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Chemical characterization of liquid residues from aqueous enzymatic extraction of soybean oil

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ABSTRACT

The aim of this study was the chemical characterization of liquid residues obtained from the extraction of soybean oil, through enzyme processing. Protein content was approximately 32 g/100 g at dry weight basis (dwb) with pH adjustment and 52 g/100 g (dwb) without pH control, and the total carbohydrate content was around 31 g/100 g. The methionine concentrations in the liquid waste were between 23 and 66 mg/L. The electrophoretic profile of the total protein showed the presence of several peptides with molecular weight (MW) lower than 25 kD when enzymatic extraction was carried out at pH 4.5. These characteristics suggest important nutritional applications to this by-product.

1. Introduction

International efforts toward the minimization of petroleum derivates, to improve consumer health and control residue generation, have led to the search for a processing technique for vegetal oil extraction without the use of petroleum derivate solvents. The use of enzymes in some steps of the solvent extraction process or in the hydraulic press system was initially described by Sherba, Steigerwalt, Faith, and Smythe (1972).

The first studies on extracting oil from soybean or other raw material via an enzymatic pathway studied the oil extraction process but does not characterize derivates or byproducts (Dominguez, Nunez, & Lema, 1994; Dominguez, Nunez, & Lema, 1995; Kashyap, Agrawal, Sarkar, & Singh 1997; Lamsal, Murphy, & Johnson, 2006; Lusas, Lawhon, & Rhee, 1982; Rosenthal, Pyle, & Niranjan, 1996; Singh, Sarker, Kumbhar, Agrawal, & Kulshreshtha, 1999; Wu, Johnson, & Jung, 2009).

The use of enzymes is known to produce good quality oils, reduce byproducts and avoid adverse operating conditions due to the solvents. The process has also been applied to extract oil from products such as avocado (Buenrostro & Lopez-Munguia, 1986), coconut (Mcglone, Lopez-Munguia, & Carter, 1986), corn germ (Karlovic, Bocevska, Jakovlevic, & Turkulov, 1994), canola (Sarker, Singh, Agrawal, & Gupta, 1998), soybean (Dominguez et al., 1994; Kashyap et al., 1997), and sunflower (Dominguez et al., 1995; Singh et al., 1999).

The most used enzymes are glycosidases. The association of glycosidases with proteases facilitates access to the vacuoles and increases the yield (Rosenthal et al., 1996). The action of glycosidases is specifically on the cellular wall. The action of proteases is linked to the hydrolysis of proteosomes and to a reduction in the emulsification (Hamada & Marshall, 1989).

Solid residue of this process was studied by Rovaris et al. (2012), showing important potential as source of fiber, mineral and protein. The aim of this study was to evaluate the effect of the pH control to hydrolyze proteins and carbohydrates for soybean oil extraction without the use of organic solvents. The liquid residues were characterized with the aim of providing future recommendations on their uses.

2. Material and methods

2.1. Chemical and materials

Expanded soybean grains were donated by Bunge Alimentos (Gaspar, Santa Catarina, Brazil). Alcalase 2.4 L (2.4 AU g^{-1}) (AU –

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Anson Units), Celluclast 1.5 L (700 EGU g^{-1}) (EGU – Endoglucanase Units) and Viscozyme L (100 FBG g^{-1}) (FGB – Fungal β-glucanase Units), by Tovani Benzaquen (Sao Paulo, Brazil). The group of amino acids commonly present in soybean protein were purchased from Sigma Co. (St. Louis, MO, USA) and analyzed: L-asparagine, L-histidine, L-glycine, L-alanine, L-glutamic acid, L-valine, L-methionine, L-isoleucine, L-tryptofan, L-phenylalanine, L-leucine, L-lysine and L-cysteine (Aguirre, Garro, & De Giori, 2008; Grieshop & Fahey, 2001; Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). Other reagents were of analytical grade.

2.2. Capillary electrophoresis

The capillary electrophoresis (CE) assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector set at 420 nm, a temperature control device maintained at 25 °C and acquisition and data treatment software supplied by the manufacturer (HP ChemStation, rev. A.06.01). An uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used, with dimensions of 48.0 cm total length, 39.5 cm effective length, an inner diameter of 75 μm and an outer diameter of 375 μm. The background electrolyte (BGE) was composed of 20 mmol/L sodium tetraborate (STB) at pH 9.3 containing 50 mmol/L sodium dodecyl sulfate (SDS). Standard solutions and samples were injected hydrodynamically (at 5 kPa for 3 s). The applied separation voltage was 23 kV with positive polarity. The amino acid composition of the liquid residues was determined by the Weber and Buck (1994) method.

