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Low temperature dry extrusion and high-pressure processing prior to enzyme-assisted aqueous extraction of full fat soybean flakes

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ABSTRACT

Oil, protein and solid extraction yields obtained during aqueous extraction processing (AEP) of full fat soybean flakes (FFSF), FFSF extruded at a die temperature of 100 °C and FFSF pressurised at 200 and 500 MPa for 15 min at 25 °C, were compared to those obtained during enzyme-assisted aqueous extraction processing (EAEP) using 0.5% of protease Protex 7L. Without enzyme addition, pretreatment of the FFSF with extrusion and 500 MPa increased and decreased, respectively, the oil extraction yield while protein extraction yield was significantly decreased after both treatments. The best treatment in terms of oil and protein recovery was EAEP of extruded flakes with 90% and 82% of oil and protein extraction yield, respectively, and 17% of free oil. Addition of protease during extraction significantly decreased the yield of isolated soy protein (ISP) due to an increased solubility of the proteins at pH 4.5. ISP from extruded EAEP had higher solubility at pH 7.0 and better functionality. The DSC results, combined with the protein extraction yields, showed that a proportion of the proteins became insoluble after extrusion and 500 MPa treatment, while only those extracted from 500 MPa FFSF had a reduced native state.

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1. Introduction

Aqueous extraction processing (AEP), which uses water as an extraction medium, is an alternative method for solvent oil extraction that has been applied to various oilseeds, including soybean, rapeseed and peanut (Rosenthal, Pyle, & Niranjan, 1996). The first reports on this process dated from the 1950s, with major work done in the 1970s. Since then, investigations on this topic have continued, but interest in this environmentally-friendly process has recently re-emerged, mainly because of increased regulatory concerns and potential opportunities to add value to the co-products (fibre and protein). This technology has other inherent advantages, including lower capital investment requirements and increased safety compared to hexane extraction, and might therefore correspond to the need to construct smaller processing plants in developed and developing countries (Lusas, Lawhon, & Rhee, 1982; Olsen, 1982). Hexane and aqueous extraction from oilseeds have in common that broken cells enhance/accelerate oil recovery (Nelson, Wijeratne, Yeh, Wie, & Wei, 1987; Wiese & Snyder, 1987). Cell wall disruption, which accelerates mass transfer and extraction kinetics, can be obtained either by mechanical or enzymatic treatment, or a combination of both. Interestingly, it is the combinatory pretreatment of soybean flakes by extrusion, followed by protease-assisted aqueous processing, that is the more efficient process to date for aqueous extraction of soybean oil (Freitas, Hartman, Couri, Jablonka, & Carvalho, 1997; Lamsal, Murphy, & Johnson, 2006). Extrusion is a mechanical process exposing material to high temperature, shear force and pressure over a short period of time (Nelson et al., 1987; Phillips, 1989; Rhee, Kuo, & Lusas, 1981). This combination of extreme processing conditions promotes both cell disruption and protein denaturation that seems to favour aqueous extraction of oil and proteolytic attack (Freitas et al., 1997; Lamsal et al., 2006). However, the exact mechanisms of this efficient combinatory process are still unclear. Better understanding of it would be beneficial to its transfer to other oilseeds and could provide co-products with desired properties.

Pretreatments other than extrusion, such as ultrasonication, pulsed electric fields, microwave heating and γ-irradiation, were applied in an attempt to improve oil extraction yield for various oilseeds prior to AEP, enzyme-assisted aqueous extraction (EAEP), or solvent extraction (Guderjan, Elez-Martinez, & Knorr, 2007; Shah, Sharma, & Gupta, 2005; Valentova, Novotna, Svoboda, Schwartz, & Kas, 2000). High-pressure processing (HPP) of food, which consists of applying pressures up to 600 MPa (~87,000 psi), is a newly developed, non-traditional technology. This process has mainly been investigated as an efficient non-thermal treatment to increase shelf-life of food products and inactivate food deterioration enzymes, while maintaining product nutritional characteristics and appearance (Norton & Sun, 2008). In few cases HPP has been studied for its potential to promote the release of compounds from their matrix (Kato, Katayama, Matsubara, Omi, & Matsuda,

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2000). There are currently no studies investigating the extractability of oil during aqueous extraction of soybean flakes pretreated with high pressure.

Maximum recovery of the oil fraction has been the driver for AEP optimisation; however, the simultaneous extraction of the soy proteins constitutes another major advantage of the process. The pretreatment applied to the soybean flakes to improve the oil extraction yield during aqueous extraction processing may affect the properties of the extracted proteins. In other words, a pretreatment that may be beneficial to a maximum oil extraction yield may be detrimental to the properties of the extracted proteins. Soy protein isolates obtained from defatted soybean material are often treated with protease to improve their functionality, particularly to increase their solubility over a wider pH range (Henn & Netto, 1996; Puski, 1975). On the other hand, protein denaturation/precipitation is promoted during extrusion. Therefore, the combination of pressure, high shear and high temperature applied to FFSF during extrusion, followed by the addition of protease during AEP, could be potentially detrimental or beneficial, respectively, to the functionality of the protein fractions recovered from the process. Besides, extensive literature is available on the properties of isolated proteins from defatted soy meal, which is the co-product obtained from soybean oil extraction, and the starting material used by the industry to produce edible soy product ingredients. Scarce information is available on the properties of soy proteins obtained from full fat soybean. The objectives of this study were to first compare the extraction yields of oil, protein and solids during aqueous extraction, assisted or not with protease treatment, of FFSF pretreated with dry extrusion and high-pressure processing. AEP and EAEP of FFSF served as baseline controls for evaluating effects of each pretreatment. Second, the effects of processing on the recovery and composition of the isolated soy proteins and their physicochemical and functional properties were determined.

