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Improvement of Surface Functionalities, Including Allergenicity Attenuation, of Whole Buckwheat Protein Fraction by Maillard-Type Glycation with Dextran

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ABSTRACT: The purpose of the current study was to determine the effects of the introduction of polysaccharide chains onto the molecular surface of buckwheat proteins on buckwheat protein surface functionality. The whole buckwheat protein fraction (WBP) was prepared using 50 mM phosphate buffer (pH 7.5) containing 0.5 M NaCl and covalently linked with 6 kDa, 17.5 kDa, 40 kDa, 70 kDa, or 200 kDa dextran by Maillard-type glycation through controlled dry-heating at 60°C and 79% relative humidity for two weeks. Conjugation with 40 kDa dextran improved the water solubility and emulsifying properties of WBP without causing a serious loss of available lysine; 84.9% of the free amino groups were conserved. In addition, we found that the introduction of dextran chains onto the molecular surfaces of WBP attenuated the antigenicity of WBP.

Keywords: whole protein fraction, buckwheat, dextran, Maillard-type glycation, surface functionality

INTRODUCTION

Buckwheat is attracting much attention as a health food due to its excellent nutritional properties, well-balanced amino acid composition, and richness in essential amino acid such as lysine, methionine, and tryptophan (1). Buckwheat proteins have been shown to protect against 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by reducing cell proliferation and cure chronic human diseases by protecting against the effects of blood cholesterol and hyperpiesia (2,3). However, buckwheat proteins cause potent allergic symptoms, including anaphylaxis, in hypersensitive patients (4). These symptoms include anaphylactic reactions such as urticaria eruptions, gastrointestinal disorders, and asthmatic attacks, etc. (4). Several studies have revealed that 8∼9 kDa, 17 kDa, 50 kDa, and 100 kDa buckwheat proteins display robust binding activity against the sera of buckwheat allergic patients (5-7). More recently, food allergy researchers have become aware that other fractions of buckwheat proteins also produce a typical allergic reaction (8-12). Thus, the allergenicity of buckwheat proteins limits their use as a general food source and additive.

A Maillard reaction-mediated modification technique was used to address this issue in the present study. Previous work has indicated that controlled dry-heating (i.e., Maillard-type glycation) can be used to introduce polysaccharide chains to the molecular surface of target molecules, which drastically reduces the allergenicity of food proteins without the use of chemical reagents (13,14). In addition, glycation with polysaccharides could favorably alter the surface functionalities of target proteins (15-18). The safety of newly-developed compounds formed by Maillard-type glycation has been confirmed (18). There is concern that this type of modification may reduce available lysine residues because the conjugation occurs by covalent binding of the ε-amino groups of proteins to the reducing-end of polysaccharides. Therefore, we determined the number of free amino groups occupied by carbohydrate moieties introduced onto the protein molecules of the whole buckwheat protein fraction (WBP). In addition, we assessed the potential use of WBP as a food-grade ingredient (i.e., emulsifier). The present study demonstrates that Maillard-type glycation of WBP with dextran improves its surface functionalities (e.g., water solubility and emulsifying properties) and attenuates its allergenicity.

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MATERIALS AND METHODS

Materials

Common buckwheat (*Fagopyrum esculentum* Moench) flour was obtained from the Education and Research Center of Alpine Field Science in Shinshu University (Ina, Nagano, Japan). Dextrans with five different molecular masses, 6 kDa (DX6), 15~20 kDa (DX17.5), 40 kDa (DX40), 50~90 kDa (DX70), and 200 kDa (DX200) were purchased from MRC Polysaccharide Co., Inc. (Toyama, Japan). Human sera were obtained from four buckwheat-allergy subjects (one male and three females; 12 to 45 years old; Table 1). The Sephacryl S-300 column and Q Sepharose FF were obtained from GE Healthcare (Tokyo, Japan). Goat anti-human IgE labeled with HRP was obtained from MorphoSys UK Ltd. (Oxford, UK). All other reagents were of biochemical grade.

