

## THE AMINOACID CONTENT IN PROTEIN MINERAL CONCENTRATES OBTAINED AT DIFFERENT ELECTROPHYSICAL PROCESSING REGIMES OF WHEY

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### Abstract

*It was investigated the amino acid content in protein mineral concentrates obtained during electrophysical processing of different types of whey at membrane electrolyzer EDP-4 at current density  $j=10$  mA/cm<sup>2</sup> and  $J=20$  mA/cm<sup>2</sup>. It was established that the degree of amino acids isolation in the protein mineral concentrates during electrophysical processing of whey depends on the: current density, duration (time) of processing, and on the type of whey. Varying these parameters, the content of essential and functional amino acids in the protein mineral concentrates during electrophysical processing can be modeled. The maximum degree of isolation of free amino acids, especially of essential amino acids, is recorded during electro-physical processing of whey after the manufacture of the granulated cottage cheese „Grauncior” (company JLC) at current density  $j=20$  mA/cm<sup>2</sup> in the 10th min of processing. The most important functional amino acids such as immunoactive, sulfur containing and branched-chain amino acids have the same character of content variations in the protein mineral concentrates as essential amino acids during electrophysical processing of whey.*

**Key words:** whey, amino acids, electrophysical processing.

### INTRODUCTION

The aminoacid composition is the most important factor in defining food protein quality, followed by the digestibility of the protein and the bioavailability of its amino acids. Due to their aminoacid composition the main bovine milk proteins – caseins and whey proteins, can be regarded as a complete source of aminoacids (Sindayikengera and Xia, 2006; Arghiriade et al., 2013). It is known that whey proteins make up about 20% of the milk proteins. The remaining 80% is casein. But milk whey proteins have been considered superior to casein from the nutritional point of view (Farrell et al., 2011). These proteins present the amino acid profile superior to casein, being similar to human milk (Hambræus, 1982; Pennings et al., 2011). Whey proteins are also leaders in the content of essential amino acids (EAAs) among other important sources of EAAs and are also rich in the branched chain amino acids (BCAAs) that are physiologically extremely important and confer whey proteins an important biological value and thought to play a role as metabolic

regulations in protein and glucose homeostasis, and in lipid metabolism (Morato et al., 2006; Smithers, 2008).

The main whey protein fractions also differ by aminoacids profile. In particular,  $\beta$ -lactoglobulin ( $\beta$ -Lg), which represents one of the main whey protein fractions, is rich in cysteine residues, an aminoacid bearing a key role in stimulating the synthesis of glutathione (Tavares and Malcata, 2013). The main amino acids in quantitative terms of  $\beta$ -Lg are Ala, Asp, Glu, Ile, Leu, Lys, Val. Another whey protein fraction –  $\alpha$ -lactalbumin ( $\alpha$ -La), is commercially used in food supplements for babies because of its similarity in structure and composition to human milk proteins. The higher content of all EAAs and BCAAs in  $\alpha$ -La makes it also an ingredient of choice in supplements for sportsmen (Tavares and Malcata, 2013). The most significant amino acids contained in  $\alpha$ -La are Trp and Cys, Leu, Ile and Val (Etzcel, 2004). Together with  $\alpha$ -La,  $\beta$ -Lg is a major source of EAAs and BCAAs (Etzcel, 2004). In the other whey protein fraction – glycomacropeptide (GMP) a higher amount of Glu, Ile, Pro, Ser, Thr, and Val in

comparison with  $\alpha$ -La and  $\beta$ -Lg was revealed (Etzel, 2004).

The protein and respectively amino acid content in whey protein concentrates or isolates depend on the methods of their obtaining (manufacturing).

The whey proteins are isolated by application by different physical, chemical and microbiological methods (principles). The most known of whey protein products are: whey protein concentrates, whey protein isolates and whey protein hydrolysates.