2. Materials and methods

2.1. Full fat soy flakes preparation

FFSF were prepared at the Center for Crops Utilization Research at Iowa State University from variety 92M91 soybeans harvested in 2006 in Iowa, USA. The soybeans were cracked in a roller mill (Model: 10X 12SGL, Ferrell-Ross, Oklahoma, OK) and aspirated by using a cascade aspirator (Kice Metal, Wichita, KS) to separate the hulls. The de-hulled soybeans were conditioned to 60 °C using a triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH), and were flaked using a smooth-surfaced roller mill (Roskamp Mfg Inc., Waterloo, IA) to ~0.30 mm thickness. The flakes were sealed and stored in plastic bags at 4 °C prior to use. Before extrusion and high-pressure processing, the soy flakes were adjusted to the desired moisture level of ~15% by adding water to the flakes in a Gilson mixer (Model: 59016A, St. Joseph, MO). The moisture-adjusted flakes were then placed in double polyethylene bags and kept at 4 °C until used. These flakes contained 19% oil (dry basis) and 32% crude protein (dry basis).

2.2. Extrusion

The extrusion was carried out in a Micro 18 twin-screw extruder (American Leistritz Extruders, Somerville, NJ, USA). The unit was equipped with a die having one 4 mm diameter, 12 mm long opening. The length and diameter of each screw were 540 and 18 mm, respectively. The screw configuration used in the experiments consisted of conveying elements (L/D = 10), kneading element (L/D = 4.4), conveying element (L/D = 1.6), kneading element (L/D = 4.4), conveying element (L/D = 1.6), kneading element (L/D = 2.2) and a conveying element (L/D = 5). The barrel consisted of six independently controlled heating barrels. The barrel temperatures, measured via Fe–CuNi thermo elements inserted in the bottom of each barrel, during the process were: feed barrel (30 °C), barrel 1 (70 °C), barrel 2 (100 °C), barrel 3 (100 °C), barrel 4 (100 °C) and barrel 5 (100 °C). Soy flakes were fed into the unit with an Accu-rate dry material feeder (Accu-rate Inc., Whitewater, WI, USA) and processed via the intermeshing co-rotating screw at a constant rpm of 100. Processed material was fed through until equilibrium conditions were reached before material was collected for experimental use. Extruded soy pellets (~80 g) were collected directly in half the amount of the water needed for extraction. The water was at room temperature.

2.3. High-pressure processing

One hundred grammes of soy flakes and 300 g of distilled water were transferred into a polyester bag (Sealpaks, KAPAK, Minneapolis, MN) and sealed such that the headspace was kept to a minimum (Multivac Inc., Kansas City, MO). The samples were pressurised at 200 and 500 MPa at an initial temperature of 25 °C for a dwell time of 15 min using a Food-Lab 900 High-Pressure Food Processor (Stansted Fluid Power Ltd., Stansted, UK). The sample holder had a 6.5 cm internal diameter and 23 cm height. The rates of pressurisation and depressurisation were 260 and 500 MPa/min, respectively. The pressurisation fluid was a 1:1 mixture of 1,2 propanediol to water (GWT Global Water Technology Inc., Oakbrook Terrace, IL). The temperature increase of the pressurisation fluid due to adiabatic heating was ~3 °C/100 MPa. Each treatment was conducted in triplicate.

2.4. Aqueous extraction and separation of the different fractions

Extractions were conducted in a temperature-controlled water bath (Acrylic open bath with Isotemp 2150 circulator, Fisher Scientific, USA) at a constant speed of 300 rpm with lab-stirrer LR400C (Fisher Scientific, USA) at a flake to water ratio of 1:10. After 1 h of reaction at 50 °C and pH 7.0, the pH was raised to 8.0 and the extraction was carried out for another 15 min. This procedure constituted the standard aqueous extraction process (AEP). For the enzyme-assisted aqueous extraction processing (EAEP), Protex 7L was added at a dose of 0.5% (w/w, on the dry basis of soy flake). Protex 7L (EC 3.4.21.62 and EC 3.4.24.38) is a bacterial neutral protease preparation, with mainly endopeptidase activities, derived from the controlled fermentation of a non-genetically modified strain of *Bacillus amyloliquefaciens*. Its optimum pH varied from 6.0 to 8.0, and optimum temperature from 40 to 60 °C. This enzyme was kindly provided by Genencor International Inc. (Rochester, NY). During AEP and EAEP, the pH was maintained at a constant value with the addition of 2 N NaOH. Separation of the liquid and insoluble residue was carried out by centrifugation with a JS 4.0 swinging bucket rotor (Beckman Coulter Inc., USA) at 3000×*g* for 15 min at room temperature. After centrifugation, the insoluble fraction was oven-dried at 130 °C overnight and the liquid fractions (cream, free oil layer and skim) were transferred into a funnel and stored at 4 °C overnight. The skim was then separated from the cream and free oil layer (Fig. 1). The cream and free oil fractions were transferred into 30 ml tubes and allowed to decant again at 4 °C overnight. When a small amount of residual skim appeared at the bottom of the cream layer after refrigerated overnight storage, it was collected and added to the previously collected skim fraction.

2.5. Proximate analysis

Oil, crude protein and solids analysis were carried out on the skim, insoluble fraction, cream layer and FFSF. Total oil and crude

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