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Demulsification of oil-rich emulsion from enzyme-assisted aqueous extraction of extruded soybean flakes

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ABSTRACT

Extraction of soybean oil from flaked and extruded soybeans using enzyme-assisted aqueous extraction processing (EAEP) is a promising alternative to conventional hexane extraction. The efficiencies of four proteases releasing oil from extruded material were compared. Protex 51FP, Protex 6L and Protex 7L each extracted 90% of the total oil available while Protex 50FP gave similar extraction yield as the control (without enzyme treatment). During EAEP, however, a stable emulsion forms that must be broken in order to recover free soybean oil. The potential of various proteases and phospholipases to destabilize the emulsion was determined. Two enzymes, a phospholipase A2 (LysoMax^{TM}) and a protease (Protex 51FP) were selected to determine the effect of enzyme concentration on demulsification. Although at a 2% concentration (w/w, enzyme/(cream + free oil)), each enzyme tested was effective in totally destabilizing the cream; the protease released significantly more free oil than did the phospholipase at concentrations less than 2%. At 0.2% concentration, 88 and 48% of free oil were obtained with the protease and phospholipase, respectively. Reducing the pH of the cream also destabilized the cream with maximum demulsification at the isoelectric point of soy proteins. These results provide destabilization strategies for the oil-rich emulsion formed during aqueous extraction processing of extruded flakes and significantly contribute to the development of this environmentally–friendly technology.

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1. Introduction

Hexane extraction process is the most cost-effective means of recovering oil from soybeans; however, hexane has substantial safety issues, including the risk of fire and explosion, as well as exposure risks to personnel. Furthermore, hexane extraction process requires expensive plants that are equipped to handle the solvents, as well as to comply with appropriate safety measures. Moreover, the emission of volatile *n*-hexane into the atmosphere contributes to the formation of ozone, which is restricted by the US Environmental Protection Agency. Therefore, the soybean crushing industry is actively looking for alternative processes.

Aqueous extraction processing (AEP), which uses water as an extraction and separation medium, has regained considerable interest during the past decade as an environmentally–friendly approach for extracting oil from oilseeds. However, AEP, which is based on the insolubility of oil in the extraction medium rather than its dissolution as applied in hexane extraction, is less efficient in achieving high oil yields (Johnson and Lusas, 1983; Rosenthal et al., 1996). Early studies have reported soybean oil extraction yield from 65 to 75% via AEP in comparison to higher than 95% via hexane extraction (Johnson, 2000; Rosenthal et al., 1996). Size of the starting soybean material is one of the most important parameters affecting oil extraction when using AEP; reducing the oil droplet size from 400 to 100 μm in the soy flour increased oil extraction from 33 to 64% (Rosenthal et al., 1998). Disrupting cell walls of soybeans is important to release oil from oil bodies; flaking and then extruding was reported to be the most effective physical treatment increasing oil extraction to 71%. This value, however, indicates that rupturing the cell walls or severely diminishing cell wall integrity of all the cells by flaking and extruding does not completely eliminate all barriers to extraction. To further increase the oil extraction yield, enzymes are needed to break down the proteins in the cell wall and pseudo-membranes surrounding the oil bodies to eliminate/reduce barriers to oil extraction. This process is called enzyme-assisted aqueous extraction processing (EAEP) or aqueous enzyme extraction (AEE). Proteases improved oil extraction from both soybean flour and extruded soybean flakes, but combining extrusion with enzyme treatments yielded more free oil than enzyme treating flour alone (Freitas et al. 1997; Rosenthal et al., 1996). Thermoplastic extrusion facilitates the accessibility of enzymes to proteins surrounding oil bodies (Freitas et al., 1997). Oil extraction yield of 88% has been reported for extrusion-aided EAEP when using a mixture of Celuclast 1.5L and Alcalase 2.4L (Freitas et al., 1997) achieved a similar extraction level, but at a much lower

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enzyme dosage using a single protease and for a significantly shorter incubation time, i.e., 90 min versus 6 h. Therefore, different proteases showed different oil extraction efficiencies.

While recent studies are reporting oil extraction efficiencies that are competitive with hexane extraction, there is another challenge to overcome before the industry will consider AEP. Unlike hexane extraction, much of the oil extracted with AEP is present in the form of a stable cream (oil-in-water emulsion). Many studies carried out on AEP extraction have reported oil extraction efficiencies, but few mentioned free oil recovery. Soybean proteins, which are co-extracted with soybean oil, are known as good emulsifiers (Aoki et al., 1980). Soybeans also contain phospholipids, particularly lecithin, which is an excellent emulsifier. These compounds probably act as surfactants and participate in the formation and the stabilization of this emulsion. The objectives of the present study were to compare the efficiencies of different proteases in extracting oil during AEP of extruded soybean flakes and to identify strategies to destabilize the emulsion and recover free oil.

2. Methods

2.1. Materials

Full-fat soy flakes were prepared from variety IA1008 soybeans harvested in 2002. The soybeans were cracked in a roller mill (model 10X 12SGL, Ferrell–Ross, Oklahoma, OK, USA) and aspirated by using a cascade aspirator (Kice Metal, Wichita, KS, USA) to remove the hulls. The dehulled soybeans were conditioned to 60 °C using a triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH, USA) and were flaked using a smooth-surfaced roller mill (Roskamp Mfg, Inc., Waterloo, IA, USA) to ~0.30 mm thickness. The flakes contained 23.8% oil (dry basis) and 9% moisture. The flakes were stored in sealed plastic bags at 4 °C until use. Before extruding, 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, USA). The moisture–adjusted flakes, i.e., conditioned full-fat flakes, were then placed into double polyethylene bags and kept at 4 °C until use.

