OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 2, ISSUE 1
2021

BIOLOGICAL SCIENCES

OPEN ACCESS

PECULIARITIES OF EXTRACTION OF β-LACTOGLOBULINE IN PROTEIN MINERAL CONCENTRATES AT ELECTROACTIVATION OF WHEY

Mircea BOLOGA[1], Elvira VRABIE[1], Irina PALADII[1], Olga ILIASENCO[1], Tatiana STEPURINA[2], Valeria VRABIE[3], Albert POLICARPOV[1], Catalina SPRINCEAN[1]

[1]Institute of Applied Physics, Chisinau, Republic of Moldova
[2]Moldova State University, Chisinau, Republic of Moldova
[3]Institute of Physiology and Sanocreatology, Chisinau, Republic of Moldova

Corresponding author: Elvira Vrabie, e-mail: vrabie657@yahoo.com

DOI: 10.38045/ohrm.2021.1.06 UDC: 637.344.8

Key words: *whey, protein-mineral concentrates, β-lactoglobulin, electroactivation.*

Introduction. *Whey is a by-product and an excellent source of proteins that is rather aggressive due to a large amount of organic substances it contains. The electro-activation of whey applied in the experiments is a wasteless method that allows the valorification of all whey components. β-lactoglobulin (β-Lg) makes up 50% of the whey proteins and 12% of the total protein content in milk.*

Material and methods. *The recovery of β-Lg in protein-mineral concentrates (PMC) by electro-activation processing of different types of whey with different initial protein content was investigated in seven configurations. The recovery of protein fractions in the PMCs were analyzed via electrophoresis with sodium dodecyl sulfate (SDS-PAGE) and 15% non-denaturing polyacrylamide gel (PAAG).*

Results. *Whey electro-fractionation and the obtaining of PMCs with predetermined protein content, namely of β-Lg, were studied on three whey types, processed at different treatment regimens and in seven configurations. The proper management of electro activation by varying the treatment regimens will allow the electro-fractionation of different types of dairy by-products.*

Conclusions. *The maximum amount of β-Lg recovered in PMCs on electroactivation is 66-71% depending on the processed whey and on the treatment regimens. Obviously, the extraction of β-Lg from initially lower protein content shows a higher recovery degree of β-Lg. The registered temperatures allows formation of PMCs without thermal denaturation.*

Cuvinte cheie: *zer, concentrate proteice, minerale, β-lactoglobulină, electroactivare.*

PARTICULARITĂȚILE EXTRAGERII β-LACTOGLOBULINEI ÎN CONCENTRATE PROTEICE MINERALE LA ELECTROACTIVAREA ZERULUI

Introducere. *Zerul, ca unul dintre subprodusele lactate, fiind o sursă excelentă de proteine, se prezintă, de asemenea, ca un produs agresiv, din cauza substanțelor organice deținute în cantități mari. Electroactivarea zerului este o metodă non-reziduală care permite valorificarea tuturor componentelor din zer. β-lactoglobulina (β-Lg) reprezintă 50% din proteinele din zer și 12% din conținutul total de proteine ale laptelui.*

Material și metode. *Extragerea β-Lg în concentrate proteice minerale (CPM), la electroactivarea diverselor tipuri de zer, cu un conținut proteic initial diferit, a fost cercetată în 7 configurații. Fracțiile proteice extrase din zer în CPM au fost analizate prin electroforeză cu dodecil sulfat de sodiu (SDS-PAGE) și concentrația gelului de poliacrilamida ne denaturant (PAAG) de 15%.*

Rezultate. *Electrofracționarea zerului și obținerea CPM cu un conținut proteic predeterminat, și anume cu β-Lg, a fost demonstrată la prelucrarea a trei tiputi de zer, în regimuri de tratament diferite, cercetate în 7 configurații. Gestionarea corectă a electroactivării, cu variația regimurilor de tratament, permite electrofracționarea diferitelor subproduse lactate.*

Concluzii. *Cantitatea maximă de β-Lg, extrasă în CPM la electroactivare, constituie 66-71%, în funcție de zerul procesat și de regimurile de tratare. Este evident că volumul de extragere a β-Lg din zerul cu un conținut proteic inițial mai mic, în CPM este mai mare. Temperaturile înregistrate permit formarea CPM fără denaturare termică.*

INTRODUCTION

Primary milk processing allows the production of different types of cheese, of large quantities of various secondary dairy products: whey, buttermilk, skim milk, caseinate, etc., with various solid contents. Global whey production is estimated at about 180-190·10^6 tons/year, of this quantity only 50% is processed. Development of wasteless technologies and processing of whey in a closed cycle is one of the major global challenges (1).

Whey is a by-product and an excellent source of proteins that is also aggressive due to a large amount of organic substances it contains. The manufacture of healthy and environmentally safe whey products requires an upgrade of methods and techniques for whey processing. The electroactivation of whey is a wasteless method that allows the valorification of all whey components. Besides, this type of processing allows controlling the whey protein content in the obtained concentrates, depending on the processing regime (2).

It is known that whey proteins make up about 20% of milk proteins. The four major whey protein components, namely β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA), and immunoglobulin (Ig) make up 90% of whey proteins. The remaining 10% are proteins such as lactoperoxidase, serum transferrin, lactoferrin, lactolin, and proteo-peptone fraction (fig. 1).

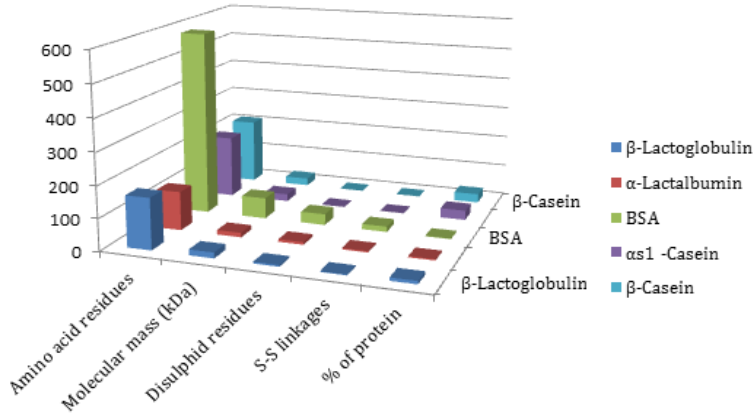

Figure 1. Composition and features of some major milk protein fractions (3).

Whey proteins are well structured proteins with secondary and tertiary stable structures. The main forces involved in assembling and maintaining the globular tertiary structure are disulfide bonds, hydrophobic interactions, hydrogen bonds, and the Van-der-Waals interactions (4).

Whey proteins are highly soluble in milk at a wide range of pH values due to the amino acid composition and the free radicals arrangement during formation of tertiary structures.

This is because of the arrangement of hydrophilic free radicals and a large number of disulphide bonds on the surface of whey proteins with globular structures (5). However, the globular structure induces resistance to proteolysis of those proteins. The four main whey protein fractions are presented below.

β-lactoglobulin. β-lactoglobulin (β-Lg) makes up 50% of the whey proteins and 12% of the total protein content of milk. Native β-Lg is a small globular protein with a molecular mass of 36.6 kDa with defined secondary and tertiary structures. The β-Lg molecule consists of an α-helix, β-sheet and random coil structures represented in a ratio of 10 to 15%, 43% and 47%, respectively (6-8). In aqueous solutions at pH 5-7 and room temperature, this protein is a dimmer of two identical subunits. Each subunit has a molecular mass of 18.3 kDa and is formed of 162 amino acids, of which 84 are essential amino acids and four cysteine residues. Cysteine (a semi-essential proteinogenic amino acid, a component of β-Lg) forms the disulfide bonds at Cys66-Cys160 and Cys106-Cys119 levels (9, 10).

In addition, β-Lg contains a free sulfhydryl SH group (Cys 121) within the native protein, which becomes active after the protein denaturation by various agents (including heating) and can interact with other proteins through disulfide bonds (6, 10-13). These interactions occur at pH 7 and at temperature range of 60-65°C. The free SH group of Cys121 is the most reactive, thus initiating the interchange of intermolecular reaction

with Cys66-Cys160 forming a new S-S bond between Cys121-Cys160, Cys121-Cys66, and Cys160-Cys160, resulting with a new dimmer that contains a new reactive SH group. The new reactive SH group initiates the formation of polymers through S-S bridges interacting with other monomers and oligomers. Cys160 is also active and initiates the interchange reactions SH/S-S, probably, due to its location near C terminus, which makes it more accessible for intermolecular reactions once the S-S bond is broken (fig. 2) (9, 14, 15).

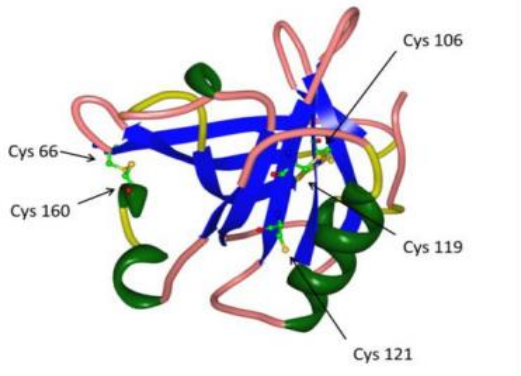

Figure 2. β-Lg structure with 5 cysteine residues and disulfide bonds (16).

