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The effects of limited enzymatic hydrolysis on the physicochemical and emulsifying properties of a lentil protein isolate

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ABSTRACT

The physicochemical and emulsifying properties of lentil protein isolates (LPI) were investigated as a function of their degree of hydrolysis (DH of 4, 9 and 20%) following exposure to trypsin/heat. Specifically, interfacial tension, surface characteristics (charge and hydrophobicity) and intrinsic fluorescence were determined. These parameters were then related to changes in the emulsification activity (EAI) and stability indices (ESI) of unhydrolyzed (u-LPI) and hydrolyzed LPI (h-LPI) in a flaxseed oil–water emulsion. Interfacial tension was found to decrease from ~6.5 to ~6.1 mN m^{-1} for u-LPI and h-LPI (DH 4–20%), respectively. A similar trend was observed for surface hydrophobicity, which declined from ~30 to ~24 for the u-LPI and h-LPI (DH 4–20%), respectively. In contrast, surface charge values were similar for all materials (~−37 mV). Intrinsic fluorescence as a function of emission wavelengths (300–400 nm) indicated a slight change in the tertiary conformation of LPI upon hydrolysis, where the magnitude of fluorescence intensity declined relative to that of u-LPI. Changes in physicochemical properties upon hydrolysis had a detrimental effect on EAI and ESI values, which declined from ~51 to ~47 m^2 g^{-1} and ~12 to ~11 min for u-LPI and h-LPI (DH 4–20%), respectively.

1. Introduction

In general, food proteins are effective at stabilizing oil-in-water emulsions due to their amphiphilic nature (i.e., possessing both hydrophilic and hydrophobic reactive sites on their surface) and interfacial film-forming abilities. Emulsions are defined as dispersions of two (or more) immiscible liquids which are inherently thermodynamically unstable and tend to phase separate overtime via creaming, flocculation and/or coalescence. Emulsion stability is highly dependent upon liquid droplet size and distribution, emulsion processing conditions (i.e., homogenization rates), protein characteristics (i.e., size, conformation, surface reactivity, concentration and solubility), solvent conditions (i.e., pH, salts and temperature), phase volume ratio and continuous phase viscosity (Can Karaca, Low, & Nickerson, 2011a,b; McClements, 2007). During emulsion formation, proteins diffuse from the bulk solution to the oil–water interface, where they unravel and re-orient to form a viscoelastic interfacial film around the dispersed oil droplets which acts as a physical and/or electrostatic barrier towards destabilization (Damodaran, 2005). Emulsion stability can arise from electrostatic repulsive forces between neighbouring droplets (depending on the salt and pH conditions) or steric hindrance induced by hydrophilic protein segments extending into the continuous phase that physically restricts coalescence (Can Karaca et al., 2011a).

In contrast to animal-derived proteins (e.g., whey, casein or ovalbumin), the emulsifying properties of plant proteins including legumes, have not been as extensively studied and their mechanism(s) of action are less well understood. Plant protein-based emulsifiers are attractive to the food industry for both product development and re-formulation, based on their low cost, nutritional benefits and greater consumer/market acceptability (Can Karaca et al., 2011a; Duranti, 2006). The emulsifying properties of a group of legume proteins have been studied and include those extracted from pea (Ducel, Richard, Popineau, & Boury, 2004), cowpea, (Kimura et al., 2008), faba bean (Galazka, Dickinson, & Ledward, 1999), soy (Martinez, Sanchez, Patino, & Pilosof, 2009) and lentil (Bora, 2002). Research arising from our laboratory compared the emulsifying properties of protein isolates derived from chickpea, lentil, faba bean, pea and soy produced by both isoelectric precipitation and salt extraction (Can Karaca et al., 2011a). Findings indicated that lentil protein isolates, produced by isoelectric precipitation gave the best emulsifying properties, having the highest emulsion capacity of the legumes tested, and comparable emulsifying activity/stability indices and creaming behavior to soy. Emulsion capacity refers to the maximum amount of oil that can be dispersed within a solution of emulsifiers (e.g., proteins) without the emulsion phase separating or undergoing an inversion from an oil-in-water emulsion to a water-in oil emulsion (McClements, 2007). The emulsifying activity index relates to the emulsion forming properties of the protein (Hill, 1996), and provides an estimate of the interfacial area stabilized by a given weight of protein based on turbidimetric analysis of a diluted emulsion (Pearce & Kinsella, 1978). The emulsifying stability index provides a measure of stability over a defined period of time for the same diluted emulsion (Pearce & Kinsella, 1978).

