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ENRICHMENT OF BIOACTIVE MATERIAL BY ENZYMATIC DEGRADATION AND MEMBRANE SEPARATION

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ABSTRACT: The aim of the present investigation was to examine the applicability of ultrafiltration and enzyme degradation process in enhancing of bioactive components. Skimmed milk was treated with Chy-Max™ Plus (which is produced by fermentation of a 100% chymosin rennet enzym) and the effect of the enzyme concentration, the time of the enzymatic degradation, and the cut off value of the membrane on the protein retention, flux, membrane fouling and gel formation were measured.
KEYWORDS: ultrafiltration, skimmed milk, enzymatic degradation, whey, bioactive components

INTRODUCTION

The bioactive materials have more effective biological properties than the well known nutrients, and therefore they have health-protective and health care effects. The bioactive materials are special macro-and micronutrients; they protect the human body, and the human health better than the other nutrients. These materials contribute to the harmonious functioning of the human body, promote health and disease prevention, and also they have an important role in the treatment of disease, to speed up the healing processes of diseases (Szakaly et al., 2001).

The biologically active materials can be found in large quantities in the skimmed milk. The a-casein and B-casein makes up the largest component in it. A high proportion of trace elements such as calcium and phosphorus can be found in these proteins. The biologically active materials have poor solubility in water, and therefore only in the form of suspensions can be found in the milk. The whey proteins and casein proteins have different physiological effects because they have different physical property. The biggest differences between the two types of proteins are in the rate of digestion and in the speed of the absorption.

Nowadays a lot of different techniques are known to extracting the whey proteins from the valuable components of the milk. Among them, the membrane technology is a current solution (ATRA et al., 2005; Yorgun et al., 2008; Fachin et al., 2005). The natural properties of milk proteins - solubility, foaming and emulsifying capacity, and gelling capacity - are retained after the application of membrane technology, and the separation process is protected the product (Saxena, 2009; Poulin, 2008).

The ultrafiltration is a prevalent separation process among the pressure driven membrane separation techniques to separate proteins of the milk. Several research groups have investigated the effectiveness of ultrafiltration of skimmed milk (Rinaldoni et al., 2009, Salvatore et al., 2011; Hodúr et al., 2009) however, the separation of the proteins followed ultrafiltration is not possible, since the sizes of the protein molecules are very close to each other (for example, B-lactoglobulin and a-lactalbumin) (Metsamuuronen et al., 2011).

The protein because of their molecular size may be rented in the concentrate phase meanwhile the lactose and the other mineral substances can separated through the membrane (Yee et al., 2007; Cheang & Zydney, 2004; Rektor &Vatai, 2004). The milk and whey protein clogs relatively quick the membrane therefore a pre-treatment or other method would be useful before the ultrafiltration of whey or milk (Rektor & Vatai, 2004).

Chymosin is an enzyme which is recovered in rennet and is used in the cheese manufacturing. The most of the used chymosin enzyme is produced recombinant in different bacteria and fungi for example E. coli, or Aspergillus niger (Rawlings & Barret, 1995). The chymosin enzyme is produced by gastric chief cells in the new born animals. The human body is used another type of enzymes to digest milk such as pepsin or lipase. The chymosin enzyme is important in the cheese production because if this aspartic peptidase enzyme can cleave efficiently the κ-cazein in their sensitive region, at the

Phenil-alanin 105 - Methionin 106 bond, the cheese production can become more economical (Kappeler et al., 2006). The chymosin releasing the κ-cazein's negatively charged C-terminus, which is the first step in the separation process of whey and curd (Langholm Jensen et al., 2013). This enzyme has found in three different forms in the animals, the A, B and the C form. The activity measurements were demonstrated that the chymosin A has increased the milk clotting ability than the other forms (Williams et al., 1997).

The extent of membrane fouling is highly depend on parameters such as: share force on the surface (Konrad et al., 2012; Karlsson et al., 2007), therefore the use of vibrated membrane modules appropriate may be increased the membranes life (Al-Akoum et al., 2005; Jaffrin, 2012; Kertész et al., 2010).