Under physiological conditions, β-Lg is a dimer of two β-Lg protein subunits, with the balance to the monomer form, which allows the conversion of the dimer into native monomers (17, 18). The equilibrium towards monomer formation from the dimmer occurs at low β-Lg concentrations, at a low ionic strength, and at pH lower than 7 (19). Thus, β-Lg can associate and dissociate, depending on the pH of the environment. At the pH typical for milk, β-Lg is a dimmer. Due to a high electrostatic repulsion at pH 3.5, β-Lg reversibly dissociates into monomers (6). On the other hand, some authors have shown that β-Lg is a monomer at pH below 3.5 (7, 20) and also above 7.5 (21). The researchers (7) also reported that at pH 3.7-6.5, the β-Lg dimmer reversibly associates into an octamer.

It was established elsewhere that β-Lg can exist as tetrameric, octameric, and multimeric aggregates, in various environmental conditions.

β-Lg solutions form gels under different conditions when the native structure is destabilized to the extent that aggregation occurs. At the long-term thermal processing (prolonged heating) at a low pH and low ionic strength, the translucent "fine-stranded" gel is formed in which the protein molecules assemble into long and rigid fibers. The mechanisms of denaturation/aggregation of β-Lg protein were described by several researchers, for example (22). At neutral pH and room temperature, β-Lg is a stable noncovalent dimer. With an increase of temperature, the β-Lg dimmer dissociates into monomers that also undergo conformational changes (23).

Critical changes in the conformation of β-Lg consist in exposing hydrophobic residues and sulfhydryl groups on the β-Lg protein surface, making it available for intermolecular interactions (24, 25). A new β-Lg configuration was defined as the “molten globules” (22, 26). It is considered that the molten globules are intermediate states in the folding and unfolding globular proteins, but their characteristics are difficult because they are present in a transient state (27, 28).

Some proteins can form stable molten globule structures after destabilizing changes, such as removal of ligands, point mutation, mild-denaturant conditions, and the formation of non-native disulfide bonds throughout intramolecular rearrangements (28, 29).

At the initial stages of β-Lg denaturation/aggregation, caused by high temperatures, the non-native monomers are formed. The exact nature of these non-native forms has not been described so far. The formation of native monomers at high temperatures is due to the intermolecular disulfide bonds interaction Cys121 sulfhydryl/ Cys106-Cys119. At least, two monomers of β-Lg are present in heated solutions – one has a native sulfhydryl group of Cys121 exposed to solvent (Mcys121) and the other – disulfide non-native Cys106-Cys121 bonds and a free and exposed sulfhydryl nonnative group of Cys 119 (Mcys 119). While Mcys121 is reversible to the native form of β-Lg, Mcys119 has a stable conformation, which makes the return to the native form upon cooling impossible (18).

Peculiarities of whey proteins behavior under the action of high temperature and pH variations

Whey proteins are heat-labile. According to the research data (27), high temperatures decrease their stability in the following order: PP>α-La>β-Lg>BSA>Ig. Thermal denaturation of the whey proteins is a two-step process. In the first phase,

unfolding occurs, which may be reversible or irreversible and includes aggregation that usually follows the irreversible unfolding (9). Heat treatment causes significant deterioration of the protein structure, leading to the modification of physicochemical properties, including solubility, water-holding capacity, emulsifying, foaming and gelling (30). Unlike casein, whey proteins are completely denatured after 5 minutes of heating at a temperature over 90°C. In fact, whey protein denaturation begins at 65°C, especially during the heating of milk at temperatures over 80°C (31). It was also demonstrated elsewhere that heating at 85°C is critical for whey protein denaturation (32). The degree of protein denaturation is determined by the degree of β-Lg denaturation, since it makes up 50% of total whey proteins (30, 33).

Influence of high temperatures and pH on the behavior of β-Lg

As reported elsewhere, thermal denaturation of β-Lg occurs in two stages (12, 33-35, 36). In the first stage, dimmers dissociate and form four monomers, and then they interact via sulfhydryl groups and form small aggregates. Aggregate formation takes place at about 70°C (12, 33, 36), whereas the maximum speed is reached at 80-85°C (29, 37).

In the second stage (called non-specific), small aggregates interact through non-specific binding and high molecular weight (MW) aggregates are formed. According to some researches (34, 35), this stage occurs at temperatures higher than those of the first stage.

Such factors as pH, concentration of salts, sugar, and proteins have a significant influence on the thermal behavior of β-Lg. Some authors established the pH-dependent thermal denaturation of β-Lg (7, 9, 34, 35, 38).

β-Lg is most sensitive at pH 9.0, when denaturation is triggered at 43°C and destruction of the secondary structure is induced at 51°C. Thermal denaturation occurs at pH 6.0 and at 78°C. Denaturation takes place at pH 5.5 and at 76°C (9).

The maximal heat stability of β-Lg was recorded at pH 3.0 (7).

Dynamic aggregation of proteins results from the intermolecular association of various forces and bonds. Since the isoelectric point of β-Lg is at pH 5.3, a decrease in the pH up to 5.5 will minimize the intermolecular electrostatic repulsion, thus increasing the molecular aggregation via Van der Waals forces and hydrophobic chemical bonds. Aggregates and polymers that are formed at low pH may be heterogeneous, thus contributing to light diffusion and turbidity of the solution.

The pH dependent aggregation of proteins apparently differs from the real protein denaturation. It was demonstrated elsewhere that in low ionic strength solutions, an increase in pH from 5.5 to 6.5, might decrease the temperature of denaturation from 80°C to 70°C. Although β-Lg denatures at pH≥6.0 rather than at pH<5.5, an increase of pH up to ≥6.0 will increase the protein-water interaction, favoring thereby protein solubility. This effect will contribute to the rejection of protein charges and will weaken the protein-protein interactions. It was found elsewhere that the solubility of whey protein decreases markedly during heating at pH values between 5.0 and 5.5, but not greater than 6.0.

The main purpose of this work was to study the extraction of β-lactoglobulins in protein-mineral concentrates (PMCs) during the electrophysical processing of different types of whey, using different processing regimes, as well as pH and temperature variations.

MATERIAL AND METHODS

Three types of initial whey (IW) products were used during the experiments on electroactivation, which were provided by the "JLC" Joint Stock Company, Chisinau, R. Moldova, including the following: granulated cottage cheese „Grăuncior" – 1; "Cottage cheese", 2% fat content – 2; and „Curd product", 18% fat content – 3.

For convenience the, types of processed whey will be named according to their initial proteins content:

1 – whey with high proteins content (WHPC);
2 – whey with medium proteins content (WMPC);
3 – whey with low proteins content (WLPC).

The isolation of the casein powder does not influence the degree of proteins isolation; however, in this case, a lower quantity of proteins is isolated from each type of whey.

The quantitative modifications of the initial protein content before and after the isolation of

casein powder are shown in Figure 3.

In all the experiments, the electrophysical processing of whey was performed within the membrane electrolyzer EDP-2 (with a short distance between the electrodes and V/S=1.4), at a stationary regime and at a current density of 10 and 20 mA/cm^2, which remained constant during processing, and in a stationary regime of discharge of the working liquid (different types of whey discharged in the cathode cell) and of the secondary liquid (2% $CaCl_2$ solutions discharged in the anode cell).

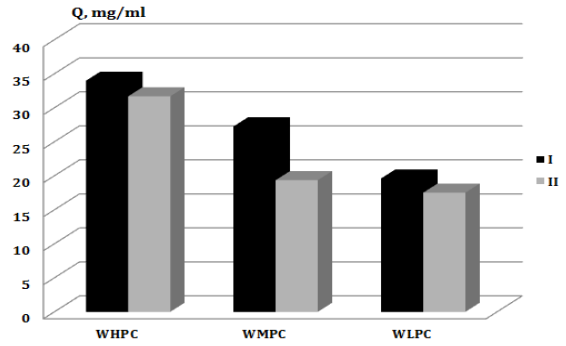

Figure 3. Initial protein content of 3 types of whey: I - IW without isolation of casein powder; II - IW with isolation of casein powder.

The PMCs at certain treatment periods were collected, whereas the results of experiments were reported by the authors earlier (39).

The quality of the IW and of the products obtained after electroactivation was determined according to the following physico-chemical and biochemical parameters:

- The pH of whey and the oxidation-reduction potential (ORP) were determined using a pH meter 766 (Knik, Germany).
- The temperature was determined in two phases: liquid and foaming. The chemical thermometer in the cathode cell was used in the liquid phase, while the pH-meter 766, Calimatic (Knick) was used in the foaming phase.

Protein content. The degree of protein recovery in the PMCs was determined by calculating the difference between the protein content in the IW and the remained deproteinized whey (DW), as shown in formula 1, separated in the field of mass forces by centrifugation at 1500 G via the Warburg method with the SF-56 spectrophotometer (40):

$$Q = Q_{ZI} - Q_{ZD}, \% \quad (1),$$

whereas:

Q – the protein content in the PMCs;
Q_{ZI} – the protein content in the IW;
Q_{ZD} – the protein content in the DW.

Protein fractions isolated from whey into the PMCs were analyzed with different concentrations of polyacrylamide gel via electrophoretic methods and three types of buffer solutions:

- **Buffer solution (I):** phosphate-citrate buffer (Me Ilvane) 0.5 M NaCl, 0.5 mM ethylenediaminetetraacetic acid (EDTA) (0.04% NaN_3) pH 5.6, used in the isolation of proteins from the PMCs in the studied whey via the electrolyzer EDP-2, during the processing of WHPC and WMPC.
- **Buffer solution (II):** 0.05 M Tris-HCl buffer 0.5 M NaCl, 0.5 mM EDTA (0.04% NaN_3), pH 8.0, used in the isolation of proteins from the PMCs in the studied whey via the electrolyzer EDP-2, during the processing of WHPC and WMPC.
- **Buffer solution (III)**: 0.025M Tris-glycine buffer pH 8.47 used in the isolation of proteins from the PMCs in the studied whey via the electrolyzer EDP-2, during the processing of WHPC and WMPC.

