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Improved emulsifying properties of soy proteins by acylation with saturated fatty acids

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ABSTRACT

Effects of acylation on emulsifying properties of soy proteins were investigated using a variety of saturated fatty acids. Beta conglycinin (7S), glycinin (11S), and acid-precipitated protein (APP) were acylated with activated fatty acid esters (6C–18C) to form covalent linkage between the carboxyl group of the fatty acid and the free amino groups of the protein. Reduction in the free amino groups of acylated 7S, 11S and APP resulted into the dissociation of the protein, indicating a structural change, as evidenced by the fluorescence spectra and the degree of modification. It was shown that the emulsifying activity (EAI) and emulsion stability (ES) of 7S and 11S were significantly improved ($p < 0.05$) upon acylation with all saturated fatty acids, whereas no change in EAI and ES for the acylated APP was observed upon attachment of short and long chain fatty acids. The fluorescence intensity was also remarkably affected by acylation showing significant changes in protein structure. Covalent attachment of fatty acids resulted into 1.4–2.2 and 1.1–1.8-fold increase in the oil binding capacity (OBC) of 7S and 11S respectively, however no changes in acylated APP. Acylated 7S showed 3.0–9.4-fold increase in the water binding capacity (WBC), with no change in acylated 11S, while acylated APP with longest chains showed low WBC. The surface hydrophobicity of 7S was significantly improved ($p < 0.05$) by acylation; no changes were observed in the acylated 11S. Furthermore, acylation decreased the surface hydrophobicity of APP. Thus, it was demonstrated that saturated fatty acids with adequate chain length are suitable candidates for the preparation of functional lipoproteins from soy proteins.

1. Introduction

Soybean protein has been used as a source of nutrients in Asian countries for many decades. Soybean protein is well known for its nutritional value, different functional properties and also as a potential source of bioactive peptides. However, soy globulins (7S, 11S and APP) have poor surface functionality, which makes it difficult to be directly utilised in functional food processing. Proteins exist in a compact folded structure with buried hydrophobic groups and exposed hydrophilic groups. The hydrophobic groups are necessary for the surface properties of protein. The large variety of hydrophobic groups that might be incorporated into the protein molecules opens a very wide range of possibilities for obtaining improved surface activity (Magdassi & Toledano, 1996).

For the past few decades, different modification techniques have been used to alter the protein structure, changing its physical and chemical properties, hence influencing its structure–functional properties. Acylation has been used among other chemical methods for protein modification. Acylation of the free amino groups on a polypeptide chain involves the utilisation of reagents that react covalently at the amino sites of the proteins. The mechanism, depending on the nature of the modifying agent, can affect the charge balance of the molecule in three different ways, namely, by preserving the positive charge on the amino groups, by abolishing the charge and bringing it to neutrality, or by imposing a negative charge as a substitute for the original positive charge (Lakkis & Villota, 1992).

The need for multiple functional food products or ingredients has increased the pressure to food industries and researchers to develop different modification techniques to enhance and diversify the protein functionalities, such as emulsifying, water and oil binding. Attachment of hydrocarbon chains may modify the charge and structural properties of soy proteins, increasing their hydrophobicity, thus improving their surface functionality. The acylation of the ε-amino groups of the lysine residues, particularly with succinyl groups, markedly enhances several properties of proteins, including their emulsifying properties (Aoki, Taneyama, Orimo, & Kitagawa, 1981; Malabat, Sanchez-Vioque, Rabiller, & Gueguen, 2001; Mirmoghtadaie, Kadivar, & Shahedi, 2009; Wong, Nakamura, & Kitts, 2006); water and oil binding (Lawal & Adebowale, 2004; Mirmoghtadaie et al., 2009) and surface hydrophobicity

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(Kim & Kinsella, 1986; Wanasundara & Shahidi, 1997). Improved physico-chemical functionalities of soy proteins will provide an insight into functional food formulation and processing. In this study, the effects of acylation on the emulsifying properties of soy proteins were assessed using a variety of saturated fatty acids. Beta conglycinin, glycinin and acid-precipitated protein were acylated with activated fatty acid esters (6C–18C); the importance of the chain length of saturated fatty acids in the acylated soy proteins was investigated to expand their use as food ingredients and in functional food formulations.

2. Materials and methods

2.1. Materials

Soy protein isolates (SPI) was obtained from Fuji Oil Co., Ltd (Osaka, Japan). Caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid and stearic acid were purchased from Wako Co., (Tokyo, Japan). Ortho-phthalaldehyde (OPA), N-hydroxysuccinimide, tetrahydrofuran (THF) and dicyclohexy carbodiimide (DCC) were also from Wako Co., (Tokyo, Japan). *cis*-Parinaric acid (CPA) and corn oil were from molecular probes (Leiden, The Netherlands) and Nisshin Oil Co., (Tokyo, Japan) respectively. The other chemicals used in this study were of analytical grade.

