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Effects of partial hydrolysis on structure and gelling properties of oat globular proteins

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ABSTRACT

The effects of partial hydrolysis and the environmental conditions (pH and temperature) on the gelling properties of oat protein isolate (OPI) were investigated. OPI was treated with flavourzyme, alcalase, pepsin and trypsin. The changes in protein structure were observed by SDS-PAGE, size exclusion high performance liquid chromatography (SE-HPLC) and amino acid analysis. Gel mechanical properties were evaluated by textural profile analysis (TPA). The results revealed that the acidic polypeptides (12S-A) of oat globulin exerted great influence over the gelling ability of oat protein. Partial hydrolysis by flavourzyme and trypsin could significantly improve oat protein gel strength, especially at pHs 8–9 by modulating the balance between the electrostatically repulsive force and the hydrophobic attractive force among polypeptide chains during the gelling process. The gels prepared with flavourzyme and trypsin treated oat proteins have comparable or higher mechanical strength than soy protein gels at neutral pH. At pH 9 the gel made of trypsin treated oat protein even showed comparable mechanical strength to egg white protein gels under the same pH. Both oat protein and its hydrolysate gel exhibited excellent water-holding capacity at neutral or mildly alkaline conditions. The results of this study indicate that oat protein has a promising potential to be used as new and cost-effective gelling ingredient of plant origin to provide texture and structure in food products.

1. Introduction

Gelation is one of the most important functional properties of proteins as it provides texture and support in foods. Generally, thermal gelation of globular proteins involves unfolding of the protein molecules by heating, which leads to exposure of hydrophobic amino acid residues. Later, unfolded molecules re-arrange and aggregate irreversibly via disulfide bridges, hydrogen bonds, hydrophobic and/or van der Waals interactions. Finally, aggregation carries on with association of protein particles and if the protein concentration is sufficiently high, a three-dimensional network is created (Lefèvre & Subirade, 2000). This process only takes place in the presence of adequate environmental conditions, such as pH, temperature and ionic strength (Totosaus, Montejano, Salazar, & Guerrero, 2002).

Plant proteins are normally considered inferior to animal proteins in terms of gelling properties. Gelatin, egg white and whey proteins are widely used as gelling agents in the food industry, particularly in meat and dairy based systems. In recent years, proteins derived from plant sources are becoming one of the food industry's fastest-growing and most-innovative ingredient segments owing to health (no Bovine Spongiforme Encephalopathy concern), religious and cost reasons. For a long time, soy protein has been the major plant protein gelling ingredient in the market. Yet there is an opportunity for other novel gelling ingredients of plant origin to meet the increasing market requirement for different functionalities and sensory attributes. Canada is the third largest producer of oat in the world, with an annual production of approximately 2.7 million tons (Statistics Canada, 2012). Canadian oat is commonly used as an animal feed and only a small percentage of the grain is currently used for human consumption. Recently the human food market for oat has been gaining momentum mainly due to the growing public awareness of the health benefits of β-glucan. This soluble dietary fiber component of oat is known to reduce blood cholesterol (Braaten, Wood, & Scott, 1994), and regulate blood glucose levels (Wood, Scott, Riedel, Wolynetz, & Collins, 1994). Several techniques have been developed to isolate β-glucan from oat grain as a health ingredient in food products. The remaining components such as protein and starch are awaiting research to develop their full value (Inglett, Lee, & Stevenson, 2008).

Oat has the highest protein level (12–20%) (Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009) among cereals with a superior amino acid profile due to higher amounts of limiting amino acids lysine and threonine (Klose & Arendt, 2012). This is related to the fact that in most cereals the major storage proteins are alcohol-soluble prolamines whereas in oat, globulins represent 70–80% of the total protein fraction (Robert, Nozzolillo, Cudjoe, & Altosaar, 1983). The major fraction in oat protein is the 12S globulin, which consists of two major subunits with molecular weight of about 32 and 22 kDa called the A- and B-subunits, where the A-subunit is an acidic polypeptide and the B-subunit is a basic polypeptide. The A- and B-subunits are disulfide

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bonded in the native globulin, forming a dimer with a molecular weight of 54 kDa, which further associates into a hexamer through non-covalent forces (Burgess, Shewry, Matlashewski, Altosaar, & Miflin, 1983). The 7S and 3S are the minor fractions. 7S globulins are polypeptides with molecular weight of 55 kDa, and some minor components with a molecular weight of 65 kDa are also present. The 3S fraction entails at least two major components with molecular weight of about 15 and 21 kDa (Klose & Arendt, 2012).

Oat 12S globulin resembles the structure of 11S globulin of soy (glycinin), which has demonstrated good gelling properties; hence oat protein has the potential to act as a gelling agent. Identification and development of such key functionality from oat protein can significantly promote its utilization in the food market. Ma et al. have significantly contributed to this area of research. However such efforts are still limited compared to those on dairy and soy proteins. Two previous publications demonstrated that oat protein could form gels (Ma & Harwalkar, 1987; Ma, Khanzada, & Harwalkar, 1988). But at acidic and neutral pH, very weak gels with poor water holding capacity were obtained. The gel properties improved after pH 8, but strong gels could only be prepared at pHs 9–10. The gel hardness was greatly increased by both acetylation and succinylation (Ma & Wood, 1986, 1987). The authors suggested that the changes in the functional properties of oat protein after modification resulted from altered conformation and increase in net charge (Ma, 1984, 1985; Ma & Wood, 1986, 1987). This was later confirmed with the study of the thermal aggregation of oat globulin by Raman spectroscopy (Ma, Rout, & Phillips, 2003). In this work, changes in protein interactions and conformation were induced by the addition of protein structure modifying agents such as chaotropic salts, sodium dodecyl sulfate or dithiothreitol, which can either enhance or inhibit thermal gelation of oat globulin.