Preparation of whole buckwheat protein fraction

The WBP was prepared according to Urisu et al. (8) with some modifications. Common buckwheat flour was defatted using acetone and air-dried for one hour to completely remove the solvent. The resulting powder was dissolved in distilled water, 50 mM phosphate buffer, pH 7.5 (PBS), PBS containing 0.1 M NaCl, or PBS containing 0.5 M NaCl and stirred overnight at 4°C. The extract was centrifuged at 6,000 *g* for 15 min at 4°C, and the supernatant was filtered through No. 5A filter paper once (Advantec Co. Ltd., Tokyo, Japan). The proteins were then precipitated out of the solution by stirring overnight at 4°C with 80% saturated ammonium sulfate. The resulting precipitate was collected by centrifugation, dialyzed against distilled water, lyophilized, and used as WBP powder.

Preparation of WBP-dextran conjugates

Maillard-type glycation was used to prepare WBP-dextran conjugates according to the method described by Kato et al. (17). Briefly, WBP was mixed 1:1 with DX6, 1:3 with DX17.5, 1:6 with DX40, 1:12 with DX70, or 1:30 with DX200. These mixtures were incubated at 60°C and 79% relative humidity for 2 weeks. The resulting WBP-dextran conjugates were separated from free proteins and carbohydrates using size exclusion chromatography with a Sephacryl S-300 column followed by ion exchange chromatography with Q Sepharose FF and used for further experiments.

Table 1. Characteristics of human sera used in this study

Serum No.	Allergy intensity[1)]	Age (years)	Sex
1	1~2	12	Female
2	2	28	Female
3	3	45	Male
4	ND	18	Female

[1)]Allergy intensity is evaluated as follows: 0, negative; 1, pseudo-positive; 2~6, positive expressed numerically; ND, not determined.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was conducted according to the method described Laemmli (19) using a 15% (w/w) acrylamide separating gel with a 5% (w/w) stacking gel containing 1% (w/v) SDS. Samples were heated at 100°C for 5 min in Tris-glycine buffer (pH 8.8) containing 1% SDS and 1% (v/v) 2-mercaptoethanol. Electrophoresis was carried out at a constant current of 15 mA for 3 h using an electrophoretic buffer of Tris-glycine containing 0.1% SDS. After electrophoresis, the gels were stained for protein and carbohydrate with 0.025% (w/v) Coomassie brilliant blue R-250 solution and 0.5% (w/v) periodic acid-Fuchsin solution (20), respectively.

Determination of free amino groups

2,4,6-trinitrobenzene sulfonic acid (TNBS) was used to quantify the free amino groups present in the WBP-dextran conjugates according to the method of Haynes et al. (21).

Determination of solubility

The solubility of WBP in distilled water, PBS, PBS containing 0.1 M NaCl, or PBS containing 0.5 M NaCl was assessed by measuring the absorbance (at 280 nm) of the supernatants of 1 mg protein/mL solutions after centrifugation at 6,000 *g* for 20 min at 4°C.

Measurement of emulsifying properties

The emulsifying properties of WBP were determined according to the method described by Pearce and Kinsella (22). Samples were dissolved in PBS at a concentration of 0.1%, and 3 mL of the sample solution was homogenized with 1.0 mL of corn oil using a Polytron PT3100 (Kinematica AG, Luzern, Switzerland) homogenizer at 6,000 *g* for 1 min at 20°C to prepare an O/W-type emulsion. One-hundred microliter aliquots of the emulsion were taken from the bottom of the test tube after standing for 0 min, 1 min, 3 min, 5 min, 10 min, and 20 min. Each aliquot was diluted with 5.0 mL of 0.1% SDS solution. The absorbance of the diluted emulsions was measured at 500 nm. The relative emulsifying activity of each sample is presented as the absorbance measured at 500 nm immediately after emulsion formation. The emulsion stability was estimated by measuring the half-life time for emulsion decay while standing for 20 min.

