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Oil-in-water emulsions stabilized by tyrosinase-crosslinked potato protein

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ABSTRACT

Potato protein (PP) holds great promise as a non-allergenic food ingredient with high nutritional value. Attempts to modulate its functional properties by crosslinking have not been reported to date. The effect of tyrosinase-mediated crosslinking of PP on the properties of o/w emulsions was studied in the present work. Among the various PPs, protease inhibitors were efficiently crosslinked by the enzyme as determined by SDS-PAGE analysis. Concentrated emulsions comprising 40% olive oil and 6.1% PP (w/w) were fabricated by a shear stress homogenizer. The PP-stabilized emulsions were evaluated after one and 4 h of incubation with tyrosinase. Emulsions were characterized by their droplet size distribution, rheological behavior, creaming resistance and microstructure. The crosslinked emulsion had a self-standing elastic gel-like structure after 1 h of incubation. Unlike the Newtonian non-crosslinked emulsion, the crosslinked emulsion exhibited a shear-thinning behavior with a 20-fold increase in viscosity. The longer incubation time coupled with shaking at 250 rpm for up to 4 h resulted in the disruption of the droplets structure and led to a 2-fold decrease in viscosity, compared to the 1-hour crosslinked emulsion. Droplet size distribution showed formation of large particles in the crosslinked emulsion. Microscopy imaging demonstrated formation of aggregated and dense emulsion droplets network, which also contributed to the emulsion gel-like behavior. In the case of the non-crosslinked emulsion, severe flocculation and coalescence was observed, regardless of incubation time. The results suggest that tyrosinase crosslinking is a useful method to modulate the properties of PP-based food formulations.

1. Introduction

Among the various non-animal protein sources, plant proteins have been widely applied as food ingredients due to their functional properties (Vaz Patto et al., 2015) and abundant supply. Potato proteins (PP) are becoming more prevalent in food products due to their high nutritional quality comparable to the protein of whole egg (Friedman, 1996; Liedl, Kosier, & Desborough, 1987) and high essential amino acid index (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011; Pouvreau et al., 2001). They are considered GRAS and non-allergenic and can be incorporated in vegetarian, vegan and Kosher Parve products (David & Livney, 2016). Potato proteins have been found as a valuable component in fining of red wine and white musts (Gambuti, Rinaldi, & Moio, 2012; Gambuti, Rinaldi, Romano, Manzo, & Moio, 2016), cheese making (Spelbrink, Lensing, Egmond, & Giuseppin, 2015), or gluten-free bread making (Witczak, Juszczak, Ziobro, & Korus, 2017). Additionally, their emulsifying (Ralet & Guéguen, 2000; Romero et al., 2011; van Koningsveld et al., 2006), foaming (Baier & Knorr, 2015; van Koningsveld et al., 2002) and gelling (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2011) properties were previously reported. In terms of health benefits, potato patatin has been shown to possess the ability to regulate serum cholesterol levels (Liyanage et al., 2008), and to act as a potential cancer chemo-preventive agent (Sun, Jiang, & Wei, 2013). Hydrolyzed potato proteins have shown remarkable antioxidative properties (Cheng, Xiong, & Chen, 2010; Nieto et al., 2009; Udenigwe, Udechukwu, Yiridoe, Gibson, & Gong, 2016).

Potato proteins include three major classes: patatin (up to 40%), protease inhibitors (up to 50%) and others (mostly high molecular weight proteins) (Løkra, Helland, Claussen, Strætkvern, & Egelandsdal, 2008; Pouvreau et al., 2001). Patatin, a globular glycoprotein with an isoelectric point between 4.5 and 5.2, is a dimer that appears as a monomer (40–45 kDa) in the presence of the sodium dodecyl sulfate (Racusen & Weller, 1984). Protease inhibitors are the most abundant and heterogeneous group of proteins with molecular sizes varying between 5 and 25 kDa (Pots et al., 1999). The most common members are serine protease inhibitors (PI-1 and PI-2 from the Kunitz family), potato cysteine protease inhibitors (PCPI), potato aspartate protease inhibitors

