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A novel soy protein isolate prepared from soy protein concentrate using jet-cooking combined with enzyme-assisted ultra-filtration

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ABSTRACT

A novel soy protein isolate (SPI) designed to be used in infant formulas was created using alcohol-bleached soy protein concentrate (SPC). This base was processed using jet cooking (JC) and enzyme-assisted ultrafiltration (UF) to improve the quality and safety of the protein. The results indicate that the nitrogen solubility index of the SPC can be greatly improved using a JC treatment at 130 °C, which increased from 8.8% to 85.4%. This process also increased the molecular weight of the SPC and improved the soluble aggregate protein yield. Enzyme-assisted ultrafiltration reduced the phytic acid content of the products from 20.59 mg/g (SPC) to 10.11 mg/g using only the phytase and to 5.80 mg/g using a composite treatment of phytase and acid phosphatase. The results indicate using a UF membrane with the MWCO of 80 kDa improved separation efficiency and reduced membrane fouling, which produced a product that was more suitable for use in infant formulas. Furthermore, the total isoflavones in the soy protein product were decreased to 70.0 mg/kg. The SPI product obtained from this process has potential application in infant formulas due to its improved solubility and the lower content of anti-nutritional factors.

1. Introduction

Soy protein is believed to have many health benefits. Since the U.S. Food and Drug Administration (FDA) claimed that the intake of soy protein might lower the risk of heart disease in 1999, the production and consumption of foods containing soy protein has increased dramatically in Western countries (Boye and Ribéreau, 2011). Commercial soy protein isolates (SPI) that are produced via traditional isoelectric precipitation are widely used in foods due to the high protein content (above 90%) and versatile functionality. SPI produced using traditional methods contains up to 200 non-protein phytochemicals, including residual fatty acids, lecithin, phytic acid, isoflavones, saponin, pigments and minerals (Fang et al., 2004). Although these components offer health benefits, some of them are not suitable for certain individuals, including infants and young children (Bousquet and Burney, 1993). It is commonly known that the phytic acid in soy protein products greatly limits the human body's ability to absorb certain minerals, including zinc and iron. Residual minerals within SPI products are also a concern, because the magnesium and aluminum content limits its application in infant formulas (Fomon and Ziegler, 1992). Evidence suggests that soy-based infant formulas may carry a risk of aluminum poisoning for infants (Martin, 1994; Sun and Wu, 2010; Tsou et al., 1991; Weintraub et al., 1986). Furthermore, the American Academy of Pediatrics has found that soy infant formulas contains 600–1300 ng/mL aluminum, which is much higher than the 4–65 ng/mL found in human milk (Fomon and Ziegler, 1992; Hawkins et al., 1994). Aluminum can compete with calcium for absorption, thus lowering bone calcification and affecting the central nervous system of infants. This is of particular concern for infants and children with renal dysfunction. As a kind of phytoestrogen, some negative effects of soy isoflavones have also been observed in infants and young children (Merritt and Jenks, 2004). *In vitro* experiments have shown that soy isoflavones could bind and activate human estrogen receptors (Kuiper et al., 1998). Therefore, minimizing the content of the non-protein components, including phytic acid, isoflavones, magnesium and aluminum, in soy protein products is the prerequisite for producing a SPI that can be safely used in soy infant formulas.

Alcohol leaching is commonly used in traditional SPC preparation, because it produces the light color and bland flavor that is

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usually desired in SPC products. It is also able to eliminate part of the anti-nutritional components in SPC, including soy oligosaccharides (such as stachyose and raffinose) which often cause human flatulence syndrome, as well as some soy allergens, including albumin and Kunitz trypsin inhibitor (Hathcock, 1990; Laroque et al., 2008). This indicates that commonly prepared SPC would be a good base material for the preparation of a novel soy protein product designed for sensitive individuals, including infants and children. Unfortunately, the soy protein subjected to alcohol leaching results in SPC with greatly reduced solubility. The nitrogen solubility index (NSI) of SPC was 10%, which limits its use as a food ingredient (Chajuss, 2001). Jet cooking (JC) has proven to be an effective method for re-functionalizing SPC (Wang and Johnson, 2001). When SPC is processed via JC, the steam is directly injected into the protein slurry through a restriction orifice, where the pressure and temperature are controlled using a holding tube. It had been found that the JC treatment can significantly improve the extractability, solubility, emulsification capacity, and foaming properties of the alcohol-leached SPC (Zheng et al., 2008).

In order to produce a novel SPI that was suitable for use in infant formulas, this study used alcohol-leached SPC as the base material, because of its light color and bland flavor. Then, jet cooking (JC) was used to re-functionalize the soy protein. Next, ultrafiltration (UF) using an exact cut-off of the membrane was employed to remove phytochemicals, including phytic acid and isoflavones, and to lower the content of magnesium and aluminum. There is a strong interaction between soy protein and phytic acid (Kumar et al., 2003); therefore, the protein slurry was processed with an enzymatic treatment using phytase and acid phosphatase prior to the UF process. Subsequent operations, including acid precipitation, re-dispersion, dialysis and drying, produced the novel SPI desired. Finally, the protein yield and solubility, as well as the content of the phytochemicals and residual minerals, within the resulting SPI were examined.

