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Rheological properties of soy protein hydrolysates obtained from limited enzymatic hydrolysis

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Abstract

Soy protein products hexane-defatted soy flour, extruded-expelled soy flour, soy protein concentrate and soy protein isolate, were modified by using the enzyme bromelain to 2% and 4% degrees of hydrolysis (DH). Peptide profiles, water solubility, and rheological properties including dynamic shear, large deformation, and apparent viscosities of resulting hydrolysates were determined. Protein subunits profiles for the hydrolysed isolates and concentrates were extensively altered by the treatment while only minor changes were observed for the hydrolysed flours. Water solubility profiles of all hydrolysates in the pH range of 3.0–7.0 were enhanced by hydrolysis. For the unhydrolysed controls, the isolate had the highest storage modulus (G'), followed by the concentrate, the extruded-expelled flour and the hexane-defatted flour. The hydrolysates retained some of their gelling ability even though the losses in storage modulus (G') were substantial. After heating step to 95 °C, the G' values of all substrates at 25 °C decreased with increase in DH. Texture profile analyses of the soy protein gels were also lower in hardness after hydrolysis. The Power Law model provided excellent fit to hydrolysate dispersions flow ($R^2 > 0.99$). Hydrolysis decreased the consistency coefficients of dispersion and increased flow behavior index resulting in thinner dispersions. These results suggest that limited protease hydrolysis of various soy protein meals with bromelain produce soy protein ingredients with modified rheological properties.

Keywords: Soy proteins; Limited hydrolysis; Rheology; Gelation; Texture analysis

1. Introduction

Food proteins, both animal and plant proteins, play critical roles in human nutrition. This traditional role aside, proteins in food formulations are increasingly expected to perform functional roles that are important to consumer food acceptance. Food proteins possess physico-chemical properties that govern their performance and behavior in food systems during processing, storage and consumption that are collectively termed functional properties. Such properties also provide the basis for utilizing an ingredient in food applications. Some examples of novel uses include use in snack and energy bars, hard pretzels, sport beverages, whole soy ingredients, egg replacements in bakery products, infant formulae, coating systems, tortillas and breads (Pszczola, 2005). Soy proteins undergo harsh processing conditions involving heat, shear, and exposure to acid, and may lose much of their functionalities.

Limited or controlled enzymatic hydrolysis of soy proteins could provide ingredients with desired or restored functionalities (Panyam & Kilara, 1996; Surowka, Zmudzinski, & Surowka, 2004; Surowka, Zmudzinski, Fik, Macura, & Lasocha, 2004; Jung, Murphy, & Johnson, 2005). Ingredients that form strong gels and give high viscosity are preferred for use in comminuted meat products and gravies, while yogurts, soups, and infant food formulations require less viscous product mix and weaker gelling properties. The extent of proteolysis during enzymatic hydrolysis can be established with the degree of hydrolysis (DH). Hydrolysates intended for nutritional formulations, e.g., hypoallergenic hydrolysates, are extensively hydrolysed, with 90% of peptides being <500 Da in molecular weight (Mahmoud, 1994). Hydrolysates intended for use as nutritional supplements undergo slight

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(about 90% peptides >5000 Da) or moderate (about 46% peptides >5000 Da) hydrolysis.

Enzymatic hydrolysis of protein employs various proteases, bromelain being one such protease. Bromelain is an endoprotease chiefly used for muscle tenderization (Melendo, Beltran, & Roncales, 1997; Teran, 2003; Kolle, McKenna, & Savell, 2004), and producing acid-coagulation resistant milk (Christensen, Florin, & Harris, 2002). Studies using bromelain to improve physicochemical and functional properties of proteins have focused on emulsion formation and water-holding capacity (Karakaya & Ockerman, 2002), umami chicken flavor development (Maehashi, Matsuzaki, Yamamoto, & Udaka, 1999), soy protein solubility and foaming properties (Molina-Ortiz & Wanger, 2002), and controlling of gluten network formation in low fat pastry products (Hart, 1999).