The aim of the present investigation was to examine the applicability of ultrafiltration and enzyme degradation process in enhancing of bioactive components and it was also examined what kind of correlation is between membrane characteristics and the success of enzymatic degradation, or the amount of potential bioactive components.. Skimmed milk was treated with Chy-Max™ Plus (which is produced by fermentation of a 100% chymosin rennet enzym) and the effect of the enzyme concentration, the time of the enzymatic degradation, and the cut off value of the membrane on the protein retention, flux, membrane fouling and gel formation were measured.

MATERIALS AND METHODS

The mass transfer and the fluid dynamics the flux is defined as the rate of volumetric flow across a unit area. In this case the effectiveness of a membrane separation can be determined also by the flux. (Bélafiné, 2002):

$$J = \frac{dV}{dt} \cdot \frac{1}{A} \qquad \left[Lm^{-2}h^{-1}\right] \tag{1.}$$

where J is the flux, A is the active membrane area [m^2], V is the filtrate volume[m^3] and t is the time [s]. The retention, R (retention), was calculated using the following equation:

$$R = \frac{C_f - C_p}{C_f} = 1 - \frac{C_p}{C_f} \qquad [\%] \tag{2.}$$

where C_F is the solute concentration in the feed [mg/L], and C_p is the solute concentration in the permeate [mg/L].

Determination of the degree of fouling is proportional to the power low exponent from an analysis of the flux-time functions (Hodúr et al, 2007.):

$$J = J_0 \cdot t^{-k} \qquad \left[Lm^{-2}h^{-1}\right] \tag{3.}$$

where J_0 is the initial flux [$Lm^{-2}h^{-1}$], t is the membrane filtration time [h], and the k is the fouling index.

The membrane resistance (R_M) was calculated from the following equation:

$$\mathrm{R_M} = \frac{\Delta \mathrm{p}}{\mathrm{J_w} \cdot \eta_\mathrm{w}} \qquad [\mathrm{m^{-1}}] \tag{4.}$$

where J_W is the water flux of [$m^3\ m^{-2}\ h^{-1}$] of the clean membrane, and η_w is the water viscosity at 25°C [Pas]. The fouling resistance (R_f) was determined followed the washing of the gel layer from the membrane. The fouling resistance (6) and the resistance of the polarization layer (R_g) were calculated as following (7):

$$\mathrm{R_f} = \frac{\Delta \mathrm{p}}{\mathrm{J_{WA}} \cdot \eta_{\mathrm{WA}}} - \mathrm{R_M} \qquad [m^{-1}] \tag{5.}$$

$$\mathrm{R_g} = \frac{\Delta \mathrm{p}}{\mathrm{J_C} \cdot \eta_{\mathrm{WW}}} - \mathrm{R_M} - \mathrm{R_f} \qquad [m^{-1}] \tag{6.}$$

where Δp is the pressure difference between the two sides of the membrane (Pa), η_{WA} [Pas] is the viscosity of the filtered solution and J_{WA} is the water flux after concentration tests. The gel layer resistance's mathematical formula the J_C is the constant flux and the η_{ww} is the wastewater viscosity.

The well known membrane filtration parameters were measured in both cases. The volume reduction ratio (VRR) was calculated using the following formula:

$$\mathrm{VRR} = \frac{\mathrm{V_{feed}}}{\mathrm{V_{feed}} - \mathrm{V_{perm}}} \tag{7.}$$

where V_{feed} is the volume of the feed [L] and V_{perm} is the volume of the permeate [L].

The analytical values of skimmed milk and separated samples were determined by Bentley B-150 type Milk Analyzer. Skimmed mild was used as feed in our work. It has 2.79g/100g protein fraction and 2.97g/100g total N protein, 0.02 g/100g milk fat, 4.5 g/100g of lactose and 8.09 g/100 g of dry material were measured in the skimmed milk samples. Skimmed milk samples were stored deep frozen until used.

Two different equipments were used for membrane separation; the VSEP - Vibratory Shear Enhanced Processing laboratory module and the MEUF - micellar enhanced ultrafiltration device.

