Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 1, 2020
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE PECULARITIES OF AMINO ACID MIGRATION IN PROTEIN MINERAL CONCENTRATES UNDER THE INFLUENCE OF DIFFERENT pH AND TEMPERATURE VALUES DURING ELECTRO-ACTIVATION OF WHEY

Elvira VRABIE[1], Irina PALADII[1], Catalina SPRINCEAN[1], Valeria VRABIE[2]

[1]Institute of Applied Physics, 5 Academiei Street, Chisinau, Republic of Moldova
[2]Institute of Physiology and Sanocreatology, 1 Academiei Street, Chisinau, Republic of Moldova

Corresponding author email: valvrabie@yahoo.com

Abstract

It was investigated the influence of pH and temperature under the degree of isolation of free amino acids in the protein mineral concentrates (PMCs) obtained as a result of processing of whey after the manufacture of the granulated cottage cheese "Grauncior" (company JLC) at membrane electrolyzer EDP-4 at current density j = 10 mA/cm^2 and j = 20 mA/cm^2. The highest degree of extraction of free, especially essential amino acids is recorded in the PMCs during electro-activation at current density j = 20 mA/cm^2, at 10 min of processing, when pH = 11.6 and t = 29.5ºC. The level of migration of each essential amino acid in the PMCs is varying in dependence on the time of electrophysical processing, on the current density, pH and temperature values.

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

INTRODUCTION

Milk and dairy products, including whey (whey products) are attributed to foods needed for human nutrition, as sources of protein and amino acids respectively.

The dairy industry produces ~180 to 190 million tons per year of whey, accounting for more than a half of the total solids present in the original whole milk, including whey proteins (20% of the total protein) and most of the lactose, minerals, and water-soluble vitamins (de Wit, 2001; Chandrapala et al., 2015).

Whey is known to be a rich source of essential amino acids. However, the quality of the nutrients can be affected in the process of processing the whey and obtaining the final products – protein mineral concentrates (PMCs).

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 55-65ºC (Etzel, 2004), chemical denaturation of proteins and chemical modification of amino acids (Desrosiers and Savoie, 1991; Cheftel, 1977).

One successful technological method is the electrical processing of whey using recovery of the protein mineral concentrate (PMC) and the simultaneous isomerization of lactose into lactulose using the electrochemical activation (ECA) of the liquid. This approach was developed in Chisinau, at the Institute of Applied Physics in the second half of the 1990s (Bologa et al., 1992).

Performance of this method depends on such parameters, as pH and temperature, because these factors determine the solubility and electrostatic protein interactions in the feed (Gonzalez et al., 2008; Luo and Ding, 2011; Rice et al., 2011a; 2011b), as well as current density.

Thus, the present study aimed to establish the peculiarities of amino acid migration in protein mineral concentrates under the influence of different pH and temperature values during electro-activation of whey.

MATERIALS AND METHODS

In the framework of the experiments the electrophysical processing was applied on the whey provided by the "JLC" Joint Stock Company, Chisinau, Republic of Moldova, after the manufacture of the: granulated cottage cheese "Grauncior". The electrophysical processing of whey was performed at the

membrane electrolyzer EDP-4, at j = 10-20 mA/cm^2, 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 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 amino acid analyzer AAA-339M.

The analysis is performed in the standard procedure for the determination of free amino acids using lithium buffer solutions, pH 2.90, 2.95, 3.20, 3.80 and 5.00, with a flow rate of 12.0 ml/hr. On the basis of the qualitative calculation of amino acid content in the liquid studied it is stated that the amount of an amino acid in the sample is proportional to the surface of the pick of the chromatogram. The calculation consists in the fact, that sample and standard mixture of amino acids with the same content is analyzed. The amount of amino acids dosed on the ionic column in the test sample is given by the formula below:

