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RECOVERY OF PROTEINE MINERAL CONCENTRATES FROM ACID WHEY BY ELECTRO-ACTIVATION

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Abstract. An electrophysical wasteless technology is presented based on the electrochemical activation of the acid whey in a periodic regime. Under consideration are variations of such principal parameters as the electric current density, voltage, pH, oxidation-reduction potential, temperature, specific energy consumption, and the degree of isolation of whey proteins in the protein-mineral concentrates (PMCs). It is found that the isolation of whey proteins in the PMCs makes about 80% from the first minutes of the process and remains constant during the rest of it at the current density $j=10 \text{ mA}\cdot\text{cm}^{-2}$ and the specific energy consumption by 2.5 times lower than during processing at $j=20 \text{ mA}\cdot\text{cm}^{-2}$, in the latter case, the isolation of whey proteins in the PMCs makes about 73-75%, which allows for decreasing the energy consumption during processing. However, in the liquid phase (LP), the recovery of about 84%, which means that from the first minutes, there are intensively formed protein compounds with a high molecular weight that which cannot be isolated as foam as a result of the ion flotation, remaining in the LP contained in the cathode cell. These investigations demonstrate an opportunity to properly control the processing of the acid whey, which depends on the processing regime (the electric current density) necessary for an optimal isolation of protein fractions in the PMCs, with a simultaneous decrease of the specific energy consumption per a unit of volume.

Keywords: *current density, electrophysical processing, energy consumption, membrane electrolyzer, whey proteins.*

Introduction

Nowadays, the issues of developing wasteless technologies and approaches for their implementation have received a wide-spread significance in several areas. One of them, of a primary importance for humans, is food industry, first of all, the processing one which often generates problems for the environment. At present, in the area of production, there

is an intensive process of revision of environmental requirements concerning wastes. Development of wasteless technologies and processing of whey in a closed cycle is one of the major global challenges.

Current situation at an international scale, related to the development of ecologically friendly technologies for processing dairy by-products, makes the following necessary: development of new high-tech and efficient methods, including electrophysical processing; establishment of the parameters for the process functioning; and energy-saving techniques for the process in question [1]. The analysis of technological processes related to the application of electro-activation requires making the processing more effective without using chemical reagents, at a low temperature and low energy consumption. The mode of the solution of this problem influences the advances in the area through the introduction of electrophysical techniques [2, 3].

Production of dairy food is one of such areas, first of all, concerning dairy by-products that are obtained, depending on the processing mode. One important by-product is whey – an excellence source of proteins. However, it is an ecologically non-friendly product because it contains large quantities of certain organic substances [4].

The nutritional value of milk owes its nutritive values to the milk sugar (lactose), nitrogen from protein fractions, and to an impressive content of calcium that is extremely important for cell signaling mechanisms and also beneficial for the human metabolism and skeletal system, all those and other milk ingredients making up a vital biologically active base for a human body [5]. First of all, those vital for human substances are in the dairy by-products extracted after the primary milk processing [6].

Skim milk, buttermilk, and whey are main lactic by-products that should be rationally and entirely consumed [7, 8].

The mode of the primary milk processing results in two types of whey:

- sweet (pH 5.5-6.0, manufactured during the making of several types of hard cheese), and
- acid/sour (pH 4.5-5.1) produced during the making of acid types of dairy products, such as cottage cheese or curd products).

Skim milk and buttermilk are obtained in the production of butter from milk, and caseinate is produced when making casein. When using non-standard high technologies for isolation of proteins from milk, an ultrafiltrate is formed, also considered a dairy by-product [9]. The solid content of whey is 6-8%, which makes about 50-70% of that of whole milk [10]. Still, in the respective literature, the solid content of whey is different and depends of the mode of the primary milk processing [11].

Lactose is a disaccharide sugar that is found only in milk. During primary milk processing, lactose almost completely passes into whey, making up 70 – 80 % of its solid content, depending on the primary processing mode [12].

Whey proteins by their structure are similar to those of blood, one of their functions being immune activity. Amino acids, including the principal ones, make up a substantial part of biologically active substances in foodstuffs.

Whey lipids are more dispersed than those of milk and are beneficial for the biochemical digestive processes [13].

The mineral composition of whey is very large and is optimally balanced and varied from the biological point of view. Whey contains milk vitamins, both water-soluble and liposoluble [14].

