Concentration of a vegetal enzymatic extract by microfiltration

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ABSTRACT

Milk clotting for cheese producing is usually performed using enzymes from animal sources and the most common, chymosin, is obtained from the stomach of newborn calves. Some researches have identified new sources of enzymes that have the ability of coagulating the milk like proteases from vegetal. The objective of this study was to evaluate the concentration by microfiltration of a vegetal proteolytic enzymatic extract obtained from sunflower seed. The raw material utilized was sunflower seed from Embrapa´s experimental station. Enzyme extraction was performed according to the following procedure: sunflower seed disintegration in a pilot plant mill, homogenization in an industrial blender and aqueous extraction in brine solution during 18 hours under refrigeration. The extract was centrifuged and conducted to microfiltration system composed by three ceramic membranes with 1.0 µm pore size. Permeate flux was determined along the process and samples were collected from feed, permeate and retentate, for determination of total proteins, proteolytic activity and coagulation unit. Average permeate flux was 33 L/hm^2 and concentration factor was equal to 5.0. As expected the coagulation unit was lower in retentate fraction (4.72 CU/mL) than in feed (10.93 CU/mL) and no milk coagulation was verified with the permeate sample. No proteolytic activity was detected in permeate fraction and total protein content was 0.29 mg/mL. Proteolytic activity increased from 1.43 U/mL in raw extract to 2.18 U/mL in retentate and protein content has increased from 1.69 mg/mL to 6.21 mg/mL. Both total protein and proteolytic activity were concentrated in retentate fraction although they were not in the same range of the concentration factor, probably due to the protein denaturing and loss of enzymatic activity. The obtained results suggest a potentiality for the concentration of the sunflower seed protease by microfiltration, although it is needed further studies to optimize the process.

Keywords: protease; membrane process, enzyme; cheese making

INTRODUCTION

Proteolytic enzymes have been used for many applications including some traditional ones like bread and cheese production, in special at biotechnological and food industry [1, 2], as milk clotting enzymes that use casein as preferential substrate [3]. Among the main biochemical changes that occur during cheese maturation the proteolysis stands out as it affects aroma, texture and flavor of the product [4].

Milk clotting for cheese producing is usually performed using enzymes, such as aspartic proteases, from animal sources, and chymosin, obtained from the stomach of newborn calves, is the most common one, usually known as rennet. However, the low availability of the raw material and the increase in cheese production have contributed to the lack of chymosin in world market, resulting in the search for new sources, like the vegetal ones [5, 6].

Microbial enzymatic extracts obtained by recombinant bacteria has also proved to be adequate for substitution of animal rennet and some researches have already identified new sources of enzymes that have the ability of coagulating the milk although few of these bacterial enzymes are commercially used [7].

An example of new application is the use of proteases from vegetal origin that will also attend people with some religious or philosophical demands. There are different plants that contain milk clotting proteases although most of them are not adequate for cheese production due to a bitter flavor formation and low cheese yield [8]. Artichoke flowers extracts are an exception as they do not present this unintended characteristic and are applied for cheese production from sheep milk.

Egito et al. [6] have shown that sunflower extracts can also be used for cheese production with desirable flavor and that the enzymes have the same site of hydrolysis of chymosin when in presence of casein. For

obtaining the sunflower enzymatic extract a large amount of water is used which results in a low concentration extract that presents a low coagulant activity.
Many techniques may be used for protein concentration like centrifugation, ion exchange, chromatography and membrane processes. The membrane technique is potentially attractive for this objective as it occurs at room temperature, it is easily operated and the separation presents high specificity [9]. In addition, the main advantage is related to its pure mechanical characteristic with no phase change that causes low impact on proteins functional and nutritional properties [10]. Reverse osmosis, nanofiltration, ultrafiltration and microfiltration are the membrane processes most used in food industries.
The objective of this study was to evaluate the concentration by membrane processes of a vegetal proteolytic enzymatic extract obtained from sunflower seed, which can be used on cheese industry.

MATERIALS & METHODS

Raw material
The raw material was sunflower seed from experimental station of Embrapa Soy.

Chemicals
Sodium chloride P.A. was used for preparing the brine extraction solution.

Experimental procedure
The enzymatic extract was obtained according to the steps showed in Figure 1 and described as follows: disintegration of sunflower seed in a pilot plant mill, homogenization in an industrial blender, aqueous extraction in brine solution containing 1% (w/v) NaCl during 18 hours under refrigeration and centrifugation in a basket centrifuge (Bellinox, Brazil) at 35g, using a 150µm nylon screen filter medium.

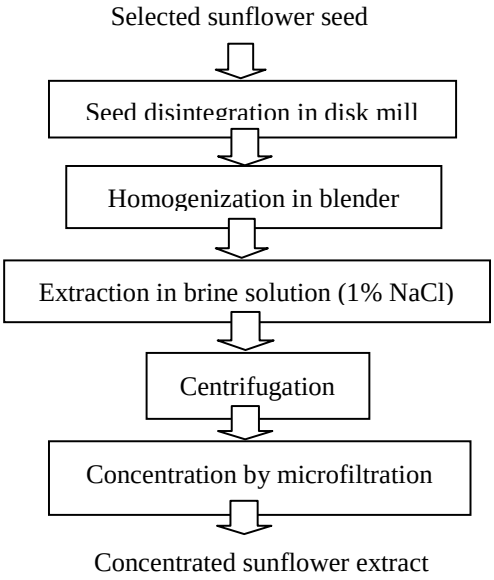

Figure 1. Block diagram for obtaining the concentrated sunflower seed extract

The extract was conducted to the microfiltration system composed by three ceramic membranes with 1.0µm pore size and 0.0165m^2 of filtration area. Process was carried out at 25^{o}C and 2 bar of transmembrane pressure, in batch mode with recirculation of retentate fraction and continuous recovery of permeate. Permeate flux was determined along the process and samples were collected from the three fractions, feed (raw extract), permeate and retentate (concentrated extract), for determination of total proteins, proteolytic activity and coagulation unit.