The amount of soluble protein concentrates (Q_s, %) obtained from the above mentioned two types of whey, extracted with various types of buffer solutions is different, showing a higher solubility amount in buffer solution (II)-0.05 M Tris-HCI 0.5 M NaCI, 0.5 mM EDTA (0.04% NaN_3), pH 8.0 (fig. 4).

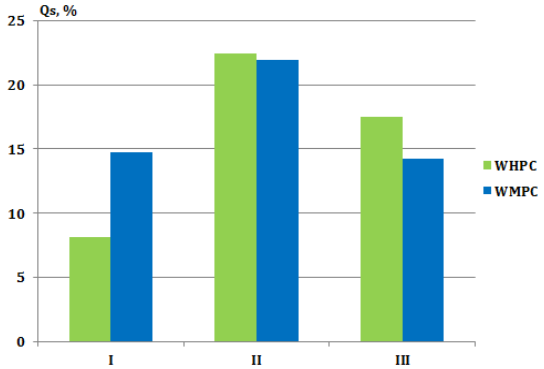

Figure 4. Content of soluble protein fractions (Q_s, %) of WHPC and WMPC recovered with buffer solutions (I-III).

The total content of soluble proteins (Q_s, %) recovered from the PMCs in the buffer solutions

mentioned above was determined by the micro-biuret method based on the interaction of peptide bonds with Cu^{2+} in a strongly alkaline medium, which results in the formation of a colored complex (41). The method is considered to be rapid, strict, and specific for protein estimation by using an alkaline copper sulfate reagent at the concentration of protein in the solution of 0.02 to 0.5 mg/mL. The sensitivity of this method is 0.003 mg/mL.

Reagents:

I - 0.28% $CuSO_4 \cdot 5H_2O$ solution in 30% NaOH

In order to avoid precipitation of $Cu(OH)_2$, when preparing the reagent, the solution of $CuSO_4 \cdot 5H_2O$ (1.4 mL of aqueous solution with 0.28g of $CuSO_4 \cdot 5H_2O$) was added dropwise in the alkaline solution (in 100 mL of 30% NaOH), and then vigorously stirred with a magnetic agitator.

II - 30% NaOH solution.

Procedure:

$A_{c.}$ – (control) 1 mL of reagent I is added to 2 mL of distilled H_2O.
A – 1 mL of reagent I is added to 2 mL of the investigated protein solution.
$B_{c.}$ – (control) 1 mL of reagent II is added to 2 mL of distilled H_2O.
B – 1 mL of reagent II is added to 2 mL of the investigated protein solution.

The prepared solutions were carefully stirred, kept for 15 minutes and then the optical density/absorption (A_{310nm}/E_{310nm}) in 1cm quartz cells at 310 nm on the spectrophotometer SF-56 was measured: A against A_c and B against B_c. The color of the prepared solution is maintained for 2.5 hours.

Further calculations were carried out on the difference of optical densities/absorptions, using formula 2:

$$\Delta A_{310nm} = A - B \quad (2)$$

The protein concentration is calculated on the previously made calibration curve (fig. 5). The calibration curves constructed for the aqueous solutions of BSA (from Serva) that were prepared by diluting the initial solution containing 1 mg/mL BSA (0.1% p-p).

The protein content via the micro-biuret assay was calculated as given below, using formula 3:

$$Y\ (mg/mL) = 0.77914 * X(\Delta A_{310nm}) + 0.00322 \quad (3),$$

whereas,

E – the optical density registered at the wavelength of 750 nm;
C – the standard protein concentration ABS, µg/mL;
R^2 – the approximate degree (R^2= 0.9999).

The approximation error is 1- R^2.

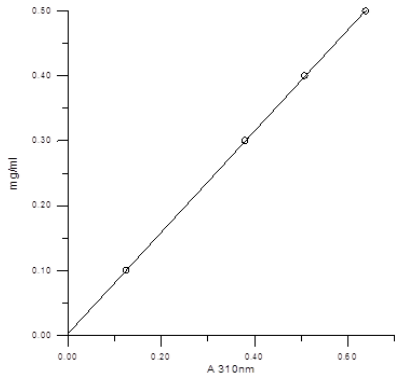

Figure 5. Calibration curve to determine soluble protein content isolated from PMCs via micro-biuret assay- BSA with concentration of 0.1-0.6 mg/mL.

Soluble proteins transferred in the buffer solution (protein buffer) were analyzed via electrophoresis with the sodium dodecyl sulfate (SDS-PAGE) and the 15% non-denaturing polyacrylamide gel (PAAG), according to (42).

The obtained results were scanned through the HP Scanget 3800 with the software Microsoft Photo Editor and analyzed with the Phoretix 1D Advans in order to determine the quantity of the major fractions in the PMC.

The recovery of β-Lg in the PMCs at electroactivation of different types of whey with different initial protein content was studied in the following configurations:

Configuration 1.
Electroactivation of WHPC at j=10 mA/cm², by collecting of whey in the form of foam at every 10 minutes (10-30 min – processing time) and CC is the content of the cathode cell, which represents the liquid phase.

Configuration 2.
Electroactivation of WHPC at j=20 mA/cm², by collecting of whey in the form of foam at every 5 minutes (5-15 min – processing time) and CC is the content of the cathode cell, which represents the liquid phase.

Configuration 3.
Electroactivation of WHPC at j=20 mA/cm², by collecting of whey in the form of foam at every

10 minutes (10-20 min – processing time) and CC is the content of the cathode cell, which represents the liquid phase.

Configuration 4.
Electroactivation of WMPC at j=10 mA/cm², by collecting of whey in the form of foam at every 10 minutes (10-30 min – processing time) and CC is the content of the cathode cell, which represents the liquid phase.

Configuration 5.
Electroactivation of WMPC at j=20 mA/cm², by collecting of whey in the form of foam at every 10 minutes (10-20 min – processing time) and CC is the content of the cathode cell, which represents the liquid phase.

Configuration 6.
Electroactivation of WLPC at j=10 mA/cm², by collecting of whey in the form of foam at every 10 minutes (10-30 min – processing time) and CC is the content of the cathode cell, which represents the liquid phase.

Configuration 7.
Electroactivation of WLPC at j=20 mA/cm², by collecting of whey in the form of foam at every 5 minutes (5-20 min – processing time), and CC is the content of the cathode cell, which represents the liquid phase.

RESULTS

Extraction of whey proteins and obtaining PMCs of a high value by electroactivation, as well as avoiding the direct usage of chemicals is an advantageous process based on modern principles, which ensures the finite cycle of the simultaneous processing of whey sugars (isomerization of lactose into lactulose) by separating them from the DW (fig. 6), (43).

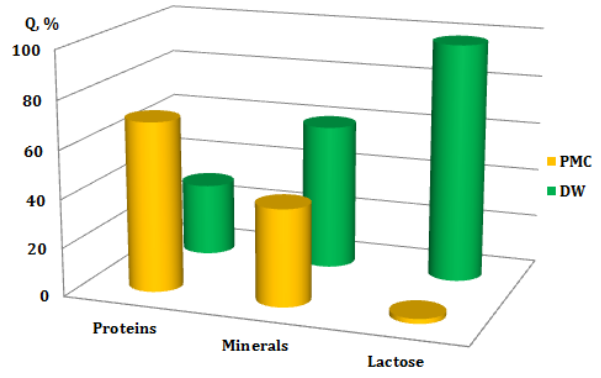

Figure 6. Electro-fractionation of DW by electroactivation.

The PMCs were investigated by electrophoretic techniques and processed via a membrane electrolizer EDP-2 at different current densities, by using different regimes of flow in different whey types, isolated with aforementioned buffer solutions.

Traditionally, these fractions make up four groups:

- high weight proteins (HWP), in which 2-5 fractions show various MW 54-249 kDa, containing the BSA with MW 66 kDa, lactoperoxidase with MW 70 kDa and lactoferine with MW 80 kDa, as well as protein complexes with MW of about 200-249 kDa;
- caseins (CSNs), in which 2-3 fractions: α-CSN, β CSN, and κ-CSN were identified, with MW 37, 33, and 46 kDa, respectively;
- β-Lg with a MW 18.4 kDa whose isolation is significant in all the collected samples;
- α-La with a MW 14.2 kDa that were isolated almost uniformly during the entire processing.

The electroactivation of WHPC, WMPC, and WLPC under all conditions mentioned above, and the identification of the content of soluble protein fractions in the 0.05 M Tris-HCl buffer 0.5 M NaCl, 0.5 mM EDTA (0.04% NaN_3), at pH 8.0, by using electrophoresis SDS-PAGE 15%, after major fractions mentioned above, demonstrated variations of the protein content.

The recovery of whey proteins in the PMCs (Q, %) from the IW varied depending on the solid content of each type of whey, on the processing regime (different current densities, the amount of the processed IW), on various electrical, thermal and physico-chemical parameters, and on the duration of treatment.

Whey proteins were most intensively recovered from WHPC, which had the highest initial protein content (the degree of recovery of whey proteins exceeds 60% at j=20 mA/cm² and 10 min of treatment), then followed by WMPC, showing an over 50% protein recovery in the PMCs, and WLPC, containing more than 50% whey proteins recovered in the PMCs (fig. 7, 8).