The protein composition of milk whey (MW) is represented by the major protein fractions: β -lactoglobulins, α -lactalbumins, immunoglobulins, and bovine serum albumin (BSA) [16, 17].

β -lactoglobulins are the quantitatively major protein fraction of MW (50-55%); they occur only in the milk of ruminants. These proteins are a good source of essential and branched chains amino acids; β -lactoglobulins are not allergic in the hydrolyzed state and are used in different formulas for infant feeding; α -lactalbumins account for about 20-25% of the MW proteins and are the main proteins of breast milk. Thanks to the balanced amino acids composition, they supply essential amino acids into a child's body (particularly, tryptophan and cysteine), and are able to bind calcium as well as zinc; they accelerate their absorption in the alimentary process; α -lactalbumin is known to enter into the composition of lactose synthetase [18]. BSA makes up 5-10% of the MW proteins, which is notable for a sufficiently large size of its molecules (see Table 1), its balanced amino acids composition, and it can be bound by lipids.

Table 1

Chemical composition of whey resulting from processing of cow's milk, % [15]

Components	Sweet whey	Acid whey
Water	93 - 94	94 - 95
Solids	6 - 7	5 - 6
Lipids	0 - 0,3	0 - 0,1
Proteins	0,8 - 0,1	0,8 - 0,1
Lactose	4,5 - 4,9	3,8 - 4,2
Minerals	0,5 - 0,7	0,7 - 0,8
Lactic acid	traces	0,8

The experiments with radioactive carbon have shown that the BSA gets into milk from blood. The immunoglobulins of MW (10-15%) also get into milk from blood (Table 2). They have the activity of antibodies against the corresponding antigens and, being the major protein fraction of colostrum, promote strengthened immunity in new-born babies.

Table 2

Content of main protein fractions in MW

Protein	Content in MW, g/l	Molecular weight, kDa	Isoelectric point
α -lactalbumin	0.7	14.1	4.8
β -lactoglobulin	3.0	18.2	4.9-5.4
BSAovine serum albumin	0.3	66	4.8
Lactoferrin	0.1	78-80	8.0-8.8
Lactoperoxidase	0.04	78-80	8.6-9.6
Immunoglobulin	0.5	150-900	5.8-7.3

In order to explain the physical-chemical properties of whey proteins and their biological roles, it is necessary to know the peculiarities of their structure. At the same time, knowing the peculiarities of the structure of these proteins can make it possible to explain

the phenomena of their behavior under the action of various factors such as temperature and pH, for example.

Thus the main whey protein fractions have a complex structure; therefore under the action of temperature and pH certain complex intermolecular interchange reactions take place, which leads to changes in the initial structure and properties [19].

There are different types of whey protein products: whey protein isolates (WPI), whey protein concentrates (WPC), whey protein hydrolysates (WPH), and protein mineral concentrates (PMC).

Whey protein isolates (WPI) - allow the extraction of proteins in the purest form of whey and containing at least 90% protein [20].

Whey protein concentrates (WPC) have a low level of fats and carbohydrates, but their protein content, depending on concentration, is 30% - 90%, the latter containing the most healthy nutrients from whey [21].

Whey protein hydrolysates (WPH) - are obtained at partial hydrolysis of whey proteins, which has faster protein absorption properties and are used in health supplements and children's formulas due to a reduced allergen potential [22].

There are different techniques to process dairy by-products for obtaining protein concentrates that are used in various beneficial/dietary supplements and in pharmaceutical products as biologically active substances. Of special interest is usage of proteins isolated from whey. Still, usage of whey proteins for the mentioned purposes requires working under certain specific strict conditions – special technological regimes to ensure a high degree of purity and maintenance of natural qualities [23].

Manufacture of healthy and environmentally safe products of whey requires upgrading of methods and techniques for whey processing. The electrophysical whey processing applied in our experiments is a wasteless method that allows the valorification of all whey components. Besides, this type of processing allows controlling the content of whey proteins in the obtained concentrates, depending on the processing regime [24].

The isolation of whey proteins and obtaining protein-mineral concentrates (PMCs) of a high value under the action of an electric current and avoiding the direct usage of chemicals is an advantageous process based on modern principles, which assures the finite cycle of the simultaneous processing of whey sugars (isomerization of lactose into lactulose), too, through separating them from the deproteinized whey. The PMCs allow the simultaneous extraction of whey proteins and minerals, increasing the biological value of the concentrates obtained, used both in the pharmaceutical and food industries [25].