Amino acid profile of whey protein concentrate (WPC) 80 and its hydrolysates consist in a high content of sulfur-containing amino acids – Met and Cys; a high concentration of Leu, Ile, Lys, Thr; and a relatively low content of the aromatic amino acids Phe and Tyr. Generally, high digestibility of milk protein is due to Tyr, Phe, Ile, Leu, and Lys content. By comparing essential amino acid acids pattern of human milk with that of WPC, sodium caseinate and their hydrolysates, it is apparent that WPC 80 and its hydrolysates are more similar to human milk than sodium caseinate and its hydrolysates (Sindayikengera and Xia, 2006).

Whey processing operations could potentially affect the content of amino acids. Amino acid profiles showed that excessive heating of whey (121°C, 5000s) destroys a significant part of Cys at, Lys, and Arg. Excessive heating also decreases the availabilities of Lys, Pro, Asp, Glu, Thr, Ala, Gly and Ser. Severe heating decreased the availabilities of Cys, Tyr and Arg, probably as a result of structural modifications of the protein upon heating (Desrosiers and Savoie, 1991).

Thus the manufacture of protein concentrates requires certain rules in order to maintain a high degree of purity in the protein native form, namely, to exclude thermal denaturation, which, in the case of whey proteins is 55-65°C (Etzel, 2004), and chemical denaturation of proteins and chemical modification of amino acids (Desrosiers and Savoie, 1991; Cheftel, 1977).

It was established that electrophysical processing of whey 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. Extraction of whey proteins and obtaining of 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 exclude the thermal and chemical denaturation of proteins (Bologa et al., 2009, 2010; Vrabie et al., 2018).

The aim of this study was the investigation of the content of amino acids in protein-mineral concentrates during the electrophysical whey processing at different processing regimes.

## MATERIALS AND METHODS

In the framework of the experiments of electrophysical processing two types of whey provided by the “JLC” Joint Stock Company, Chisinau, Republic of Moldova, after the manufacture of the: granulated cottage cheese “Grauncior”; “Cottage cheese”, 2% fat content were used. The electrophysical processing of whey was performed at the experimental membrane electrolyzer, at  $j=10-20$  mA/cm<sup>2</sup>, in the stationary regime, specially designed for collecting the samples so as to study amino acids (Maximuc et al., 2008). All PMCs were collected every 5 minutes and in the cathode cell (CC) (Bologa et al., 2009).

The determination of the content of amino acids in the studied samples was done by the ion-exchange chromatography (Moore et al., 1958) at aminoacid analyzer AAA-339M.

## RESULTS AND DISCUSSIONS

In our experiments, 18 proteinogenic aminoacids out of 20 were detected: Asp includes both Asp and Asn and Glu includes both Glu and Gln (in the process of detection Asn is combined with Asp and Gln with Glu and so they have the identical picks that reflect the quantity of extraction).

To determine what is the best (optimal) regime to remove a higher amount of aminoacids, especially essential ones, from two types of whey - after the manufacture of the granulated cottage cheese “Grauncior” and of the „Cottage cheese”, 2% fat content – the dynamic of variation of the aminoacid content at different current density:  $j=10$  mA/cm<sup>2</sup> and  $j=20$  mA/cm<sup>2</sup> was compared (Figure 1).

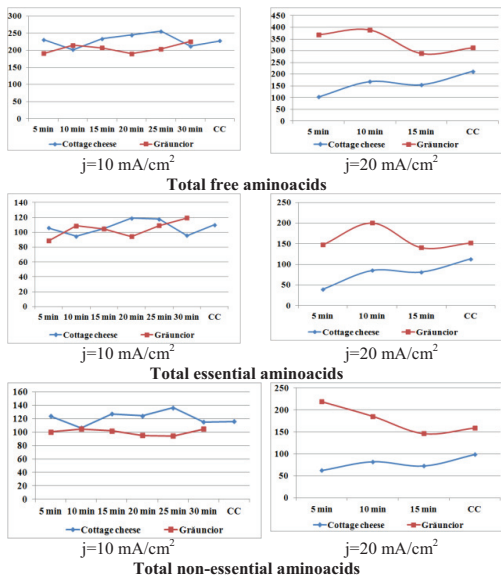


Figure 1. Variations of total free amino acids of whey after manufacture of granulated cottage cheese “Grauncior” and “Cottage cheese”, 2% fat content during electrophysical processing, in stationary regime, at current density  $j=10 \text{ mA/cm}^2$  and  $j=20 \text{ mA/cm}^2$

