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Effect of acetylation and succinylation on physicochemical properties and structural characteristics of oat protein isolate

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ABSTRACT

The effects of acetylation and succinylation on physicochemical properties and structural characteristics of oat protein isolate (OPI) were investigated. The degree of N-acylation rapidly increased prior to O-acylation, due to higher reactivity of ε-amino groups than hydroxyl groups. The acylation was able to decrease zeta potential of OPI at neutral pH, and succinylated OPI had lower zeta potential than acetylated OPI. The surface hydrophobicity (H_0) of OPI was changed significantly by acylation treatment, which varied with the type and level of applied anhydrides. The succinylation led to a remarkable increase in the molecular weight of OPI determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The secondary structure and tertiary conformation of proteins in the OPI was analyzed by Fourier transform infrared (FTIR) and intrinsic fluorescence spectroscopy. The acylation could result in the transformation of β-sheet to α-helix and random coil, and less compact tertiary conformation, especially succinylation.

1. Introduction

Oat has higher nutritive value than many other cereals by the reason of its soluble fiber, unsaturated fatty acids and antioxidant [1]. In addition, as a potential source of low-cost proteins, oat has the highest level of protein within the scope of 15% to 20% with well-balanced amino acid composition among all the cereals. Oat protein has higher nutritive and biological value resulting from lack of anti-nutritional factors [2] and relatively abundant restrictive amino acids such as lysine [3]. Ma and Khanzada [4] reported that proteins in the oat provided good water and fat binding capacities, solubility and emulsifying activity, which could be used as natural high molecular material applying in food processing and the cosmetic industry. However, more functional and physicochemical properties of oat protein need to be further improved through appropriate modifications. Some chemical, physical and enzymatic modification methods, such as acylation [5], deamidation [6], high pressure [7], enzymatic cross-linking [8] and enzymatic hydrolysis [9], have been used to improve emulsifying, gelling, solubility and foaming properties of food proteins to a certain extent.

Chemical modification has the advantages of high efficiency and strong controllability for improving the functional and physicochemical properties of proteins, which is easier to implement large scale production for industrialization than physical and enzymatic modification. Acylation reaction of food proteins is a kind of typical chemical modification such as succinylation and acetylation, which occurs between amino acid residues and anhydrides including succinic anhydride and acetic anhydride. In the previous researches, Ma and Wood [10] reported that succinylation and acetylation could improve gelling properties of proteins in the oat. Gilles et al. [11] found that acylation could obviously enhance the digestibility of oat protein, which would not change its nutritional value. The emulsifying properties and water solubility of oat protein were improved by acylation which decreased water holding capacity, compared with those of native proteins, according to the research of Mirmoghtadaie et al. [12]. The functional properties of protein may be related to its physicochemical properties and spatial structure. However, there are fewer studies about physicochemical properties and structural characteristics of oat protein after acylation including acetylation and succinylation.

In this paper, the acylation of oat protein isolate was studied, which included the measurement of zeta potential and surface hydrophobicity of acylate proteins. The electrophoresis, infrared and fluorescence

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spectroscopy were analyzed. The effect of acetylation and succinylation on physicochemical properties and structural characteristics of the proteins in oat protein isolate has been investigated, which could provide a theoretical basis for the processing of protein polymer materials.

2. Materials and methods

2.1. Materials and chemicals

Oats were purchased from a local retail market and passed 80 mesh after milled, which were defatted by using hexane. Acetic and succinic anhydrides, 1-anilino-8-naphthalene-sulfonate (ANS) and 2,4,6-trinitrophene sulphonic acid (TNBS) were purchased from Sigma Company (St. Louis, MO, USA). All other chemicals were analytical or better grade.

2.2. Preparation of oat protein isolate (OPI)

OPI was prepared according to the procedure of Zhang et al. [13], with some modifications. The defatted oat flour was mixed with distilled water at a flour: solvent ratio of 1:10 (w/v) to obtain the slurry, which was constantly stirred at ambient temperature for 2 h keeping the pH at 9.0 before centrifugation at 3500*g* for 20 min. The supernatant was adjusted to pH 4.5 and recentrifuged to obtain the precipitate, which was re-dissolved after washed to neutral using distilled water. The protein solution was neutralized to pH 7.0 and finally lyophilized to produce OPI powder. The content of protein in OPI was 91.42 ± 0.39% as measured by Kjeldahl method using a nitrogen conversion factor of 6.25.

