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Recovery of protein from brewer's spent grain by ultrafiltration

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ABSTRACT

Application of ultrafiltration in recovery of protein from brewer's spent grain (BSG) was studied in this work. The effectiveness in removing water and salts was evaluated. Results indicated that increasing of cross-flow rate could improve the limiting flux. More than 92% of the protein was retained by the membranes with both MWCO of 5 and 30 kDa. The protein contents in the final product were 20.09 ± 1.40% and 15.98 ± 0.58%, respectively by 5 and 30 kDa membranes compared with that of 4.86 ± 0.61% concentrated by rotary evaporation. It indicated that ultrafiltration had good ability in the removal of salts in the extract solution and improved the quality of final products. The 5 kDa membrane had a little higher protein retention capacity than that of 30 kDa.

1. Introduction

Brewers' spent grain (BSG) is the major by-product of the brewing industry, representing around 85% of the total by-products generated. BSG has high content of protein and protein content more than 20% on dry weight basis is reported [1]. BSG is of low cost and high nutritive value. The ingestion of BSG, or its derived products, has health benefits. Incorporation of BSG into rat diets is beneficial to intestinal digestion, alleviating both constipation and diarrhoea. Such effects were attributed to the content of glutamine-rich protein, and to the high content of non-cellulosic polysaccharides and smaller amounts of β-glucans [1].

For a long time, the main application of BSG has been limited as animal feed along with utilization of BSG in increasing bricks porosity [2], removal of Cu(II) ions from aqueous solutions [3], and as brewing yeast carrier [4,5]. The incorporation of BSG into ready-to-eat snacks was also studied [6,7]. Due to the presence of many beneficial components in BSG, separation of BSG into its individual components for both food and non-food applications is found important. These researches included valorization of BSG to recover valuable compounds such as α-tocopherol by supercritical fluid extraction (SFE) technology coupled with pretreatment processes [8], recovery of ferulic acid in BSG by sequentially extracting with alkali of increasing strength [9], solublilization carbohydrates from BSG by microwave radiation to 160 °C in the presence of 0.1 M HCl [10], extraction of ferulic and *p*-coumaric acids by alkaline hydrolysis of BSG [11], recovery of lignin from BSG [12] and production of oligosaccharides [13].

For the separation of protein, alkaline extraction and protein precipitated by the addition of ammonia sulphate [14] or by acidification to pH 4.5 using 4N HCl [15] were commonly employed. Salts residue was removed by dialyzing against distilled water. Celus et al. [16] used alkaline (17%, w/v) extraction with 0.1 M NaOH at 60 °C. After 60 min of extraction, samples were filtered (180 μm) and the proteins in the filtrate were precipitated by acidification to pH 4.0 using 2.0 M citric acid. The precipitated protein was obtained after centrifugation at 10,000 × *g* for 10 min at 4 °C and was finally freeze-dried. Diptee et al. [17] extracted protein from BSG using 0.6% Na_2HPO_4 solution, and ethanol was added to precipitate protein. But it was difficult to obtain protein from BSG in our previous experiment since it was easily denatured by temperature during processing. And the salt remained in the extract solution could not be efficiently removed by dialysis. Membrane separation has many advantages such as absence of phase change of water and reduced energy consumption for the removal of water and small molecular size compounds, such as salts. Since no heat was added to the co-product stream, quality of protein in the co-products should not be compromised [18]. In recent years, ultrafiltration has been used for the separation of a wide range of compounds [18,19] and membranes are used extensively throughout the production

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of biotechnology products [20]. Application of membrane process might overcome the problem met in BSG protein isolation.

However, the major problems of ultrafiltration process are concentration polarization and fouling which reduce the permeate flux far below the theoretical capacity and change membrane selectivity. Both concentration polarization and fouling strongly depend on operation conditions and membrane characteristics, such as feed properties, membrane molecular weight cut off (MWCO), trans-membrane pressure (TMP) and cross-flow rate. In the present work, application of ultrafiltration in removing water and salts in the recovery of protein from BSG was investigated and the effectiveness was evaluated.

2. Materials and methods

2.1. Preparation of protein solution

BSG (73.8% moisture, 7.6% protein, Kjeldahl N × 6.25, wet weight basis) was obtained from Guangzhou Zhujiang Brewery Group Co., Ltd., China. It was kept in −20 °C and thawed at 4 °C overnight before extraction. The BSG extract was prepared by ultrasound-assisted extraction using sodium carbonate buffer (pH 10), which was found good in extraction of protein from BSG in our lab (unpublished data). The protein solution was prepared by extraction of BSG (100 g) using 1000 ml of extractant for 1 h and then filtered through nylon cloth and the filtrate solution was centrifuged (10,000 × *g*) at 4 °C. The supernatant was collected and used as feed solution.