Native, globular proteins are generally resistant to enzyme hydrolysis due to their compact tertiary structures that protect many of the peptide bonds (Adler-Nissen, 1976). Denatured proteins, like most processed soy proteins, have exposed peptide bonds available for enzymatic cleavage. Enzymatic hydrolysis can be responsible for (1) a decrease in hydrolysate molecular weight, (2) an increase in ionizable group number, and (3) exposure of previously concealed hydrophobic groups (Panyam & Kilara, 1996). Changes in functional properties, including rheological characteristics, are a direct result of the above-mentioned changes. Structural modifications to the two major soy protein components, namely glycinin and β-conglycinin, by proteases (endo or exo-peptidases) dictate the resultant hydrolysate properties (Jung et al., 2005). Gel hardness and fracturability of soy proteins are attributed to glycinin, whereas β-conglycinin contributes to gel elasticity (Utsumi, Matsumura, & Mori, 1997).

Extensive literature is available on the enzymatic modification of soy proteins from moderate to high DH by proteolytic enzymes and effects on hydrolysate functional properties (Kim, Park, & Rhee, 1990; Achouri, Zhang, & Shiying, 1998; Calderon-de-la Barca, Ruiz-Salazar, & Jara-Maarini, 2000; Hrckova, Rusnakova, & Zemanovic, 2002; Tsumura et al., 2005). The effects of limited hydrolysis on rheological properties of soy proteins, however, are not understood. The aim of the present study was to determine the rheological properties (gelation and viscosity) of soy protein products, which have undergone limited protease hydrolysis (up to 4% DH).

2. Materials and methods

2.1. Substrates

Four commercial soy protein products were used as starting materials; a hexane-defatted soy flour (SF, Nutrisoy 7B), a soy protein concentrate (SPC, Acron S) and a soy protein isolate (SPI, Profam 955), which were acquired from Archer Daniels Midland Co. (Decatur, IL) and an extruded-expelled soy flour (EEF), which was acquired from Iowa Soy Specialities (Vinton, IA). The crude protein contents for the substrate as determined in our laboratory were: SF, 54 g/100 g, EE, 49 g/100 g, SPC, 77 g/100 g, and SPI, 93 g/100 g on as is basis. Protein dispersibility index (PDI) was determined externally by Eurofins Scientific Inc. (Des Moines, IA) and were 88, 68, 71, and 11 for SF, EE, SPC, and SPI, respectively.

2.2. Enzymatic hydrolysis

The food-grade enzyme bromelain, an endopeptidase derived from pineapple stems (Bio-Cat Inc., Troy, VA) was used to carry out the hydrolysis. The enzyme activity was specified as 2000 gelatin digestion units (GDU)/g protein. Hydrolysis was performed at 50 °C and pH 7.0. DH was controlled and determined by using the pH-Stat method (Adler-Nissen, 1986), which determines the %DH on the basis of the number of free titratable amino groups produced by hydrolysis of peptide bonds. Compared to other methods, the pH stat method allows direct monitoring of DH in real time. DH was calculated using the following equation:

$$DH = [(V_{NaOH} \times N_{NaOH})/(\alpha \times MP \times h_{tot})] \times 100\%,$$

where α is the degree of dissociation of α-amino groups, MP is the mass of protein (g), h_{tot} is the number of peptide bonds in the substrate (meqv/g protein), concentration of the base (NaOH) was 2 mol/l, α value was 0.44, and h_{tot} was 7.8 (Adler-Nissen, 1986).

Hydrolysis of the 10 g/100 g substrate dispersion was carried out in a 250-mL temperature-controlled glass reactor with constant stirring. pH during hydrolysis was controlled by using a titrator (718 STAT Trition, Brinkmann, Westbury, NY). Appropriate enzyme-to-substrate ratios were selected to reach 2% or 4% DH values, wherein a plateau in DH over time was achieved. Freezing the hydrolysates, which were later freeze-dried, quickly stopped enzymatic reaction. The unhydrolysed control dispersion was prepared at the same pH, temperature and reaction time as was necessary to achieve 4% DH and similarly freeze-dried. Crude protein contents of the freeze-dried hydroylsates were the same as those of the corresponding substrates.

2.3. Sodium dodecyl sulfate polyacryalamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on unhydrolysed controls and enzyme hydrolysates to determine the effects of the enzyme treatments on the protein polypeptide profiles. SDS-PAGE was carried out using a SDS-Tris-glycine buffer system with 4% stacking gels and 13% resolving gels (Mini Protean II Gel, Biorad Inc., Hercules, CA) following methods of Jung et al. (2005). A low-range molecular weight (MW) marker ranging from 66 to 6.5 kDa (M3913, Sigma Chemical Co., St. Louis, MO) and laboratory-purified β-conglycinin and glycinin were used as standards.