The major protein fractions (HWP, CSN, β-Lg, α-La) recovered in the PMCs by electroactivation of three types of whey were determined under

all the aforementioned conditions, using the electrophoretic analysis with SDS-PAGE 15%. The soluble proteins (Q_s, %) were studied in buffer solution (II) at pH 8.0, which allowed a more intense extraction compared to the use of buffer solution (I) with pH 5.6.

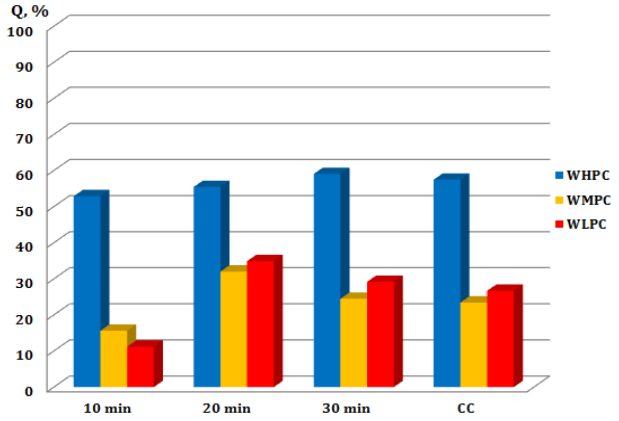

Figure 7. Recovery of whey proteins in PMCs, at processing of 3 types of whey at j=10 mA/cm^2.

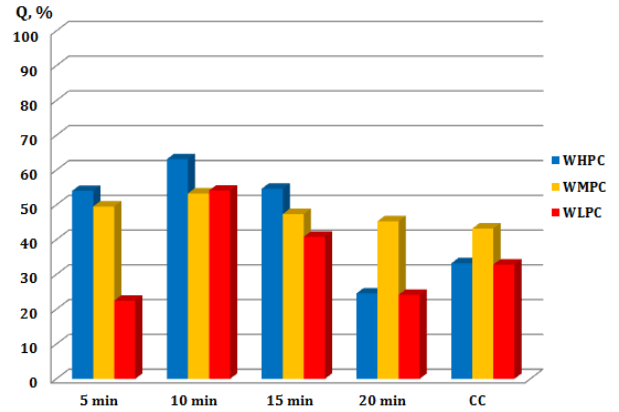

Figure 8. Recovery of whey proteins in PMCs, at processing of 3 types of whey at j=20 mA/cm^2.

Configuration 1.

The total content of soluble proteins (Q_s, %) recovered with buffer (II) pH 8.0, of the PMCs extracted from WHPC at j=10 mA/cm^2 varied depending on the processing time, however being almost twice lower than the total protein content recovered in the PMCs. This fact was due to the tangled electro-bio-chemical processes occurring in the formation of protein-mineral compounds and that could not be extracted with this type of buffer solution (fig. 9).

Electrophoretic analysis with SDS-PAGE 15% of soluble proteins under the mentioned conditions demonstrated a non-uniform extraction of major protein fractions (HWP, CSN, β-Lg, α-La), conditioned by both high protein content of the IW and total solid contents, as well as due to the electrohydrodynamic and electro-biochemical processes, including the ionic flotation, activated by electrochemical interaction (fig. 10, 11).

The whey studied in Configuration 1 allowed extracting the following β-Lg: 46.58% at 10 min of processing, 31.36% at 20 min and 42.89% at 30 min; whereas in the liquid phase – in 37.74%. β-Lg extraction also depends on various pH and temperature values.

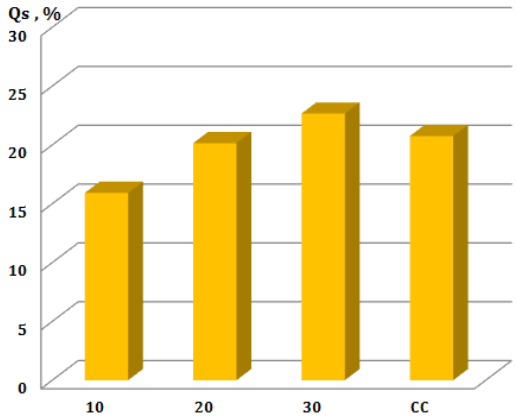

Figure 9. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, by electroactivation of WHPC at j=10 mA/cm^2, being processed for over 10-30 min.

On electroactivation, the protein molecules exhibited an oscillating electric field due to their amphoteric properties. The temperature gradient inside the cathode cell was non-uniform, increasing from 19 to 24°C, however, the temperature at the cathode surface may be higher than inside the cell, causing protein denaturation.

High temperatures were not registered in this configuration, thus excluding the thermal denaturation of β-Lg. Such factors as pH, concentration of minerals, carbohydrates, and proteins have a significant impact on the thermal behavior of β-Lg.

The pH value in the researched configuration increased on electroactivation from 4.53 to 9.20 in the first 10 minutes. The isoelectric point of β-Lg was – pH 5.3, causing a partial sedimentation of β-Lg in PMC.

It was mentioned elsewhere that β-Lg is the most sensitive at pH 9.0 and at temperature 43°C, which is the starting point for denaturation,

whereas at 51°C, the destruction of the secondary structure occurs (9).

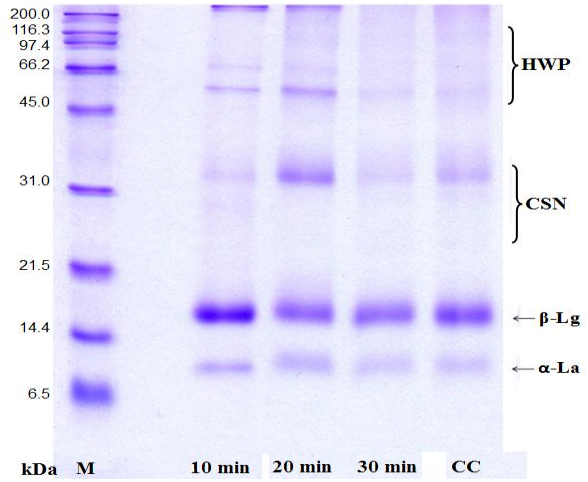

Figure 10. SDS-PAGE 15% of soluble protein concentrates of WHPC isolated with buffer solution (II), pH 8.0, by electroactivation at j=10 mA/cm², with marker M; over a 10-30 min processing time.

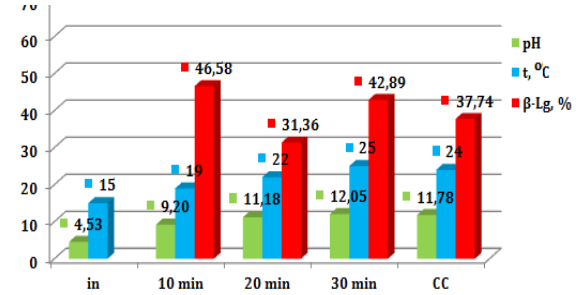

Figure 11. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WHPC, isolated with buffer solution (II), at pH 8.0, analyzed with SDS-PAGE 15%, by electroactivation at j=10 mA/cm²; over a 10-30 min processing time.

The aggregation of whey proteins involves the interaction of free-SH groups with the S-S bonds of cysteine-containing proteins such as β-Lg, k-CSN, α-La, and BSA through the – SH/S-S interactions (44).

These interactions between proteins lead to an irreversible aggregation of proteins into protein complexes of different molecular sizes, which depend on the heating conditions and the protein composition.

On electroactivation, it is assumed that β-Lg unfolding occurs and the hidden – SH groups become available on the surface of the molecule under these conditions, possibly as in the model described in (45). Since α-La and BSA are also present in whey, the reaction scheme could be extended by an extrapropagation step to accommodate the reaction between β-Lg and α-La or BSA (45).

A considerable increase of the pH values by the treatment causes the activation of the – SH/S-S groups and the extraction of β-Lg in the PMCs.

Configuration 2.

The total content of soluble proteins (Q_s, %) of the PMCs obtained from WHPC, recovered with buffer solution (II), at pH 8.0, being electroactivated at j=20 mA/cm², followed by the collection of PMCs at every 5 minutes, allowed a more detailed study of the extraction of protein fractions in the PMCs, by reducing the registration step.

The amount of soluble proteins varied from 20 to 25%, which confirmed the formation of protein compounds with a high MW, which could not be solved with this type of buffer solution (fig. 12).

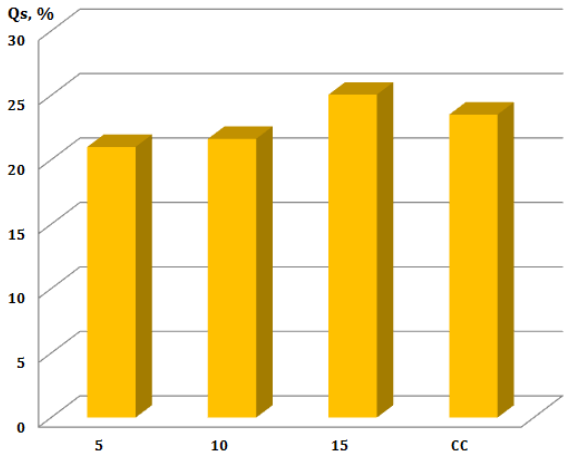

Figure 12. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, by electroactivation of WHPC, at j=20 mA/cm², and over a 5-15 min processing time.