A higher degree of total free amino acids isolated in the PMCs was established during electrophysical processing of whey:

- after the manufacture of the “Cottage cheese”, 2% fat content, at current density  $j=10 \text{ mA/cm}^2$ ; and
- after the manufacture of the granulated cottage cheese “Grauncior”, at current density  $j=20 \text{ mA/cm}^2$ .

The maximum content of essential and non-essential amino acids in PMCs was also recorded during electrophysical processing of whey:

- after the manufacture of the “Cottage cheese”, 2% fat content, at current density  $j=10 \text{ mA/cm}^2$ ; and
- after the manufacture of the granulated cottage cheese “Grauncior”, at current density  $j=20 \text{ mA/cm}^2$ .

Thus, the greatest amount of free, essential and non-essential amino acids is established at the whey after the manufacture of the granulated cottage cheese “Grauncior”, at current density  $j=20 \text{ mA/cm}^2$  in the 5<sup>th</sup> and 10<sup>th</sup> minutes of electrophysical processing.

The variation of the content of essential amino acids in the PMCs during electrophysical processing with membrane electrolyzer of the

granulated cottage cheese “Grauncior” in the stationary regime of treatment, at a current density of  $10 \text{ mA/cm}^2$  is shown in Figure 2.

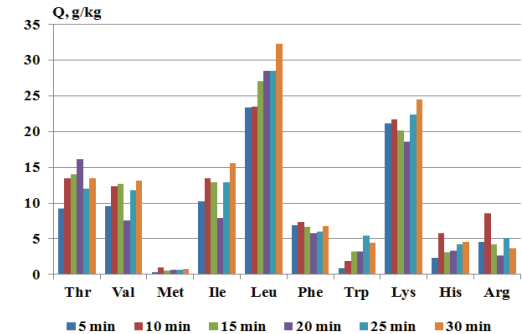


Figure 2. Variations of content of essential amino acids in PMCs during electrophysical processing of whey after manufacture of granulated cottage cheese „Grauncior” in stationary regime, at current density  $j=10 \text{ mA/cm}^2$

From the presented data in Figure 2 it is seen that during electrophysical processing of whey after the manufacture of the granulated cottage cheese “Grauncior” at a current density of  $j=10 \text{ mA/cm}^2$ , all essential amino acids are extracted in the PMCs, but the degree of isolation of each amino acid in the PMCs varies depending upon the time of electrophysical whey processing: four essential amino acids (Met, Phe, His and Arg) have the maximum degree of isolation at 10 minutes of electrophysical processing, two (Thr and Leu) – at 20 min of processing, two (Trp and Leu) – at 25 min (amount of isolated Leu is identical at 20 and 25 min of processing) and three amino acids – Val, Ile and Lys – have the maximum degree of isolation at 30 min of electrophysical processing.

The quantitative spectrum of essential amino acids of whey after the manufacture of the “Cottage cheese”, 2% fat content, during electrophysical processing with membrane electrolyzer, at a current density of  $j=10 \text{ mA/cm}^2$  is shown in Figure 3.

It is established that two out of ten essential amino acids have the highest degree of isolation at 5 min (His and Arg), five – at 20 min of processing (Thr, Val, Met, Ile, Leu), two – at 25 min (Phe and Trp) and one – at 30 min (Trp). Met has the same maximum quantity at 20 and 25 min of processing and Trp – at 25 and 30 min. The maximum content of Lys is detected in the cathode cell, although a higher

degree of this amino acid is recorded at 20 and 25 min of electrophysical processing.