Immuno-dot blotting assay

A nitrocellulose membrane sheet was soaked in distilled water overnight, and then 5 μL sample solutions of WBP and WBP conjugates were spotted on to the membrane. Human sera were added to the membrane, the membrane was blocked with 0.3% skim milk, and the mem-

brane was incubated for 1 h at 25°C. Then, the incubation solution (i.e., human sera with 1.0% skim milk) was removed, and the secondary antibody, goat anti-human IgE labeled with HRP, was added. The membrane was incubated for 1 h at 25°C. Finally, bands were visualized with Ez-West-Blue solution (Atto Co. Ltd., Tokyo, Japan) containing 3,3',5,5'-tetramethylbenzidine and H_2O_2. PBS containing Tween-20 was used as washing buffer throughout the immuno-dot blotting assay.

Quartz crystal microbalance analysis

Antigen-antibody interaction was also determined by quartz crystal microbalance (QCM) analysis (14,23). Briefly, 2 μL of a serum mixture from four buckwheat allergy patients was immobilized on a gold electrode surface, and the frequency of the electrode was measured with a 27-MHz QCM machine equipped with a frequency counter (Affinix QN system, Initium Inc., Tokyo, Japan). After stabilization, 0.04 mg protein/mL, 0.2 mg protein/mL, 1.0 mg protein/mL, and 5.0 mg protein/mL concentrations of WBP or WBP conjugate dissolved in 50 mM phosphate buffer (pH 7.0) were added to the mixing chamber every 600 s for 3,000 s. Changes in the 27-MHz frequency at the electrode were monitored to determine the interaction between buckwheat antibody and WBP.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using the Student's *t*-test (Microsoft Excel 2010) for comparing differences between treatment means.

RESULTS AND DISCUSSION

Preparation of whole buckwheat protein fraction and dextran conjugates

Protein solution was prepared from defatted buckwheat flour using distilled water, PBS, PBS containing 0.1 M NaCl, or PBS containing 0.5 M NaCl, and applied for SDS-polyacrylamide gel electrophoresis. As shown in

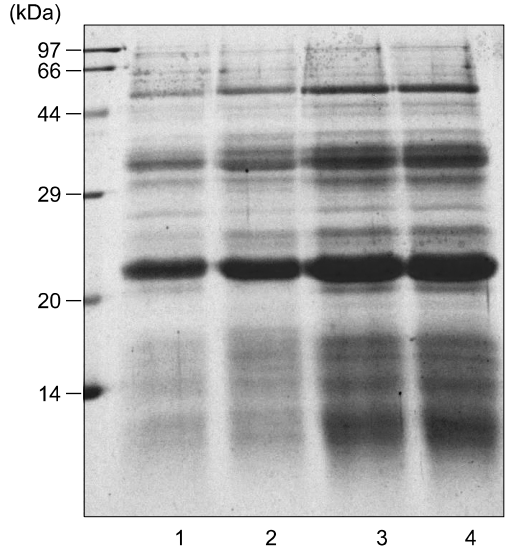

Fig. 1. SDS-polyacrylamide gel electrophoretic patterns of whole buckwheat protein fractions prepared using different salt concentration. After electrophoresis, gel sheet was stained with Coomassie brilliant blue (CBB). Lane 1, whole buckwheat protein fraction (WBP) prepared using distilled water; lane 2, WBP prepared using 50 mM phosphate buffer, pH 7.5 (PBS); lane 3, WBP prepared using PBS containing 0.1 M NaCl; lane 4, WBP prepared using PBS containing 0.5 M NaCl.