Methodological part

The electrophysical whey processing requires certain technical conditions to assure the control over the technological process, which are taken into account in the construction of a membrane electrolyzer (Figure 1) constructed by the authors [26]. The electrolyzer frame is from a dielectric material; the cathode - from AISI 304/1.4301 stainless steel (considered safe for usage in the dairy, food, and pharmaceutical industries); the anode – from solide graphite plates so as to exclude its dissolving during electrochemical activation. The cathode and the anode cells are separated by a cation-exchange membrane an MK-40 cation-exchange membrane. The electrolyzer is connected to the electric current source TEC – 5020.

The acid whey was obtained from the milk collected at the Dairy Training Farm of the Chair of Biotechnologies in Zootechnology, of the State Agrarian University of Moldova. That milk was first subjected to fermentation at room temperature and then skimmed.

First, the acid whey is cooled, then separated from the casein powder, next - processed electrophysically in the cathode cell of the membrane electrolyzer (see Figure 1), with a shorter distance between the electrodes and with the ratio of the volume of the processed whey (V , ml) on the electrode (cathode) surface (S , cm^2) - $V/S = 1.4$), at current densities $j = 10$ and $20 \text{ mA}\cdot\text{cm}^{-2}$.

The 2% CaCl_2 solution was used as secondary liquid in the anode cell in order to maintain the conductivity of the processed liquid and the delivery of bivalent ions into the cathode cell via the MK-40 membrane.

The processed whey collected as foam in different processing times: 5-10-15-20 min, was called the foamy phase (FP), while the liquid phase (LP) was considered to be the content of the cathode cell after processing (CC). Next, the collected foam was centrifuged at 1500G, thus separating the PMCs from the deproteinized whey (DW).

The deproteinized whey already contains the isomerized lactulose that is processed later. The PMCs enriched with certain protein fractions are dried at lower temperatures, for instance, via lyophilization, so as to exclude thermally caused changes in the isolated protein fractions. The second liquid from the anode cell is collected and kept in a reservoir later re-cycled. The following parameters were registered: electric: voltage U , V; thermal: temperature t , $^{\circ}\text{C}$, in the LP and in the FP; physical-chemical: pH and oxidation-reduction potential (ORP), mV; biochemical: the degree of the isolation of the total protein content in the PMCs (Q , %). In addition, the energy consumption was calculated, A ($\text{W}\cdot\text{h}$) as well as, and the specific energy consumption per a unit volume A_v ($\text{W}\cdot\text{h}\cdot\text{ml}^{-1}$).

In order to assess the quality of whey and whey products obtained after electrophysical processing, the following principal physical-chemical parameters were considered:

1. the pH of whey (active acidity) and of the ORP. The measurements were made on a 766 Laboratory pH meter, 5 minutes after the sample collection;
2. protein content, determined under the Warburg method, with a spectrophotometer CФ-56, at a wave length of 278 nm (the reference solution was bovine serum albumin, the standard determination coefficient - $k = 1.77$) [27].

The degree of the isolation of whey proteins in the PMCs (Q , %) was calculated as the difference of the protein content in the initial whey and that which remained in the deproteinized whey.

All measurements were carried out in three consecutive series; the graphs and diagrams were made in Excell, with the presentation of mathematical relations, with the approximation level R^2 and the approximation error $1-R^2$.

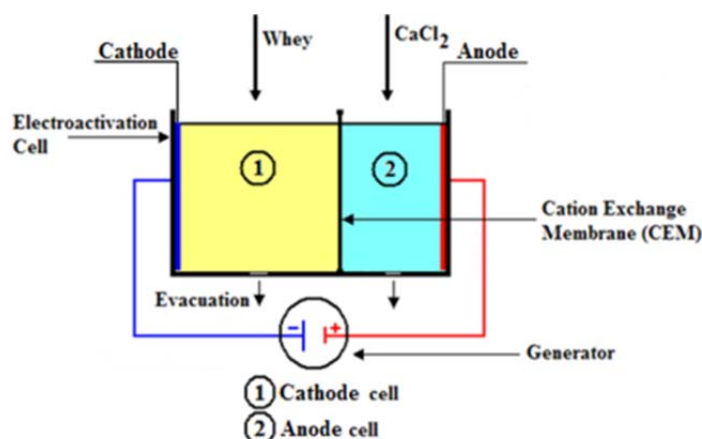


Figure 1. Layout of experimental membrane electrolyzer.

