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Cheese whey protein recovery by ultrafiltration through transglutaminase (TG) catalysis whey protein cross-linking

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ABSTRACT

In whey ultrafiltration (UF) production, two main problems are whey protein recovery and membrane fouling. In this study, membrane coupling protein transglutaminase (TG) catalysis protein cross-linking was investigated under different conditions to find out the best treatment. We found that the optimal conditions for protein recovery involved catalyzing whey protein cross-linking with TG (40 U/g whey proteins) at 40 °C for 60 min at pH 5.0. Under these conditions, the recovery rate was increased 15–20%, lactose rejection rate was decreased by 10%, and relative permeate flux was increase 30–40% compared to the sample without enzyme treatment (control). It was noticeable that the total resistance and cake resistance were decreased after enzyme catalysis. This was mainly due to the increased particle size and decreased zeta potential. Therefore, membrane coupling enzyme catalysis protein cross-linking is a potential means for further use.

1. Introduction

In dairy industry, cheese production effluent is one source of organic contamination (Prazeres, Carvalho, & Rivas, 2012). However, organic waste such as protein, lactose, fat can still be recycled by various biotechnological processes and reused as a food component because they are valuable natural resources (Baldasso, Marczak, & Tessaro, 2014). It has been reported that whey protein powder helps promote weight loss, lower cholesterol and reduce blood pressure as dietary protein supplement (Patel, 2015). In modern society, an increasing number of people attempt to develop muscle via exercise and whey protein powder intake, especially males.

Membrane technology is practical for handling a considerably large amount of production (1–2 kg of cheese yields 8–9 kg of whey), and it is able to separate protein, lactose, salt and water from cheese whey. In industry, ultrafiltration is widely used to concentrate whey protein because it can exclude impurities to some extent (Baldasso, Barros, & Tessaro, 2011a, 2011b). Some membrane recovery whey protein methods exist, such as the diafiltration, vibratory shear-enhanced processing system (VSEP) and forward osmosis (FO). Diafiltration (DF) is used to produce high whey-protein concentrate (WPC), which requires high purification and high level of performance. However, diafiltration requires large amount of water and recycled permeate needs to be modified to achieve the desired flow rate and composition, which is originally imported from the ultrafiltration plant. Therefore, it is necessary to develop methods to reduce diafiltration water consumption and recycle times during the production of whey protein concentrates. One problem is whey protein fouling during membrane operation, two main proteins, α-lactoglobulin and β-lactoalbumin, get stuck in membrane pores during ultrafiltration, which leads to reduce efficiency. HPLC illustrated that not only peptides but also two native proteins (α-lactoglobulin and β-lactoalbumin) permeate the filter during ultrafiltration (Barba, Beolchini, Cifoni, & Veglio, 2001). Butylina, Luque, and Nyström (2006) investigated fractionation and further isolated the typical peptides and proteins in sweet whey by ultrafiltration and nanofiltration. The whey protein was rejected by ultrafiltration and the peptides were rejected by nanofiltration. The combination of UF and NF achieved the maximum recovery of whey protein and peptides. Another method to recover whey is the use of a forward osmosis (FO) and reverse osmosis (RO) integrated membrane system with a NaCl draw solution that is separately utilized for whey powder production and water recovery. It was found that compared to the UF/RO system, FO/RO for whey processing could also be effectively employed considering the economic performance (Aydiner et al., 2014). Hodúr, Kertèsz, Csanádi, and Szabó (2009) also assessed the performance of a vibratory shear-enhanced processing system (VSEP) for whey concentration to reduce

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membrane fouling by high shear rate. It was found that the VESP system has high flux reduction ratios and low total resistances compared with a classical, cross-flow, plate and frame membrane configuration system (3DTA) with the same membrane.

A series of studies have been performed that considered membrane-fouling and protein-stick problem during ultrafiltration, i.e. α-lactoglobulin and β-lactoalbumin. Many studies found that large sized proteins have less influence than small protein, the latter of which are absent from the fouling layer after long term filtration (Tolkach & Kulozik, 2006). Norazman et al. (2013) also found that large proteins were easier to clean than small protein during whey protein fouling membrane cleaning. In this study, the TG enzyme was used to catalyze whey protein cross-linking to increase the whey protein molecular size, with the hope of reducing the membrane fouling and increasing the membrane flux and whey protein recovery rate during ultrafiltration. There are several whey protein cross-linking enzymes, including horseradish peroxidase (Heijnis, Wierenga, Janssen, van Berkel, & Gruppen, 2010; Saricay, Wierenga, & de Vries, 2013), tyrosinases (EC 1.14.18.1) (Ercili-Cura et al., 2012), laccase (Tantoush et al., 2011) and transglutaminase (EC 2.3.2.13) (Agyare & Damodaran, 2010; Gauche, Vieira, Ogliari, & Bordignon-Luiz, 2008). Of all these cross-linking enzymes, the TG enzyme is able to catalyze α-lactoglobulin, β-lactoalbumin and even the caseinomacropeptide (Tolkach & Kulozik, 2005) cross-linking by forming intra- and intermolecular ε-(γ-glutamyl)lysine crosslinks, leading to a high molecular weight polymer in cheese whey. Furthermore, the TG enzyme can catalyze protein cross-linking in the absence of mediator addition to help enzyme catalysis. Therefore, TG enzyme was chosen to catalyze whey protein cross-linking in this study from an economic and catalytic standpoint. In whey protein research, the TG enzyme catalyzes protein cross-linking mainly on α-lactalbumin and β-lactoglobulin. The α-lactalbumin structure has 8 glutamine residues and 12 lysine residues, while β-lactoglobulin has 16 glutamine residues and 15 lysine residues in its protein chain. However, not all of these residues are available for enzymatic reaction with transglutaminase, due to the globular structure of whey proteins (Gauche et al., 2008). This research found that a maximum of five lysines and five glutamine residues on α-lactalbumin can be modified by transglutaminase depending on the temperature, pH, and presence or absence of calcium (Nieuwenhuizen et al., 2003). For application in food, there have been some studies showing that transglutaminases are used to improve the functional properties of whey proteins, such as elasticity, water holding capacity, heat stability, foaming and emulsifying activity. Therefore, the cross-linking whey protein catalyzed by the TG enzyme can be added to foods as functional ingredients and improve some functional properties of food products in food industry (Anuradha & Prakash, 2009).