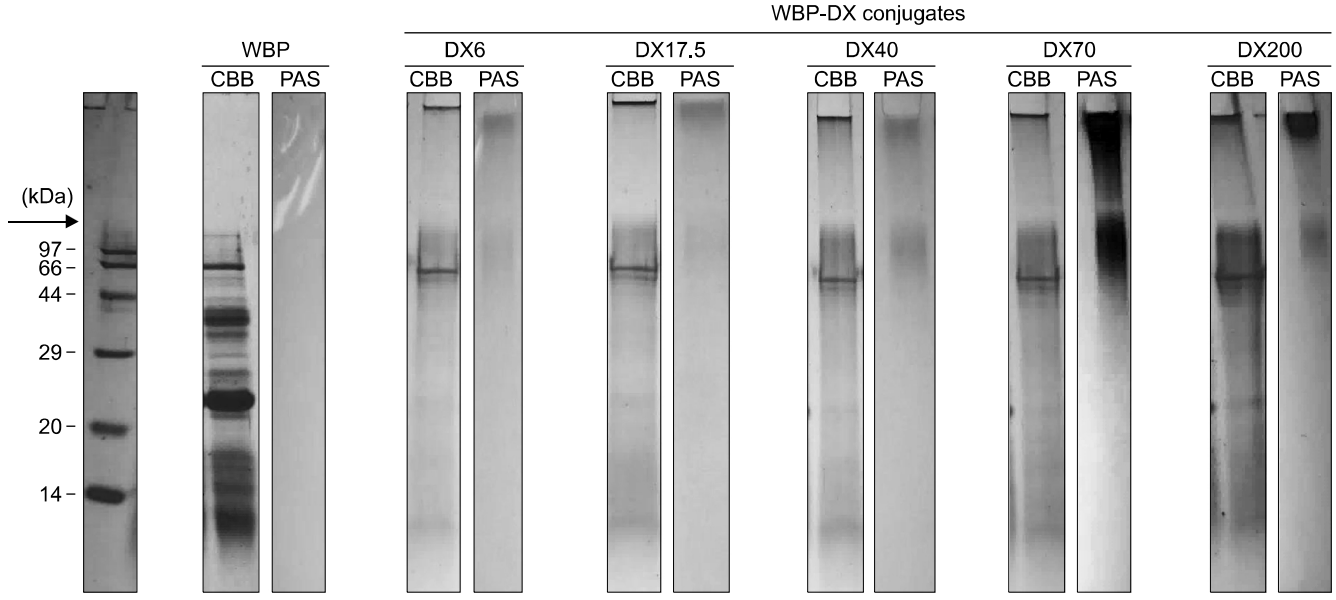

Fig. 2. Electrophoretic patterns of WBP-dextran conjugates. After electrophoresis, gel sheets were stained with Coomassie brilliant blue R-250 solution (CBB) and periodic acid-Fuchsin solution (PAS) for protein and dextran, respectively. Arrow indicates the boundary between stacking and separating gels. WBP, native WBP; DX6, WBP-DX6 conjugate; DX17.5, WBP-DX17.5 conjugate; DX40, WBP-DX40 conjugate; DX70, WBP-DX70 conjugate; DX200, WBP-DX200 conjugate.

Fig. 1, the largest amount of proteins containing 24 kDa globulin named Fag e 1 (11) and 16 kDa albumin named Fag e 2 (12) was obtained by using PBS with 0.5 M NaCl in which the yield was 4.39%. Choi and Ma stated that buckwheat seed storage proteins are rich in salt-soluble globulin and albumin (24). Thus, we made use of the proteins as WBP for further experiments.

WBP was mixed 1:1 (molar : molar) with DX6, 1:3 with DX17.5, 1:6 with DX40, 1:12 with DX70, or 1:30 with DX200, dissolved in 50 mM PBS, and lyophilized. The resulting powder was incubated at 60°C and 79% relative humidity for 2 weeks to generate a naturally occurring Maillard-type glycation between the ε-amino acid groups of proteins and the reducing-end of polysaccharides. As shown in Fig. 2, this process resulted in the formation of WBP-dextran conjugates. The WBP-dextran conjugates can be visualized as newly emerged broad bands that are adjacent to the boundary area between the stacking gel and the separating gel. The location of these bands does not appear to be related to the molecular size of the dextran used. WBP-dextran conjugates were purified with a Sephacryl S-300 column followed by ion exchange chromatography with Q Sepharose FF.