Results and discussion

The electrophysical processing of the acid whey, with the initial pH 4.60, was carried out in the membrane electrolyzer (see Figure 1), and the current densities 10 and 20 mA·cm⁻².

It was found that voltage varies much more at $j=20$ mA·cm⁻² than at $j=10$ mA·cm⁻², provoking more rapid processes at a higher energy consumption (Figures 2, 3).

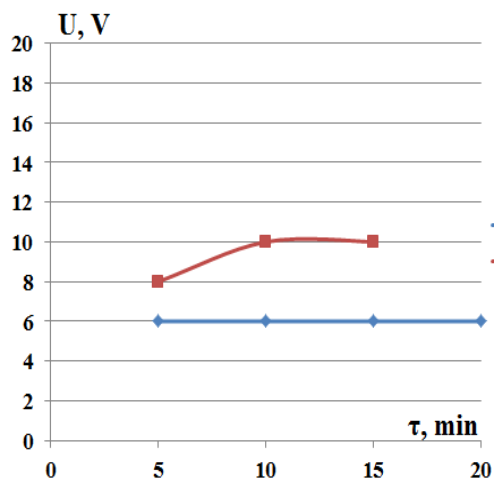


Figure 2. Voltage variations at processing at: 1- $j=10$ mA·cm⁻²; 2- 20 mA·cm⁻²

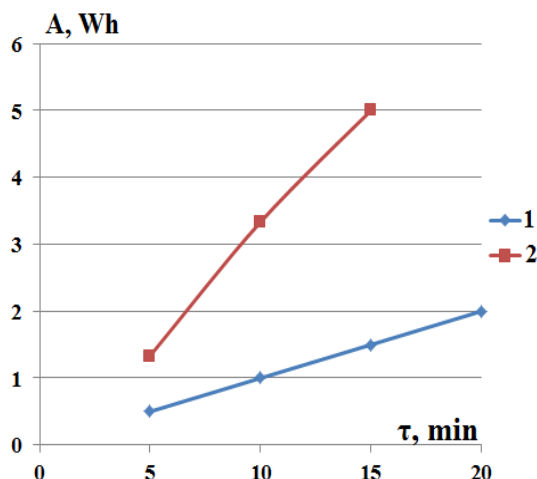


Figure 3. Variations of energy consumption at processing at: 1- $j=10$ mA·cm⁻²; 2- 20 mA·cm⁻²

Voltage variations, stationary regime, at electrophysical processing of the acid whey, at current densities of $j=10, 20$ mA/cm², can be presented as the following relations (1-2):

$$\text{For } j=10 \text{ mA/cm}^2 \quad y = -0.0007x^3 + 0.03x^2 - 0.4333x + 8; \quad R^2 = 0.99 \quad (1)$$

$$\text{For } j=20 \text{ mA/cm}^2 \quad y = -0.04x^2 + x + 4; \quad R^2 = 0.99 \quad (2)$$

where: y – voltage U , (V); x – time of processing τ (min); R^2 – degree of approximation; $1-R^2$ – error of approximation.

Variations of energy consumption A (Wh), in the stationary regime, at electrophysical processing of the acid whey, at current densities of $j=10, 20$ mA/cm² and, 20 mA/cm², can be presented as the following relations (3-4):

$$\text{For } j=10 \text{ mA/cm}^2 \quad y = 0.1x - 14; \quad R^2 = 0.99 \quad (3)$$

$$\text{For } j=20 \text{ mA/cm}^2 \quad y = 0.367x - 0.44; \quad R^2 = 0.99 \quad (4)$$

where: y – energy consumption A (Wh); x – time of processing τ (min); R^2 – degree of approximation; $1-R^2$ – error of approximation.

The growth of temperature both in the LP and the FP is not over the threshold of denaturation of whey proteins (55-65 °C). At the current density $j=20$ mA·cm⁻², the temperature growth is faster than at $j=10$ mA·cm⁻² because of the Joule effect, thus contributing to at the isolation of protein fractions in the PMCs in line with their typical characteristics at higher temperatures (Figures 4, 5).