An electrophoretic analysis with SDS-PAGE 15% of the content of protein fractions extracted from the PMCs with buffer solution (II), pH 8.0, by electroactivation of WHPC at j=20 mA/cm², at every 5 minutes of treatment, allowed the registration of the extraction of β-Lg fractions, which made up about 31.36% in the first 5 minutes of processing and 31.92% over 10 minutes of processing, whereas the total extraction of β-Lg made up 63.28%, compared to the results in

Configuration 1, where the total extraction of β-Lg was 46.58% over 10 min which confirms the "unfold-ding" of β-Lg, according to the models previously described (fig. 13, 14).

Both the temperature and pH values increased faster, leading to a more abundant total extraction of β-Lg over the first 10 minutes.

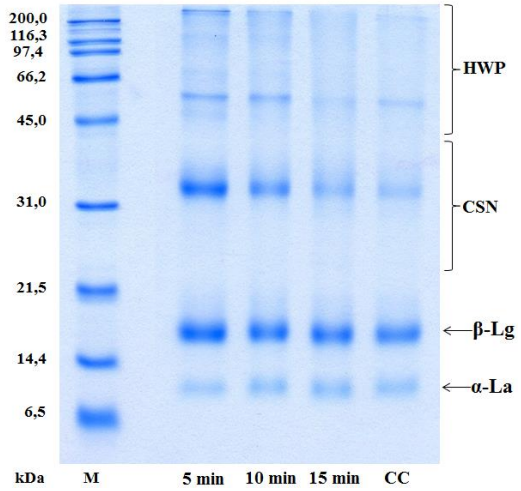

Figure 13. SDS-PAGE 15% of soluble protein concentrates isolated with buffer solution (II), at pH 8.0, by electroactivation of WHPC, at j=20 mA/cm², with marker M, over a 5-15 min processing time.

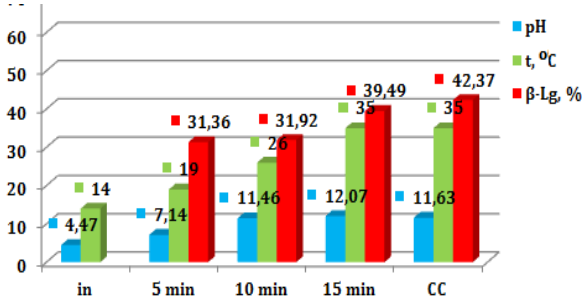

Figure 14. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WHPC, isolated with buffer solution (II), at pH 8.0, assayed by SDS-PAGE 15%, by electroactivtion at j=20 mA/cm², over a 5-15 min processing time.

Configuration 3.

The increased PMCs collection over 10 minutes allowed increasing the extraction of protein fractions in the PMCs.
The increasing electric current density, allowed increasing both the extraction of protein fractions in the PMCs, as well as the specific energy consumption per unit of processed volume.

The total content of soluble proteins (Q_s,%) extracted with buffer solution (II), pH 8.0, from the PMCs obtained from WHPC, electroactivated at j=20 mA/cm², PMCs being collected at every 10 minutes does not exceed 25%, which was considerably lower than the total protein content extracted in the PMCs (54-62%, see fig. 8), (fig. 15).

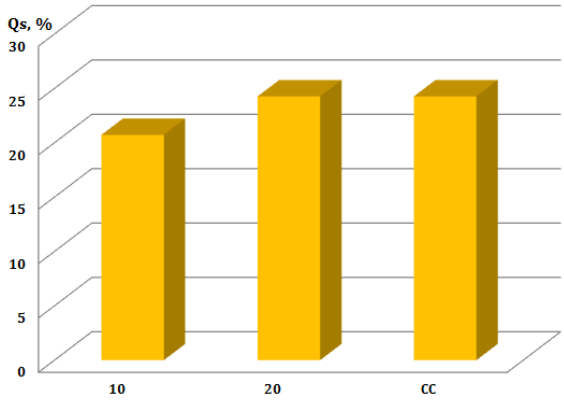

Figure 15. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, by electroactivation of WHPC at j=20 mA/cm², over 10-20 min processing time.

The electrophoretic analysis with SDS-PAGE 15% of the content of protein fractions of WHPC extracted from PMC with buffer solution (II), at pH 8.0, by electroactivation at j=20 mA/cm², at every 10 minutes of treatment, allowed the registration of an increased extraction of protein fractions in the PMCs, particularly of the amount of β-Lg is 54% over the first 10 minutes, however it showed a decreasing character during the processing.

Thus, the energy consumption was twice higher than while processing this type of whey at a j=10 mA/cm² current density, followed by an intensive formation of protein compounds with a high MW, which did not allow, however, their extraction with the mentioned buffer solution, as well as the electrophoretic assay with SDS-PAGE 15% (fig. 16, 17).

The temperature increased more intensely, compared to Configurations 1 and 2, reaching up to 47-48°C. The pH with intensely alkaline values caused the unfolding of proteins and the activation of the – SH/S-S groups, which facilitated the extraction of β-Lg, according to the described models, which also acted in the case of electroactivation.

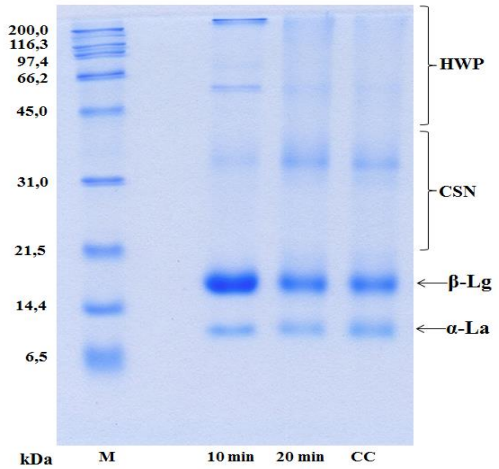

Figure 16. SDS-PAGE 15% of soluble protein concentrates isolated with buffer solution (II), pH 8.0, by electroactivation of WHPC at j=20 mA/cm², with marker M, over 10-20 min processing time.

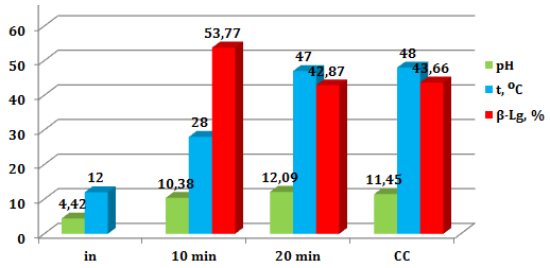

Figure 17. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WHPC, isolated with buffer solution (II), pH 8.0, assayed by SDS-PAGE 15%, by electroactivation at j=20 mA/cm², over 10-20 min processing time.

Configuration 4.

The whey electro-fractionation and PMCs formation with predetermined protein content is more obvious by electroactivating the WMPC.

The total content of soluble proteins (Q_s, %) extracted with buffer solution II, at pH 8.0, from the PMCs obtained by electroactivating WMPC at j=10 mA/cm² and the PMCs collection at 10 min intervals did not exceed 20%, which was considerably lower than the total protein content extracted in the PMCs (see fig. 7), (fig. 18).

The electrophoretic analysis with SDS-PAGE 15% of the content of protein fractions of WMPC, extracted from the PMC with buffer solution (II), pH 8.0, at j=10 mA/cm² and over a 10 min interval of treatment, allowed registering an increased rate of extracted protein fractions in the PMCs, particularly an increased amount of β-Lg (approximately 70%) was registered from the first minutes of processing, which however showed a decreasing tendency towards the end of the process (fig.19, 20).

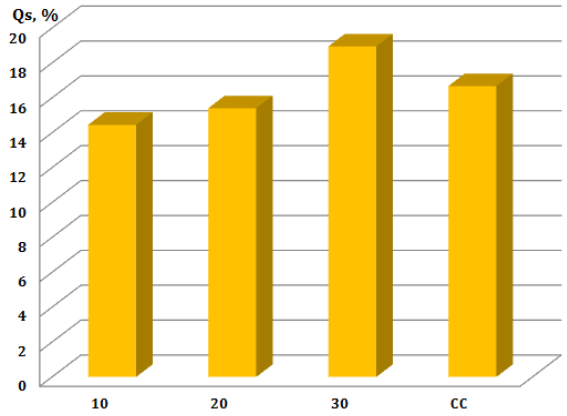

Figure 18. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, by electroactivation of WMPC at j=10 mA/cm², over 10-30 min processing time.

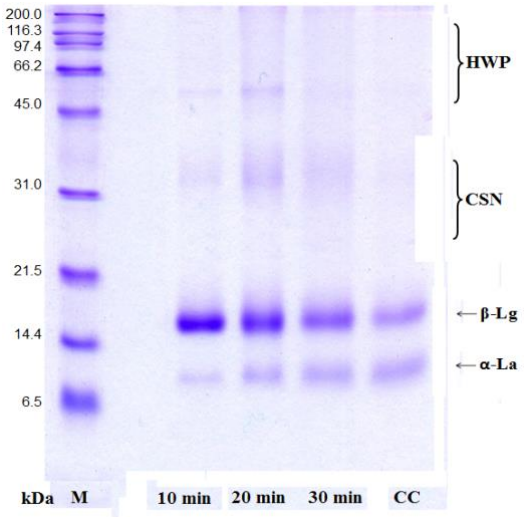

Figure 19. SDS-PAGE 15% of soluble protein concentrates isolated with buffer solution (II), pH 8.0, at electroactivation of WMPC at, j=10 mA/cm², with marker M, over 10-30 min processing time.