2. Materials and methods

2.1. Materials

The SPC was purchased from Qinhuangdao Goldensea Grain & Oil Co., Ltd. (China) and the defatted soy flour was obtained from Shandong Yuwang Industrial Co., Ltd. (China). The protein content of these two soy products were 63.6 ± 0.3% and 47.5 ± 2.1%, which was determined using the Dumas method ($N \times 6.25$, wet base) in a nitrogen/protein analyzer (Rapid N Cube, ElementarAnalysensysteme GmbH, Hanau, Germany). Phytase (20,000 U/g) was purchased from Zhuhai Yiduoli Bioengineering Co., Ltd. Acid phosphatase (10,000 U/g) was purchased from Shanghai EKEAR Biological Technology Co., Ltd. Papain (800 U/g) was purchased from Jiyun Biological Technology Co., Ltd. (China). Standards of sodium phytate, daidzin, glycitin, genistin, daidzein, glycitein and genistein were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The proteins (conalbumin, aldolase and thyroglobulin) used for the calibration of the molecular weight distribution curves in the size exclusion chromatography (SEC) analysis were purchased from GE Healthcare UK Ltd. (UK). All other chemicals were of analytical grade.

2.2. Preparation of typical SPI and novel SPI

The typical SPI was prepared from the defatted soy flour using the method described by Wang et al. (2012). The protein was extracted in the alkaline condition (pH 8.0) and then the isoelectric precipitation (pH 4.5) was performed to collect the soy protein fraction. Redispersion, dialysis and lyophilization were performed to complete the typical SPI preparation process.

The procedure used for producing the novel SPI is shown in Fig. 1. The SPC was suspended in deionized water, with a protein content of 6% (w/v), and then adjusted to pH 9.0 with 2 N NaOH. The initial volume of the solution was 1000 mL. After stirring for 2 h at room temperature, the slurry was processed in a colloid mill (LABOR-PILOT 2000/4, IKA, Staufen, Germany) with an angular speed of 1000 r/m, using the 0.15 mm grinding hole. The slurry was centrifuged at 3000*g* (Himac CR 22 G High-Speed Refrigerated Centrifuge, HITACHI, Japan) at 25 °C for 10 min to remove the insoluble matter. It was then subjected to the JC system (Nan-Liang Food Machine Company Co. Ltd., Guangzhou, China) at different temperatures (120, 130 and 140 °C) for 90 s. The JC treatment involved injecting steam into the protein slurry using a hydroheater to achieve transient heating and high shear. A tube was placed after the hydroheater to provide a 90-s residence time for the slurry. The slurry was then cooled to room temperature and adjusted to pH 5.0, which is the working pH for the subsequent enzymatic treatment. Phytase (1000 U/g protein) and acid phosphatase (0.5 U/g protein) were added and the slurry was incubated at 50 °C for 2 h. After the pH was adjusted to 8.0, it was subjected to a UF system that was composed of a peristaltic pump (ZT60-600, Baoding Longer Precision Pump Co., Ltd., China) and JM-S0522W hollow fiber membranes (Guangzhou Jason Membrane Technology Co., Ltd., China). Two polyethersulfone (PES) membranes with the molecular weight cut-offs (MWCO) of 10 and 30 kDa were used in the UF system. A polystyrene (PS) membrane with a MWCO of 80 kDa was also used. The total surface area of the membranes was 0.4 m^2. The transmembrane pressure was 0.2 MPa. The UF process was conducted at room temperature. The UF process was monitored using the volume concentration ratio (VCR) and the resulting score was a VCR of 4. The total permeation volume of the UF treatments was 2000 mL. The resulting supernatant was adjusted to a pH of 4.5 and centrifuged at 3000*g* (Himac CR 22 G High-Speed Refrigerated Centrifuge, HITACHI, Japan) at 25 °C for 15 min. The precipitate obtained using the novel method was washed twice and re-dispersed in deionized water (1:7, w/v), and neutralized to pH 7.0 with 2 N NaOH. It was then dialyzed against deionized water at 4 °C for 48 h through a dialysis membrane with an 8–14 kDa/mol molecular weight cut off and lyophilized. The resulting product was referred to as SPC-JCEU. The product processed with each of the steps used in the novel method except the UF process was referred to SPC-JCE. The product that had been produced without using either the enzymatic treatment or the UF process was referred to SPC-JC.

Membrane flux was jointly determined by the membrane's external force, resistance and properties. Membrane flux (J, L/m^2 h) was calculated as

$$J = \Delta V/(\Delta T \times A) \quad (1)$$

where ΔV was the sampling volume (L), ΔT was the sampling time (h), and A was the membrane's effective area (m^2). The record of the sampling volume started 5 min after the flow was stable. At the beginning, the sampling volume was recorded every minute. After 10 min, it was recorded every 10 min.

The protein retention rate (PRR) was calculated as

$$\mathrm{PRR} = (1 - C_p/C_o) \times 100\% \quad (2)$$

where C_p was the protein content in the permeate fluid (μg/mL), and C_o was the original solution (μg/mL).

2.3. Characterization of soy protein products

The protein content of the novel SPI was determined using the Dumas method ($N \times 6.25$). The NSI was determined using the AOCS