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Importance of size and charge of carbohydrate chains in the preparation of functional glycoproteins with excellent emulsifying properties from tofu whey

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ABSTRACT

The importance of size and charge of carbohydrate moiety in tofu whey–carbohydrate conjugates, formed through Maillard-type glycosylation, was investigated. Tofu whey (TW) ultrafiltered fractions (UFTW > 3 kDa and UFTW < 3 kDa), acid-precipitated protein (APP), β-conglycinin (7S) or glycinin (11S) were covalently conjugated with carbohydrates (galactomannan (GM), *okara* polysaccharides, xyloglucan (XG), chitin and chitosan oligo), through naturally-occurring Maillard-type glycosylation at controlled temperature (60 °C), relative humidity (65%) and pH (7.0) for 7-days. The UFTW > 3 kDa fraction conjugated to polysaccharides or oligosaccharides showed improved emulsifying activity index (EAI) and emulsion stability (ES) ($p < 0.05$). The UFTW < 3 kDa, APP, 11S and 7S–oligosaccharide conjugates showed almost 3.0 times improvement in ES but no significant difference in EAI. In addition, UFTW < 3 kDa, APP, 7S, and 11S–chitosan oligo conjugates showed 5.0 times lower EAI and ES than those conjugates with XG or chitin oligo, due to electrostatic interaction between the negatively-charged groups of soy protein and positively-charged groups of chitosan oligo. Tofu whey fraction–carbohydrate glycoproteins showed both high and low water or oil-binding capacities, implying that different carbohydrates utilised in the preparation of glycoproteins possess different properties and mechanism in water or oil-binding capacity.

1. Introduction

Tofu is the main processed soybean product in the world. Tofu whey is the liquid that oozes out of soybean curd during processing and is considered as a soybean processing by-product. Tofu wastes deteriorate very quickly because of their high water content (~80%) and their high content of nutritious substances for bacteria. Although, tofu wastes contain high quantities of beneficial nutrients, most tofu by-products are used as animal feeds, fertiliser or simply disposed of.

Since tofu whey has poor surface functionalities, structural modification through Maillard-type glycosylation is expected to improve its functional properties. Glycosylation of protein is expected to overcome its instability to heating and to further improve its functional properties (Kato, Sasaki, Furuta, & Kobayashi, 1990). The Maillard reaction can be used as a simple and environmentally friendly way of recycling wastes into useful food ingredients. For more than two decades, the Maillard reaction has been employed in the preparation of functional glycoproteins between protein and carbohydrate. The excellent emulsifying properties of glycoproteins basically depend on the physiochemical properties of the proteins and carbohydrates. The introduction of a carbohydrate chain onto the protein molecular surface may change the physiochemical properties, e.g., emulsion properties (Jing & Nakamura, 2005), of the protein.

Improvement of molecular surface functionalities of proteins by Maillard-type conjugation with polysaccharides or oligosaccharides has been reported, with regard to solubilities, antimicrobial effects, antioxidative action and allergenicities (Nakamura, Kato, & Kobayashi, 1990; Nakamura, Ogawa, Nakai, Kato, & Kitts, 1998; Nakamura et al., 2008; Song, Babiker, Usui, Saito, & Kato, 2002; Usui et al., 2004). It is considered that the molecular surface properties of the protein and carbohydrate, including electrostatic interaction, play a great role in glycoprotein formation, as well as in the formation and stabilisation of emulsions. In addition, the combination of charge and size between protein and carbohydrates moieties seems to be an important factor for the preparation of functional glycoproteins from tofu whey. These findings will bring more insight into the further utilisation of tofu wastes in functional food processing, as well as minimising quantities of waste produced, thus reducing waste management and disposal problems. Also, the developed glycoprotein could find application in the beverage and meat industries as a food additive or ingredient. This

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study demonstrates the importance of size and charge of carbohydrate moiety in tofu whey–carbohydrate conjugates formed through Maillard-type glycosylation.

2. Materials and methods

2.1. Materials

Liquid tofu whey, soybean coat and *okara* were from Asahimatsu Foods Co. Ltd. (Nagano, Japan). Acid precipitated protein (APP), glycinin (11S) and β-conglycinin (7S) were prepared from soy protein isolate (Fuji Oil Co. Ltd., Osaka, Japan). Xyloglucan (Tamarind xyloglucan-1400 Da) was a gift from Dainippon Seiyaku Co. Ltd. (Osaka, Japan). Chitin and chitosan oligosaccharides (800 Da) were from Yaizu Suisankagaku Industry Co. Ltd. (Shizuoka, Japan). UF membranes MWCO 3000 Da were purchased from Millipore Corporation (Bedford, MA). LMW calibration kit for SDS electrophoresis was purchased from Amersham Pharmacia Biotech Ltd. (Uppsala, Sweden). All reagents used in this study were of analytical grade.