Configuration 5.

The total content of soluble proteins (Qs, %), extracted with buffer solution (II), at pH 8.0, of the PMCs obtained from WMPC, by the electroactivation at j=20 mA/cm² and the PMCs collection at every 10 minutes interval varied from 15% to 20%, which was considerably lower compared to the total protein content extracted in the PMCs (50-54% see fig. 8) (fig.21).

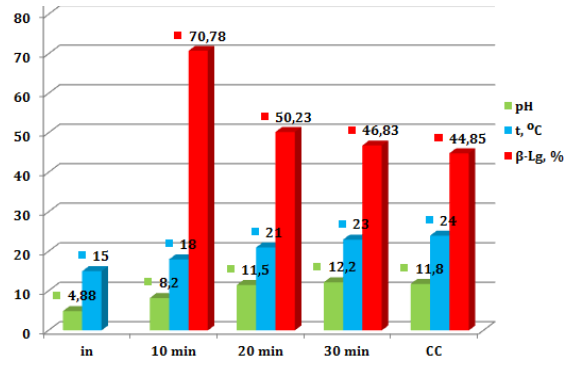

Figure 20. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WMPC, isolated with buffer solution (II), pH 8.0, assayed by SDS-PAGE 15%, by electroactivation at j=10 mA/cm², over 10-30 min processing time.

In this configuration, the pH values increased more slowly, and the amount of β-Lg (70.8%) was extracted at low alkaline values, which might be due to several mechanisms such as passing through the isoelectric point, the formation of dimers – oligomers of cystine, protein salination, etc.

At lower energy consumption, however, the β-Lg protein fraction did not exhibit a fast protein/protein interaction, which led to the formation of high-molecular polymers, thus allowing the extraction of a higher amount of β-Lg (46).

Thermal denaturation was excluded in this configuration and did not exceed 24°C. An abundant extraction in the first 10-20 mi-nutes of processing led to a reduced β-Lg content in the PMCs, obtained towards the end of the process.

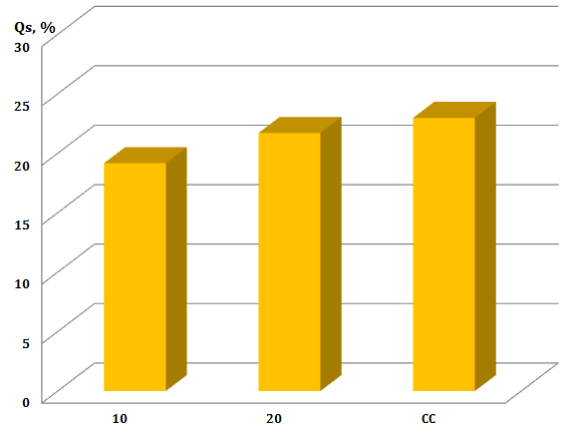

Figure 21. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, at electroactivation of WMPC at j=20 mA/cm², over 10-20 min processing time.

The SDS-PAGE 15% electrophoretis of the protein content, extracted from the PMCs with buffer solution (II), at pH 8.0, by electroactivation of WMPC at j=20 mA/cm² at every 10 min of treatment, allowed the extraction of β-Lg of 57-60%, which differed from that processed at j=10 mA/cm², under the same treatment conditions, the content of β-Lg being 71% (fig. 22, 23).

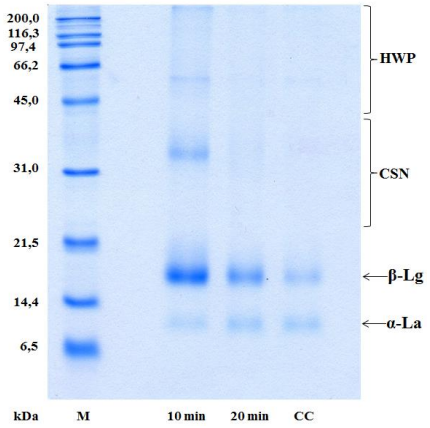

Figure 22. SDS-PAGE 15% of soluble protein concentrates isolated with buffer solution (II), pH 8.0, by electroactivation of WMPC at j=20 mA/cm², with marker M, over 10-20 min processing time.

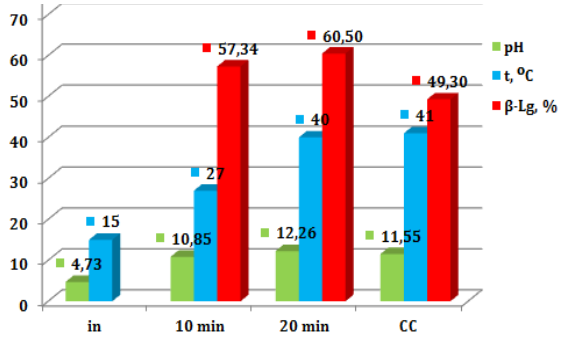

Figure 23. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WMPC, isolated with buffer solution (II), pH 8.0, by with SDS-PAGE 15% analysis, by electroactivation at j=20 mA/cm², over 10-20 min processing time.

Evidently, there was a very intense aggregation with other proteins, forming highly molecular protein compounds. The pH values increased considerably over the same processing time from pH 10.85 in the first 10 min to pH 12.26 at 20 min of treatment, which contributed to protein/protein aggregation (46). The high temperature did not cause thermal denaturation of the proteins, the maximum values being 40-41°C.

Configuration 6.

The electroactivation of WLPC at 10 mA/cm² obviously demonstrated different extraction degrees of whey protein fractions in the PMCs, which depended both on the processing regime, energy consumption, amount of processed whey, processing time, the pH variations, and on the type of the whey processed, which, in turn, depended on the primary processing of dairy products.

The total content of soluble proteins (Q_s, %), extracted with buffer (II), at pH 8.0 from the PMCs obtained from WLPC, electroactivated at j=10 mA/cm², varied non-uniformly during treatment, having a maximum value of 18% at the first 10 minutes. The amount of soluble proteins was lower than with the two types of whey described above, possibly, due to a lower protein content in the IW (fig. 24).

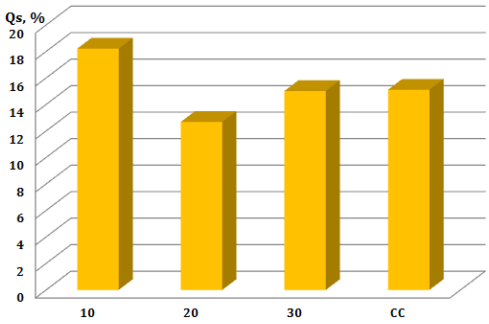

Figure 24. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, by electroactivation of WLPC at j=10 mA/cm², over 10-30 min processing time.

The SDS-PAGE 15% electrophoreris of the protein content of WLPC, extracted from the PMCs with buffer (II), at pH 8.0, by electroactivation at j=10 mA/cm², at every 10 minutes of treatment, registered an increased extraction degree of protein fractions in the PMCs.

The extraction of one of the major fractions of whey, β-Lg, was registered in the first 10 minutes of processing (51.35%), which takes place at neutral pH values (6.80), followed by a decrease of its content to intense alkaline values of pH 11.55-12.10. The processing temperature did not exceed the denaturation point (fig. 25, 26).

Configuration 7.

The total content of soluble proteins (Q_s, %) extracted with buffer solution (II), pH 8.0, of the PMCs obtained from WLPC, being electroactivated at j=20 mA/cm² and registered at every 5 min interval varied uniformly during treatment. The amount of soluble proteins was 20-25% (fig. 27).

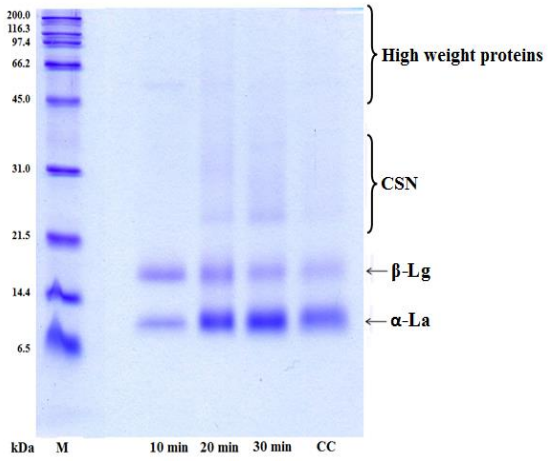

Figure 25. SDS-PAGE 15% of soluble protein concentrates isolated with buffer solution (II), pH 8.0, by electroactivation of WLPC at j=10 mA/cm², with marker M, over 10-30 min processing time.

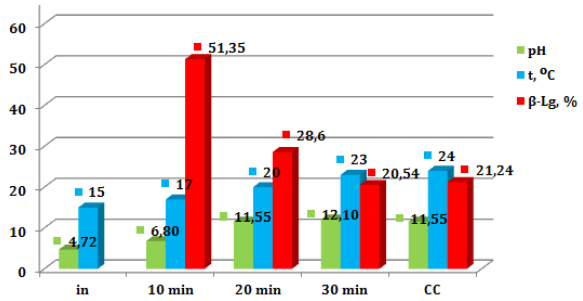

Figure 26. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WLPC, isolated with buffer solution (II), pH 8.0, by SDS-PAGE 15% analysis, by electroactivation at j=10 mA/cm², over 10-30 min processing time.