Abbreviations: TyrBm, tyrosinase from *Bacillus megaterium*; PP, potato protein; SPB, sodium phosphate buffer; PFJ, potato fruit juice

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(PAPI), other Kunitz type protease inhibitors (PKPI) and potato carboxypeptidase inhibitors (Pouvreau et al., 2001). PI-2 is a dimeric protein with one disulfide bridge and molecular mass of 22 kDa, while PI-1 is a pentameric protein composed of five 7–8 kDa isoinhibitor protomers (Pouvreau et al., 2001). PCPI is considered to be a beta-II protein based on structural properties which are similar to those of potato serine protease inhibitors and Kunitz type soybean trypsin inhibitors (Pouvreau, Gruppen, van Koningsveld, van den Broek, & Voragen, 2005). Potato protease inhibitors vary in their inhibiting effects among the groups, but generally inhibit trypsin, chymotrypsin and human leukocite elastase (Pouvreau et al., 2001).

It has been shown that potato proteins as a crude extract exhibited poor emulsification properties (Ralet & Guéguen, 2000), thus different methods have been used to improve its emulsification ability and emulsion stability such as: hydrolyzation (Nieto et al., 2009), pH treatment or ionic strength modulation (Delahaije, Gruppen, van Nieuwenhuijzen, Giuseppin, & Wierenga, 2013), succinylation (Delahaije, Wierenga, Giuseppin, & Gruppen, 2014), phosphorylation (Miedzianka & Pęksa, 2013), addition of chitosan (Calero, Muñoz, Cox, Heuer, & Guerrero, 2013), addition of κ-carrageenan (O'Sullivan, Kurukji, Norton, & Spyropoulos, 2017), addition of guar gum (Santos, Calero, Guerrero, & Muñoz, 2015), and conjugation with galactose, galactooligosaccharides, or galactan (Seo, Karboune, & Archelas, 2014). Furthermore, it has been reported that fractionated PP isolate possess better emulsifying properties than the raw PP mixture (Ralet & Guéguen, 2000).

Modifying the properties of proteins by enzymatic crosslinking, by introducing new covalent bonds, can expand their applications. The enzymatic approach provides a safe biological technique to improve food properties (Zorn & Li, 2017). Protein crosslinking by tyrosinases from different origins have been demonstrated to increase gel firmness in meat products (Lantto, Puolanne, Kruus, Buchert, & Autio, 2007) and improve the physical stability of milk protein emulsions (Selinheimo et al., 2007).

Bacterial tyrosinase from *Bacillus megaterium* (TyrBm) was isolated, characterized and crystalized in our lab (Goldfeder, Kanteev, Isaschar-Ovdat, Adir, & Fishman, 2014; Sendovski, Kanteev, Ben-Yosef, Adir, & Fishman, 2011). Tyrosinases are dicopper enzymes, responsible for generation of melanin by converting the native substrate L-tyrosine to dopaquinone which spontaneously undergoes polymerization to melanin. Recently, it was found that TyrBm-mediated crosslinking improved soy glycinin emulsion physical stability (Isaschar-Ovdat, Rosenberg, Lesmes, & Fishman, 2015). There are no previous reports describing the modulation of PP properties by chemical or enzymatic crosslinking. The goal of this study was to evaluate the ability of tyrosinase to crosslink PP and investigate the effect of the enzymatic treatment on emulsion stability.

2. Experimental

2.1. Materials

Ammonium sulfate, sodium metabisulfite, and Nile red were obtained from Sigma Chemical Co. (Rehovot, Israel). Refined olive oil and raw potatoes were purchased from the local supermarket (Haifa, Israel), while the commercial Solanic® potato proteins 206P (patatin; 80 kDa) and 306P (protease inhibitors; 5–25 kDa) were obtained from Avebe (Avebe A.U., Foxhol, Netherlands).