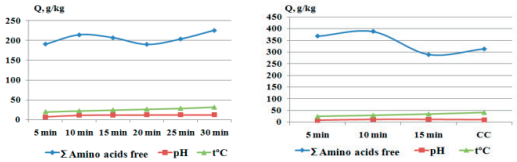
$C_{i(doz.)} = k \cdot n \cdot S_{i(prob.)}/S_{i(st.)} \cdot M_i \cdot 10^{-6}$ (mg),

Where: $C_{i(doz.)}$ - the ionic concentration of amino acids in the volume of the dosed node; n - the amount of the amino acids in the analyzed mixture; $S_{i(prob.)}$ - the tip(pick) surface of the amino acids in analyzed mixture; $S_{i(st.)}$ - the tip (pick) surface of the amino acids in standard mixture; k - correction coefficient considered to be changing the detector sensitivity; M_i - the ionic molecular weight of the amino acid. The automatical analyzer AAA-339M detects ninhydrin positive components within 1-100 nanomoles concentration. The duration of the analysis of the physiological fluids is 3.5 hours.

RESULTS AND DISCUSSIONS

In the frame of investigation, it was studied the influence of pH and temperature on the migration of amino acids in protein minerals concentrates (PMCs) during electro activation of whey at different processing regimes.

The variations of total free amino acid content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior" in the stationary regime of treatment, at current density j = 10 mA/cm^2 and j = 20 mA/cm^2, depending on the variations of pH and temperature, are presented in the Figure 1 (A and B).



A **B**

Figure 1. Variations of total free amino acid content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of free amino acids is kept at the same level with increasing pH and temperature during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" at current density j = 10 mA/cm^2 (Figure 1 A).

Electrophysical processing of whey at current density j = 20 mA/cm^2, with increasing pH and temperature, more drastically affect the isolation of free amino acids in the PMCs (Figure 1 B).

Thus, during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" the greatest amount of free amino acids is extracted at first 5-10 min of processing, and with increasing temperature and pH, the decreasing of their contents is attested.

The variations of the essential amino acid content in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", in the stationary regime, at current density j = 10 mA/cm^2 and j = 20 mA/cm^2, depending on the variations of pH and temperature, are presented in Figure 2.

Electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" at current density j = 10 mA/cm^2 revealed that the highest amount of essential amino acids is obtained in the PMCs at 10 min of processing, then the content is kept stable at 15 min and decreases at 20 min of processing. Then, at 25 and 30 min of electrophysical processing, the content of essential amino acids

reach its maximum values in the PMCs when pH and temperature also have higher values (Figure 2 A).

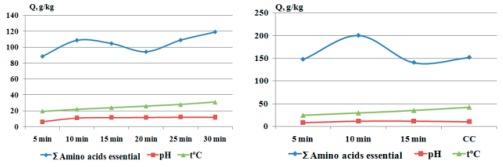


Figure 2. Variations of essential amino acid content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

Electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, revealed approximately the same variations of the essential amino acid content as at current density j = 10 mA/cm^2: the maximum content is attested at 10 min of electrophysical processsing, then the content decreases (Figure 2 B).

Next is represented the variation of each essential amino acid content in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior" in the stationary regime, at current density j = 10 mA/cm^2 and j = 20 mA/cm^2, depending on the variations of pH and temperature.

The essential amino acids for humans are: threonine (Thr), methionine (Met), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tryptophan (Trp), lysine (Lys), histidine (His), arginine (Arg). Each essential amino acid plays an important biochemical and functional role in maintaining vital processes in living organism (Reeds, 2000).

The degree of Thr isolation in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-20 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, then decreases at 25 min and slightly increases at 30 min, with a rise of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2 the maximum degree of isolation of Thr was established at 10 min when pH and temperature were growing (Figure 3).

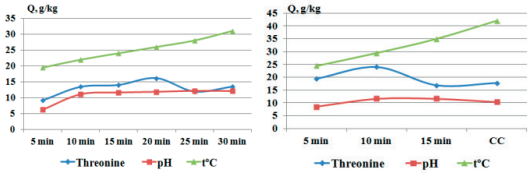


Figure 3. Variations of threonine (Thr) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)** ,depending on variations of pH and temperature

The content of Val extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-15 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, then decreases at 20 min and increases again at 25 and 30 min of electrophysical processing, with the rise of temperature and pH (Figure 4).