The SDS-PAGE 15% electrophoresis of protein content of WLPC, extracted from the PMCs with buffer solution (II), at pH 8.0, electroactivated at j=20 mA/cm² and at a 5 min interval of treatment, registered an increased recovery of protein fractions in the PMCs. An increased amount of β-Lg (66%), extracted in the PMCs from WLPC was recorded over the first 5 min of treatment, showing the highest value compared to those of the two types of whey previously studied (WHPC and WMPC) under similar processing conditions, which also decreased during processing (fig. 28, 29).

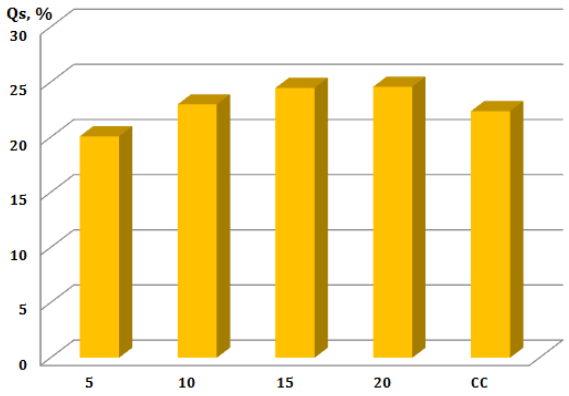

Figure 27. Variations of content (Q_s, %) of total soluble proteins recovered with buffer solution (II), pH 8.0, by electroactivation of WLPC at j=20 mA/cm² and over 5-20 min processing time.

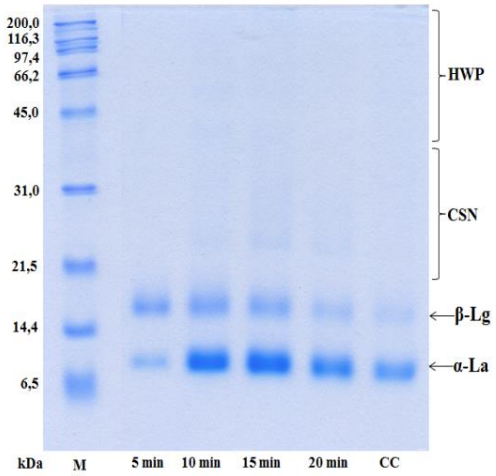

Figure 28. SDS-PAGE 15% of soluble protein concentrates isolated with buffer solution (II), pH 8.0, by electroactivation of WLPC at j=20 mA/cm², with marker M, over 5-20 min processing time.

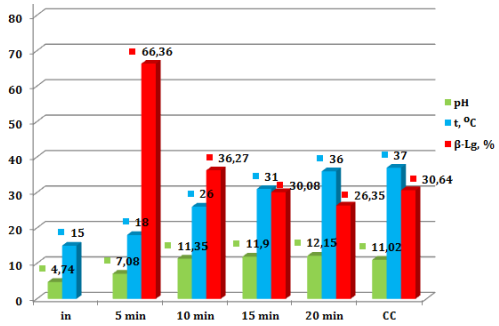

Figure 29. Variations of pH values, temperature and soluble protein fraction content (β-Lg, %) of WLPC, isolated with buffer solution (II), pH 8.0, analyzed by SDS-PAGE 15%, by electroactivation at j=20 mA/cm² and over 5-20 min processing time.

The maximum amount of β-Lg was extracted at neutral pH values, compared to Configuration 2, likely due to a higher initial content of β-Lg in this type of whey.

The processing temperature did not exceed the denaturation point. Moreover, the temperature-increasing rate was lower than in Configuration 2 due to a lower initial protein content (see fig. 8), which reduced the resistance of the processed medium, thus leading to a decrease of the Joule effect.

Whey electro-fractionation and PMCs formation with predetermined protein content was more clearly demonstrated on WLPC. The correct management of electroactivation by using different treatment regimens will allow the electro-fractionation of different types of dairy by-products.

DISCUSSIONS

Various and non-uniform isolation of protein fractions in the PMCs by electroactivation of the three types of whey was determined. First, depending on the properties of each fraction separately and then on their behavior resulting from the electrochemical action.

The explanation of the physical-chemical and biochemical properties of the whey proteins and the reasoning support of the formation of the PMCs requires a clear information on their structure. The phenomena and behavior of certain structural peculiarities of whey proteins might account for the action of some factors, such as temperature, pH, and ORP.

The whey PMCs formation depends on the electroactivation regime and the following parameters: electric current density, energy consumption per volume unit, variations of the pH and ORP values, temperature variations and Joule heat. Multiple inter- and intra-molecular processes occur under the action of the electric current on the solid shear of whey on about 6% and over 200 ingredients, which cause variations in the isolation of protein fractions in the PMCs

Whey proteins have a high solubility within a wide range of pH values owing to the composition of amino acids and the arrangement of their free radicals during the formation of tertiary structures. The pH increase is also compulsive due to processes generated by electroactivation.

It is due to the arrangement of hydrophilic radicals and of a large number of disulfide bonds on the surface of globular structures of whey proteins, which is similar to other research data. Furthermore, the globular structure provides resistance of those proteins to proteolysis.

The experimental data on the isolation of β-Lg viz. the most abundant whey protein, within the first 5-10 min, when the pH was kept within the neutral range (pH 7.0-8.0), at a low temperature registered in the volume of the CC, but which can be higher on the cathode surface, allowed concluding that reactive sulfhydryl groups (thiols) play an important role in the isolation of whey proteins by cysteine radicals.

Upon the oxidation of sulfhydryl groups of two cysteine radicals and the formation of covalent disulphide bonds (–S–S–) in proteins, a cystine is formed, which is a dimer of cysteine that jointly with hydrogen, ionic, and hydrofolic bons maintains the spatial structure of protein molecules. Similarly, intermolecular bridges are formed between cysteine radicals, dissociated both as a result of electroactivation and due to the increase inpH up to 8.3. The study results have proved that cysteine radicals participate in the PMCs formation of whey proteins, by blocking of sulfhydryl groups with Sodium iodoacetate. Introducing it into the initial whey reduces the isolation of proteins via the elimination of agregation of prot eins according to the mechanism described. The aggregation of whey proteins via various mechanisms and modes has been mostly presented earlier (47).

Sedimentation of proteins in their isoelectric point (pI) is one of the mechanisms that influences the extraction of β-Lg in PMCs. The variation of the active acidity in the cathode cell is not homogeneous, being conditioned both by its size/dimensions and by the initial whey flow at treatment, which increases the pI by certain protein fractions and obviously their sedimentation in pI during processing.

Isolation of whey proteins in the PMCs via the processing regimens depends on the action of several mechanisms of formation of protein compounds and on coagulation. An important role of Ca in the isolation of proteins has been confirmed by lowering its amount in deproteinised whey during processing and its presence in the mineral composition of the PMCs.

During electroactivation of whey, the concentration of ions in the electrolyzer zone might be several times higher (at the level of an order of magnitude) than in the initial solution, thus creating conditions for proteins salinity, which was indirectly confirmed by an intensive foaming in the cathode zone and by their later isolation supported by ionic flotation due to electroactivation. Protein salinization is one of the main mechanisms in the recovery of β-Lg in the PMCs.

The maximum amount of β-Lg recovered in the PMCs on electroactivation is 66% from WLPM and 71% from WMPC, which differed depending on the treatment regimes. Obviously, that the recovery of β-Lg is higher while extracting β-Lg from whey with a lower initially protein content.

The temperature registered in the cathode cell was not over 55-60°C, hence permitting PMCs formation without undergoing thermal denaturation.

CONCLUSIONS

The correct management of the electroactivation with various treatment regimens will allow the electro-fractionation of different types of dairy by-products.

1. The recovery of β-Lg in protein-mineral concentrates (PMCs) by electroactivation of whey depends on the electric current density, initial protein content, temperature, and pH values.

2. Electroactivation of whey with high initial protein content allows the extraction of a lower amount of β-Lg in PMCs due to intense electro-bio-chemical interactions, leading to the formation of protein-mineral compounds with a high molecular weight.

3. The recovery of β-Lg in PMCs, at the electroactivation of whey with medium and low initial protein content was more obvious, registering the maxima of 71% and 66%, respectively, due to a lower protein content in the initial whey, which allowed a more intense recovery.

4. The maximum amount of β-Lg recovered in PMCs was extracted within the first 5-10 minutes of treatment at low alkaline pH 7.00-8.00.

5. The temperature in the cathode cell was not over 55-60°C, hence permitting PMCs formation without undergoing thermal denaturation;

6. The main mechanisms that allow the recovery of β-Lg in PMCs are as following:
- Activation of the –SH/S-S groups and the extraction of β-Lg in the PMCs while increasing the pH values during treatment.
- Sedimentation of proteins in their isoelectric points.
- Protein salinization is the major mechanisms in the recovery of β-Lg in the PMCs.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest

ACKNOWLEDGMENT

This research was carried out within the STCU project 6011 and partially in the state program ANCD 20.80009.5007.06 (2020-2023).

