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Improvement of functional properties of whey soy protein phosphorylated by dry-heating in the presence of pyrophosphate

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ABSTRACT

The physicochemical and functional properties of a whey soyprotein (WSP), which was phosphorylated by dry heating, were investigated. The phosphorus content of WSP was 0.85 g/100 g by dry-heating at pH 4.0 for 5 days in the presence of pyrophosphate. ^{31}P NMR spectral data suggested that the serine and sugar of WSP were phosphorylated. Although changes in the random coil structure of WSP measured by circular dichroism were mild, increased surface hydrophobicity and decreased tryptophan fluorescence intensity were observed following phosphorylation. Phosphorylation improved the stability against heat-induced insolubility, emulsifying properties, foaming properties, water absorption capacity, and oil absorption capacity of WSP. These results suggested that dry-heating phosphorylation is a useful method for improving the functional properties of WSP.

1. Introduction

The effective use of protein in food processing system is dependent on tailoring the protein's functional characteristics to meet the complex needs of manufactured food products. It is expected that improvements in the functional properties of food proteins could lead to the maximization of their effective use in the food industry.

Phosphorylation is a useful method for improving the functional properties of food proteins (Matheis & Whitaker, 1984). Several phosphorylation methods have been reported over the past few decades (Aoki et al., 1997; Kato, Aoki, Kato, Nakamura, & Matsuda, 1995; Seguro & Motoki, 1989; Vojdani & Whitaker, 1996). However, because of some issues concerning phosphorylation methods, their practical application remained largely complicated (Li, Ibrahim, Sugimoto, Hatta, & Aoki, 2004; Li, Salvador, Ibrahim, Sugimoto, & Aoki, 2003).

We have phosphorylated egg white proteins (EWP) by a dry-heating process in the presence of phosphate (Enomoto et al., 2007; Li, Hayashi, et al., 2005; Li et al., 2004, 2003). This method significantly improving the heat stability, emulsifying properties, and gelling properties of EWP. Furthermore, the calcium phosphate solubilizing ability of EWP can be enhanced by phosphorylation. Other animal food proteins have also been successfully phosphorylated by dry-heating in the presence of pyrophosphate, thereby proving that phosphorylation improves some functional properties of proteins (Enomoto et al., 2007; Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005). However, to our knowledge, the effects of phosphorylation by dry-heating on the functional properties of proteins derived from plant sources have not yet been investigated.

Whey soy protein (WSP), is a byproduct of the manufacture of soy protein isolate or from the process of making tofu (soy curd). Residual whey from these processes contains a range of proteins, including lipoxygenase, β-amylase, lectin, Kunitz trypsin inhibitors, and cytochrome *c* (Iwabuchi & Yamaguchi, 1987; Rackis, Wolf, & Baker, 1986). These WSPs exhibit some different characteristics. For example, the lipoxygenase is involved in the oxidation of polyunsaturated fatty acids, a reaction that gives rise to some constituents responsible for off-flavor; it has a molecular weight (MW) of 100 kDa and an isoelectric point (pI) of 5.68 and is readily inactivated by heat (Wolf & Corvan, 1975). β-Amylase from soybeans has a MW of 57 kDa and a pI of 5.85; there are no disulfide bonds in this enzyme (Morita, Yagi, Aibara, & Yamashita, 1976). Soybean lectin, a tetramer (MW, 120 kDa) composed of identical subunits (MW, 30 kDa each), lacks disulfide bridges but has two saccharide binding sites and a pI of 5.81. Kunitz trypsin inhibitor

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has a MW of 20 kDa and two disulfide bridges (Koide & Ikenaka, 1973). Some WSPs, such as trypsin inhibitors, contribute to the nutritional quality of soy by virtue of their relatively high half-cystine content (Tan-Wilson & Wilson, 1986).

There has been an increasing market demand for soy foods due to their reported beneficial effects on nutrition and health (Peñas, Préstamo, & Gomez, 2004). However, most WSPs generated from food processing are usually simply discarded even if they could be used as a good source of proteins (Peñas et al., 2004). Hence, the increased demand for soy protein products makes the recovery of WSP more attractive. This suggests that some incentives exist to improve the functional properties of WSPs and consequently, allowing their efficient utilization in the food industry. In the present study, some physicochemical and functional properties of the phosphorylated WSP (PP-WSP) were characterized. Lastly, we investigated the improvement in the functional properties including emulsifying properties, foaming properties, water absorption, and oil absorption of PP-WSP.

2. Materials and methods

2.1. Materials

Defatted and solvent-free soy flour purchased from Zhengzhou Tong Chuang Yisheng Food Co., Ltd. (Henan, China) was prepared under controlled conditions (not thermally inactivated to avoid denaturation). 1-Anilino-8-naphthalene sulfonate (ANS) and 5, 5-Dithiobis-(2-nitrobenzoic acid, DTNB) were purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan). All other chemicals were of reagent grade.