Analytical methods
Total extracellular proteins

Total protein content was determined according to Bradford [11] colorimetric method that is based on the interaction of basic and acid groups of proteins with dissociated groups of organic dyes, resulting in colored compounds.

Proteolytic activity

The determination of the proteolytic activity was based on the formation of colored components in basic medium from the digestion of an azoprotein solution. It expresses the enzyme action on a proteic substrate.

One unit of proteolytic activity (U/mL) was defined as the amount of enzyme that produces a 0.01 variation in absorbance between blank and sample, per minute, at established reaction conditions [12].

Coagulation unit

The ability of clotting milk was performed according to Berridge [13] by adding the enzymatic extract to milk at pH 6.5 and determining the time needed for coagulation. On unit of coagulation (CU/mL) was defined as the amount (mL) of the extract able to coagulate 1mL of reconstituted skim milk powder during 1 min at 37°C.

RESULTS & DISCUSSION

The permeate flux behavior of microfiltration process is showed in Figure 2, where it is possible to observe its stability along more than 200 minutes. The average flux value was 33 L/hm^2 and a volumetric concentration factor equal to 5.0 was obtained in the process. Preliminary studies using ultrafiltration membranes (pores between 0.001 to 0.1 µm) have showed a very low permeate flux and its sharp drop with time (data not shown). This flux decline has a negative influence on the economics of membrane operation and should be prevented. For this reason ceramic microfiltration membranes (pores between 0.1 to 10 µm) were evaluated and a reasonable value of permeate flux was obtained.

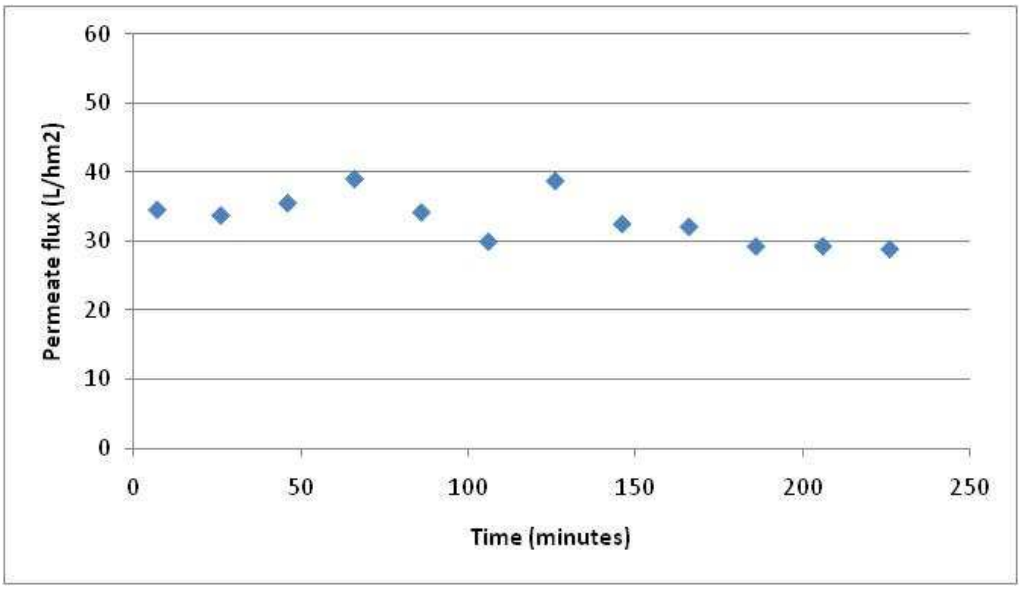

Figure 2. Permeate flux behaviour of sunflower extract microfiltration.

The results of total protein, proteolytic activity and coagulation unit are presented at Table 1.

Table 1. Total protein, proteolytic activity and coagulation unit of the three fractions during microfiltration of sunflower enzymatic extract

Parameter	Total protein (mg/mL)	Proteolytic activity (U/mL)	Coagulation unit (CU/mL)
Feed (raw extract)	1.69 ± 0.17	1.43 ± 0.07	10.93 ± 0.09
Permeate	0.29 ± 0.02	0	nc
Retentate	6.21 ± 0.24	2.18 ± 0.20	4.72 ± 0.01

nc – no coagulation

As expected, the coagulation unit was lower in the retentate fraction (4.72 CU/mL) than in the feed (10.93 CU/mL) and no milk coagulation was verified with the permeate sample. No proteolytic activity was detected in the permeate fraction and the total protein content was 0.29 mg/mL. Proteolytic activity has increased 1.5 times, while total protein content has increased about four fold. Both total protein (3.7 times) and enzymatic activity (1.5 times) were concentrated in retentate fraction although they were not in the same range of the concentration factor, probably due to the protein denaturing and enzyme inactivation. Neves et al. [14], whose have studied the squid protein concentration by ultrafiltration, has attained a lower concentration factor (1.8) although they have gotten to concentrate total proteins at the same range.

CONCLUSION

The obtained results suggest a potentiality for the concentration of the sunflower seed protease by microfiltration, although this process needs further studies to reduce enzymatic activity losses.

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