2.3. Chromatography conditions

High-performance size-exclusion chromatography coupled with multi-angle, laser-light scattering, refractive index detection (HPSEC-MALLS/RI) was used to analyze the liquid residue using equipment comprised of an HPLC pump (model Waters 515, Milford, MA, USA), an injector, four ultrahydrogel columns – 120, 250, 500 and 2000 – with exclusion limits of $5{\cdot}10^3$, $8{\cdot}10^4$, $4{\cdot}10^5$ and $7{\cdot}10^6$, respectively; *DAWN DSP* Light Scattering (Wyatt Technology, Goleta, CA, USA) and a differential refraction index detector (model 2410 Waters, Milford, MA, USA). The eluent used was a solution of 0.1 mol/L $NaNO_2$, with 200 mg/L of NaN_3. Before the analysis, the samples were filtered in cellulose acetate membranes with a pore size of 0.22 μm. The filtrate was analyzed in a Shimadzu (Columbia, MA, USA) HPLC chromatograph with control unit CBM-10A, oven CTO-10A, pump LC-10AD, refraction index detector RID-10a, a Supelcogel Pb column (Supelco Sigma–Aldrich, Bellefont, PA, USA) of 30 cm × 7.8 mm and Supelcogel Pb pre-column of 5 cm × 4.6 mm. The analysis temperature was 80 °C, flow 0.1 mL/min, and water eluent.

2.4. Gas chromatography

Alditol acetate derivatives were analyzed using a Trace GC Ultra (Thermo Electron Corporation, Austin, TX, USA) gas chromatography (GC) equipped with a capillary column DB-225 (0.25 mm × 30 m). The injector and detector by flame ionization temperatures were 250 and 300 °C, respectively. The oven temperature was programmed at 100–215 °C, with a heating rate of 40 °C/min. Helium was used as carrier gas, at the flow of 1.0 mL/min.

2.5. Extraction of soybean oil and production of residues

The extraction was carried out via four enzymatic pathways. In the first, 200 g of the expanded soybean and 400 mL of phosphate buffer pH 8.0 were mixed with the proteolytic enzyme Alcalase at a concentration of 1 g/100 mL and kept in a thermo controlled bath at 60 °C for 4 h. This medium was acidified with 0.1 mol/L hydrochloric acid to a pH 4.5 and then 200 mL of citrate buffer pH 4.5, and the enzyme Celluclast 1 g/100 mL was added. The mixture was kept in a thermo-controlled bath at 60 °C for 2 h. The pH of the material was corrected to approximately 3.0 for enzyme inactivation and centrifuged at 750× g-force for 25 min (Janetzki centrifuge, model S60). The oil and the solid and liquid residues obtained from the process were then separated. The solid and liquid residues were frozen in liquid nitrogen, freeze-dried in a Terroni (series LD) freeze dryer and then placed in polypropylene bags, vacuum sealed and kept at −18 °C until the analysis was carried out. In the second system, the enzyme Celluclast was replaced with Viscozyme L, and the same procedure described for the first system was applied. In the third and fourth systems the same enzymes used in the first and second system were employed, but the pH was not controlled during the hydrolysis and thus distilled water was used in place of the buffer solutions. The inactivation of enzymes was achieved in all systems by lowering the pH. A reference sample (Br), expanded soybean, with and without control of pH, with no enzymatic treatment, was treated following the same procedures. All experiments were carried out in triplicate.

2.6. Capillary electrophoresis analysis of amino acids

2.6.1. Sample hydrolysis

Approximately 1 g of a 10 mg/mL solution of the freeze-dried sample was weighed in a covered glass test tube, 1 mL of 8 mol/L hydrochloric acid was added and the mixture was kept in an oven at 100–110 °C for 24 h for the sample hydrolysis. After cooling, a 200 μL aliquot of the hydrolyzed product was transferred to a microtube, and 1200 μL of 1 mol/L sodium hydroxide were added. Hydrolyzed and non-hydrolyzed samples were analyzed on the CE instrument after the derivatization. The amino acids derivatization was carried out using a modified procedure based on that described in Hsieh and Chen (2007).

2.7. Physicochemical analysis

Freeze-dried samples were analyzed for ash content (923.03), total lipids (920.85), and total nitrogen (N X 5.75) (920.87) (AOAC, 2005), and the results are presented in g/100 g (dwb). The determination of reducing sugars was carried out according to Miller (1959).

The total carbohydrate content was estimated by difference. Energy values (kcal) were obtained by application of the factors 4, 9 and 4 per gram of protein, lipid and carbohydrate, respectively (Watt & Merrill, 1999).

2.8. Monosaccharide

The individual neutral sugars were quantified through GC after being reduced and converted to alditol acetates (Wolfrom & Thompson, 1963).

2.9. Uronic acid analysis

Uronic acid dosage was carried out by Blumenkrantz and Asboe-Hansen (1973) method, using as standard solution of 10–100 μg/mL galacturonic acid, evaluated in 520 nm.

2.10. Extraction of total soybean protein

Total protein was extracted from samples (20 mg) with 500 μL of 30 mmol/L Tris [Tris(hydroxymethyl)aminomethane] buffer (pH 8)