Bioresource Technology 100 (2009) 5340–5347

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Factors affecting emulsion stability and quality of oil recovered from enzyme-assisted aqueous extraction of soybeans

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ARTICLE INFO

Article history:
Received 5 November 2008
Received in revised form 12 March 2009
Accepted 14 March 2009
Available online 30 June 2009

Keywords:
Soybeans
Enzyme-assisted aqueous extraction
Enzyme demulsification
Emulsion stability
Protease

ABSTRACT

The objectives of the present study were to assess how the stability of the emulsion recovered from aqueous extraction processing of soybeans was affected by characteristics of the starting material and extraction and demulsification conditions. Adding endopeptidase Protex 6L during enzyme-assisted aqueous extraction processing (EAEP) of extruded soybean flakes was vital to obtaining emulsions that were easily demulsified with enzymes. Adding salt (up to 1.5 mM NaCl or $MgCl_2$) during extraction and storing extruded flakes before extraction at 4 and 30 °C for up to 3 months did not affect the stabilities of emulsions recovered from EAEP of soy flour, flakes and extruded flakes. After demulsification, highest free oil yield was obtained with EAEP of extruded flakes, followed by flour and then flakes. The same protease used for the extraction step was used to demulsify the EAEP cream emulsion from extruded full-fat soy flakes at concentrations ranging from 0.03% to 2.50% w/w, incubation times ranging from 2 to 90 min, and temperatures of 25, 50 or 65 °C. Highest free oil recoveries were achieved at high enzyme concentrations, mild temperatures, and short incubation times. Both the nature of enzyme (i.e., protease and phospholipase), added alone or as a cocktail, concentration of enzymes (0.5% vs. 2.5%) and incubation time (1 vs. 3 h), use during the extraction step, and nature of enzyme added for demulsifying affected free oil yield. The free oil recovered from EAEP of extruded flakes contained less phosphorus compared with conventional hexane-extracted oil. The present study identified conditions rendering the emulsion less stable, which is critical to increasing free oil yield recovered during EAEP of soybeans, an environmentally friendly alternative processing method to hexane extraction.

1. Introduction

Due to growing environmental concerns over using hexane to extract edible oil from oilseeds, aqueous extraction processing (AEP) wherein water is used as a separation medium, has regained attention. In addition to being an organic-solvent-free process and enabling simultaneous extraction of oil and protein fractions, AEP has the potential to produce oil that requires less refining (i.e., degumming may be eliminated) due to low phospholipid content in the recovered oil (Rosenthal et al., 1996; Bocevska et al., 1993; Embong and Jelen, 1977). To be competitive with the current hexane process, which is currently used to produce vegetable oil in the United States, AEP oil extraction yields need to be close to the 95% yield that is obtained with solvent extraction.

Early AEP investigations were not promising with only 60% of the total oil being extracted, but recent improvements including the pretreatment of the flakes with extrusion followed by enzyme-assisted aqueous extraction (EAEP) give high extraction yields and renew opportunities for this process. The formation of a stable cream emulsion, in which most of the oil is distributed, remains a serious challenge to the commercial viability of any aqueous extraction procedure. Total destabilization of the cream to release the oil contained in this emulsion is required.

Previous attempts to break the emulsions formed during AEP of various oilseeds (i.e., coconuts, peanuts, rapeseed, sunflower and soybeans), include using phase inversion (Sugarman, 1956; Lusas et al., 1982), freezing and thawing (Roxas, 1963; Embong and Jelen, 1977; Chabrand et al., 2008), shearing (Hagenmaier et al., 1972, 1973; Rhee et al., 1972), chilling and thawing (Gunetileke and Laurentius, 1974; Chabrand et al., 2008), microfiltration (Del Colle et al., 2007), pH adjustment (Rhee et al., 1972), and phospholipase and protease treatments (Lamsal and Johnson, 2007; Wu et al., 2009; Zhang et al., 2007).

Two strategies have been recently reported to perform well in demulsifying emulsions recovered from EAEP of extruded soy flakes: (i) acid treatment with hydrochloric acid (pH 4.5); and (ii) enzyme hydrolysis by using an alkaline serine endopeptidase (2.5%, 50 °C, 90 min) (Wu et al., 2009; de Moura et al., 2008). Optimization of the enzymatic demulsification conditions, however, has not yet been conducted. While direct effects of physical, chemical and enzyme treatments on the stability of emulsions recovered

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doi:10.1016/j.biortech.2009.03.087

during aqueous extraction of oilseeds have been reported, our understanding of the effects of extraction parameters, such as properties of the starting material and extraction conditions, on the stability of the emulsion towards demulsification is limited.

The present study aims to identify parameters that might affect both free oil recovery during the aqueous extraction step and the stability of the cream emulsion, including: (i) characteristics of the starting material (full-fat soybean flour, full-fat soybean flakes and extruded flakes) and presence of salt; (ii) presence of enzyme during extraction (AEP vs. EAEP); (iii) extraction conditions; and (iv) duration of refrigerated storage of extruded flakes (from 1 week to 3 months). Secondly, post-extraction parameters, such as presence of free oil and enzyme treatment conditions (temperature, incubation time and protease concentration) on the stability of the emulsion recovered from EAEP of extruded flakes need to be established. Finally, the partitioning of the protease activity in the different fractions recovered from the EAEP of extruded flakes needs to be determined and the qualities of EAEP-extracted oil compared to hexane-extracted oil.

