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Protein recovery and anti-nutritional factor removal from soybean wastewater by complexing with a high concentration of polysaccharides in a novel quick-shearing system

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ABSTRACT

In this study, to recover proteins and minimize anti-nutritional factor (ANF) levels in soybean wastewater, a novel quick-shearing system was used to obtain homogeneous electrostatic complexes between soybean whey protein (SWP) and a high concentration (4%, w/v) of polysaccharide. During processing, protein recovery and trypsin inhibitory activity loss induced by complexing with sulfated and carboxylated polysaccharides were investigated as function of polysaccharide type, pH, protein to polysaccharide mass ratio, and the physical state (solid, liquid). The removal of 80% of ANF levels and recovery of 90% of proteins were achieved using solid sulfated polysaccharides. Large amounts of particle complexes were observed for sulfated polysaccharides as compared to carboxylated polysaccharides by confocal laser scanning microscopy. A 100-kDa polyethersulfone ultrafiltration membrane was effective in recovering proteins (80%) from ι-carrageenan/SWP complexes, and a small amount of loss of the polysaccharides was observed for each cycle.

1. Introduction

With increasing consumption of soybean protein isolate in China, millions of tons of soybean whey are generated every year. These large quantity of soybean wastewater puts significant pressure on downstream processing steps and is challenging to dispose of, because its biochemical oxygen demand and chemical oxygen demand values range between 5000 and 8000 mg/L and 8000–20000 mg/L, respectively (Lv et al., 2013). Soybean wastewater contains Kunitz trypsin inhibitor (20 kDa), Bowman-Birk protease inhibitor (7.9 kDa), and soybean agglutinin (120 kDa), which are usually described as anti-nutritional factor (ANF). Although heat treatment is often used to inactivate ANF (Friedman et al., 1991), the huge volume of wastewater and the high thermal stability of the Bowman-Birk protease inhibitor limits its feasibility (DiPietro and Liener, 1989). There is also some evidence that the removal and recovery of ANF in soybean by-products may be environmentally significant, as it can help to reduce the biochemical oxygen and chemical oxygen demand values of wastewater.

The recovery of the proteins present in soybean wastewater, along with a reduction or elimination of ANF, could provide an attractive alternative resource with potential therapeutic value. It is well known that different forms of Bowman-Birk protease inhibitor (concentrated and purified forms) exhibit anti-cancer or cancer-preventive activity (Armstrong et al., 2000; Wang and Shen, 2000). Protein-polysaccharide interactions have long been studied as an eco-friendly method for protein recovery (Wibowo et al., 2005), protein purification (Du et al., 2014; Xu et al., 2011), and for bioactive compound delivery (Zhang et al., 2016).

However, neither protein recovery nor removal of ANF from soybean wastewater have been very successful. For example, chitosan, a well-studied cationic polysaccharide, has been widely used for protein recovery from wastewater, including milk whey (Savant and Torres, 2000), tofu whey (Jun et al., 1994), and seafood wastewater (Savant et al., 2003). However, a low recovery efficiency (< 50%) was observed when chitosan was added to soybean wastewater, due to the large amounts of uncomplexed soybean agglutinin and β-amylase (Li et al., 2017). Smith et al. (1962) obtained a relatively good recovery of proteins from soybean whey using negatively charged polysaccharides, including alginic acid, gum karaya; however the experiment was carried out in very dilute solutions, and has limited industrial applications. Therefore, the present study involves the use high concentration of a natural anionic polysaccharide solution to avoid the significant increase in the total volume of wastewater, which was beneficial in recovering proteins and removing ANF on a large scale.

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The choice of an appropriate protein-polysaccharide system is helpful in protein recovery and ANF removal. For instance, the recovery efficiency was low for carboxymethyl cellulose when the degree of substitution was approximately 0.8; whereas 70% of the protein recovery was obtained when carboxymethyl cellulose having a degree of substitution greater than 1.7 (Hill and Zadow, 1978). Protein can bind to sulfated polysaccharides at a neutral or alkaline pH (i.e., above of isoelectric point of protein); complexation with carboxylated polysaccharides at such pH values is weak or non-existent, and the system has also been reported to have thermodynamic incompatibility (Dickinson, 2003). Frequently, the same polysaccharide with a different charge density and chain flexibility has shown a varying protein binding ability; for example, Stone and Nickerson (2012) reported that λ-carrageenan formed stronger complexes with proteins than that of ι- and κ-carrageenan. Therefore, when different types of polysaccharides bind to proteins, the complexation behavior, such as phase separation, and complexes structures, is expect to vary with the polysaccharide properties such as the concentration and viscosity.

One challenge that was expected in this study is the fact that high concentration of polysaccharides might take long time to dissolve and form complexes with proteins, which could further affect the protein recovery and ANF removal. Therefore, a laboratory-assembled quick-shearing system was used in this study to promote protein-polysaccharide complexation and generate homogeneous electrostatic complexes. Then, the protein recovery and ANF levels as affected by polysaccharide type, pH, protein/polysaccharides (Pr:Ps) mixing weight ratios were investigated. Confocal laser scanning microscope (CLSM) was used to build the relationship between the microstructure of complexes and protein recovery; while ultrafiltration (UF) was used to separate the proteins from the protein-polysaccharide complexes.