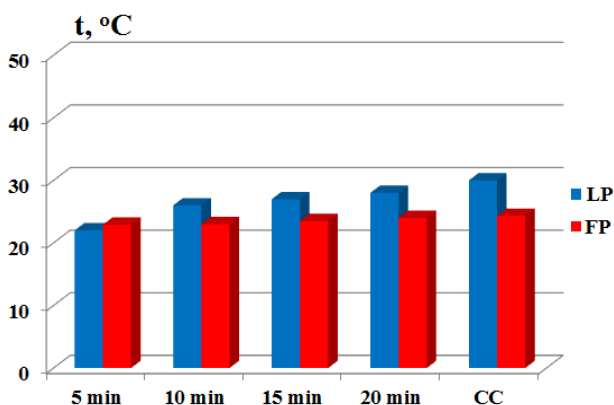


Figure 4. Temperature variations in LP and FP, at $j = 10 \text{ mA}\cdot\text{cm}^{-2}$.

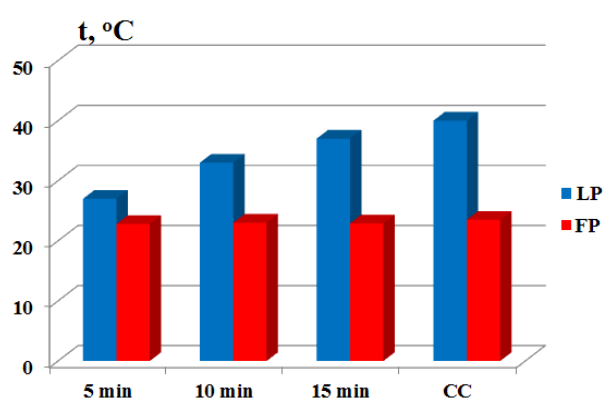


Figure 5. Temperature variations in LP and FP, at $j = 20 \text{ mA}\cdot\text{cm}^{-2}$.

Variations of temperature in LP and FP, in the stationary regime, at electrophysical processing of the acid whey, at current densities of $j=10 \text{ mA}/\text{cm}^2$ and, $20 \text{ mA}/\text{cm}^2$, can be presented as the following relations (5-8):

$$\text{For LP, } j=10 \text{ mA}/\text{cm}^2: y = 0.001x^3 - 0.0708x^2 + 1.6044x + 15.699; \quad R^2 = 0.99 \quad (5)$$

$$\text{For FP, } j=10 \text{ mA}/\text{cm}^2: y = -0.0003x^3 + 0.014x^2 - 0.139x + 23.27; \quad R^2 = 0.99 \quad (6)$$

$$\text{For LP, } j=20 \text{ mA}/\text{cm}^2: y = 0.0013x^3 - 0.08x^2 + 2.1667x + 18; \quad R^2 = 0.99 \quad (7)$$

$$\text{For FP, } j=20 \text{ mA}/\text{cm}^2: y = 0.0013x^3 - 0.046x^2 + 0.497x + 21.3; \quad R^2 = 0.99 \quad (8)$$

where: y – temperature in LP and FP t ($^{\circ}\text{C}$); x – time of processing τ (min); R^2 – degree of approximation; $1-R^2$ – error of approximation.

The variations of the pH values and the respective decrease of the ORP values at both current densities applied in our experiments demonstrate the passing of aquacomplexes into hydrocomplexes. However, this process is faster at $j=20 \text{ mA}\cdot\text{cm}^{-2}$ when the formation of hydrocomplexes starts at the beginning of the processing. It can be accounted by both electro-chemical activation and rapid formation of hydroxyl ions of the processed whey, as well as by the activation, in particular, of protein fractions which interact with minerals thus contributing to the formation of the PMCs (Figures 6, 7).

Variations of the pH values, in the stationary regime, at electrophysical processing of the acid whey, at current densities of $j=10 \text{ mA}/\text{cm}^2$ and, $20 \text{ mA}/\text{cm}^2$, can be presented as the following relations (9-10):

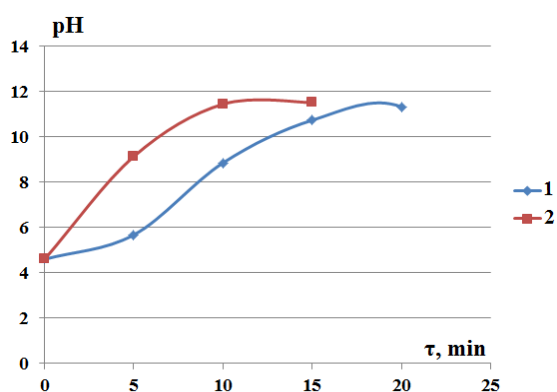


Figure 6. Variations of pH at: 1- $j=10 \text{ mA}\cdot\text{cm}^{-2}$; 2- $20 \text{ mA}\cdot\text{cm}^{-2}$.