2. Methods

2.1. Materials

Full-fat soy flour was obtained from Natural Products Inc. (Grinnell, IA, USA). Full-fat soy flakes were prepared from variety IA 92M91 soybeans. Protex 6L (alkaline serine endopeptidase, optimum pH and temperature of 8.0 and 50 °C, respectively) and LysoMax™ (E.C. 3.1.1.4, phospholipase A2, pH and temperature optima of 8.0 and 40 °C, respectively) were provided by Genencor International, Division of Danisco (Rochester, NY, USA). Hexanes (H302-4), ethyl alcohol (A407-1), ethyl ether (E138-4), NaOH (S318-3), HCl (A144-500), NaCl (S271-500) and $MgCl_2$ (M33-500) were from Fisher Scientific (Fair Lawn, NJ, USA). Petroleum ether (9272-03) was from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Deionized water was used in all experiments unless specified otherwise. Milli-Q water was prepared with Milli-Q reagent water system (Millipore Corp., Billerica, MA, USA).

2.2. Preparing full-fat soy flakes

The soybeans were cracked by using a corrugated roller mill (model 10X12SGL, Ferrell-Ross, Oklahoma City, OK, USA) and aspirated in a multi-aspirator (Kice, Wichita, KS, USA) to separate into meats and hulls fractions. The meats were conditioned to 60 °C using a triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH, USA). The conditioned meats were flaked by using a smooth-surfaced roller mill (Rosskamp Mfg Inc., Waterloo, IA, USA) to approximately 0.25-mm thickness and 3–5 mm width. For satisfactory extrusion, the flakes were adjusted to 15% moisture content by spraying water onto the flakes while tumbling in a Gilson mixer (model #59016A, St. Joseph, MO, USA). The moistened flakes were then placed into double polyethylene bags and kept at 4 °C until used.

2.3. Extrusion

The moistened and tempered flakes were extruded using a Micro 18 twin-screw extruder (American Leistritz Extruders, Somerville, NJ, USA). The unit was equipped with one 4-mm diameter and 12-mm long opening die. The length and diameter of each screw was 540 and 18 mm, respectively. The screw configuration consisted of conveying elements (length/diameter (L/D) = 10), a kneading element (L/D = 4.4), a conveying element (L/D = 1.6), a kneading element (L/D = 4.4), a conveying element (L/D = 1.6), a kneading element (L/D = 2.2), and a conveying element (L/D = 5). The barrel consisted of six independently controlled heating sections. The barrel sections had jackets in which air was circulated at controlled flow rates via solenoid valves to achieve constant temperatures during processing (the feed barrel was cooled with water instead of air). The temperatures of each barrel section, measured via Fe–CuNi thermo elements inserted into the bottom of each barrel section, were: feed section, 30 °C; section 1, 70 °C; section 2, 100 °C; section 3; 100 °C, section 4, 100 °C; and section 5, 100 °C. Soy flakes were fed into the unit using an Accu-rate dry material feeder (Accu-rate Inc., Whitewater, WI, USA) and extruded using intermeshing co-rotating screws at 100 rpm speed.

2.4. Storing extruded flakes

After extrusion, extruded flakes were immediately used for extraction or stored in a refrigerator at 4 °C and in an incubator at 30 °C for up to 96 days.

2.5. Aqueous extraction

About 300 g of extruded flakes were collected in a 4-L flask containing 1500 g of water at room temperature. Once transferred to a 3- or 4-L jacketed glass reactor with a bottom drain valve (Chemglass, Vineland, NJ, USA), additional hot water was added to achieve 1:10 flakes-to-water ratio. The temperature was raised to 50 °C via a water circulator (HAAKE Phoenix P1, Thermo HAAKE, Portsmouth, NH, USA). The pH was adjusted to 9.0 with 2 N NaOH. Unless stated otherwise, Protex 6L was added to the slurry at 0.5% w/w (g enzyme/g flakes, as is) and the slurry was stirred via an external stirrer at 600 rpm for 1 h. The pH of the slurry was kept nearly constant by adding 2 N NaOH. When the same operations were conducted without enzyme, the procedure was termed AEP. After the 1-h holding period, the AEP or EAEP slurry was then cooled in a 4 °C refrigerator for about 1 h.

$MgCl_2$ and NaCl at 0%, 0.5%, 1.0% and 1.5% (w/w) concentrations were added to full-fat soy flour, full-fat soy flakes and extruded flakes. The slurry was prepared with Milli-Q water following the procedures reported above.

2.6. Fractionation prior to demulsification optimization experiments

Once cooled, the slurry was centrifuged at 20 °C and 3000×g for 15 min (Avanti J-20 XPL, Beckman Coulter, Fullerton, CA, USA) to separate the insolubles fraction from the other liquid fractions (skim, cream and free oil). The liquid fractions were transferred to separatory funnels and stored overnight at 4 °C. The skim and the [cream + free oil] fractions were then separated and the [cream + free oil] fractions were combined in a separatory funnel and stored overnight at 4 °C. Any remaining skim after the overnight holding period was removed. The [cream + free oil] was mixed well and a sample taken to determine oil content. To determine the effects of free oil on demulsification, the free oil fraction was removed prior to mixing and a sample was taken to determine oil content.

The oil extraction yield was determined as

$$\text{Oil extraction yield } (\%) = 100 - \left[\frac{(\text{oil(g) in starting material, db}) - (\text{oil(g) in insoluble fraction, db})}{\text{oil(g) in starting material, db}} \times 100\right] \quad (1)$$