2. Materials and methods

2.1. Materials

Cooled defatted soybean meal was provided by the Yuwang Ecological Food Industry Co., Ltd. (Dezhou, Shandong, China). It had a protein content of 55.4% (N × 6.25, dry basis) and a nitrogen solubility index of 85%. The sulfated polysaccharides, i.e. dextran sulfate, ι-carrageenan and λ-carrageenan were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Carboxylated polysaccharides: xanthan gum was purchased from Sigma Chemical Co. (St. Louis, MO, USA); carboxymethyl cellulose, arabic gum and sodium alginate were provided by Sinopharm Chemical Reagent Co. (Shanghai, China). All other reagents used were of analytical grade.

2.2. Preparation of soybean wastewater

According to our previous work (Li et al., 2016), soybean wastewater was prepared as follows: crushed soybean meal was added to deionized water at a solid/liquid ratio of 1:10, adjusted to pH 7.0 and stirred well for 30 min. Then, the mixtures were centrifuged at 4000 g for 30 min to remove the insoluble curd. The supernatant was adjusted to pH 4.5 with 1.0 M hydrochloric acid, and centrifuged again to remove the protein isolate. The remaining supernatant (soybean wastewater) was stored at 4 °C after 0.02% sodium azide was added as a preservative.

2.3. Quick-shearing system setup

A quick-shearing system (Fig. 1) was used to produce protein-polysaccharide complexes under controlled conditions, which was mainly consisted of a high-shear homogeneous mixer, a raw wastewater tank and a polysaccharide tank. The high-shear homogeneous mixer with the dimensions of 47.7 × 12.0 × 12.2 cm (L × W × H) was adapted from a commercial high-shear dispersing emulsifier (FLUKO, FA30 model, Shanghai, China). It consists of a high speed motor (a), which mixes and homogenizes materials through a dispersing agitator (b, 3.0 × 27.8 cm, diameter × length) into a stainless steel chamber (c) without jacket, connected to a polysaccharide tank and raw wastewater tank, as shown in Fig. 1. The soybean wastewater is introduced in the raw wastewater tank. The high concentration polysaccharide solution is introduced in the polysaccharide tank, and they are homogenized in the high-shear homogeneous mixer. Before starting the quick-shearing system, 1.0 L of soybean wastewater was firstly filled into raw wastewater tank, then the speed of high-shear homogeneous mixer was set to 18000 rpm, and the system was started. After 1 min running, the quick-shearing system was filled with raw wastewater, and then the desired amount of polysaccharide was added into the system through polysaccharide tank. Then the system was kept running for another 3 min, followed by the stop of the system, finally the produced protein-polysaccharide complexes were collected from the outlet placed on the base of the raw wastewater tank. The process is running in batch by batch.

2.4. Formation of protein-polysaccharide complexes

The protein-polysaccharide complexes was prepared using the quick-shearing system at desired weight ratios (from 1:1 to 7:1) of soybean whey protein to polysaccharide under different pH (pH 2.5, 3.5, 4.5) conditions. The produced mixtures was collected and centrifuged at 4000 g for 20 min to obtain the precipitated complexes.

2.5. Protein recovery and protein component analysis

The bicinchoninic acid method was used to determine protein content (Smith et al., 1985). As a control, the sample solution was replaced by the same volume of deionized water. All experiments were performed in triplicate.

Protein complexes and supernatant were analyzed by the Tricine–SDS-PAGE electrophoretic system (Schagger, 2006). Briefly, protein samples were dissolved at sample solutions and reduced with 100 mM DTT in boiling water bath for 3 min. After preparing for gel electrophoresis, 10 μL of the sample solution was loaded, and consecutive electrophoresis was carried out at a constant voltage of 30 V for the upper gel (4%) and 100 V for the lower gel (16%). After electrophoresis, Coomassie brilliant blue G-250 solution was used as the staining solution, and the gel was destained in 10% (v/v) acetic acid solutions.

2.6. Trypsin inhibitory activity assays

Trypsin inhibitory activity (TIA) was measured according to the method of Liu and Markakis (1989). Using Nα-benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPA) at 0.04% (w/v) as the substrate, 2 mL of trypsin 0.01% (w/v) in 0.04 mM HCl solution was added to start the reaction at 37 °C for 10 min. Then, 1 mL of 30% (w/v) acetic acid was added to terminate the reaction, and the absorbance was monitored at 410 nm using with a spectrophotometer (UV 2000, Unocal, Japan). Trypsin inhibitor units were calculated as the amount of inhibitor that reduced the absorbance per minute of the standard reaction by 0.01. For accuracy, the reaction was measured in the linear portion, in the 40%–60% inhibition range.

2.7. Confocal laser scanning microscopy

The microstructure of the soybean whey protein (SWP)-polysaccharides complexes were acquired with laser confocal microscopy (Leica TCS SP8, Heidelberg, Germany). Staining of the complexes was carried out by mixing a 1 mL aliquot of the complexes with 20 μL of fluorescein isothiocyanate (FITC, 1 mg/mL in ethanol) and 20 μL of Nile Red (1 mg/mL in ethanol), kept in the dark for 30 min. The samples were then placed on a microscope stage and the droplet distribution