Enzymatic hydrolysis is a preferable tool to alter functional properties of proteins because of milder processing conditions required, easier control of reaction and minimal formation of by-products (Mannheim & Cheryan, 1992). Recent research has reported the effect of enzymatic hydrolysis over the gelling properties of proteins including soy protein (Hou & Zhao, 2011), rice bran protein (Yeom, Lee, Ha, Ha, & Bae, 2010), sunflower protein (Sanchez & Burgos, 1997), and canola protein (Pinterits & Arntfield, 2007). Results from these studies indicate that improvement of the gelling capacity is highly enzyme specific. The gelling properties of oat protein treated with trypsin were studied in previous work (Ma & Wood, 1986, 1987), however, weak gel structure was obtained due to the short size of the protein molecules, which may no longer be able to associate to form a strong gel matrix. Since the final composition and thus the use of the hydrolysates will depend on the type of enzyme used and the hydrolysis conditions (Benítez, Ibarz, & Pagan, 2008). A systematic investigation of the effect of various proteases over the gelling capacity of oat protein is required. Till now, such information is not available, however important for the development of new modification strategy to improve oat protein gelling properties.

Modification of protein conformation can also be achieved through limited hydrolysis, as changes in the secondary and tertiary structure can be produced. This can alter the surface exposure of reactive amino acids, leading to an increase in interactions favoring aggregation (Foegeding & Davis, 2011) and three-dimensional network formation. Our preliminary trials have demonstrated that partial enzymatic hydrolysis can improve oat protein gelling properties under specific conditions. Thus it is hypothesized that oat protein and its hydrolysates could form gels of plant origin with similar properties as those from animal proteins such as egg white. Therefore, this work aims to complete a systematic study of the thermal gelation of oat protein and its hydrolysates under different environmental conditions with an emphasis on the gel mechanical strength and water-holding capacity which are the most important gel characteristics for food applications. If the defined gel physical properties would be in the range of similar properties of animal protein derived gels, value-added opportunities would exist for oat protein to be used as a new gelling ingredient in food formulations such as meat binder and fat replacer to create food with improved quality and nutritive value, or used in meat analogues for vegetarian foods. In this way, additional revenue return could be generated to oat producers and processors to enhance their sustainability.

2. Materials and methods

2.1. Materials

Naked oat grains (*Avena nuda*) were purchased from Wedge Farms Ltd., Manitoba, Canada. The protein content was 17.2%. Flavourzyme (≥500 U/g), alcalase (2.4 U/g), pepsin (≥250 U/mg), trypsin (1462 U/mg), sodium dodecyl sulfate (SDS) and Tri-nitro benzene sulfonic acid (TNBS) were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). E-Z run pre-stained protein ladder/marker was purchased from Fisher Scientific (Whitby, ON, Canada).

2.2. Protein extraction

Oat grains were ground to flour using a mill (Ultra Centrifugal ZM 200 Retsch, PA) equipped with a 0.5 mm screen. The flour was then defatted with hexane at room temperature. Globular protein was extracted from the defatted oat flour according to the method reported by Wu, Sexson, Cluskey, and Inglett (1977) with some modifications. Briefly, defatted oat flour was dispersed in an alkali solution adjusted to pH 9.2 using sodium hydroxide at a flour-to-solvent ratio of 1:6 and mixed for 1 h at room temperature. The slurry then passed through a 300 μm wire mesh and the permeated mixture was centrifuged at 7000 ×*g* for 15 min. Then, the supernatant was collected and pH was adjusted to 5 with 1 M HCl, followed by centrifugation at 7000 ×g for 15 min. The pellet corresponding to the precipitated protein was washed with distilled water and freeze-dried for later use. Protein content of the extracted oat protein was determined using the Leco nitrogen analyzer (FP-428, Leco Corporation, St Joseph, MI) and a nitrogen to crude protein conversion factor of 6.25 was used.

2.3. Enzymatic hydrolysis of oat protein

A 2% (w/v) protein suspension was prepared with distilled water. The pH and temperature of the suspension were adjusted to the optimum condition for each enzyme. Hydrolysis with flavourzyme was carried out at pH 7 and 50 °C, alcalase at pH 8 and 50 °C, pepsin at pH 2 and 37 °C and trypsin at pH 8 and 37 °C. The enzyme/substrate ratio was set at 10/100 for all treatments. Over the hydrolysis period (30 min) the pH was kept constant with 1 M HCl or 1 M NaOH. At the end of the hydrolysis, the solution was heated at 90 °C for 10 min to inactivate the enzyme. Hydrolysate samples were collected, freeze-dried and stored for further experiments. The protein content of the dried hydrolysates was also determined using the Leco nitrogen analyzer (FP-428, Leco Corporation, St. Joseph, MI).

2.4. Characterization of oat protein and its hydrolysates

Degree of hydrolysis (DH) was determined by the TNBS assay (Adler-Nissen, 1979). Total number of amino groups was determined in a sample completely hydrolyzed with 6 N HCl at 110 °C for 24 h. The DH was calculated with the following equation

$$DH = \frac{h}{h_{tot}} \times 100$$

where h (hydrolysis equivalents) is the amount of peptide bonds cleaved during hydrolysis, which is expressed as millimole equivalents per gram of protein (mmol/g of protein) and h_{tot} is the total amount of peptide bonds in the protein substrate. L-Leucine (0–1.5 mM) was used to generate a standard curve ($R^2 = 0.99$).