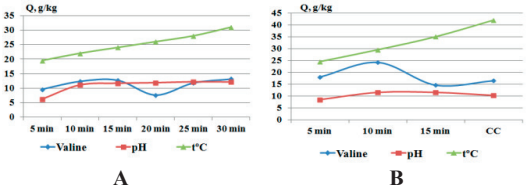


Figure 4. Variations of valine (Val) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese „Grauncior" in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

During electrophysical processing at current density j = 20 mA/cm^2 the maximum degree of isolation of Val was established at 10 min, when pH and temperature were higher (Figure 4).

The degree of Met isolation in the PMCs is one of the smallest among essential amino acids extracted in the PMCs during electrophysical processing of the studied whey. But during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" with electrolyzer EDP-4 at current density j = 10 mA/cm^2, an increase of Met in PMCs is noted at 10 min of processing, a slight decrease at 15 min and then its content is kept

stable during electrophysical processing. During electrophysical processing at current density j = 20 mA/cm^2, the same variation of Met content in the PMCs is established, with increased values of pH ant temperature (Figure 5).

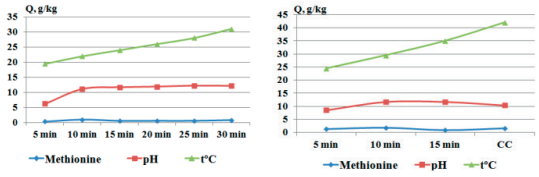


Figure 5. Variations of methionine (Met) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of Ile extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-15 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, then decreases at 20 min and increases again at 25 and 30 min of electrophysical processing, with the rise of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of Ile was established at 10 min, when pH and temperature were growing (Figure 6).

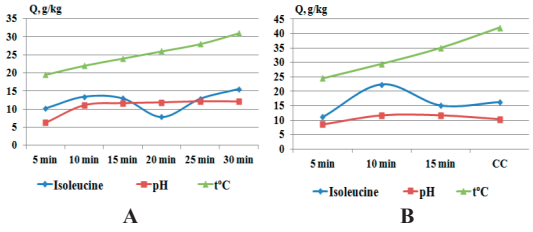


Figure 6. Variations of isoleucine (Ile) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of Leu extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during the entire period of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, and reaches its maximum content at 30 min of processing, when pH and temperatures have the maximum values. During electrophysical processing at current density j = 20 mA/cm^2 the maximum degree of isolation of Leu was established at 10 min, when pH and temperature are growing (Figure 7).

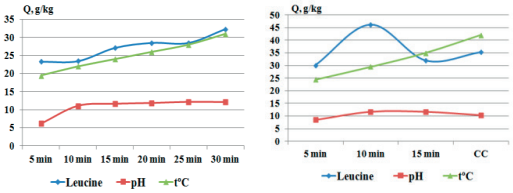


Figure 7. Variations of leucine (Leu) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The content of the essential amino acid Phe, extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" during electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, is low in comparison with other essential amino acids. A relatively high amount of this amino acid is noted at 5 and 10 min of processing, then its level decreases, although the values of pH and temperatures are growing during the entire electrophysical processing. The maximum degree of isolation of Phe in the PMCs is recorded at 10 min of electrophysical processing, at current density j = 20 mA/cm^2 (Figure 8).

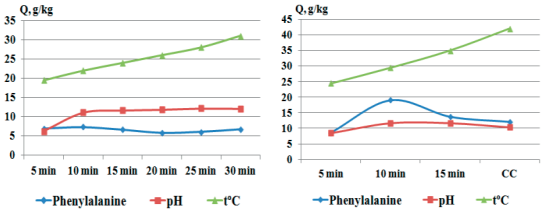


Figure 8. Variations of phenylalanine (Phe) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature during electrophysical processing

The degree of Trp isolation in the PMCs increases during electrophysical processing of whey after the manufacture of the granulated cottage cheese "Grauncior" with electrolyzer EDP-4 at current density j = 10 mA/cm^2. During electrophysical processing at current density j = 20 mA/cm^2 a high degree of Trp extraction in the PMCs is established at 10 and 15 min of electrophysical processing, with increased values of pH and temperature (Figure 9).