Changes in free amino group content

Fig. 3 shows the relative number of free amino groups present in the WBP-dextran conjugates. There was a tendency for the small molecular size dextrans to link with the target protein more effectively. WBP conjugates with DX40, DX70, and DX200 had relatively high numbers of free amino groups (i.e., 84.9%, 86.1%, and 87.7%, respectively). Since Fag e 1 and Fag e 2 contain nine and twelve lysine residues, respectively (11,12), at least seven lysine residues on Fag e 1 and nine lysine residues on Fag e 2, remained available after conjugation of WBP with dextran.

Changes in surface functionalities, including water solubility

Table 2 summarizes the differences in the solubility of WBP prepared with different salt concentrations. The solubility of all WBP-dextran conjugates was higher than that of native WBP in distilled water and in PBS without NaCl. Previously, we reported that Maillard-type glycation partially alters the tertiary structure and amphiphilicity of proteins due to the introduction of hydrophilic

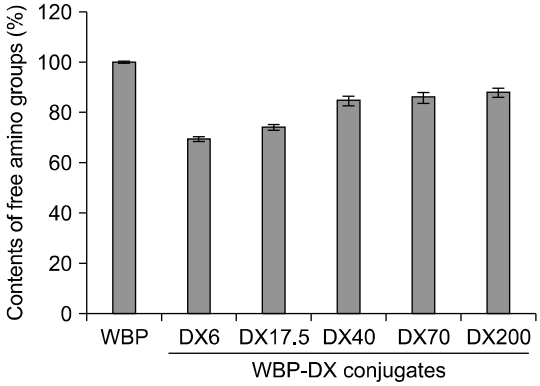

Fig. 3. Free amino acid groups in WBP-dextran conjugates. Determination of free amino acid group was done based on trinitrobenzene sulfonic acid (TNBS) test. Data show mean±SD (n=5) from three independent experiments. WBP, native WBP; DX6, WBP-DX6 conjugate; DX17.5, WBP-DX17.5 conjugate; DX40, WBP-DX40 conjugate; DX70, WBP-DX70 conjugate; DX200, WBP-DX200 conjugate.

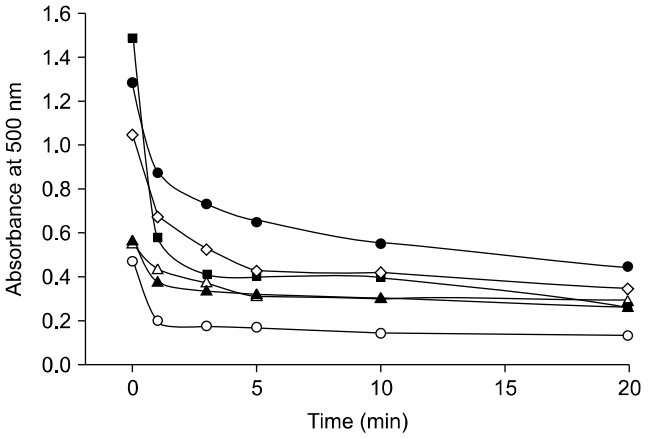

Fig. 4. Emulsifying properties of WBP-dextran conjugates. Data are from a representative experiment repeated three times with similar results. ○, native WBP; △, WBP-DX6 conjugate; ▲, WBP-DX17.5 conjugate; ●, WBP-DX40 conjugate; ■, WBP-DX70 conjugate; ◇, WBP-DX200 conjugate.