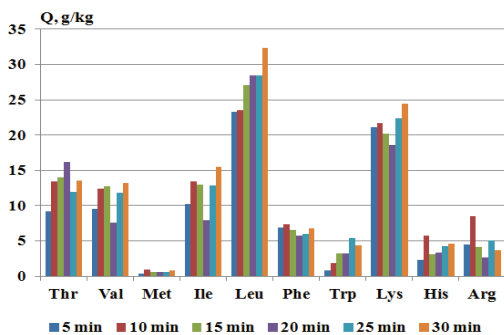


Figure 3. Variations of content of essential amino acids in PMCs during electrophysical processing of whey after manufacture of “Cottage cheese”, 2% fat content, in stationary regime, current density  $j=10 \text{ mA/cm}^2$

Data analyses of variation of essential amino acids content in the whey after the manufacture of the granulated cottage cheese “Grauncior” at a current density of  $20 \text{ mA/cm}^2$  is shown in Figure 4.

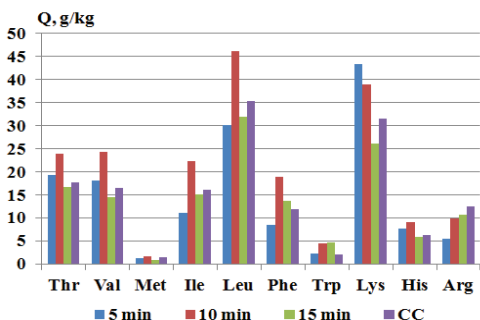


Figure 4. Variations of content of essential amino acids in PMCs during electrophysical processing of whey after manufacture of granulated cottage cheese “Grauncior”, in stationary regime, at current density  $j=20 \text{ mA/cm}^2$

Results revealed that in this type of whey, at current density  $j=20 \text{ mA/cm}^2$ , the highest degree of isolation at 10 min of processing have, such essential amino acids as Thr, Val, Met, Ile, Leu, Phe, Trp; at 5 min – Lys; at 15 min – Trp and Arg (Trp was extracted in the same extent at 10 and 15 min of processing, but a higher amount of Arg is found in the cathode cell).

At electrophysical processing of whey after manufacturing of the „Cottage cheese”, 2% fat content, at current density  $j=20 \text{ mA/cm}^2$ , the

majority of essential amino acids (9 out of 10 – Val, Met, Ile, Leu, Lys, His and Arg) have a higher content in the cathode cell, with the exception of Trp that has the highest degree of isolation at 15 min of processing.

If we compare the process only during 5 min, 10 min and 15 min, then there appears the second peak of quantity that reflects the degree of isolation of Val, Met, Ile, Leu, Lys, His and Arg observed at 10 min of processing (Figure 5).

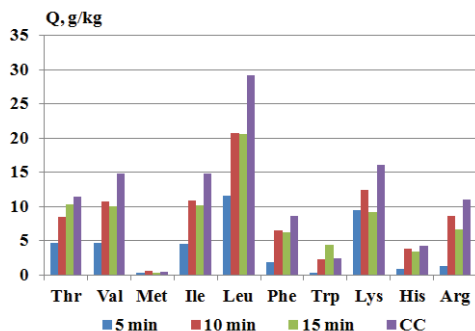


Figure 5. Variations of content of essential amino acids in PMCs during electrophysical processing of whey after manufacture of “Cottage cheese”, 2% fat content, in stationary regime, at current density  $j=20 \text{ mA/cm}^2$

From the obtained data it is clear that the current intensity differently influences the degree of extraction of essential amino acids in the PMCs.

In addition to the essential amino acids, non-essential amino acids were also analyzed, which also have a significant role in nutrition and health. Currently the notion of functional amino acids is utilized. Within this category of amino acids there are both essential and the non-essential amino acids such as Glu, Pro, Cys, Tyr, etc.

