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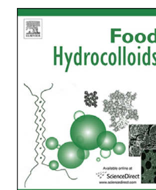

Adsorption of oat proteins to air–water interface in relation to their colloidal state

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The colloidal size and charge of oat protein isolate (OPI) at pH 7.2 and 9.0 with/without transglutaminase (TG) treatment were investigated and related to its surface activity at air–water interface. OPI was prepared from defatted oat flour. It was dispersed in water (pH 7.2), or in buffer (pH 9.0) and the soluble fraction (supernatant after centrifugation at 10 000 × g) was used for particle size, ζ-potential and dynamic surface tension measurements. Dispersions were found to be electrostatically stable (ζ-potential > −35 mV) with average particle sizes of ~70 nm at pH 7.2 and ~30 nm at pH 9.0. When diluted at pH 7.2, dissociation and re-association of the particles occurred resulting in increased polydispersity and increased size, while at pH 9.0 particle size and ζ-potential were unchanged after dilution. Dynamic surface tension measurements revealed slower adsorption dynamics and higher final surface tension values at pH 9.0 than at pH 7.2. TG-treatment of OPI dispersion resulted in formation of inter-molecular covalent linkages and led to decreased average particle size and increased stability against dilution in OPI dispersions at neutral pH. TG-treated proteins showed increased negative charge at pH 7.2 and resulted in higher surface tension values compared to the untreated samples. The topography images of adsorbed or spread layers of OPI at a/w interface revealed that oat globulins were effectively adsorbed and existed as monomeric particles or formed aggregates at the interface depending on pH.

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1. Introduction

Increasing cost of animal husbandry to the environment and the ever increasing world population necessitate increased supply of sustainable protein sources. Today, the use of plant protein ingredients in the human diet is restricted due to their lower nutritional value compared to the animal-sourced proteins and poor technological functionality. The only plant protein exploited in significant volumes as an ingredient for human nutrition is soy (Frost & Sullivan, 2012). However, alternative sources like legume or cereal protein isolates or concentrates are emerging in the food ingredient market.

Oats (*Avena sativa* L.) is a distinct cereal owing to its higher protein (12–25% of the groats), unsaturated fatty acids, soluble fiber (β-glucan) and antioxidant contents compared to other cereals (Lasztity, 1996). The unique gluten-free protein composition of oats makes it an allowed food ingredient for celiac patients in many countries (Fric, Gabrovska, & Nevoral, 2011). While alcohol-soluble prolamins are the main storage protein in other cereals, the major storage proteins in oats are the salt-soluble globulins comprising 70–80% of the total proteins (Robert, Nozzolillo, Cudjoe, & Altosaar, 1983). Oat globulins show high similarity with soy glycinin (more generally the legumin-like storage 11S globulins) in terms of amino acid sequence, molecular weight and the quaternary structure. Identification of salt extracts of oat grain revealed existence of 3S, 7S, and 12S globulins, 12S being the major fraction. Oat 12S globulin is an oligomeric protein (322 kDa) consisting of six subunits linked together by noncovalent interactions. A subunit is comprised of two polypeptide chains, α (acidic) and β (basic), (~32 and ~22 kDa respectively) which are linked together with a single disulphide bond (reviewed by Klose & Arendt, 2012; Lasztity, 1996). The pI of oat globulins was reported to be around pH 5.5 but a basic fraction (pI at pH 8.0–10.0) was also observed (Ma & Harwalkar, 1984). Oat prolamins (avenins) belong to the S-rich prolamins. They are rich in glutamic acid and proline residues and low in basic amino acids.

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Avenins are generally extracted with 70% (v/v) ethanol without reduction of inherent disulphide bonds and they are characterized with molecular weights 17–34 kDa and show pI at the range of pH 5.0–9.0. Most of the metabolically active proteins (mostly enzymes) in oats are in the water-soluble albumin fraction. Oat albumins contain high levels of lysine, in fact, the highest among the cereal albumins and they contain the lowest levels of glutamine–glutamic acid among the other oat proteins. They are a group of proteins with molecular weights at around 15 kDa (major) and 22–35 kDa (minors) and pI ranging from pH 4.0 to 7.0 (Klose & Arendt, 2012; Lasztity, 1996; Ma & Harwalkar, 1984).

