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Single-step enzyme processing of soybeans into intact oil bodies, protein bodies and hydrolyzed carbohydrates

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ABSTRACT

To maximize value and minimize waste in soy processing, all major soybean components, i.e., oil, protein and carbohydrate, should be collected and utilized. Existing processing was originally designed to maximize oil extraction. It tends to make protein and carbohydrate separation from remaining meal more difficult and reduce their value. In this study, a single-step sonication-assisted enzyme processing was developed to separate and collect intact oil bodies, protein bodies and hydrolyzed carbohydrates from soybeans. Confocal laser scanning microscopic observations confirmed enzymatic destruction of cell wall and separation of oil bodies and protein bodies by centrifugation. Distributions of oil, protein and carbohydrate were quantitated in the top oil/cream layer, bottom protein-enriched precipitates, and the middle aqueous solution. Effects of the soybean particle size and the concentration of an *Aspergillus niger* enzyme were investigated. A pulsed ultrasonic treatment at 1.5 W/ml, for 5 min every 3 h during the enzyme processing, was found to significantly improve the performance and separation of oil bodies from protein. 87% of oil bodies were collected from cracked particles of 0.42–1.19 mm in size using, per g particles, 2 ml enzyme that contained 0.62 FPU/ml cellulase, 93 U/ml xylanase, 5.8 U/ml pectinase and 6.4 U/ml α-galactosidase.

1. Introduction

A soybean consists of four parts: a soybean hull (outside coat) (8%), the plumule and hypocotyl-axis (2%), and two large cotyledons (90%) [1]. The cotyledons comprise of cylindrical (30 μm × 70 μm) cells filled with protein and oil [2]. Each cell has a protective wall that is made up of polymeric carbohydrates such as cellulose, hemicellulose and pectin [3]. Protein is mostly packaged as nearly spherical protein bodies of 2–10 μm in diameter. Oil is stored as much smaller oil bodies, approximately 0.2–0.5 μm in diameter (also referred to as oleosomes and spherosomes by researchers) [4]. Oil bodies fill the space between protein bodies. Oil bodies are stabilized by a phospholipid layer interspersed with the amphipathic proteins oleosins.

Accordingly, soybeans contain three main component groups: about 18–20% oil, 40% protein and 25–30% carbohydrate (12–18% insoluble and 12–15% soluble) [1]. It is desirable to make use of all these components; however, because soybean uses have evolved historically in different parts of the world, various processes have been developed that make a wide range of soy products from different main component groups [5] and leave the other components in different chemical and/or physical forms [6,7]. These remaining materials have high contents of biodegradable organics. If not recovered for uses, they still need to be processed as waste prior to disposal at substantial costs. Because of their different properties [8,9], these byproducts or wastes from diverse processes may need different series of processing methods [10–12]. Customizing these processing methods can be difficult and expensive. There are significant benefits to develop a simple, unified soy processing that effectively recovers and separates all major components in the soybeans.

Current large-scale soy processing typically collects oil by hexane extraction. Besides being from the non-renewable petroleum source, hexane is highly flammable and poses significant hazards to personnel and property. Hexane-free enzyme-assisted aqueous processes have been investigated recently for oil extraction from soybeans [13,14] and other oilseeds [15,16]. For soybean oil, the starting materials used in these studies were full-fat soy flakes and flour [17,18]. Protease was a common enzyme used to assist extraction [13], presumably because it was thought that breakdown of protein, the major component, would facilitate release of the oil dispersed and trapped in the solid mixture. Protease indeed improved oil extraction [17] but it also caused substantial protein loss and the associated economic penalty. There are also reports on using carbohydrase to improve oil extraction. Passos et al. extracted grape seed oil by treatment with an enzyme cocktail of cellulase, protease, xylanase and pectinase [19]. In another report, the

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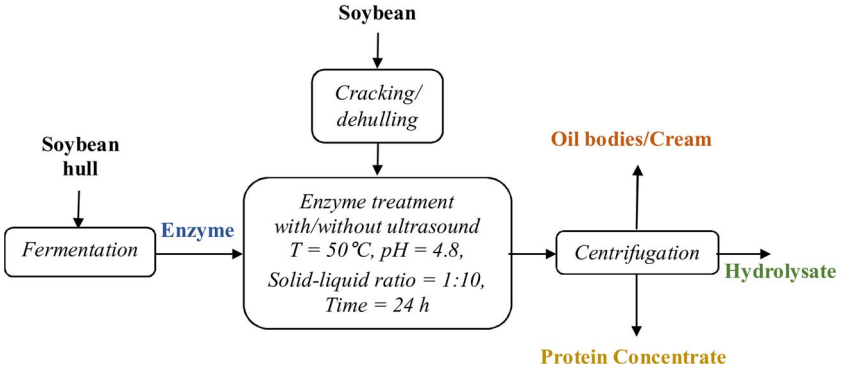

Fig. 1. A block diagram of the enzyme-based soybean processing developed in this study for collection of oil bodies/cream, hydrolysate and soy protein concentrate.

aqueous extraction of canola oil was improved by enzyme treatment with protease, pectinase and cellulase [20]. These studies were often done for increasing oil extraction, without emphasis on collection of intact oil bodies. Kapchie et al. [21] reported improved oil body extractability from soy flour after 20 h incubation with a mixture of cellulase, hemicellulase and pectinase. Collection of other important components (protein and carbohydrate) was however not studied. Intact oil bodies can find many high-value uses that involve stable oil-in-water systems, such as in food, cosmetic and pharmaceutical applications [22]. Oil bodies provide excellent stability of oil/water mixtures that otherwise requires addition of high levels of surfactants [23].

