathode cell content)

To understand the process of high extraction of α-La from the first minutes of processing in the electrolyzer EDC-pilot, the investigation of the lactose solution (4%) under the same conditions was carried out, which explains the isomerization of lactose into lactulose by the L-A-transformation mechanism [26].

The investigation of the isomerization of lactose solution (4%) in the electrolyzer EDC-pilot allowed to identify the formation of a complex between the isomerized lactulose and calcium ions due to a higher amount of Ca^{2+} ions in the anode cell (3.75 times), compared to that in the electrolyzer EDP-2 (Figures 14-16).

The polarization angle in the 4% lactose solution indicates positive values α°=2.45, tube length l=100 mm.

The registration of the polarization angle α° after electroactivation of the 4% lactose solution was carried out during relaxation, maintaining it in the range 0.54°-0.48° until 90 min after processing. The decrease and transition of α° to negative values starts at 96 min after processing, having unstable character until 108 min.

The polarization angle α° increases, and at a certain point, very rapidly, a precipitate is formed (at 118 min after processing), which makes it difficult to determine the polarization angle (Figures 15, 16).

After 118 min and an intense sediment formation, α° values were not possible to be registered. The sample was filtered off the sediment formed, and α° remained stable for a long time, α°=0.43-0.45.

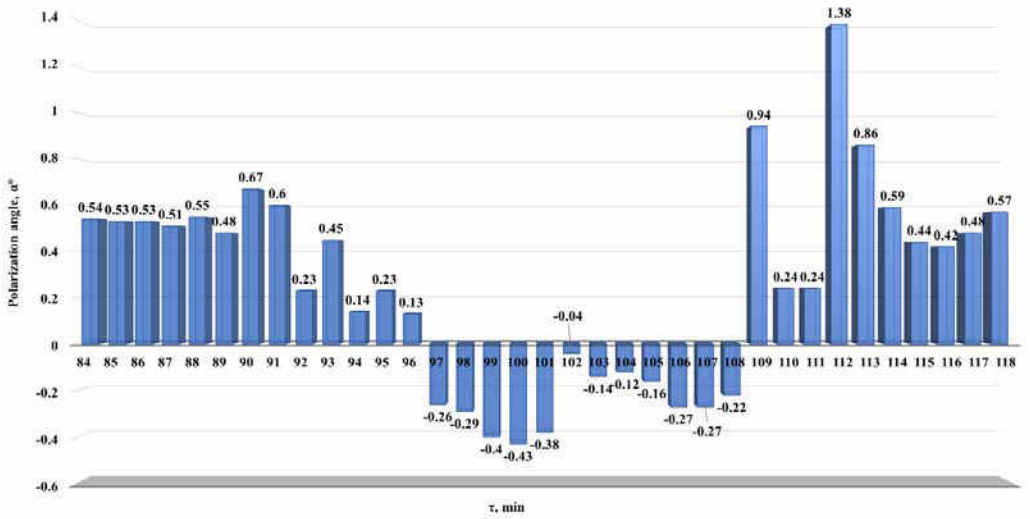

Figure 14. Variations of the polarization angle α° after electroactivation of lactose solution 4% in the electrolyzer EDC-pilot (84-118 min after processing), j=20 mA/cm^2.

Figure 15. Formation of a calcium sediment with lactulose after electroactivation of lactose solution (4%) in the electrolyzer EDC-pilot, j=20 mA/cm^2.

Figure 16. Solution after filtration containing lactulose.

The maximum extraction of α-La (see Figures 12, 13) at the electroactivation of whey with medium protein content, in the EDC-pilot, stationary regime, at j=10 mA/cm^2, was registered from the first minutes, but the polarization angle α° did not show negative values (Figure 17).

The property of calcium ions to form a complex with lactulose by the LA - transformation mechanism, which was demonstrated in the electroactivation of the lactose solution (4%), and a higher amount of Ca^{2+} ions in the anode cell in the EDC- pilot compared to EDP-2, allowed a more intense formation of this complex also via the electroactivation of whey. The surface of activation is bigger in the EDC-pilot compared to EDP-2, creating conditions for both rapid isomerization of lactose into lactulose and hydrolysis of whey proteins, followed by activation of the amino groups which participate intensively at the Amadori rearrangement.

Isomerized lactulose "releases" α-La from the lactose synthase complex and interacts rapidly with calcium ions; α° does not record negative values during storage after processing in EDC-pilot.

Hypothetically, the mentioned transformations are very fast and intense, which do not allow to “capture” the moment of transition of the polarization angle α° to negative values,

typical of lactulose. Variations of pH values in relaxation after the electroactivation and variations of the polarization angle α° the isomerization of lactose into lactulose via the electroactivation of whey in the EDC-pilot at j=10mA/cm^2 during relaxation after processing is shown in Figures 17, 18.

Variations of pH values during relaxation after electroactivation and variations of the polarization angle α°, which characterize the isomerization of lactose into lactulose at the electroactivation of whey in EDC-pilot, at j=10mA/cm^2 during relaxation (after processing) are shown in Figures 17, 18.