It may well be stated that the solubility of oat proteins is limited around neutral or slightly acidic pH environments which are applicable for most food products. In fact, they are scarcely soluble at molecular level but exist as colloidal aggregates. Stability of protein aggregates (particles) in liquid media is critical for their use in food applications as foaming or emulsifying agents or formation of gelled structures. There are several studies where oat proteins emulsifying (Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009; Wu, Sexson, Cluskey, & Inglett, 1977) and foaming (Kaukonen et al., 2011; Konak et al., 2014; Ma & Harwalkar, 1984; Mohamed et al., 2009) properties were explored. Also the effects of chemical or enzymatic modifications such as acetylation (Mohamed et al., 2009), succinylation (Mirmoghtadaie, Kadivar, & Shahedi, 2009; Mohamed et al., 2009), deamidation (Ma & Khanzada, 1987; Mirmoghtadaie et al., 2009) and enzymatic cross-linking (Mohamed et al., 2009; Siu, Ma, & Mine, 2002; Siu, Ma, Mock, & Mine, 2002) on functional properties were reported. In most of those studies, emulsifying and foaming capacities and stabilities were assessed and compared to that of soy proteins or plant proteins from other sources. Such comparisons, however, are complicated due to variation in composition of the raw materials, processing histories and protein extraction procedures. Since plant proteins are not truly soluble but form colloidal dispersions, determination of their colloidal state prior to assessment of functionality is needed particularly for development of networks and interfaces and for control of macroscopic phase separation in multiphasic food systems. Recent interest in applying soft matter physics for foods has highlighted the importance of the whole range of structural scales (Ubbink, Burbidge, & Mezzenga, 2008). In mesoscale assembly, interactions of proteins are governed by a complicated balance of forces, where electrostatic and hydrophobic effects play a crucially important role. The functionality of plant proteins has mainly been associated with their solubility and the possibility of deriving structural building blocks from oligomeric assemblies remains largely unexplored. de Folter, van Ruijven, and Velikov (2012), has recently shown formation of oil-in-water Pickering emulsions by using water-insoluble zein particles offering consideration of plant protein natural colloidal aggregates as food-grade, biocompatible materials for particle stabilized emulsions and foams. More insight on the molecular and colloidal properties of plant proteins in conditions relevant to food materials is necessary for design of new plant-based protein ingredients as unique functional and nutritive additives in foods.

Functional properties of oat proteins have previously been assessed by emulsion and foam making at various conditions, however, only few of those studies included characterization of the physicochemical and the colloidal properties of the protein particles. In the present work, we investigated the colloidal size and charge properties of naturally existing oat protein particles at different pH environments and after enzymatic modification by transglutaminase. The aim was to relate the colloidal state to the surface activity of oat proteins at the air–water interface. Oat protein isolate was extracted from a high protein oat fraction which is a side stream from β-glucan enrichment process (Sibakov et al., 2011).

2. Materials and methods

Oat protein isolate (OPI) was produced from oat protein concentrate (OPC) powder by alkaline extraction followed by isoelectric precipitation as described in Liu et al. (2009). OPC was obtained from supercritical CO_2 extracted oat grits as described in Sibakov et al. (2011). It is a fine powder containing around 60% protein (the rest being starch ~30% and lipids ~2%). Prepared OPI was stored in a desiccator until use.

2.1. Differential scanning calorimetry (DSC)

Mettler Toledo DSC820 (Dietikon, Switzerland) equipped with liquid nitrogen cooling system was used to determine peak temperatures and enthalpies of the OPC and OPI in excess water. An amount of 10 ± 1 mg of each sample was weighed in 100 μL stainless steel pans, followed by adding water (75% water content). Samples were equilibrated for two hours before measurements. Heating of each sample was carried out from 0 to 130 °C at a rate of 10 °C/min. Peak temperatures and enthalpies were determined using Mettler Toledo analysis software. Average of two measurements was reported.

2.2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed to analyse the protein compositions in OPC and OPI and also to analyse the enzymatic oligomerisation of oat proteins upon TG treatment. For analysis of the protein compositions of OPC and OPI, both powders were dispersed in water and pH was adjusted to 10 for high protein solubility. SDS-PAGE was performed under reducing and non-reducing conditions according to Laemmli (1970). A Bio-Rad electrophoresis unit (Bio-Rad Laboratories, Richmond, CA, USA) was used with pre-cast 12% Tris–glycine polyacrylamide gels (PAGEr Gold Precast gels, Lonza, Rockland, ME, USA). Pre-stained SDS-PAGE standards (broad range, Bio-Rad, Richmond, CA, USA) were used for molecular weight estimation and the gels were stained with coomassie for visualization of the protein bands.

2.3. Preparation of OPI solutions

OPI was dispersed in water or sodium phosphate (10 mM, pH 7.2) or borate (10 mM, pH 9.0) buffers at a protein concentration of 5 mg/mL by magnetic stirring at room temperature for 1 h. The dispersion was centrifuged at 10 000 × *g* for 15 min (at 20 °C) and the supernatant was kept as the stock solution. Stock solutions were stored in the refrigerator until use within a few days. Protein concentration of the stock solutions was determined using the Bio-Rad DC protein assay kit (Bio-Rad, Richmond, USA) using bovine serum albumin as the protein standard. The stock solutions were then diluted in sodium phosphate (10 mM, pH 7.2) or borate (10 mM, pH 9.0) buffers to the protein concentrations of 0.1 and 0.01 mg/mL and left at room temperature for 30–60 min prior to the measurements. The change in the buffer molar concentration after the dilutions was negligible.

2.4. TG treatment

The commercial transglutaminase (TG) preparate Activa® WM (Ajinomoto, Tokyo, Japan) was used after removal of the maltodextrin as described in Lantto, Puolanne, Kalkkinen, Buchert, and Autio (2005). The activity of the TG preparation was assayed by the colorimetric hydroxymate method (Folk, 1970) using 0.03 M N-carbobenzoxy-L-glutaminylglycine (Sigma Aldrich) as substrate at

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