TG catalysis conditions can be determined by the amount of substrate, which influences the size of product and whey protein deposits on the ultrafiltration membrane surface, leading to membrane resistance. Therefore, the whey protein rejection rate was different under TG enzyme catalysis conditions. In this research, the investigation included the ultrafiltration performance and TG catalysis conditions (e.g. enzyme concentration, pH, temperature and reaction time). The optimized ultrafiltration condition were selected to detect membrane resistance, particle size and zeta potential.

2. Materials and methods

2.1. Materials

Polyethersulfone membranes (PESs) with a molecular weight cut-off of 10 kDa were obtained from Sepro Co. America. Chemical Co. *N*-carbobenzoxy (CBZ)-glutaminyl-glycine, hydroxamic acid and Foline-phenol were purchased from sigma. The transglutaminase (EC 2.3.1.13) was supplied by Taixing Yiming biological Co., Ltd. (Jiangsu, China). Whey was obtained from cheddar cheese production in our laboratory.

2.2. Methods

2.2.1. Experimental procedure

Whey was obtained from the production of cheddar cheese from skim milk. TG enzyme catalysis whey protein cross-linking under different enzyme concentration, pHs, temperatures and reaction times was performed pre-filtration. Then, the samples were poured into the ultrafiltration cup. The transmembrane pressure was 0.15 MPa. The ultrafiltration operation efficiency was investigated according to the whey protein recovery rates, rejection rates, lactose rejection rates, VCF and relative membrane flux. Then, the optimized ultrafiltration (UF) conditions were selected to detect membrane resistance, particle size and zeta potential.

2.2.2. The effect of enzyme catalysis concentration on whey ultrafiltration recovery

Different TG enzyme concentrations (20, 40, 60 and 80 U/g of whey proteins) were added to whey and kept at temperature of 40 °C for 60 min and pH 6.0. Then, whey protein recovery rates, rejection rates, lactose rejection rates, VCF and membrane flux were assessed at different ultrafiltration times. The no enzyme addition whey sample was used as a control.

2.2.3. The effect of TG catalysis pH on whey ultrafiltration recovery

Different TG enzyme catalysis pHs (5.0, 6.0, 7.0 and 8.0) were investigated at an enzyme concentration 40 U/g of whey protein and 40 °C for 60 min. Then, the whey protein recovery rates, rejection rates, lactose rejection rates, VCF and membrane flux were assessed at different ultrafiltration times compared to the control.

2.2.4. The effect of TG catalysis temperature on whey ultrafiltration recovery

Different TG enzyme catalysis temperatures (30 °C, 35 °C, 40 °C, 45 °C and 50 °C) were investigated at an enzyme concentration 40 U/g of whey protein, pH 5.0 and 40 °C reacting 60 min. Then, the whey protein recovery rates, rejection rates, lactose rejection rates, VCF and membrane flux were assessed at different ultrafiltration times compared to the control.

2.2.5. The effect of TG catalysis time on whey ultrafiltration recovery

Different TG enzyme catalysis times (30 min, 60 min, 90 min and 120 min) were investigated at an enzyme concentration 40 U/g of whey proteins, pH 5.0 and 40 °C. Then, the whey protein recovery rates, rejection rates, lactose rejection rates, VCF and membrane flux were assessed at different ultrafiltration time compared to the control.

2.2.6. TG enzyme activity measurements

The transglutaminase (EC 2.3.1.13) activity measurements were assayed following the procedure described procedure (Folk & Cole, 1965). A reagent: 100 mg N - CBZ - Gln - Gly was dissolved in 2 ml of 0.2 N NaOH solution and then 4 ml of 0.2 N Tris HCl buffer, 2 ml of 0.01 N reduced glutathione and 2 ml 0.1 N hydroxylamine hydrochloride were added. B reagent: 3 N hydrochloric acid, 12% trichloroacetic acid and 5% ferric chloride hexahydrate were blended in an equivalent volume. Hydroxamic acid (at a concentration of 10, 20, 40, 60, 80 and 100 μmol/ml) was set as the substrate instead of enzyme, drawing the enzyme calculation standard curve. We added 0.5 ml A reagent to 0.05 ml substrates at 37 °C after blending for 10 min and then added the B reagents to induce