Torsional vibration reduced fouling in both dead-end and cross flow modes. Increasing vibration amplitude decreased membrane fouling, increased rejection of most solutes and changed the morphology of the scales from a tightly packed layer to a more scattered distribution of particles. The VSEP system consists of disk-shaped flat-sheet membranes. This laboratory module attached to a central shaft. The shaft was rotated a short distance at a frequency of 54 Hz. Under examination 503 cm^2 active filtering surface, regenerated cellulose,, flat-sheet ultrafiltration membranes were used at controlled temperature and pH. The cut-off values of applied membranes were 100 kDa and 10kDa. The trans-membrane pressure difference was 0.5 MPa.

The MEUF equipment has 15 cm^2 active filtering surface. A regenerated cellulose based flat-sheet UF membranes were used with a cut-off value at 10 kDa. During the measurements 0.5 MPa pressure was applied and the feed side was stirred with a magnetic stirrer at 200 rpm to keep the cross-flow on the membrane surface.

The permeate of skimmed milk's 100 kDa separation (F10) was treated by Chy-Max™ Plus enzyme (which is produced by fermentation of a 100% chymosin rennet enzyme). The enzimatic degradation were made on 43°C and 1cm^3/L enzyme concentration was used. The untreated samples, i.e. permeat of 100 kDa separation added enyme (P10E0) and samples taken every third hours of degradation procedure (P10E3, P10E6) were filtered on a 10 kDa cut-off value ultrafiltration regenerated cellulose membrane in the MEUF equipment.

RESULTS AND DISCUSSION

The flux - time functions of the skimmed milk membrane separation are presented in Figure 1. The flux was increasing at the first 20 min of the separation and it was decreasing slowly follows. This interesting and unusual behaviour could explain with the effect of vibration.

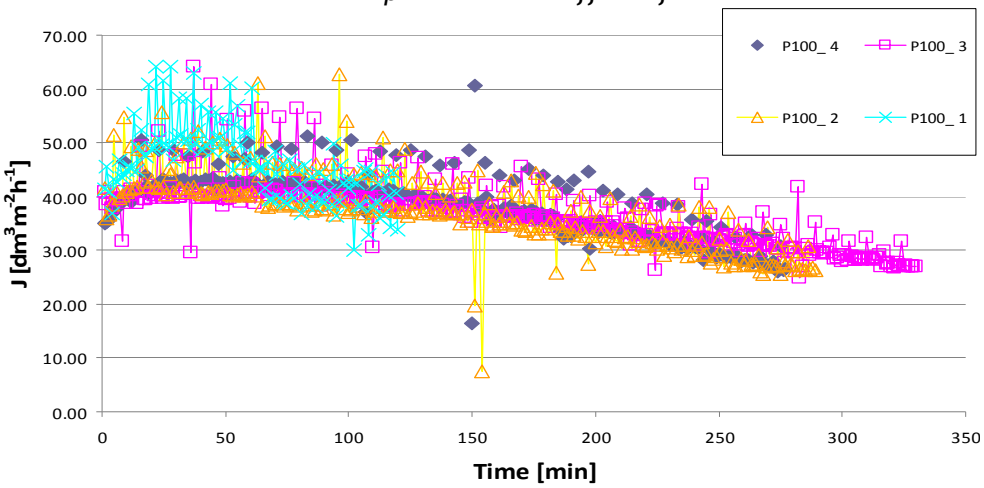


Fig. 1: Flux of the skimmed milk as a function of time (RC membrane with cut off 100 kDa, vibration frequency: 54 Hz , TMP:0.5 MPa)