2.2. Preparation of Maillard-type glycoproteins of tofu whey

As carbohydrate candidates, naturally-occurring water-soluble polysaccharides were prepared from soybean coat and *okara*. One hundred grams of soybean coat powder were extracted two times with hot distiled water (100 °C) at a ratio of 12:1, according to Whistler and Saario (1957), with slight modification. Polysaccharide mixture was homogenised and filtered through a cloth. The filtrate was adjusted with 1.0 N HCl to pH 4.5, poured into three volumes of absolute ethanol and centrifuged at 7500 rpm for 30 min. The precipitate was resuspended four times in absolute ethanol and centrifuged. The precipitate was dissolved in distiled water, lyophilised and used as galactomannan (GM). *Okara* polysaccharide was prepared according to the method of Yoshii, Furuta, Maeda, and Mori (1996), with some modifications. Thirty grams of *okara* powder were placed in a beaker, two volumes of 1% $CaCl_2$ solution were added, and the pH of the slurry was adjusted to 4.5 with 1.0 N HCl. The beaker was covered with aluminium foil and autoclaved at 120 °C for 60 min. Forty grams of hydrolysed *okara* were taken, centrifuged at 7500 rpm, at 40 °C for 30 min. The supernatant was lyophilised and used as *okara* polysaccharide. In addition, one litre of liquid tofu whey was freeze-dried into 0.2 g of tofu whey powder. Tofu whey powder 2.0% w/v was ultrafiltered through a 3000 Da MWCO membrane. Two fractions, retained (UFTW > 3 kDa) and permeate (UFTW < 3 kDa), were collected. The fractions were dialysed against deionised water, lyophilised and used for further investigation.

The UFTW > 3 kDa fraction was conjugated with polysaccharides (GM and *okara* polysaccharide) or oligosaccharides (XG, chitin and chitosan), through natural-occurring Maillard-type glycosylation (Nakamura, 2007). The UFTW > 3 kDa fraction was mixed with polysaccharides or oligosaccharides in 50 mM phosphate buffer pH 7.0, at a weight ratio of 1:1 and lyophilised. On the other hand, the UFTW < 3 kDa fraction APP, 11S or 7S were mixed with oligosaccharides in distiled water at a weight ratio of 1:1. The pH of the mixture was adjusted to 7.0 with 1.0 N HCl or 1.0 N NaOH. The mixture was stirred at 4 °C overnight and lyophilised. The lyophilised powders were dry-heated at 60 °C, 65% RH in a desiccator containing saturated KI solution in its base for 7-days. Free proteins and carbohydrates were then separated, using size-exclusion chromatography (Sephacryl S-300 or S-500HR column; 1.6 × 60 cm; GE Healthcare, Uppsala, Sweden) for UFTW > 3 kDa–polysaccharide or oligosaccharide conjugates, and pH adjustment (Katsuya, Makoto, Tadashi, Shin, & Koji, 2007) for UFTW < 3 kDa, APP, 11S or 7S–oligosaccharide conjugates. The pH of the conjugates were adjusted to 4.5 with 1.0 N HCl and stirred overnight at 20 °C. The solution was centrifuged at 7500 rpm for 30 min. The supernatant was dialysed against deionised water at 4 °C for 48 h and lyophilised to conjugate powder. The resulting conjugates were stored at 4 °C for further analysis.

2.3. SDS–polyacrylamide gel electrophoresis analysis

Confirmation of conjugates was carried out using SDS–polyacrylamide gel electrophoresis (SDS–PAGE) analysis, according to the method of Laemmli (1970) in 15% separating and 5% stacking gels. Samples (20 μl, 0.25%) were loaded on the gels and electrophoresis was carried out using Tris–HCl buffer (pH 8.3) containing 0.1% SDS, at 30 mA for 2.5 h. The gels were stained with Coomassie brilliant blue (CBB) R-250 (0.025% CBB, 0.5% methanol, containing 0.1% acetic acid) for proteins or 0.5% periodic acid-Fuchsin (PAS) for carbohydrates. The gels were destained with 0.25% methanol containing 0.07% acetic acid for protein or washed with 10% sodium bisulphite solution containing 1.0 N HCl for PAS-stained gels (Zacharius, Zell, Morrison, & Woodlock, 1969).

2.4. Emulsifying properties

The emulsifying properties of the conjugates were determined, according to the method of Pearce and Kinsella (1978). A 1.0 ml aliquot of corn oil was added to 3.0 ml of 0.1% sample solution dissolved in 50 mM phosphate buffer, pH 7.0, and then homogenised at 12000 rpm for 1 min at 20 °C. One hundred microlitres of emulsion were taken from the bottom of the test tube at 0, 1, 2, 5, 10 and 20 min and diluted with 5.0 ml of 0.1% SDS solution. The absorbances of the diluted emulsions were measured at 500 nm. The relative emulsifying activity was taken as the absorbance at 500 nm taken immediately after emulsification. The emulsion stability (ES) was expressed as an estimate of the half-life of the absorbance of the emulsion formed 10 min after emulsification. EAI was calculated by the method of Chove, Grandison, and Lewis (2001) and used to measure the emulsifying properties of the conjugates.

2.5. Water-holding and oil-binding capacity

Water-hydration and oil-binding capacity were assessed according to Sosulski (1962, 1976) with some modifications. For the water holding experiment, distiled water was added to a sample powder and the contents were stirred at 1800 rpm for 30 s after every 10 min for 40 min. The contents were centrifuged at 1300*g* for 25 min and free water was removed carefully. The centrifuge tube with contents was dried in a desiccator for 15 min. The amount of absorbed water was determined by weight difference. For the oil-binding experiment, commercial corn oil (Ajinomoto Co., Tokyo, Japan) was added to the sample powder, and shaken vigorously at 2500 rpm for 30 s after every 5 min for 30 min. The contents were centrifuged at 15,000*g* for 25 min, free oil was decanted and the amount of absorbed oil determined by difference. The values obtained were taken as water-holding and oil-binding capacity of glycoproteins.

2.6. Statistical analysis

Statistical analysis to determine significant differences between means of groups was done using paired *t*-test (Microsoft Office Excel 2003). A value of $p < 0.05$ was considered as statistically significant. The results were expressed as means with standard deviation of triplicates.