Table 2. Effect of salt concentration on WBP solubility

	Distilled water	50 mM phosphate buffer (pH 7.5)		
		Without NaCl	With 0.1 N NaCl	With 0.5 N NaCl
WBP	0.498±0.013	0.654±0.012	0.713±0.006	0.785±0.008
DX6	0.524±0.007	0.793±0.006	0.820±0.005	0.883±0.004
DX17.5	0.689±0.008	0.848±0.009	0.967±0.011	1.011±0.086
DX40	0.759±0.006	0.922±0.005	1.002±0.013	1.150±0.037
DX70	0.711±0.008	0.913±0.002	0.976±0.002	1.019±0.001
DX200	0.512±0.010	0.724±0.005	0.762±0.007	0.829±0.006

Values show the absorbance of the sample supernatant at 280 nm (mean±SD, n=6). WBP, native WBP; DX6, WBP-DX6 conjugate; DX17.5, WBP-DX17.5 conjugate; DX40, WBP-DX40 conjugate; DX70, WBP-DX70 conjugate; DX200, WBP-DX200 conjugate.

moieties on the molecular surface of the target proteins (16). A similar phenomenon was observed in all of the WBP-dextran conjugates prepared in this study. Notably, a remarkable improvement in solubility was observed when WBP was conjugated with DX40; the water solubility of the WBP-DX40 conjugate was 1.5 times greater than the water solubility of native WBP.

Fig. 4 shows the emulsifying properties of WBP-DX conjugates. We found that the emulsifying properties of WBP were substantially improved by conjugation with dextran. A well-balanced improvement in emulsifying activity and emulsion stability was observed for the WBP-DX40 conjugate. The emulsifying activity (OD at 500 nm without standing) of the WBP-DX40 conjugate was 2.7 times higher than that of native WBP, and the emulsion stability (half-life time of emulsion) of the WBP-DX40 conjugate was 6.0 times higher than that of the native WBP. In WBP conjugates, partially denatured proteins may attach at the oil-water interface during physiological emulsification to form a coherent layer with oil droplets, where hydrophilic dextran with an appropriate chain length (i.e., 40 kDa) provide colloid stability through their thickening behavior in the aqueous phase (15-18). Thus, we concluded that the introduction of dextran chains to WBP may be responsible for the observed improvements in solubility and emulsifying properties.

Changes in antigenicity

Immuno-dot blotting and QCM analyses were used to evaluate dextran conjugation-related changes in the antigenicity of WBP. We used human sera from four subjects who tested positive for the buckwheat-specific IgE antibody. As shown in Fig. 5, the introduction of polysaccharide chains decreased the reactivity of sera against WBP. The relative signal intensity of each dot was measured with a Java-based image processing program, Image J 1.47 (http://imagej.nih.gov/ij/); differences among the attenuation levels of DX6, DX17.5, DX40, DX70, and DX200 conjugates were not detected. Therefore, we used the QCM technique to determine the binding activity between WBP and the IgE antibody in sera.

As shown in Fig. 6, the 27-MHz frequency measured for native WBP on the QCM electrode remarkably decreased when the protein concentration was increased every 600 s. This change may provide evidence for an irreversible binding between WBP and the specific antibody against WBP because antigen-antibody interactions on the QCM electrode decrease the frequency of the electrode. However, the 27-MHz frequency was not changed by the addition of the WBP-DX40 conjugate, even at high concentrations. Table 3 shows the 27-MHz frequency values of WBP and WBP-DX conjugates at the final concentration of 5.0 mg-protein/mL (i.e., at 3,000 s). The frequency value of the WBP-conjugates was not lower than that of native WBP. Dextran chains on the molecular surface of WBP may prevent IgE antibody cross-linkage. In addition, the partial denaturation induced during Maillard-type glycation with dextran may cause conformational alterations that are accompanied

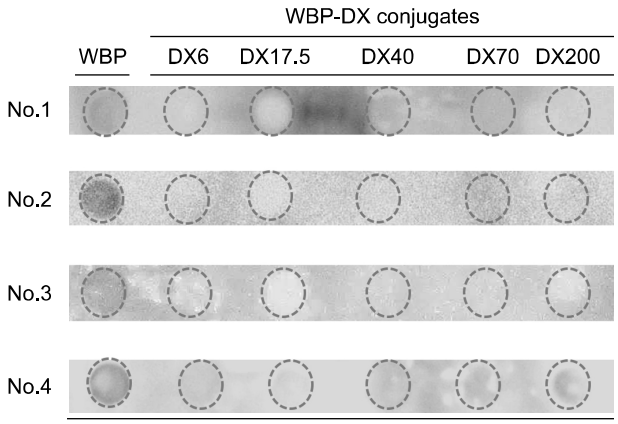

Fig. 5. Immuno dot-blotting profiles of WBP-dextran conjugates. WBP, native WBP; DX6, WBP-DX6 conjugate; DX17.5, WBP-DX17.5 conjugate; DX40, WBP-DX40 conjugate; DX70, WBP-DX70 conjugate; DX200, WBP-DX200 conjugate.