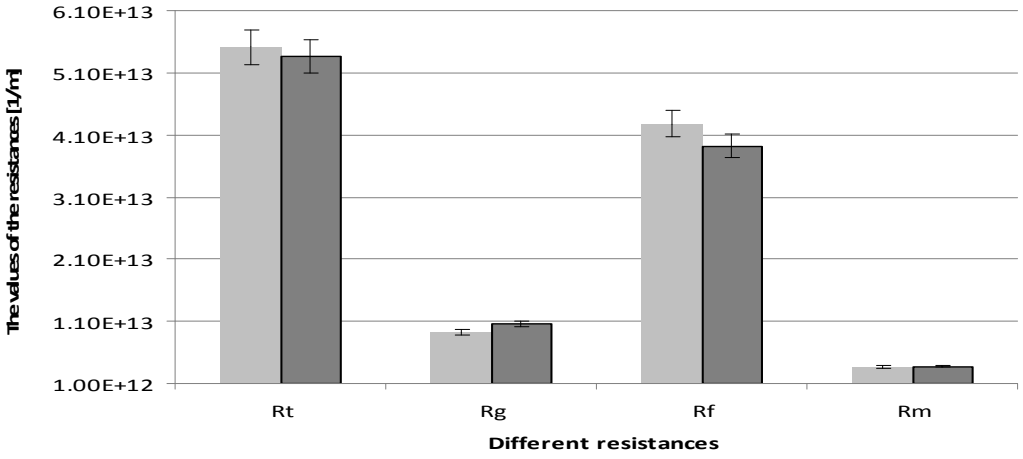


Fig. 2: Resistances of the skimmed milk ultrafiltration (RC membrane with cut off 100 kDa, vibration frequency: 54 Hz , TMP: 0.5 MPa)

The different resistance values were calculated: the membrane resistance (Rm), the resistance of the gel layer on the surface of the membrane (Rg), the fouling resistance (Rf) and the total resistance (Rt) (Fig. 2). The resistances values showed there is no significant differences between the measured values of the samples demonstrated that the skimmed milk samples weren't changed during

the deep frozen storage. The gel layer resistance is smaller than the fouling resistance similarly as it was published by Shi & Benjamin (2009) and as it is known from the other publication about the vibrated membrane separation. The proportion the resistances enhanced the well known data: vibrated systems reduce the gel layer resistance.

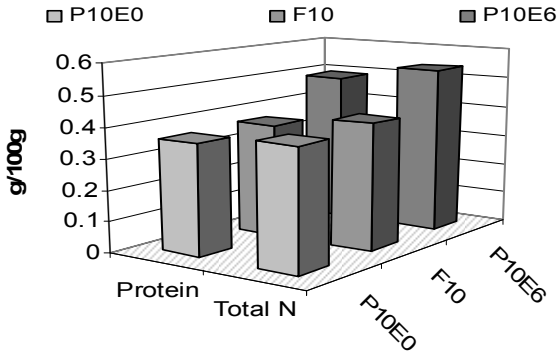


Fig. 3: Analytical values of the 100 kDa permeate as a Feed (F10) and the enzyme treated (P10E6) and untreated (P10E0) permeate samples

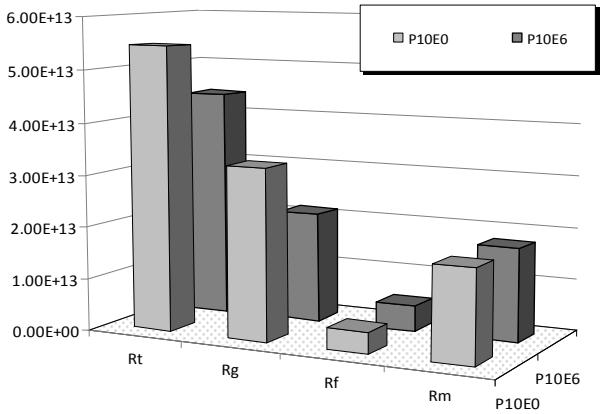
The analitical values of the feed (F10) and the untreated (P10E0) and treated (P10E6) permeate fractions were demonstrated in the Fig. 3. The protein and total nitrogen contents were higher in the treated samples than in the untreated samples which means that the enzymatic degradation of protein was functioned (Fig. 3).

The retention was calculated on the base of protein and the total N content and it was found that the permeate of enzyme treated samples had more protein and total nitrogen, which also proofs the enzyme degradation was successful.

The resistance values of the ultrafiltration in the case of the enzyme treated and untreated samples are shown in the Fig.4. The total resistance and the gel layer resistance are smaller and the fouling resistance is higher in the case of enzyme-degraded samples, it means that the smaller protein fractions increased during the 6 hour long treatment.