The degree of isolation of non-essential amino acids of whey after the manufacture of the granulated cottage cheese “Grauncior” at current density  $j=10 \text{ mA/cm}$  is heterogeneous during electrophysical processing: the higher content of Pro was noted at 5 min of processing; Ser, Gly and Glu – at 10 min; Tyr and Cys – at 15 min; Ala and Asp after 30 min of electrophysical processing (Figure 6).

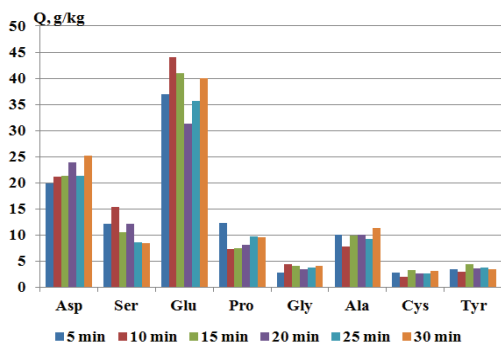


Figure 6. Variations of content of non-essential aminoacids in PMCs during electrophysical processing of whey after manufacture of granulated cottage cheese “Grauncior”, in stationary regime, at current density  $j=10 \text{ mA/cm}^2$

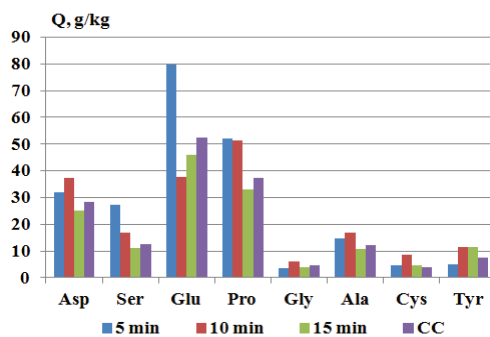


Figure 8. Variations of content of non-essential aminoacids in PMCs during electrophysical processing of whey after manufacture of granulated cottage cheese “Grauncior”, in stationary regime, at current density  $j=20 \text{ mA/cm}^2$

The character of non-essential aminoacid isolation during electrophysical processing of whey after the manufacture of the “Cottage cheese”, 2% fat content, at current density  $j=10 \text{ mA/cm}^2$ , is also heterogeneous as the whey after the manufacture of the granulated cottage cheese “Grauncior” (Figure 7).

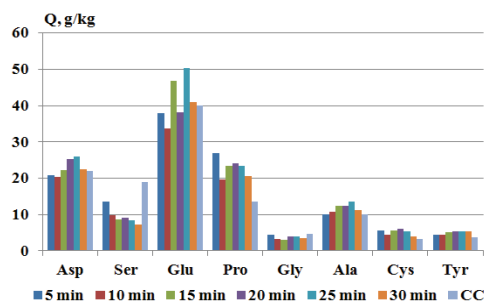


Figure 7. Variations of content of non-essential aminoacids in PMCs during electrophysical processing of whey after manufacture of “Cottage cheese”, 2% fat content, in stationary regime, at current density  $j=10 \text{ mA/cm}^2$

After 5 min of electrophysical processing a higher degree of isolation of Ser, Pro and Gly; after 20 min – of Asp, Cys and Tyr; after 25 min – of Glu and Ala was noted (Asp has approximately the same degree of isolation at 20 and 25 min of processing). Also such aminoacids as Ser and Gly have the highest content in the cathode cell (Figure 7).

The variation of non-essential aminoacid content of whey after the manufacture of the granulated cottage cheese “Grauncior” at current density  $j=20 \text{ mA/cm}^2$  is shown in figure below.

A higher degree of non-essential aminoacids isolation is revealed at 5 and 10 min of processing: at 5 min a larger content of Ser, Glu and Pro; at 10 min – of Asp, Gly, Ala, Cys and Tyr was noted.