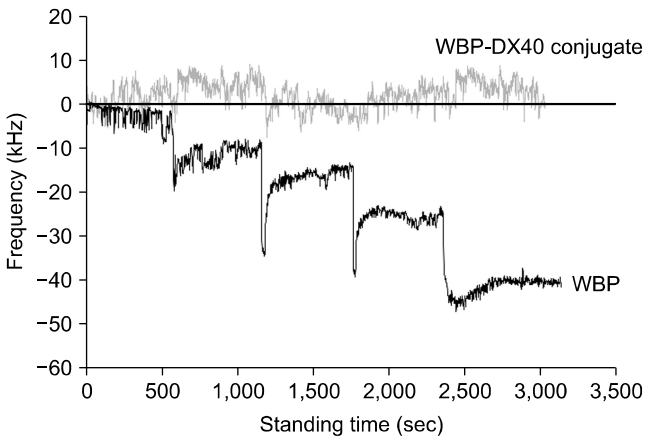

Fig. 6. Change in QCM profile of WBP by the conjugation. Data are from a representative experiment repeated three times with similar results.

Table 3. 27-MHz frequency values at the final concentration of 5.0 mg protein/mL

	WBP	DX6	DX17.5	DX40	DX70	DX200
Values	−41.1	−1.78	−0.146	−0.0629	−0.224	−0.188

WBP, native WBP; DX6, WBP-DX6 conjugate; DX17.5, WBP-DX17.5 conjugate; DX40, WBP-DX40 conjugate; DX70, WBP-DX70 conjugate; DX200, WBP-DX200 conjugate.

by the destruction of WBP epitopes.