For $j=10 \text{ mA}/\text{cm}^2$:

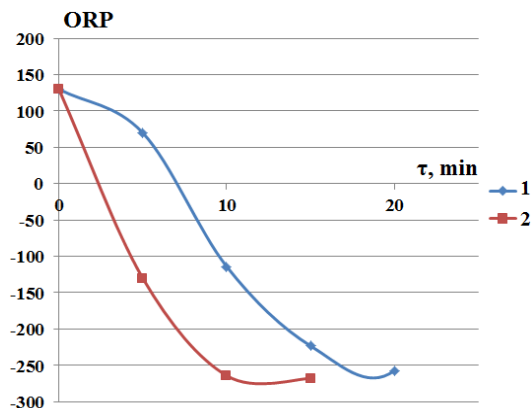


Figure 7. Variations of ORP at: 1- $j=10 \text{ mA}/\text{cm}^2$; 2- $20 \text{ mA}/\text{cm}^2$.

$$y = 0.0002x^4 - 0.0114x^3 + 0.1725x^2 - 0.3912x + 4.6; \quad R^2 = 0.99 \quad (9)$$

$$\text{For } j=20 \text{ mA/cm}^2: y = -0.044x^2 + 1.13x + 4.6; \quad R^2 = 0.99 \quad (10)$$

where: y – the pH values; x – time of processing τ (min); R^2 – degree of approximation; $1-R^2$ – error of approximation.

Variations of the oxidation-reduction potential (ORP (mV), at electrophysical processing of the acid whey, at current densities of $j=10 \text{ mA/cm}^2$ and, 20 mA/cm^2 , can be presented as the following relations (11-12):

$$\text{For } j=10 \text{ mA/cm}^2: y = 0.001x^5 - 0.0619x^4 + 1.515x^3 - 16.192x^2 + 38.217x + 130; \quad R^2 = 0.99 \quad (11)$$

For $j=20 \text{ mA/cm}^2$:

$$y = -0.0021x^4 + 0.0693x^3 + 1.8533x^2 - 62.733x + 130; \quad R^2 = 0.99 \quad (12)$$

where: y – the oxidation-reduction potential (ORP, mV); x – time of processing τ (min); R^2 – degree of approximation; $1-R^2$ – error of approximation.

The degree of the isolation of whey proteins in the PMCs (Q , %) from the initial acid whey makes up ca. 80-84%. A greater isolation of whey proteins in the PMCs, first of all, is due to the solids content (mostly proteins) of the initial whey and is a direct consequence of the primary milk processing (Figure 8).

Intensive foaming from the first minutes of processing is an indicator of the formation of quite stable PMCs. The degree of the isolation of protein fractions in the PMCs at $j=10 \text{ mA}\cdot\text{cm}^{-2}$ is high enough and makes up about 80% at the first minutes of processing and remains as such during the whole processing period. At $j=20 \text{ mA}\cdot\text{cm}^{-2}$, the degree of the whey proteins isolation in the PMCs from the FP depends on the time and is the following: after 5 min – 75.91%, after 10 min – 74.58%, and after 15 min – 73.40%. As to the LP (CC), there is registered about 84%, which means that, in the first minutes, there are intensively formed protein compounds with a high molecular weight which cannot be isolated as foam as a result of the ion flotation, remaining in the LP contained in the CC. This is also confirmed by the variations of the pH values, which means a rapid growth that stipulates the formation of high molecular weight protein compounds at $j = 20 \text{ mA}\cdot\text{cm}^{-2}$.