2.3. Preparation of acylated OPI

Acylated OPI samples were prepared by using the method of Franzen and Kinsella [14] with slight modifications. 2% (w/v) of OPI dispersions were prepared by suspending the lyophilized OPI powders in phosphate buffer (0.1 mol/L, pH 9.0), and stirred at ambient temperature for 1 h. The succinic anhydrides were slowly added to the protein dispersions to give the anhydride-to-protein ratio of 0.2, 0.4, 0.6, 0.8 and 1.0 (g/g). The mixture dispersions were constantly stirred for 1 h at ambient temperature, and the pH was stabilized at 9.0 by adding 1 mol/L NaOH in the process of reaction. After neutralizing pH to 7.0, the dispersions were dialyzed for 24 h at 4 °C and lyophilized to obtain the succinylated OPI samples. The acetylated OPI samples were prepared using acetic anhydride instead of succinic anhydride, and the other procedures were the same as the succinylated OPI samples. The native OPI was not treated as control.

2.4. Measurement of degree of N-acylation

The measurement for degree of N-acylation was using the method of Habeeb [15] with slight modifications. The protein samples were dispersed in phosphate buffer (50 mmol/L, pH 8.5) which contained 50 mmol/L NaCl to form the 1% (w/v) of protein dispersions, and mixed with 0.1% (w/v) TNBS solution at a ratio of 1:1 (v/v). 2 mL of mixtures were heated for 2 h at 60 °C, and then cooled down to room temperature. The reaction was stopped by addition of 0.5 mL of 1 mol/L HCl and 1 mL of 10% (w/v) SDS. The determination for the absorbance at 335 nm was carried out by a UV–vis spectrophotometer, and the degree of N-acylation was expressed by percent decrease in absorbance of acylated proteins compared with the native protein.

2.5. Measurement of degree of O-acylation

Degree of O-acylation of protein in sample was determined according to the method of Gounaris and Perlmann [16] with slight modifications. The reaction reagent was prepared by mixing the distilled water, 3.5 mol/L NaOH and 2 mol/L NH_2OH-HCl at the volume ratio of 1:1:2, and mixed with 1 mL of 5 mg/mL sample solution before thermal treatment for 2 h at 40 °C. The termination of reaction was performed by adding 1 mL of 3 mol/L HCl. 1 mL of 0.37 mol/L $FeCl_3$ was added for coloration for 15 min before centrifugation. The absorbance at 540 nm of the supernatant was determined using a spectrophotometer, which represented the degree of O-acylation.

2.6. Measurement of zeta potential

Zetasizer Nano ZS (Malvern Instruments Ltd., UK) was used to determine the zeta potential of samples. The protein sample was dissolved in 0.01 mol/L phosphate buffer (pH 7.0) to give a concentration of 0.5% (w/v), and injected into the apparatus. The averages of three measurements were reported as zeta potential.

2.7. Measurement of surface hydrophobicity (H_0)

H_0 of samples was measured by the ANS method as described by Yuksel et al. [17] with slight modifications. The samples were dispersed in 0.01 mol/L phosphate buffer at pH 7.0 to obtain 1 mg/mL protein dispersions, which were constantly stirred at ambient temperature for 1 h. The protein concentrations in the dispersions were diluted to the range of 0.05–1 mg/mL with the same buffer. 2 mL of the sample diluent was mixed with 20 μL of 8.0 mmol/L ANS solution (prepared by the same buffer). Perkin-Elmer 2000 fluorescence spectrophotometer was used to determine the fluorescence intensity, which was carried out at wavelengths of 390 nm (excitation) and 470 nm (emission) with a constant slit of 5 nm for both excitation and emission. The index of H_0 was expressed by the initial slope of fluorescence intensity versus protein concentration.

2.8. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out using 4% stacking gel and 12% separating gel by the method of Yadav et al. [18]. The sample buffer consisted of 0.5 mol/L Tris-HCl buffer mixing with 5% (v/v) glycerol, 20% (w/v) SDS, 0.1% (w/v) bromophenol blue and 2% (v/v) 2-mercaptoethanol. The protein sample was dissolved in the sample buffer to obtain 2 mg/mL dispersion, which was heated at 95 °C for 5 min before electrophoresis. After running, gels were fixed and stained with Coomassie Blue R250 dye.

2.9. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of protein sample was carried out by the procedure of van der Venc et al. [19] using a BioRad FTS-60A FTIR spectrometer (Bio-Rad Laboratories, Richmond, CA, USA). The protein samples were mixed with dried KBr, and ground under the infrared lamp. The FTIR spectra ranging from 500 to 4000 cm^{-1} were recorded with 4 cm^{-1} resolution.

2.10. Intrinsic fluorescence spectroscopy

The intrinsic fluorescence spectra of the protein samples were measured according to the method of Li et al. [20]. The protein samples were dispersed in 0.01 mol/L phosphate buffer at pH 7.0 to obtain 1.5 mg/mL dispersions, which were analyzed by Hitachi F-2000 fluorescence spectrophotometer (Hitachi, Ltd, Tokyo, Japan). The excitation wavelength was 290 nm and the emission spectra between 300 and 400 nm was recorded with a constant excitation and emission slit of 5 nm.