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In order to further enhance the emulsifying properties of proteins, some researchers have explored limited enzymatic hydrolysis by proteases (e.g., trypsin) as a means to enhance functionality. Limited hydrolysis can lead to a partial unravelling of the protein structure (altering its conformational stability) to expose more ionizable and hydrophobic groups, decrease protein mass, and release polypeptides/peptides into solution (Panyam & Kilara, 1996). A limited degree of protein hydrolysis (DH) (<10%) has been found by some to enhance protein functionality, however above a critical point a detrimental effect can be seen (Govindaraju & Srinivas, 2006; Guan, Yao, Chen, Shan, & Zhang, 2007; Karayannidou et al., 2007; Panyam & Kilara, 1996). Karayannidou et al. (2007) reported that trypsin-treated sunflower protein isolate (DH~10%) showed improved emulsifying and foaming properties relative to the non-hydrolyzed material. Guan et al. (2007) reported enhanced solubility, water holding, emulsifying and foaming properties for trypsin-treated oat bran protein (DH~4–8%) relative to the native protein. Also, Ventureira, Martinez, and Anon (2010) reported that trypsin-treated amaranth protein (DH of 2.2%) showed enhanced oil–water emulsion stability relative to the native form. In contrast, Govindaraju and Srinivas (2006) reported that the hydrolysis of arachin protein (DH~19%) using papain, alcalase and fungal protease resulted in a significant decrease in emulsification and, foaming capacity and stability in an oil–water emulsion. Chabanon, Chevalot, Framboisier, Chenu, and Marc (2007) reported that a 15% hydrolysis of canola protein with alcalase resulted in lower foaming capacity and stability and emulsion activity and stability in an oil–water emulsion in comparison to that of the unhydrolyzed protein.

The overall goal of this research was to investigate structure-function relationships associated with the limited enzymatic hydrolysis of a lentil protein isolate (LPI), as it related to its surface characteristics, protein conformation and emulsifying properties. Lentil proteins are primarily comprised of globulins (~49%) and albumins (~16.8%) (Boye, Fatemeh, & Pletch, 2010). The salt-soluble globulin fraction consists mainly of legumin (11S, S — Svedberg Unit; ~340–360 kDa) and vicilin (7S; ~175–180 kDa) (Swanson, 1990). Legumin is a hexameric protein comprised of ~60 kDa subunits of α (~40 kDa) and β (~20 kDa) chains, whereas vicilin is a trimeric protein comprised of ~5060 kDa subunits (Swanson, 1990). In contrast, the water-soluble albumins range in size from 5 to 80 kDa, and include protease and amylase inhibitors, and lectins (Boye et al., 2010).

2. Materials and methods

2.1. Materials

Whole green lentil seeds (CDC Grandora) and flaxseed oil were provided by the Crop Development Centre (Saskatoon, SK, Canada) and Bioriginal Food and Science Corp. (Saskatoon, SK, Canada), respectively. The following materials were purchased from Bio-Rad (Mississauga, ON, Canada): Bio-Rad Broad Range Marker, Bio-Rad Tris–HCl gel (15%T), Coomassie blue R-350 and Laemmli Sample Buffer. Hexane was purchased from Fisher Scientific (Ottawa, ON, Canada). Picrylsulfonic acid (trinitrobenzenesulfonic acid (TNBS)) and trypsin (10,600 units/mg) were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). The following chemicals were purchased from VWR (Edmonton, AB, Canada): hydrochloric acid, sodium dihydrogen phosphate, sodium dodecyl sulfate, sodium hydroxide, and sodium monohydrogen phosphate. All chemicals used in this study were of reagent grade except for sodium dodecyl sulfate which was ultrapure. The water used in this research was produced from a Millipore Milli-Q™ water purification system (Millipore Corp., Milford, MA, USA).