All enzymes, LysoMax™ (E.C. 3.1.1.4, phospholipase A2, optimal pH 8.0, optimal temperature 40 °C), G-ZYME® G999 (E.C. 3.1.1.32, lysophospholipase, optimal pH 4.5, optimal temperature 50 °C), Protex 6L (alkaline serine endopeptidase, optimal pH 8.0, optimal temperature 50 °C), Protex 50FP (acid fungal endopeptidase–exopeptidase complex, optimal pH 4.5, optimal temperature 50 °C), Protex 51FP (neutral fungal endopeptidase–exopeptidase complex with high aminopeptidase activity, optimal pH 8.0, optimal temperature 50 °C), and Protex 7L (neutral metallo endopeptidase, optimal pH 7.0, optimal temperature 50 °C) were provided by Genencor Division of Danisco (Rochester, NY, USA).

Petroleum ether "A", boiling point 40–45 °C, was bought from Fisher Scientific (Pittsburg, PA, USA). All other chemicals were analytical grade and were purchased from Sigma (St. Louis, MO, USA).

2.2. Extrusion and enzyme-assisted aqueous extraction processing (EAEP)

A twin-screw extruder (18 mm screw diameter, Micro 18, American Leistritz Extruders, Somerville, NJ, USA) was used for all extrusion trials using methods already reported. In brief, conditioned full-fat soy flakes were extruded at 100 °C barrel temperature and 100 rpm rotational speed with a high-shear screw configuration. The extruded soy pellets (~200 g) were collected directly into room temperature water in about one-half the amount of water needed for extraction. Additional water was added to achieve flakes to water ratio (w/w) of 1:10. The beakers were placed into a temperature-controlled water bath and stirred to disperse the slurries. The pH and temperature of the slurries were then adjusted to their optima as recommended by the enzyme supplier and enzyme was added at a 0.5% dose (w/w, on dry weight of soy flakes). The reactions were maintained for 1 h at stable temperature and pH before centrifuging. Separation was conducted in an SLA-3000 fixed-angle rotor (Sorvall RC5B Plus, Newtown, CT, USA) at 3000×*g* for 15 min to separate insoluble materials from the each slurry. The cream, free oil, and skim fractions for the each slurry were poured into a glass separatory funnels and allowed to settle overnight at 4 °C to separate the skim from the (cream + free oil) fraction.

2.3. Demulsification procedure

Demulsification experiments were carried out on the (cream + free oil) fraction from slurry obtained by using 0.5% Protex 7L during the extraction step using the conditions described above. A large amount of (cream + free oil) fraction (~320 g) was prepared by using ~1.2 kg of soybean flakes. Aliquots of ~20 g of (cream + free oil) fraction were poured into 50 mL plastic centrifuge tubes. Once the optimum pH and temperature for the enzyme tested were reached, the enzyme was added and the reaction was carried out with constant stirring using a ThermoScientific Variomag multi-point inductive-drive stirrer with external control (Thermo Fisher Scientific, Waltham, MA, USA) submerged in a water bath (Polystat® temperature controller, Cole-Parmer Instrument Company, Vernon Hills, IL, USA) for 90 min. The control was treated with the same stirring, pH, incubation time and temperature, but without enzyme addition. To study the effects of pH on emulsion destabilization, the pH of the cream was adjusted by the addition of 2N NaOH or 2N NaOH until the desired pH value was reached, and the pH-adjusted cream was incubated at 50 °C for 15 min with constant stirring. The control corresponded to the cream at its natural pH. Following centrifugation at 100×*g* for 5 min, free oil was then collected into tared beakers by decanting as previously reported (Lamsal and Johnson, 2007). Petroleum ether "*A*" was added to the centrifuge tubes to solubilize any oil adhering to the walls of the centrifuge tubes. The solvent was evaporated and the weight of the oil was determined. The residual cream in the tubes was also weighed. Oil contents in the residual cream and other EAEP fractions were determined as described below and compared to the total oil in the extruded flakes.

2.4. Oil determination

Total oil contents of full-fat soy flour, full-fat soy flakes, extruded full-fat flakes and AEP fractions were determined in duplicate by using the Mojonnier method (AOAC, 1990 – AOAC Method 922.06 for solid samples, and AOAC Methods 995.19 and 989.05 for cream and skim fractions, respectively). The oil yield (%) was calculated by the oil amount in fraction (g) divided by the initial weight of oil in flakes and was expressed as a percentage. The total oil yield of each treatment was expressed by the addition of oil yields in skim, insolubles and cream. This value was standardized to 100%. The free oil yield (%) was the ratio between the weight of free oil obtained after demulsification and the weight of oil in the initial (cream + free oil) fraction multiplied by 100.

2.5. Oil droplet size analysis

The oil droplet size distribution in the cream emulsion was measured using a laser light scattering instrument (Mastersizer 2000S, Malvern Instruments, Ltd., Chicago, IL, USA). The refractive index ratio of 1.10 was used to calculate the oil droplet size distributions. Cream samples were analyzed immediately after funnel separation and treated samples were analyzed right after incubat-