The soy flakes and flour used in previous studies of enzyme-assisted aqueous extraction were pretreated by methods such as extrusion, heat treatment, grinding and pressing [17,18]. These pretreatments destroyed the structures of cells and subcellular protein and oil bodies, resulting in complex mixtures of all components that would tend to be more difficult to separate than if the protein and oil bodies were kept intact and not mechanically mixed by the pretreatments. Of major concerns for food and feed uses are the indigestible oligosaccharides and insoluble carbohydrate that are not removed during, e.g., protein concentrate preparation, causing lower digestibility for monogastric animals and fish. This significantly decreases the value of soy protein and carbohydrate. Soybean meal is currently used mainly in animal and aquaculture feed that does not require higher protein contents [24,25]. If protein and carbohydrate can be separately collected, they can be used for higher value purposes; protein as high quality food and feed protein ingredient and carbohydrate as fermentation feedstock [26] for production of fuel and value added chemicals [27,28]. For example, effective production of arabitol (a xylitol isomer) by yeast (*Debaryomyces hansenii*) fermentation using enzymatic hydrolysates of soybean meal and hulls has been reported recently [29]. Using high solid loadings in the hydrolysis, the hydrolysate collected is suitable for fermentation without further concentration steps. It is desirable to develop a new processing method that collects intact oil bodies and separates protein and carbohydrate effectively.

The objective of this study is to meet this need by developing a new process with single enzymatic step and none of the aforementioned pretreatments. It relies on effective enzyme to break down cell wall structures and release intact protein and oil bodies. This approach simplifies soy processing and offers the potential to maximize separate collection of all major components and preserve intact oil and protein bodies for high-value uses. Soybeans are protected by hulls, which are mainly composed of cellulose, hemicellulose and pectin [30]. Unless it is desirable to also collect the value of hull carbohydrates, hulls can be removed by a dehulling process which typically also serves to crack the beans into small pieces. After dehulling, the cotyledon cell wall is the primary barrier for enzymatic processing. Cell wall is constructed of pectin, hemicellulose, and cellulose microfibrils crosslinked with pectin [31] and mass transfers across intact cell wall only through small (20–80 nm) openings [32]. So, to collect oil and protein bodies, it is necessary to break down the cell walls. An enzyme mixture that degrades all types of cell wall carbohydrates may achieve this. We studied protein and carbohydrate separation in defatted meal [33–35] using fungal enzymes produced with soybean hulls as substrate [36]. By using such an enzyme in the new process, we hypothesized that cell walls can be hydrolyzed into soluble carbohydrates and collected in hydrolysate; oil can be collected as intact oil bodies; and protein, at a process pH near its isoelectric point (pI ~ 4.8) [26,33], can be collected as insoluble precipitate.

In this study we investigated a new, simplified and complete soybean processing to achieve multiple objectives: (1) to recover and separate all major component groups (protein, oil and carbohydrate), (2) to simplify processing, (3) to unify the product properties for more effective subsequent uses, and (4) to collect oil and protein as intact oil bodies and protein bodies. To achieve these objectives, we investigated enzymatic methods that do not involve significant mixing, blending, and/or pressing of protein and oil bodies and cell wall carbohydrate as in the existing processing. Proper ultrasonic treatment was reported to help extract oil from oilseeds [37,38]. In this study different ways of ultrasonication were also compared for effects on the new enzymatic soy processing.

2. Materials & methods

The block diagram of the entire enzyme-based process studied in this work is shown in Fig. 1.

2.1. Materials & equipment

Full-fatted, dehulled, cracked soybeans were provided by Archer Daniels Midland (Decatur, IL). $(NH_4)_2SO_4$ (granular), KH_2PO_4 (99% purity), HCl (concentrated, 37.4%) and NaOH (98.8%) were purchased from Fisher Scientific (Waltham, MA). Proteose peptone (from meat, Type I, for microbiology), $MgSO_4{\cdot}7H_2O$ (99%), $MnSO_4{\cdot}4H_2O$ (99%), $ZnSO_4{\cdot}7H_2O$ (ACS reagent grade), $CoCl_2{\cdot}6H_2O$, $FeSO_4{\cdot}7H_2O$ (reagent grade), $CaCl_2{\cdot}2H_2O$ (reagent grade), urea (98%), NaN_3 (> 99%) and dinitrosalicylic acid (DNS, 98%) were purchased from Sigma-Aldrich (St. Louis, MO). The *Aspergillus niger* (NRRL 341) seed culture was obtained from the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Culture Collection (Peoria, IL). Two 3-l Bioflo 110 fermentors (New Brunswick Scientific; Edison, NJ) were used for enzyme production by fermentation. Absorbance was measured using a spectrophotometer (UV-1601, Shimadzu; Columbia, MD). Hydrolysis experiments were conducted in a shaker (Thermo Scientific MaxQ 5000 Incubating/Refrigerating floor shaker; Ashville, NC). Two centrifuges were used: a Sorvall Legend X1R centrifuge (Thermo Scientific; Waltham, MA) and a Sorvall RC 5C centrifuge (DuPont; Wilmington, DE). The vacuum oven used was manufactured by Lab-line Instruments (Melrose Park, IL).