2.2. LPI preparation

Whole green lentil seeds were ground into a fine flour using a food processor (Cuisinart mini-prep plus grinder) (~1 min), followed by an IKA A11 analytical mill treatment (IKA Works Inc., Wilmington, NC, USA) (~1 min). The flour was then defatted by stirring in hexane (1:3 [w/v], flour: hexane) for 40 min; this procedure was repeated two additional times. Protein isolates were prepared from the defatted flour based on methods of Papalamprou, Doxastakis, and Kiosseoglou (2010) and Can Karaca et al. (2011a). In brief, 100 g of defatted flour was mixed with water at a 1:10 (w/v) ratio. The pH of the resulting suspension was adjusted to 9.00 using 1.0 N NaOH followed by mechanical stirring at 500 rpm for 1 h at room temperature (21–23 °C). The mixture was then centrifuged at 5000 ×*g* at 4 °C for 20 min using a Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA) to collect the supernatant. The resulting pellet was re-suspended in water at a ratio of 1:5 (w/v), adjusted to pH 9.00 using 1.0 N NaOH, stirred for 1 h at room temperature, followed by centrifugation (5000 ×*g*, 20 min, 4 °C). Supernatants were pooled and adjusted to pH 4.50 with 0.1 M HCl to precipitate the protein (Bora, 2002). The LPI was washed with water, frozen at −30 °C and then freeze dried using a Labconco FreeZone 6 freeze drier (Labconco Corp., Kansas City, MO, USA). Proximate analysis of the resulting LPI was conducted according to AOAC Official Methods 925.10 (moisture), 923.03 (ash), 920.85 (lipid), and 920.87 (crude protein by using %N×6.25) (AOAC, 2003). Carbohydrate content was determined on the basis of percent differential from 100%. Proximate analyses were performed on three separate protein isolate preparations with each preparation analyzed in triplicate ($n=3$).

2.3. LPI hydrolysis as a function of enzyme/substrate ratio

This study included the preparation of a LPI control, hydrolyzed LPI products, and determination of a total LPI hydrolysis value.

2.3.1. Preparation of the control sample (unhydrolyzed and heat treated)

One hundred milliliters of a 1.0% (w/v) lentil protein isolate in 35 mM sodium phosphate (pH 7.80) was stirred overnight at 4 °C. The resulting solution was transferred to a shaking (90 rpm) water bath (PolyScience, Niles, IL, USA) at 37 °C for 1 h. A 250 μL aliquot of the mixture was removed and added to 2.00 mL of 1.0% SDS in 35 mM sodium phosphate (pH 7.80) and heated at 85 °C in a water bath for 10 min and then a 250 μL aliquot was taken and added to 2.00 mL of 35 mM sodium phosphate buffer (pH 7.80). This reaction was repeated in triplicate and analyzed employing the TNBS reaction. The LPI control sample is identified as h_c in Eq. (1).

2.3.2. Preparation of trypsin catalyzed protein hydrolysates

One hundred milliliters of a 1.0% (w/v) LPI in 35 mM sodium phosphate (pH 7.80) was stirred overnight at 4 °C. The resulting solution was transferred to a shaking (90 rpm) water bath at 37 °C for 1 h. Trypsin was then added to lentil protein solutions at the following enzyme/substrate (E/S [w/w]) ratios: 1/100, 1/250, 1/500 and 1/1000. A 250 μL aliquot of each E/S ratio experiment was removed at time intervals of 5, 10, 20, 30, 40, 60, 80, 100, 120 min, and were individually added to 2.00 mL of 1.0% SDS in 35 mM sodium phosphate (pH 7.80) and heated at 85 °C in a water bath for 10 min to quench the hydrolysis reaction and then a 250 μL aliquot was taken and added to 2.00 mL of 35 mM sodium phosphate buffer (pH 7.80). A sample blank consisting of 250 μL of 1.0% SDS in 35 mM sodium phosphate (pH 7.80) and 2.00 mL of 35 mM sodium phosphate buffer (pH 7.80) was run with each batch of hydrolysis experiments. All partial hydrolysis reactions were performed in triplicate and all samples and blanks were analyzed employing the TNBS reaction. Partially hydrolyzed LPI samples are referred to as h_t in Eq. (1).

2.3.3. Preparation of total protein hydrolysates

Total LPI hydrolysis was performed based the methods of Adler-Nissen (1979), Jung, Murphy, and Johnson (2005) and Barbana and Boye (2011). In brief, ~24 mg of LPI was weighed into a