2.2. Preparation of 7S, 11S and APP from soy proteins

7S and 11S globulins were prepared from soy protein isolate (SPI) according to the method of Nagano et al. (Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992). APP was prepared through acid precipitation of SPI at pH 4.5 (Kinsella, 1979). Briefly, the SPI was dispersed in distilled water (1:15 w/w), adjusted to pH 7.5 with 2 N NaOH, stirred at 20 °C for 1 h, and centrifuged at 15,000*g*, 10 °C, for 15 min. Dry sodium bisulfate ($NaHSO_3$) 1 mM was added, and the pH was adjusted to 6.4 with 3 N HCl. The mixture was kept overnight at 4 °C. The dispersion was centrifuged at 15,000 *g*, 10 °C, for 15 min. The precipitated 11S fraction was suspended in distilled water, the pH was adjusted to 8.0 with 2 N NaOH and lyophilised. The supernatant was subjected to alkaline washing with 0.25 M NaCl, the pH adjusted to 5.0 with 4 N HCl and stirred for 1 h, then centrifuged at 15,000 *g*, 10 °C for 15 min. The supernatant was diluted with 2-fold cold water, the pH brought to 4.8 by 3 N HCl, stirred for 1 h and centrifuged at 15,000*g*, 4 °C, for 15 min. The insoluble fraction 7S was suspended in distilled water and adjusted to pH 7.5 with 2 N NaOH, dialysed and then lyophilised.

2.3. Acylation of soy proteins

Esterification was carried out according to Haque et al. (Haque, Matoba, & Kito, 1982) with slight modifications. Equimolar ratio (1:1) of fatty acid and NHS in THF with DCC, were incubated overnight at 25 °C. Dicyclohexylurea was removed through filtration, and the filtrate was dried in a vacuum evaporator and dissolved in 100 ml ethyl acetate. The solution was then washed with 10% NaCl and distilled water, filtered, dried over Na_2SO_4 column and crystallized by a vacuum evaporator. The product was re-crystallized by ethyl acetate–petroleum ether. The ester linkage formation was confirmed by means of hydroxamic acid reaction after treatment with hydroxylamine (Lapidot, Rappoport, & Wolman, 1967). One hundred mg of 7S, 11S or APP were separately dissolved in 25 ml of 1% $NaHCO_3$; activated fatty acid was dissolved at 0.035 mM in 5 ml THF and added drop wise to the sample solution. The pH of the mixture was adjusted to 9.0 and was kept constant with 1.0 N NaOH. The solution was incubated at 25 °C for 6 h while stirring. A 100 mM glycine solution was added to terminate the reaction. The reaction mixture was dialysed for 48 h with two changes of deionized water, and finally dialysed in 20 mM PBS, pH 7.0 at 4 °C for 24 h, centrifuged at 10,000 *g*, 4 °C for 20 min, and lyophilised. All samples were applied to the Bio-Rad system equipped with Superdex 200 HR 16/70 prep grade, and eluted with phosphate buffered saline (pH 7.2) at a flow rate of 1.5 ml/min, to obtain the modified soy proteins.

2.4. Determination of degree of modification

The modification rate was determined by using the o-phthalaldehyde assay. The primary amino groups in native and acylated samples were determined as described by Goodno et al., (Goodno, Swaisgood, & Catignani, 1981) with slight modifications. A fresh reagent was prepared by mixing 16.4 mg of o-phthalaldehyde (dissolved in 2.5 ml of 95% methanol), followed by 25 ml of sodium tetraborate buffer pH (9.5), 5 ml of 20% SDS, 400 μl of β-mercaptoethanol solution; the total volume was adjusted to 100 ml with distilled water. A 50 μl of 0.1% sample was mixed with 1 ml o-pthalaldehyde reagent and incubated at room temperature for 2 min. The absorbance of the reaction mixture was read at 340 nm (UV 1700 spectrophotometer Shimizu, Japan); bovine serum albumin was used as standard. The degree of modification was calculated based on the decrease in absorbance of the acylated samples in comparison to the native one.

2.5. Emulsifying properties

The emulsion properties were determined by the method of Pearce & Kinsella (Pearce & Kinsella, 1978). The absorbance of the resulting dispersion was measured at 500 nm. The emulsion activity index (EAI) was calculated according to the following equations;

$$EAI(m^2/g) = \frac{2T[(A_0 \times D_f)]}{[(C \times Q \times 10^4)]}$$

where, T, A_0, C, D_f and Q represent turbidity, absorbance immediately after emulsion formation ($t = 0$ min), initial concentration of protein 0.1 g/100 ml, dilution factor, and oil fraction, respectively. The emulsion stability of the modified sample was taken as the absorbance at the half time of the total emulsion standing time.

2.6. Water and oil binding capacities

The water binding capacity (WBC) and oil binding capacity (OBC) of native and acylated samples were determined according to Matemu et al. (Matemu, Kayahara, Murasawa, & Nakamura, 2009). Briefly, distilled water or oil was added to the sample and either stirred at 1800 rpm for 30 s every 10 min, for 40 min, or shaken vigorously at 2500 rpm for 30 s every 5 min, for 30 min, respectively. The contents were centrifuged at 1300 *g* for 25 min for water binding and 15,000*g* for oil binding, respectively. The free water or oil was removed carefully. The amount of absorbed water or oil was determined by the weight difference.

2.7. Surface hydrophobicity

The surface hydrophobicity of acylated samples was determined by the method of Kato and Nakai (Kato & Nakai, 1980), with *cis*-parinaric acid (CPA) as a fluorescence probe, which depends on the polarity of the environment inside and at the protein surface. Samples were prepared by dissolving in 0.01 M phosphate buffer (pH 7.0) to a concentration of 0.01% (w/v). A 20 μl of CPA solution