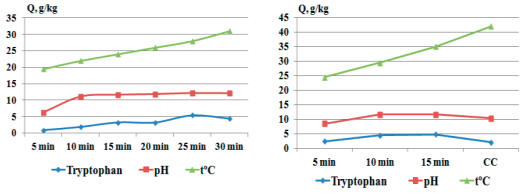


Figure 9. Variations of tryptophan (Trp) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature during electrophysical processing

The content of Lys extracted in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-10 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, decreases at 15-20 min and increases again at 25 and 30 min of electrophysical processing, with the rise of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of Ile was established at 5 min when pH and temperature are growing (Figure 10).

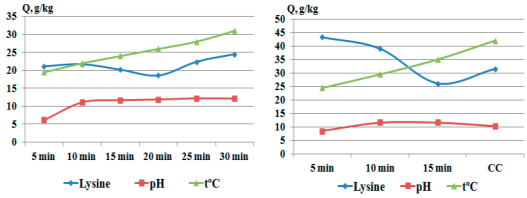


Figure 10. Variations of lysine (Lys) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 (A) and j = 20 mA/cm^2 (B),depending on variations of pH and temperature

Lys is the limiting amino acid that determines the biological value of many proteins from foodstuff.

The His content in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-10 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, decreases at 15 min and keeps stable at 20-30 min of electrophysical processing, with growth of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of His is established at 10 min, when pH and temperature are growing (Figure 11).

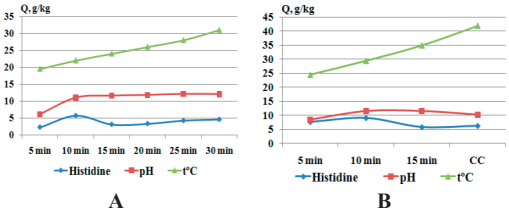


Figure 11. Variations of histidine (His) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 **(A)** and j = 20 mA/cm^2 **(B)**, depending on variations of pH and temperature

The Arg content in the PMCs of whey after the manufacture of the granulated cottage cheese "Grauncior" increases during 5-10 min of electrophysical processing with electrolyzer EDP-4 at current density j = 10 mA/cm^2, decreases at 15-20 min and then slightly increases at 30 min of electrophysical processing, with the growth of temperature and pH. During electrophysical processing at current density j = 20 mA/cm^2, the maximum degree of isolation of Arg is established at 10 min, when pH and temperature are growing, and the highest content is recorded in the cathode cell (Figure 12).

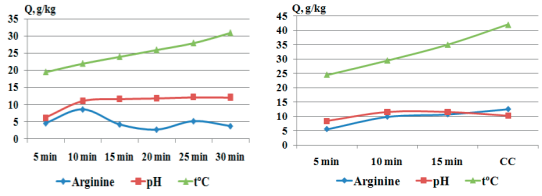


Figure 12. Variations of arginine (Arg) content in PMCs during electrophysical processing with electrolyzer EDP-4 of whey after manufacture of granulated cottage cheese "Grauncior", in stationary regime, at current density j = 10 mA/cm^2 (A) and j = 20 mA/cm^2 (B), depending on variations of pH and temperature

Thus, certain peculiarities of amino acids migration in the concentrates obtained under the influence of different pH and temperature values were determined.

The highest degree of extraction of free, especially essential, amino acids is recorded in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, when pH = 11.6 and tºC = 29.5ºC.

The level of migration of each essential amino acid in the PMCs is varying in dependence on the time of electrophysical processing, on the current density, pH and temperature values.

