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Oat protein solubility and emulsion properties improved by enzymatic deamidation

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ABSTRACT

Oat protein produced by a dry milling process was enzymatically deamidated by a food-grade protein-glutaminase (PG), and the effects on structure, solubility and emulsifying properties of oat proteins were studied. The reactions were conducted at neutral pH and low salt concentration conditions. Oat proteins were deamidated up to a deamidation degree of 59%. The solubility of proteins doubled. Oil-in-water emulsions prepared with the native and deamidated oat proteins differed as the emulsions prepared from oat proteins with high deamidation degree had a more uniform oil droplet particle size and longer stability. Fourier transform infrared (FT-IR) analysis demonstrated that oat protein secondary structure became more flexible by deamidation. Protein-glutaminase mediated deamidation appeared to be a promising technique to improve oat protein functionality such as emulsifying ability and solubility.

1. Introduction

Oats (*Avena sativa*) are a rich source of human nutrients. The protein content of oat grain is the highest among the major cereals (Hoseney, 1994). Oat proteins have a relatively good balance in the amino acid profile. The major group of protein in oats, globulin, has poor solubility in water under neutral and slightly acidic pH condition (Loponen et al., 2007). Oat globulin has a very compact molecular structure and a denaturation temperature of 112 °C that is higher than in most plant seed globulins (Marcone et al., 1998). As a result, oat globulin shows poor functionalities in aqueous foods and limits the food uses of oats and oat proteins (Ma, 1984; Loponen et al., 2007). Recently, technologies including fine milling and air-classification have enabled the industrial production of the oat protein enriched fraction to reach a production yield up to 5% with a protein concentration over 70%, and to reach higher production yields with lower protein concentration (Sibakov et al., 2011). There is a demand to improve the food-use functionality of the oat protein fraction.

Protein functionalities in aqueous food systems mainly refer to water solubility, emulsifying properties, foaming ability and gelling ability. Proteins with good solubility can produce aqueous foods with high protein content. Moreover, good solubility is normally an important precondition property for good emulsifying, foaming and gelling properties. Emulsifying ability is one of the most important functionalities of food proteins, since emulsions are common systems existing in various aqueous foods (McClements, 2005). Therefore, improvement of water solubility and emulsifying properties of oat proteins would extend their food-use applications.

Attempts have been made to improve the oat protein functionality, water solubility and emulsifying properties by using various methods such as succinylation and acetylation (Mirmoghtadaie et al., 2009; Mohamed et al., 2009), deamidation with acids (Mirmoghtadaie et al., 2009), and hydrolysis with proteolytic enzymes (Guan et al., 2007; Nieto-Nieto et al., 2014). However, there is

Abbreviations: DD, deamidation degree; DOP, deamidated oat proteins; FT-IR, Fourier transform infrared; NOP, native oat proteins; PG, protein-glutaminase; SDS, sodium dodecyl sulphate; SDS-PAGE, Sodium dodecyl sulphate - polyacrylamide gel electrophoresis; SE-HPLC, Size-Exclusion High-Performance Liquid Chromatography; TCA, trichloroacetic acid; UV, ultraviolet.

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still a need for processing methods that are food-grade, controllable, effective and specific in improving oat protein functionality. Legal issues about using succinic anhydride and acetic anhydride in food protein processing are still unclear. Acid deamidation is normally conducted at a low pH and high temperature condition, which may change the properties of the other compounds like starch and vitamins in food. Moreover, acid deamidation and proteolysis can lead to severe degradation of protein that might be problematic for causing bitter taste (Seo et al., 2008). No previous studies exist on the deamidation of oat protein without causing proteolysis at the same time.

One possible means to improve oat protein functionality without causing severe hydrolysis could be deamidating oat protein with a food grade commercial enzyme, protein-glutaminase (PG, purified from *Chryseobacterium proteolyticum*, EC.3.5.1.44), that specifically catalyzes the deamidation of the side chain amino group of protein-bound glutaminyl residues (Yamaguchi et al., 2001; Scheuplein, 2007). However, it was not clear whether PG could work on oat proteins or not. This is because the PG enzymatic treatments are normally conducted in neutral pH and low salt concentration conditions, in which oat protein can hardly be solubilized. In addition, the molecular structure of oat globulin is globular and very compact, which may result in a difficulty for the PG enzyme to attach to the oat protein.

The present work studied the modification of compact oat protein by enzymatic treatment with PG in neutral pH and low salt concentration conditions. Deamidation degree, molecular size and secondary structure, as well as the solubility and emulsifying properties of oat proteins were investigated.

2. Materials and methods

2.1. Materials

The oat protein enriched fraction was obtained from a process described by Sibakov et al. (2011). Briefly, whole oat grits (Raisio Oyj, Kokemäki, Finland) were defatted by supercritical CO_2 (NateCO_2 GmbH, Wolnzach, Germany). The defatted material was then milled with a Contraplex 250CW pin disc mill (Hosokawa Alpine AG, Augsburg, Germany) and air classified twice by a 315ATP air classifier (Hosokawa Alpine AG). As a result, the ultra-fine protein particles (D_{90} < 10 μm) were separated from the starch endosperm flour. The protein fraction contained 62.0% protein, 17.1% starch, 2.8% fat and 2.0% dietary fiber (of which 1.0% β-glucan). The enzyme, protein-glutaminase (PG) Amano 50 (50 unit/g, 1% active constituent, 99% dextrin as bulking agent), was supplied by Amano Enzyme Inc. (Japan).