Variations of the degree of the recovery

of whey proteins in the PMCs (Q , %) from the initial acid whey, in the stationary regime, at electrophysical processing of the acid whey, at current densities of $j=10 \text{ mA/cm}^2$ and, 20 mA/cm^2 , can be presented as the following relations (13-14):

$$\text{For } j=10 \text{ mA/cm}^2: y = 0.0353x^3 - 0.9005x^2 + 9.6851x + 42.17; \quad R^2 = 0.99 \quad (13)$$

$$\text{For } j=20 \text{ mA/cm}^2: y = 0.0018x^3 - 0.05x^2 + 0.1757x + 76.055; \quad R^2 = 0.99 \quad (14)$$

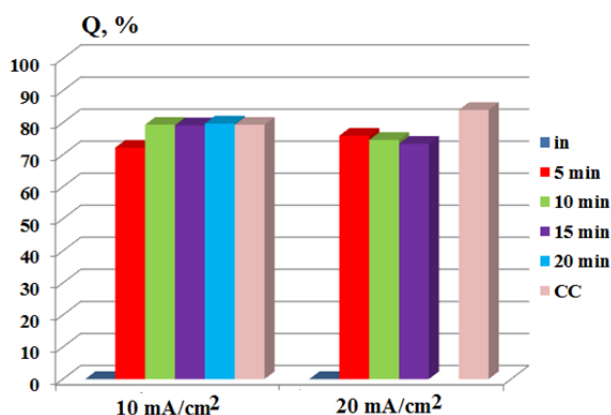


Figure 8. Variations of degree of proteins isolation in PMCs (Q , %), at different times, at: 1- $j= 10 \text{ mA}\cdot\text{cm}^{-2}$; 2- $20 \text{ mA}\cdot\text{cm}^{-2}$.

where: y – the of degree of the recovery of whey proteins in the PMCs (Q , %); x – time of processing τ (min); R^2 – degree of approximation; $1-R^2$ – error of approximation.

The variations of the specific energy consumption per a unit of volume A_v ($W \cdot h \cdot ml^{-1}$) show that it is by 2.5 times lower at $j=10 \text{ mA} \cdot \text{cm}^{-2}$ than at $j=20 \text{ mA} \cdot \text{cm}^{-2}$ (Figure 9).

The isolation of whey proteins is greater at processing at $j=10 \text{ mA} \cdot \text{cm}^{-2}$, in a periodic regime, besides, the energy consumption is lower. This is why the authors would recommend whey processing under these conditions.

These investigations demonstrate an opportunity to properly control the processing of the acid whey, which depends on the processing regime (the electric current density) necessary for an optimal isolation of protein fractions in the PMCs, with a simultaneous decrease of the specific energy consumption per a unit of volume.

Conclusions

In this study, a wasteless processing method was investigated; the object of study was the acid whey processed electrophysically in a periodic regime. During processing, the following parameters were registered: electric ones – current density and voltage; thermal ones – temperature in the LP and in the FP; physical-chemical – pH and the ORP; biochemical – the degree of the isolation of whey proteins in the PMCs. Electro-activation of the acid whey made it possible to get the degree of isolation at about 80% from the first minutes of processing and keep it during the whole period at $j=10 \text{ mA} \cdot \text{cm}^{-2}$, with the specific energy consumption by 2.5 times less than during processing at $j=20 \text{ mA} \cdot \text{cm}^{-2}$, where the degree of isolation was lower and in the following order: after 5 min – 75.91%; after 10 min – 74.58%; and after 15 min – 73.40%). However, in the LP, the recovery was of about 84%, which means that from the first minutes, there are intensively formed protein compounds with a high molecular weight which cannot be isolated as foam as a result of the ion flotation, remaining in the LP contained in the CC.

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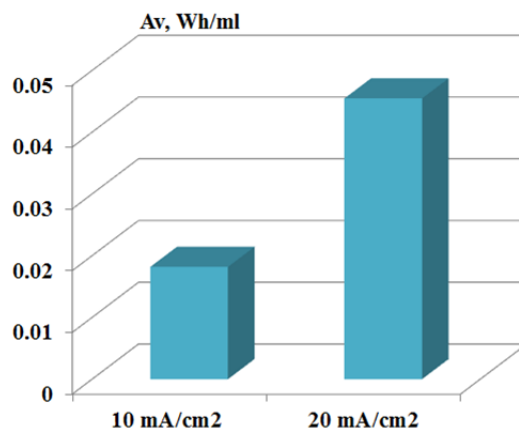


Figure 9. Variations of total specific energy consumption per unit of volume A_v ($W \cdot h \cdot ml^{-1}$) at $j=10$ and $20 \text{ mA} \cdot \text{cm}^{-2}$.

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