2.2. Preparation of WSP

WSP was prepared according to the method described by Thanh, Okubo, and Shibasaki (1975). Defatted soy meals were mixed with a 20-fold volume of 50 mmol/L Tris–HCl buffer, pH 8.0 containing 10 mmol/L of 2-mercptoethanol (2-ME) and then stirred at room temperature for 1 h. The supernatant can be obtained by centrifugation at 10,000*g* at 20 °C for 20 min. After adjusting the pH of the solution to 4.8 with 2 mol/L HCl, the supernatant was separated from the precipitate by centrifugation at 10,000*g* for 30 min. The supernatant was dialyzed against deionized water at 4 °C for 3 days, followed by lyophilization.

2.3. Preparation of phosphorylated WSP (PP-WSP) and dry-heated WSP (DH-WSP)

PP-WSP and DH-WSP were prepared according to our previously reported paper (Li et al., 2004). Briefly, the WSP was dissolved at a concentration of 20 g/L in 0.1 mol/L sodium pyrophosphate buffer at pH 4.0, adjusting the pH with 1 mol/L HCl, and the solution was lyophilized. Lyophilized sample was incubated at 85 °C for 5 days according to a previous paper (Li et al., 2004). To remove free pyrophosphate, the dry-heated sample was dissolved and dialyzed against deionized water for 3 days. The sample was then lyophilized.

Unlike in the preparation of PP-WSP, the DH-WSP at a concentration of 20 g/L was dissolved in deionized water. The pH of the solution was adjusted to 4.0 with 1 mol/L HCl, and the mixture was subsequently lyophilized and dry-heated under the same conditions, following in the preparation of PP-WSP. Finally, the dry-heated samples were dissolved and dialyzed with deionized water for 3 days. The sample was thereafter lyophilized.

2.4. Determination of phosphorus content of PP-WSP

Protein samples were digested in perchloric acid. The phosphorus in the digest was regarded as the total phosphorus (Pt) of PP-WSP. For the determination of inorganic phosphorus (Pi), 5 ml of 100 g/L trichloroacetic acid (TCA) was added to the same volume of a 10 g/L PP-WSP solution, and the solution was centrifugated at 3000*g* for 20 min. The phosphorus in the supernatant was regarded as Pi. The phosphorus content was determined by the method of Chen, Toribara, and Warner (1956). The amount of phosphorus bound to protein was estimated based on the difference of Pt and Pi contents.

2.5. ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy

^{31}P NMR spectroscopy was carried out with a Bruker Avance DRX 500 spectrometer (Bruker AG, Karlsruhe, Germany) operating at 162 MHz and 20 °C. Phosphoric acid (850 g/L) was used as an external standard. Proton-decoupled ^{31}P NMR spectrum at pH 8.0 (adjusted with 1 mol/L NaOH/D_2O solution) was obtained for the PP-WSP. Sample was dissolved in D_2O solution at a concentration of 30 g/L. The spectrum was obtained with an 80° tipping pulse and a 2-s repetition time.

2.6. Measurement of solubility

Protein samples were dissolved at a concentration of 1 g/L in 50 mmol/L Tris–HCl buffer (pH 7.0), and then centrifuged at 3000*g* for 20 min. The concentration of protein in the supernatant was determined using the method of Lowry, Rosebrough, Farr, and Randall (1951).

2.7. Measurement of disulfide group (SS)

SS content was obtained as the half of difference between the total sulfhydryl thiol (SH_t) and the free sulfhydryl thiol SH (SH_f). SH_t group was determined using Ellman's reagent DTNB after reduction of the samples according to the method of Beveridge, Toma, and Nakai (1974). Reduction was carried out as follows: a volume of 2 ml of supernatant was treated with 20 g/L 2-ME for 2 h. SH_f group was determinated by the same method without reduction. Briefly, samples (60 mg of WSP) was solubilized in 10 ml 86 mmol/L Tris-90 mmol/L glycine-4 mmol/L EDTA (pH 8.0) buffer containing 8 mol/L urea and gently stirred with a glass rod for 1 h until complete dispersion. The WSP solution was then centrifuged at 10,000*g* for 10 min. One milliliter of the resulting supernatant was added with 40 ml DTNB (4 mg/ml methanol) and mixed rapidly by inversion to read the absorbance at 412 nm 5 min later. Samples and reagent blanks were included in each determination. Protein was separated by precipitation with 125 g/L TCA for 1 h and subsequent centrifugation at 8000*g* for 10 min. The resulting precipitate was washed four times with 125 g/L TCA solution and solubilized in 7 ml urea buffer. SH_t groups were determined on 1 ml of this solution using Ellman's reagent as described previously. Both total and free SH contents were calculated using an Ellman's reagent molar absorptivity value of 13,600. The SS content was expressed as μmol/g of sample.

2.8. Evaluation of surface hydrophobicity

The surface hydrophobicity of WSP was evaluated by measuring the fluorescence intensity (FI) and initial slope (S_0) of WSP in the presence of ANS according to the method of Hayakawa and Nakai (1985). The WSP samples were dissolved in 20 mmol/L phosphate buffer (pH 7.4), containing 0.1 mmol/L EDTA to give a concentration