During electrophysical processing of whey after the manufacture of the “Cottage cheese”, 2% fat content, at current density  $j=20 \text{ mA/cm}^2$ , the main non-essential amino acids (Asp, Glu, Pro, Gly, Ala, Cys, Tyr) have the highest content in the cathode cell, with the exception of Ser, which has the maximum degree of isolation at 5 min of processing (Figure 9).

If we compare only the time of processing, then we can establish that a higher content of Asp, Glu, Pro, Gly, Ala, Cys, Tyr is at 10 min of electrophysical processing.

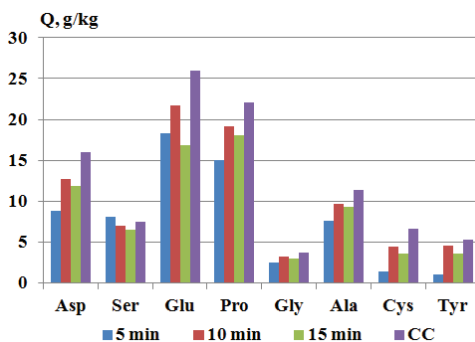


Figure 9. Variations of content of non-essential aminoacids in PMCs during electrophysical processing of whey after manufacture of “Cottage cheese”, 2% fat content, in stationary regime, at current density  $j=20 \text{ mA/cm}^2$

From the presented data it can be seen that Glu has the highest content compared with other proteinogenic amino acids. Glu is a non-essential amino acid but it was established to play a significant role in the main physiological processes of living organism and its nutritional contribution to health (Newsholme et al., 2003; Hertz, 2013). Thus Glu it is considered as a functional one. Thus, the fact that during electrophysical processing of both whey after the manufacture of the granulated cottage cheese “Grauncior” and whey after manufacture of the “Cottage cheese”, 2% fat content, with membrane electrolyzer, at current density  $j=10 \text{ mA/cm}^2$  and  $j=20 \text{ mA/cm}^2$ , a higher degree of Glu is established, denoting the biofunctional value of PMCs. Data referring to Glu include both data about Glu and Gln, because in the process of detection Glu is combined with Gln and so they have the identical picks that reflect the quantity of extraction. The Gln and Glu are closely related in a chemical sense: Gln can produce Glu through the glutamate ammonium ligase. Gln becomes conditionally essential (requiring intake from food or supplements) in states of illness or injury and it is also important for functioning of body in stress conditions (Lacey and Wilmore, 1990).

Thus, the degree of amino acids isolation in the PMCs during electrophysical processing of whey depends on the: current density, duration (time) of processing, and on the type of whey. Varying these parameters, the content of essential and functional amino acids in the PMCs during electrophysical processing can be modeled.

The maximum degree of isolation of free amino acids, especially of essential amino acids, is recorded during electrophysical processing of whey after the manufacture of the granulated cottage cheese „Grauncior” at current density  $j=20 \text{ mA/cm}^2$  in the 10<sup>th</sup> min of processing.

This allows us to consider that electrophysical processing with membrane electrolyzer at current density  $j=20 \text{ mA/cm}^2$  of whey after the manufacture of the granulated cottage cheese „Grauncior” is the optimal regime (condition) for the maximum content extraction of essential and functional amino acids in the PMCs.

## CONCLUSIONS

The level of migration of each essential amino acid and non-essential amino acid in the PMCs is varying in dependence on time of electrophysical processing and current density that can be promising investigations in the direction of the PMCs obtaining with desired amino acids content and spectrum by applying various parameters (regimes) of whey electrophysical processing.

The maximum degree of isolation of free amino acids, especially of essential amino acids is recorded during electrophysical processing of whey after the manufacture of the granulated cottage cheese „Grauncior” at current density  $j=20 \text{ mA/cm}^2$  t, at 10 min of processing.

## ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Applied Physics and Institute of Physiology and Sanocreatology and also was financed from STCU Project 6011 “*The electrophysical processing of whey for obtaining of healthy and environmental products: technology and installation*”.

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