2.2. Determination of the deamidation degree (DD)

The deamidation degree (DD) of oat proteins was determined according to the method of Yong et al. (2006), with slight modifications. Oat proteins (10 mg) were weighed in 2 mL microcentrifuge tubes. One mL of 200 mM sodium phosphate buffer (pH 7.0) containing 0.001 unit/mL, 0.01 unit/mL, 0.065 unit/mL, 0.13 unit/mL or 0.26 unit/mL of PG were added. The carefully sealed samples were mixed and incubated at 40 °C for various periods of time between 0 and 48 h. Then the samples were cooled down in an ice bath and proteins precipitated by adding 3 mL of 0.3 M trichloroacetic acid (TCA) solution and centrifuging at room temperature (21 °C, 5 min, 3220 × g). The concentration of the ammonia in the supernatant was measured using an ammonia assay kit (Megazyme International Ireland Ltd., Ireland) according to the manufacturer's instructions. Oat protein (10 mg) was totally deamidated by treatment with 1 mL of 2 N HCl at 100 °C for 3 h. The DD was expressed as the ratio of the amount of ammonia released from the PG reaction to that from the total deamidation reaction. All treatments were repeated independently and ammonia was determined in duplicate.

2.3. Preparation of protein-glutaminase (PG) deamidated oat proteins

Protein-glutaminase (PG) deamidated oat protein samples were prepared according to the method of Yong et al. (2006), with slight modifications. One gram of oat protein was dispersed in 100 mL of 200 mM sodium phosphate buffer (pH 7.0) containing different dosages of PG (0.001 unit/mL, 0.01 unit/mL and 0.26 unit/mL), and then incubated at 40 °C for 12 h. Then the suspension was dialyzed (molecular weight cut-off of 10 kDa) against 0.1 M acetic acid at 6 °C for over 72 h to remove the salts with low molecular weight. After dialysis, the suspension in the dialysis tube was lyophilized.

2.4. Size-exclusion high-performance liquid chromatography (SE-HPLC) analysis

The oat protein samples were analyzed with SE-HPLC as described by Loponen et al. (2009) with modifications. The oat protein samples were extracted 1:100 by 1.5% SDS, 50 mM sodium phosphate, pH 6.9 buffer, at 50 °C overnight and then centrifuged at room temperature (10 min, 10 000 × g). The extract was then mixed 1:1 with the elution buffer (0.1% SDS, 20% acetonitrile, and 50 mM sodium phosphate, pH 6.9) and filtered (0.45 μm). The solution was then analyzed with a SE-HPLC system equipped with two coupled size-exclusion chromatography columns Superdex Peptide 10/300 GL and Superdex 200 10/300 GL (GE Healthcare Biosciences AB, Uppsala, Sweden), a HPLC system (Hewlett Packard series 1050, Germany) and a multiple wavelength UV detector. The columns had separation ranges of 100–7000 and 10,000–600,000 for molecular weights, respectively. The operation of the SE-HPLC analysis was carried out with an injection volume of 100 μL, and a flow rate of 0.4 mL/min, at room temperature. The oat protein was detected at a wavelength of 210 nm. The molecular weight distribution of the SE-HPLC was calibrated by analyzing a series of standard protein fractions and peptides including oat globulin, prolamin peptides (9-, 19- and 33-mer) and so on using the SE-HPLC, then collecting the eluted fractions from the standard samples according to corresponding elution volume with 1 mL intervals, and finally analyzing the molecular weight of the eluents with SDS-PAGE.

2.5. Fourier transform infrared spectroscopy (FT-IR) analysis

Dry powder of the native and protein-glutaminase (PG) deamidated oat protein samples were analyzed by a Perkin Elmer Spectrum One FT-IR spectrometer according to the method of Yong et al. (2006) and Siu et al. (2002a) with modifications. Eight independent scans were averaged for each sample. The band assignment to protein secondary structural component was conducted for amide region I (1600-1700 cm^{-1}). Deconvolution of the infrared spectra was performed using FT-IR Spectrum One software (V3.02.01, Perkin Elmer, Inc., USA) according to the method of Kauppinen et al. (1981). The deconvolution gamma value (resolution enhancement factor) was set as 2.4, and the smoothing length was set as 60%. Deconvoluted spectra were then fitted with Gaussian band shapes by using the software Origin 7.5 (v7.5870, OriginLab Corporation, USA). Identification of protein secondary structure in Gaussian fitted multiple peaks was conducted according to the methods of Siu et al. (2002 b), Yong et al. (2006) and Carbonaro and Nucara (2010) with integration and slight modifications. The area of each peak indicated the quantity of each