Table 1: The retention values and the different protein and total Nitrogen values

Samples	*R [%]*	*Protein [g/100g]*	*Total N [g/100g]*
F10	*100*	*0.37*	*0.4*
P10E0	*97.5*	*0.36*	*0.39*
P10E3	*98.2*	*0.39*	*0.42*
P10E6	*97.5*	*0.49*	*0.54*



	Rt	Rg	Rf	Rm
P10E0	5.46E+13	3.27E+13	4.10E+12	1.78E+13
P10E6	4.40E+13	2.13E+13	4.90E+12	1.78E+13

Fig. 4.: Resistances on the treated(P10E6) and untreated (P10E0) samples

This tendency could follow in the Fig.5 also where the percentage of gel layer resistance and the fouling resistance are shown.

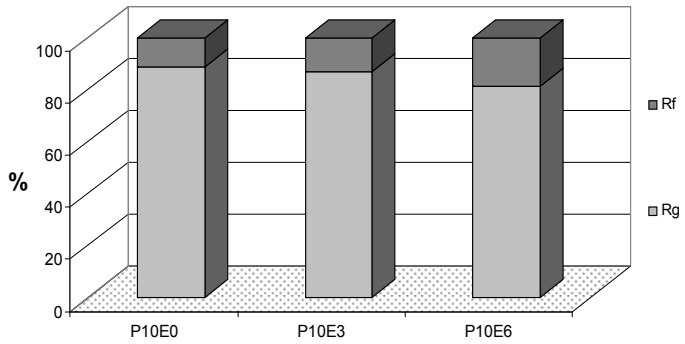


Fig. 5.: The percentage of gel layer (Rg) and fouling resistance (Rf) at untreated (P10E0), the 3 hour long treated (P10E3) and 6 hour long treated (P10E6) samples.

Presumably the lower flux values (Fig. 6) detected at the treated samples was caused by the higher fouling resistance.

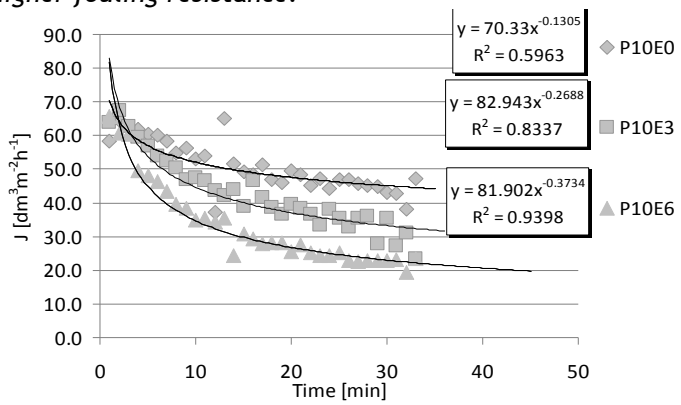


Fig. 6: The enzyme-treated (P10E3, P10E6) and untreated (P10E0) samples flux values as a function of time

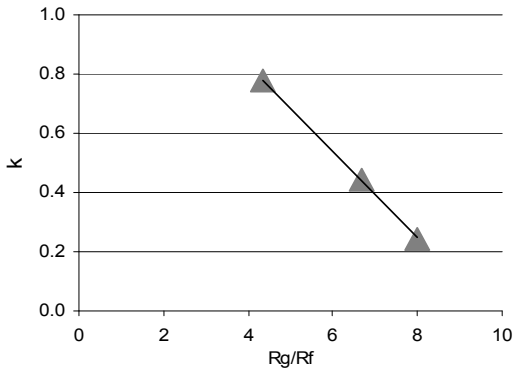


Fig. 7: Fouling index values as a function of t the gel and the fouling resistance ratio

CONCLUSIONS

This research is an initial step of the project to enhance the milk originated bioactive components in the membrane separated fractions.

In our experiment were performed the skimmed milk's filtration on regenerated cellulose membranes, and chymosin-treated ultrafiltered permeate fraction.

The different resistance values obtained chymosin enzyme treated samples showed that the enzymatic degradation of protein was functioned; therefore the protein content of the permeate phase increased and it was bigger in the 6 h degradeted samples than the 3 h degradeted ones.

Since the fouling index has a linear relationship with the ratio of the gel resistance and fouling resistance, it could be relevant parameter for expression of the degradation in adequate conditions. The next step should be the investigation of the correlation of the Rg/Rf ratio and the bioactive component content of the samples.

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