In this study, we demonstrated that Maillard-type glycation improved the water solubility, emulsifying properties, and hypoallergenicity of WBP without decreasing the number of available lysine residues. Many attempts have been made to develop non-toxic, food-based emulsifiers. Since dextran synthesized by lactic-acid bacteria such as *Leuconostoc mesenteroides* was the first microbial polysaccharide to be commercialized and receive approval for use in food in the many countries (25), it should be the most promising agent to introduce hydrophilicity into WBP. WBP-dextran conjugates would be ideal as a novel macromolecular emulsifier for food application.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Javornik B, Kreft I. 1984. Characterization of buckwheat protein. *Fagopyrum* 4: 30-38.
2. Tomotake H, Yamamoto N, Yanaka N, Ohinata H, Yamazaki R, Kayashita J, Kato N. 2006. High protein buckwheat flour suppresses hypercholesterolemia in rats and gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its low protein digestibility. *Nutrition* 22: 166-173.
3. Tomotake H, Shimaoka I, Kayashita J, Yokoyama F, Nakajoh M, Kato N. 2000. A Buckwheat protein product suppresses gallstone formation and plasma cholesterol more strongly than soy protein isolate in hamsters. *J Nutr* 130: 1670-1674.
4. Wieslander G. 1996. Review on buckwheat allergy. *Allergy* 51: 661-665.
5. Yanagihara Y, Koda A. 1980. Immunopharmacological study of buckwheat hypersensitivity. *Nihon Yakurigaku Zasshi* 10: 184-188.
6. Yano M, Nakamura R, Hayakawa S, Torii S. 1989. Purification and properties of allergenic proteins in buckwheat seed. *Agric Biol Chem* 53: 2387-2392.
7. Matsumoto R, Fujino K, Nagata Y, Hashiguchi S, Ito Y, Aihara Y, Takahashi Y, Maeda K, Sugimura K. 2004. Molecular characterization of a 10-kDa buckwheat molecule reactive to allergic patients' IgE. *Allergy* 59: 533-538.
8. Urisu A, Kondo Y, Morita Y, Wada E. 1994. Identification of a major allergen of buckwheat seeds by immunoblotting methods. *Allergy Clin Immunol News* 6: 151-155.
9. Nair A, Adachi T. 1999. Immunodetection and characterization of allergenic proteins in common buckwheat (*Fagopyrum esculentum*). *Plant Biotechnol* 16: 219-224.
10. Yoshimasu MA, Zhang JW, Hayakawa S, Mine Y. 2000. Electrophoretic and immunochemical characterization of allergenic proteins in buckwheat. *Int Arch Allergy Immunol* 123: 130-136.
11. Yoshioka H, Ohmoto T, Urisu A, Mine Y, Adachi T. 2004. Expression and epitope analysis of the major allergenic protein Fag e 1 from buckwheat. *J Plant Physiol* 161: 761-767.
12. Tanaka K, Matsumoto K, Akasawa A, Nakajima T, Nagasu T, Iikura Y, Saito H. 2002. Pepsin-resistant 16-kD buckwheat protein is associated with immediate hypersensitivity reaction in patients with buckwheat allergy. *Int Arch Allergy Immunol* 129: 49-56.
13. Arita K, Babiker EE, Hiroyuki A, Kato A. 2001. Effect of chemical and genetic attachment of polysaccharides to proteins on the production of IgG and IgE. *J Agric Food Chem* 49: 2030-2036.
14. Nakamura S, Suzuki Y, Ishikawa E, Yakushi T, Jing H, Miyamoto T, Hashizume K. 2008. Reduction of *in vitro* allergenicity of buckwheat Fag e 1 through the Maillard-type glycosylation with polysaccharides. *Food Chem* 109: 538-545.
15. Marshall JJ, Rabinowitz ML. 1976. Preparation and characterization of a dextran-trypsin conjugate. *J Biol Chem* 251: 1081-1087.
16. Nakamura S, Kato A, Kobayashi K. 1992. Bifunctional lysozyme-galactomannan conjugate having excellent emulsifying properties and bactericidal effect. *J Agric Food Chem* 40: 735-739.
17. Kato A, Murata K, Kobayashi K. 1988. Preparation and characterization of ovalbumin-dextran conjugate having excellent emulsifying properties. *J Agric Food Chem* 36: 421-425.
18. Nakamura S, Dokai K, Matsuura M, Saeki H. 2002. Food safety of functional neoglycoproteins prepared by covalent attachment of galactomannan to food proteins. *Nutraceuticals & Food* 7: 139-145.
19. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
20. Zacharius RM, Zell TE, Morrison JH, Woodlock JJ. 1969. Glycoprotein staining following electrophoresis on acrylamide gels. *Anal Biochem* 30: 148-152.
21. Haynes R, Osuga DT, Feeney RE. 1967. Modification of amino groups in inhibitors of proteolytic enzymes. *Biochemistry* 6: 541-547.
22. Pearce KM, Kinsella JE. 1978. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J Agric Food Chem* 26: 716-723.
23. Okahata Y, Niikura K, Sugiura Y, Sawada M, Morii T. 1998. Kinetic studies of sequence-specific binding of GCN4-bZIP peptides to DNA strands immobilized on a 27-MHz quartz-crystal microbalance. *Biochemistry* 37: 5666-5672.
24. Choi SM, Ma CY. 2006. Extraction, purification and characterization of globulin from common buckwheat (*Fagopyrum esculentum* Moench) seeds. *Food Res Int* 39: 974-981.
25. Sarwat F, Ul Qader SA, AmanA, Ahmed N. 2008. Production and characterization of a unique dextran from an indigenous *Leuconostoc mesenteroides* CMG713. *Int J Biol Sci* 4: 379-386.