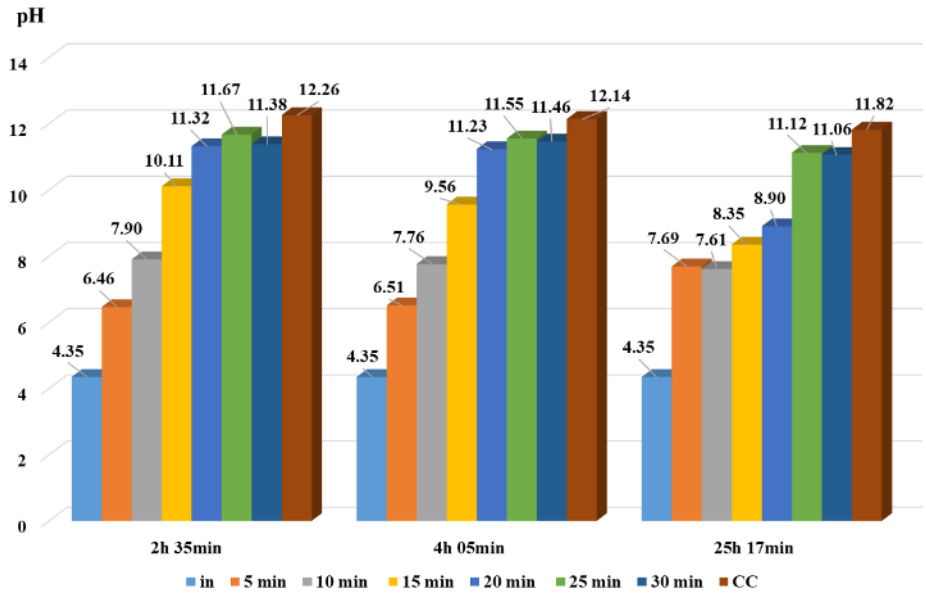

Figure 17. Variations of pH values during relaxation after the electroactivation of whey with medium protein content in EDC-pilot, at j= 10 mA/cm^2, at certain storage periods: 2 h 35 min; 4 h 05 min; 25 h 17 min; (in – initial whey, 5-30 min - processing time, CC - cathode cell content).

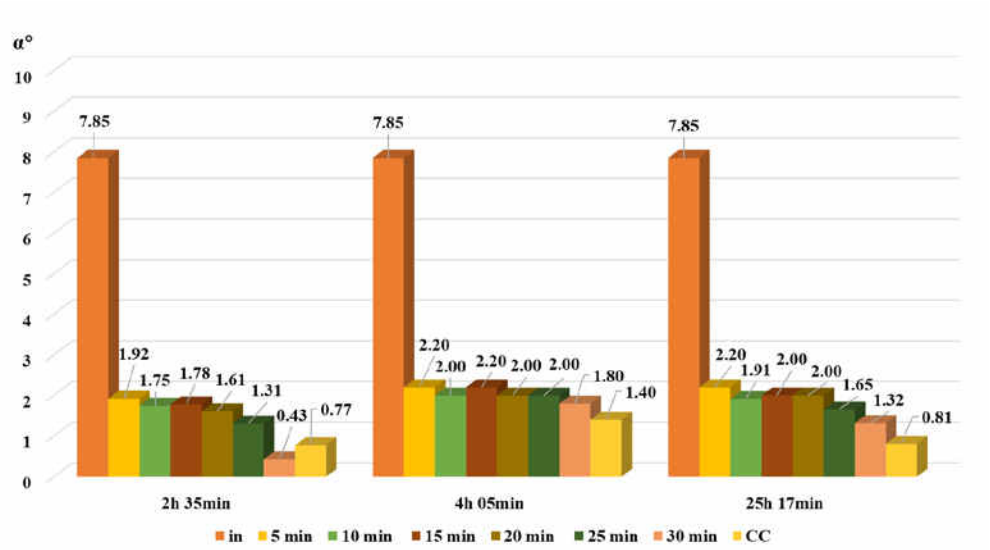

Figure 18. Variations of the polarization angle α° during relaxation after the electroactivation of whey with medium protein content in EDC-pilot, at j= 10 mA/cm^2, at certain storage periods: 2 h 35 min; 4 h 05 min; 25 h 17 min; (in – initial whey, 5-30 min - processing time, CC - cathode cell content).

The research demonstrates the importance of the amount of Ca^{2+} ions depending on the geometrical parameters of the electrolyzers used and allows to adjust the necessary concentration of calcium ions in the anodic liquid, which influences the isomerization of lactose into lactulose, both by the LA-transformation mechanism and by the Amadori rearrangement.

The semicylindrical casing electrolyzer with optimized technical parameters allows to increase the surface of activation, intensifying inter- and intramolecular interactions and a more intense extraction of α-La from the first minutes of electroactivation.

By handling both the technical and geometrical parameters of the two electrolyzers used, as well as the amount of calcium ions in the anode cell, the electroactivation of different types of whey allows the electrofractionation of whey proteins and its different recovery in PMCs, enriching it with certain protein fractions at different processing regimes, and obtaining protein concentrates with predetermined protein content, which occurs simultaneously with the isomerization of lactose into lactulose.

4. Conclusions

The different and uneven extraction of whey proteins in PMCs upon electroactivation of different types of whey in two different electrolyzers is conditioned by the properties of each protein fraction and their behavior upon electrochemical activation, by the initial solid content, especially that of proteins and minerals, by the activation conditions and by the constructive/geometrical parameters of the used electrolyzers.

The extraction of α-La at the electroactivation of whey demonstrated its recovery in PMCs upon the maximal lactose isomerization into lactulose after its „release" from the *lactose synthase* complex.

The amount of calcium ions in the anodic liquid influenced the isomerization of lactose into lactulose, contributed to the formation of a complex between calcium and isomerized lactulose, and led to an intense formation of a sediment.

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Submission of manuscripts: jes@meridian.utm.md