REFERENCES

1. Etzel MR. Manufacture and use of dairy protein fractions. *J Nutr*. 2004;134(4):996S-1002S. doi:10.1093/jn/134.4.996S
2. Sprinchan E, Bologa M, Stepurina T, Bologa Al, Polikarpov A. Peculiarities of the Electric Activation of Whey. *Surf Eng Appl Elect*. 2011;47(1):66-69.
3. Farrell HM Jr, Jimenez-Flores R, Bleck GT, et al. Nomenclature of the proteins of cows' milk--sixth revision. *J Dairy Sci*. 2004;87(6):1641-1674. doi:10.3168/jds.S0022-0302(04)73319-6
4. Singh H, Havea P, Thermal denaturation, aggregation and gelation of whey proteins. In: Fox PF, McSweeney P.L.H. (Eds). *Advanced Dairy Chemistry*. Vol.1. Proteins, 3 rd ed. Kluver Academic/Plenum Publishers, New York, 2003; p.1257-1283.
5. Dissanayake M, Vasiljevic T. Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *J. Dairy Sci.* 2009;92(4):1387-97. doi: 10.3168/jds.2008-1791
6. Swaisgood H.E. Sulphhydryl oxidase: properties and aplication. *Enzyme Microb. Technol*. 1986; 2:265-272.
7. Boye J, Ismail A, Alli I. Effects of physicochemical factors on the secondary structure of β-lactoglobulin. *Journal of Dairy Research.* 1996;*63*(1): 97-109. doi:10.1017/S0022029900031575
8. Kontopidis G, Holt C, Sawyer L. Invited review: beta-lactoglobulin: binding properties, structure, and function. *J Dairy Sci*. 2004;87(4):785-796. doi:10.3168/jds.S0022-0302(04)73222-1
9. de Wit J, Klarenbeek G. Effects of various heat treatments on structure and solubility of whey proteins. *J. Dairy Sci*. 1984;67(11): 2701-2710.
10. Caessens P, Visser S, Gruppen H. Method for the isolation of bovine β-lactoglobulin from a cheese whey protein fraction and physicochemical characterization of the purified product. *Int. Dairy J.* 1997;7(4):229-235.
11. Galani D, Owusu Apenten R.K. Heat-induced denaturation and aggregation of β-Lactoglobulin: kinetics of formation of hydrophobic and disulphide-linked aggregates. *Int. J. Food Sci. Technol*. 1999;34(5):467-476.
12. Sawyer L, Kontopidis G, Wu S.-Y. β-lactoglobulin - a three-dimensional perspective. *Int. J. Food Sci. Techn*. 1999;34(5-6):409-418.
13. Holt Carl, De Kruif C.G. (Kees). Advanced Dairy Chemistry - 1 Proteins. In: (eds.) P.F. Fox, P.L.H. McSweeney, 3rd edition, Kluwer Academic/Plenum Publishers, NY, USA. 2003; p.233-276; 10.1007/978-1-4419-8602-3_5.
14. Mulvihill D. M, Donovan M. Whey Proteins and Their Thermal Denaturation - A Review. *Irish Journal of Food Science and Technology.* 1987; 11(1):43-75.
15. Godovac-Zimmermann J, Krause I, Buchberger J, Weiss G, Klostermeyer H. Genetic variants of bovine beta-lactoglobulin. A novel wild-type beta-lactoglobulin W and its primary sequence. B*iol Chem Hoppe Seyler*. 1990;371(3):255-260.
16. Moreland JL, Gramada A, Buzko OV, Zhang Q, Bourne PE. The Molecular Biology Toolkit (MBT): a modular platform for developing molecular visualization applications. *BMC Bioinformatics*. 2005:6:21. doi:10.1186/1471-2105-6-21
17. Papiz MZ, Sawyer L, Eliopoulos EE, et al. The structure of beta-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature*. 1986; 324(6095):383-385. doi:10.1038/324383a0
18. Croguennec T, Mollé D, Mehra R, Bouhallab S. Spectroscopic characterization of heat-induced nonnative β-lactoglobulin monomers. *Protein Sci.* 2004;13(5):1340-1346.
19. Reithel F. J, Kelly M.J. Thermodynamic analysis of the monomer-dimer association of beta-lactoglobulin A at the isoelectric point. *Biochemistry.* 1971;10 (13):2639–2644.
20. Dalgalarrondo M, Chobert J. M, Dufour E, Bertrand-Harb C, Dumont J. P, Haertle T. Characterization of bovine β-lactoglobulin B tryptic pepti-

es by reversed-phase high perfomance liquid chromatography. *Milchwissenschaft.* 1990;45 (4):212-216.
21. Demetriades K, Coupland J. N, and Mcclements D. J. Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. *J. Food Sci*. 1997;62(3):462-467.
22. De la Fuente M.A, Singh H, and Hemar Y. Recent advances in the characterization of heat-induced aggregates and intermediates of whey proteins. *Trends Food Sci. Technol.* 2002;13(8):262-274.
23. Qi X.L, Holt C, Vc Nully D, Clarke D.T, Brownlow S, Jones G.R. Effect of temperature on the secondary structure of β-lactoglobulin at pH 6,7 as determined by CD and JR spectroscopy; a test of molten globule hypothesis. *Biochemical Journal*. 1997;324 (1): 341-346.
24. Hoffmann M.A.M, van Mil P.J.J.M. Heat-induced aggregation of betalactoglobulin as a function of pH. *J. Agric. Food Chem.* 1999;47(5):l898-1905.
25. Manderson G.A, Hardman M.J, Creamer L.K. Effect of heat treatment on the conformation and aggregation of β-lactoglobulin A, B, and C. *J. Agric. Food Chem.* 1998;46(12):5052–5061.
26. Iametti S, De Gregori B, Vecchio G, Bonomi F. Modifications occur at different structural levels during the heat denaturation of beta-lactoglobulin. *Eur J Biochem*. 1996;237(1):106-112. doi:10.1111/j.1432-1033.1996.0106n.x
27. Bychkova V.E, Ptitsyn O.B. The molten globule in vitro and in vivo. *Chemtracts Biochem. Molec.Biol*. 1993;4:133-163.
28. Creighton TE. How important is the molten globule for correct protein folding? *Trends Biochem Sci*. 1997;22(1):6-10. doi:10.1016/s0968-0004 (96) 20030-1
29. Hirose M. Molten globule state of food proteins. *Trends Food Sci. Technol.* 1993;4(2):48–51.
30. Donovan M, Mulvihill D. M. Thermal denaturation and aggregation of whey proteins. *Irish Journal of Food Science and Technology*. 1987;11(1):87-100.
31. Maćej OD, Jovanović ST. Complex formation between casein and whey protein in heat treated milk. *Acta periodica technologica* 2000;(31):83-93.
32. Duranti M, Pagani S, Iametti S, Carpen A. Heat-induced changes of milk proteins: rocket immunoelectrophoretic detection of α-lactalbumin. *Milchwissenschaft.* 1989;44 (3):142-144.
33. Morr CV. Functionality of heated milk proteins in dairy and related foods. *J. Dairy Sci*. 1985;68(10): 2773-2781.

Date of receipt of the manuscript: 01/06/2020
Date of acceptance for publication: 19/12/2020

Mircea BOLOGA ORCID ID: 0000-0002-5262-9666
Elvira VRABIE ORCID ID: 0000-0001-8607-8981
Irina PALADII ORCID ID: 0000-0002-5683-5248

34. Elfagm AA, Wheelock JV. Interaction of bovine alpha-lactalbumin and beta-lactoglobulin during heating. *J. Dairy Sci.* 1978a;61(1):28-32.
35. Elfagm AA, Wheelock JV. Heat Interaction between alpha-lactalbumin, beta-lactoglobulin and Casein in Bovine Milk. *J. Dairy Sci.* 1978b; 61(2):159-163.
36. Kirchmeier O, Kamal NM, Klostermeyer H. Heat treatment of milk and development of SH-groups. *Milchwissenschaft.* 1985;40(12):722-724.
37. de Rham O, Chantan S. Role of ionic environment in insolubilization of whey protein during heat treatment of whey products. *J. Dairy Sci.* 1984;67(5): 39-949.
38. Hillier RM, Lyster RJ, Cheeseman GC. Thermal denaturation of alpha-lactalbumin and beta-lactoglobulin in cheese whey; effects of total solids concentration and pH. *J. Dairy Res.* 1979;46:103-111.
39. Vrabie EG, Bologa MK, Paladii IV, et al. Electrical Processing of Whey. Role of Construction, Technological and Energy Characteristics of Reactors. *Surf. Eng. Appl. Elect*. 2019;55(2):197-209 doi: 10.3103/S1068375519020145
40. Warburg O, Christian W. Isolation and crystallization of enolase. *Biochem. Z.* 1942;310:384-421 (in German).
41. Kochetov G.A. *Practical Handbook on Enzimology*. Moscow, 1980, p.400 (in Russian).
42. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227(5259):680-685. doi:10.1038/227680a0
43. Vrabie EG, Bologa MK, Paladii IV, et al. Nanotechnological Aspects at Electroactivation of Secondary Dairy Products. *IFMBE Proceedings, Springer.* 2020;77:799-803.
44. Considine T, Patel HA, Anema SG, et al. Interactions of milk proteins during heat and high-hydrostatic-pressure treatments – a review. *Innov. Food Sci. Emerg. Technol.* 2007;8(1):1-23.
45. Roefs SP, De Kruif KG. A model for the denaturation and aggregation of beta-lactoglobulin. *Eur J Biochem*. 1994;226(3):883-889. doi:10.1111/j.1432-1033.1994.00883.x
46. Anema SG. McKenna AB. Reaction kinetics of thermal denaturation of whey proteins in heated reconstituted whole milk. *J. Agric. Food Chem*. 1996;44:422-428.
47. Sprinchan EG. Optimization of technological regimes for obtaining protein-mineral concentrated products from secondary milk raw materials. *Surf. Engin. Appl.Electrochem.* 2009;45:63-70.