The parameters for the optimal isolation of each essential amino acid in the the PMCs are:

Thr (threonine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Val (valine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Met (methionine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the "Grauncior", at current density j = 10 mA/cm^2, at 10 min of processing, pH = 11.8 and tºC = 25ºC;

Ile (isoleucine) - during electrophysical processing with electrolyzer EDP-4 of whey after themanufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Leu (leucine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Phe (phenyalanine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5 ºC;

Trp (tryptophan) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 10 mA/cm^2, at 5 min of processing, pH = 12.1 and tºC = 28ºC;

Lysine (Lys) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 5 min of processing, pH = 8.5 and tºC = 24.5 ºC;

His (histidine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 10 min of processing, pH = 11.6 and tºC = 29.5ºC;

Arg (arginine) - during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j = 20 mA/cm^2, at 15 min of processing, H = 11.6 and tºC = 35ºC.

CONCLUSIONS

The peculiarities of amino acids migration in the concentrates obtained under the influence of different pH and temperature values were established.

The highest degree of extraction of total free and essential amino acids, is recorded in the PMCs during electrophysical processing with electrolyzer EDP-4 of whey after the manufacture of the granulated cottage cheese "Grauncior", at current density j =

20 mA/cm^2, at 10 min of processing, when pH = 11.6 and tºC = 29,5ºC.

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

REFERENCES

Bologa, M., Vrabie, E., Maximuk, E. (2009). A method for whey processing. Patent MD nr. 3793. *BOPI* nr. 1.

Chandrapala, J., Duke, M. C., Gray, S.R., Zisu, B., Weeks M., Palmer, M., Vasiljevic, T. (2015). Properties of acid whey as a function of pH and temperature. *J. Dairy Sci.,* 98, 4352-4363.

Cheftel, J.C. (1977). Chemical and nutritional modification of food proteins due to processing and

Bologa, M.K., Stepurina, T.G., Lupu, E.I., Vrabie, E.G., et al. (1992). *Myasn. Molochn. Prom-st*., 6, 22-23.

storage. *In: Food Proteins. Whitaker JR and SR Tannenbaum (Eds.). Avi. Publishing Co, Westport, Connecticut*, 401-445.

de Wit, J. N. (2001). Lecturer's Handbook on Whey and Whey Products. *Eur. Whey Prod. Assoc., Brussels,* Belgium.

Desrosiers, T., Savoie, L. (1991) Extent of damage to amino acid availability of whey protein heated with sugar. *Journal of Dairy Research*, 58, 431-441.

Etzel, V.R. (2004). The emerging role of dairy proteins and bioactive peptides in nutrition and health. Manufacture and use of dairy protein fractions. *The Journal of Nutrition*, 134(4), 9965-10025.

Gonzalez, M.I., Alvarez, S., Riera, F.A., Alvarez, R. (2008). Lactic acid recovery from whey ultrafiltrate fermentation broths and artificial solutions by NF. *Desalination*, 228, 84-96.

Luo, J., Ding. L. (2011). Influence of pH on treatment of dairy waste water by NF using shear enhanced filtration system. *Desalination,* 278, 150-156.

Maximuk, E., Bologa, M., Confratenco, S., Vrabie, E. (2008). The diaphragm electrolyzer. Patent Md 3496. *BOPI* nr. 2.

Moore, S., Spackman, D.H., Stein W. (1958) Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.,* 30(1), 1185-1190.

Reeds, P.J. (2000). Dispensable and indispensable amino acids for humans. *J. Nutr.* 130(7), 1835S-40S.

Rice, G., Barber, A.R., O'Connor, A.J., Pihlajamaki, A., Nystrom, M., Stevens, G., Kentish, S.E. (2011a). The influence of dairy salts on NF membrane charge. *J. Food Eng.,* 107, 164-172.

Rice, G., Barber, A.R., O'Connor, A.J., Stevens, G.W., Kentish, S.E. (2011b). Rejection of dairy salts by a NF membrane. *Purif. Tech.,* 79, 92-102.