2.2. Methods

2.2.1. Purification of TyrBm

TyrBm was isolated and characterized in our laboratory as previously described (Shuster & Fishman, 2009).

2.2.2. Isolation of potato protein fractions

Potato proteins were isolated from fresh potatoes (*Solanum tuberosum* L.) purchased from the local supermarket (Haifa, Israel). The isolation was done according to the method of van Koningsveld et al. (2001) with slight changes. Briefly, potato tubers were peeled and cut into small pieces and frozen at − 80 °C for 30 min. The frozen potato samples were suspended in buffer (25 mM SPB, pH 7.0, 4 mM sodium metabisulfite) (1:1 ratio) and homogenized using a commercial blender on low speed for 5 min. The potato slurry was subjected to cheese cloth filtration and the turbid filtrate was centrifuged at 14,000*g* for 30 min at 4 °C. The supernatant was then filtered using 1.2 μm GF/C Whatman filters to obtain a clear yellowish filtrate which is known to be similar to industrial potato fruit juice (PFJ).

Protein precipitation was carried out at 60% ammonium sulfate saturation. The mixtures were incubated for 90 min at 100 rpm and 4 °C. The recovered precipitates upon centrifugation (14,000*g*/30 min/4 °C) were suspended in 25 mM sodium phosphate buffer (SPB, pH 7.0) and dialyzed against the same buffer for 24 h at 4 °C (100 rpm) to remove the ammonium sulfate, followed by lyophilization. The purity of the potato protein was evaluated on SDS-PAGE gel in comparison to commercial Solanic® Avebe PP isolates 206P and 306P. The total protein content was determined by the Bradford method (Bradford, 1976) and elemental analysis (FlashSmart CHNS, Thermo Scientific Inc. Massachusetts, US).

2.2.3. Water content

Water content was determined by drying lyophilized potato protein concentrates in triplicates in an oven at 150 °C for 16 h, and determining the weight difference before and after drying (Løkra, Schüller, Egelandsdal, Engebretsen, & Strætkvern, 2009).

2.2.4. Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). 20 μL of different concentrations - depending on solubility - of potato protein extracts or fractions in water was mixed with 100 μL of Folin-Ciocalteu reagent (1:10). After 7 min, 100 μL of Na_2CO_3 solution (200 g/L) was added, followed by a 60 min incubation in the dark (30 °C), and the optical density was measured at 765 nm. The total phenolic content was calculated on the basis of a calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), in milligrams per liter of the sample.

2.2.5. Enzymatic crosslinking of potato proteins

1% (w/v) PP was suspended in 25 mM SPB pH = 7 and stirred for 30 min at ambient temperature. The crosslinking reaction was carried out as previously described (Isaschar-Ovdat et al., 2015) with slight changes. TyrBm was added in a protein ratio of 1:30 and the reaction mixture was incubated at 37 °C with shaking at 250 rpm in an incubator shaker (TU-400 Orbital Shaker Incubator, MRC, Holon, Israel). Samples were taken at various times (0, 30, 60, 120, 180 and 240 min, respectively) and the reaction was stopped by directly mixing the reaction mixture with electrophoresis sample buffer (× 4) at 1:1 ratio (v/v). The samples were analyzed by SDS-PAGE.

2.2.6. Preparation of crosslinked protein solutions and emulsions

6.1% (w/v) PP was suspended in 25 mM SPB pH = 7 and stirred overnight at 4 °C to ensure full hydration. The reaction conditions were set as described in Section 2.2.5 and immediately 40% (w/v) olive oil was added and homogenized using a shear dispersing unit (Pro200, Pro-Scientific, Oxford CT, USA) for 5 min at 35,000 rpm. The emulsions were then incubated for 60 or 240 min at 37 °C with shaking at 250 rpm in an incubator shaker (TU-400 Orbital Shaker Incubator, MRC, Holon, Israel). For the non-crosslinked emulsions all details remain identical while SPB was added instead of enzyme.