2.2. Equipment and membranes

Minipore Labscale™ TFF system (Millipore, USA) was used for this study. The system consisted of a re-circulation pump, cross-flow ultrafiltration module (Pellon-XL Module, Millipore, USA) equipped with membrane of BIOMAX®. The trans-membrane pressure and cross-flow velocity were adjusted by a manual valve and pump controller. The pressure was measured by a standard pressure gauge. Membranes of MWCO of 5 and 30 kDa with a surface area of 0.05 m^2 were used in these experiments.

All experiments were operated at ambient temperature (∼25 °C). BSG extract (500 ml) was used as the feed for each experiment. The pressures were regulated using pressure gauges. The cross-flow rate and permeate solutions were measured using graduated cylinder and stopwatch.

After each run, the membrane was cleaned by alkali treatment as recommended: a solution of 0.1 M sodium hydroxide was recycled past the membrane at a cross-flow rate of 1.0 L/h for an hour with TMP of 25 psi. The storage solution recommended for this type of membrane is 0.1 M sodium hydroxide and was used in all the experiments.

2.3. Effects of membrane MWCO and operating conditions

The effects of membrane MWCO and operating conditions were studied using the total recycle mode. Both retentate and permeate were re-circulated to feed tank. The trans-membrane pressure (TMP) of 10–45 psi was used for both membranes. The cross-flow rates were controlled at 0.9, 1.8 and 2.7 L/h for both membranes. The permeate flux was measured using a graduated cylinder and a stop watch. The samples of permeate and feed bulk were collected for protein analysis.

2.4. Solution concentration

A single batch concentration was investigated for the removal of solvent. The retentate was recycled to the feed bulk while the permeate was removed from module. At each experiment, 0.5 L sample solution was concentrated. TMP was controlled at 25 psi. The cross-flow rate was controlled similar to those used in the total recycle mode. At each condition, the permeate flux was measured until permeate flux was constant. The permeate and retentate samples were collected for analysis. The concentrated solution was lyophilized to get final products.

A control concentration method of vacuum rotary evaporation was carried out at 40 °C using a rotary evaporator (RE-52CS/5299, Shanghai Yarong Ltd., China). The protein solution with volume of 0.5 L prepared from BSG was concentrated to 0.1 L, and then lyophilized to get final protein products. The experiment repeated in triplicate.

2.5. Analytical methods

Soluble protein concentration in the permeate and feed bulk was measured by Bradford method [21], using bovine serum albumin (BSA) as the standard. One milliliter of diluted sample was placed in a test-tube. Five milliliters of Bradford dye was added and mixed and allowed to stand for 1–2 h. The absorbance of the mixed sample was measured at 595 nm with a UV–vis spectrophotometer. The concentration of protein in the sample was determined using the standard curve of UV absorbance and concentration.

The average pressure experienced by the membrane surface between the feed and retentate ports is called the trans-membrane pressure (TMP) and is calculated using Eq. (1):

$$\mathrm{TMP} = \frac{P_{\mathrm{in}} + P_{\mathrm{out}}}{2} \tag{1}$$

where P_{in} is the feed pressure (psi) and P_{out} is the retentate pressure.

Membrane flux is a measure of the permeate flux taking into account the active surface area of the membrane and is calculated using Eq. (2):

$$J = \frac{1}{A}\frac{\mathrm{d}V}{\mathrm{d}t} \tag{2}$$

where J is the permeate flux (L/m^2 h), A is the area of the membrane (m^2), V is the filtrate volume (L) and t is the unit time.

Total membrane resistance (R_{m}) can be determined from Eq. (3):

$$R_{\mathrm{m}} = \frac{\Delta P}{\mu J} \tag{3}$$

where ΔP is the filtration pressure, it is equivalent to the TMP here; and μ the solution viscosity, was determined by a viscometer (DV-I Viscometer, Brookfield Engineering Labs, Inc., USA). The changes of membrane resistance for pure water were determined before and after filtration.

The protein retention ratio (R) was defined as

$$R = 1 - \frac{C_{\mathrm{P}}}{C_{\mathrm{F}}} \tag{4}$$

where C_{F} is the concentration of protein in feed stream and C_{P} is the concentration of protein in permeate.

The yield was calculated as

$$Y(\%) = \frac{P_{\mathrm{final}}}{P_{\mathrm{BSG}}} \times 100 \tag{5}$$

where P_{final} is the protein content in final product and P_{BSG} is the protein content in BSG (wet basis).

3. Results and discussion

3.1. Pure water flux

Pure water flux was measured at the beginning of the experiment. The flux increased linearly with TMP within the tested pressure range, 5–50 psi (Fig. 1), as same as reported by Chollangi