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Preparation of glycosylated zein and retarding effect on lipid oxidation of ground pork

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ABSTRACT

The focus of the present work was to investigate the glycosylation of zein, partial properties of the glycosylated zein (GZ) and its retarding effect on lipid oxidation of ground pork. Zein was glycosylated with chitosan (MW 1500 Da) by microbial transglutaminase, the reaction was verified by FT-IR. Under the optimized conditions, 97.48 mg of glucosamine was covalently conjugated to 1 g of zein, determined by HPLC. The solubility and the surface hydrophobicity of GZ were significantly improved. *In vitro* studies of GZ showed a dose-dependent scavenging activity against free radicals of DPPH, superoxide and hydroxyl radical, and the EC_{50} value for DPPH radical was 1.99 μg TE/mg protein. In addition, reducing power and Fe^{2+}-chelating capacity of it were 16.60 and 12.96 μg TE/mg protein, respectively. GZ resulted in low levels of thiobarbituric acid-reactive substances and peroxide value of ground pork. These results suggest that GZ is a potential natural antioxidant.

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1. Introduction

Corn gluten meal (CGM), a major co-product of corn wet-milling process, contains 67–71% proteins consisting of approximately 65% zein and 30% glutelin (Gioia, Cuq, & Guilbert, 1998). Zein, the predominant food grade protein in corn, is a mixture of highly hydrophobic proteins rich in proline, glutamine, leucine, and alanine (Pomes, 1971). Zein is insoluble in aqueous solutions but is readily soluble in organic solvents such as 60–70% isopropanol and 70–90% ethanol. Corn glutelin has a large number of inter- and intra-molecular disulfide bonds that create a closed compact structure, therefore it dissolves only in dilute acid or alkali solutions (Paraman, Hettiarachchy, Schaefer, & Beck, 2006). These characteristics make CGM less applicable in food industry, and it is mostly utilized as animal feed. If the protein from CGM is modified for utilizing in food industry, its value and presence in market place would be increased.

Protein glycosylation is regarded as one of the major protein post-translational modifications in cells. Glycosylation in body, an enzyme-driven process, is a crucial step in altering the biological activity of cells as it regulates the interaction between the cellular molecules (Hong, Gottardi, Ndagijimana, & Betti, 2014). Glycosylation reaction can also occur in the food system. Compared to the native protein form, incorporation of carbohydrate into protein has shown promising improvements in several aspects of functionality of glycosylated protein. The Maillard reaction, also referred to as non-enzymatic glycosylation, has been studied extensively as it can significantly improve functional characteristics of native protein, such as solubility, emulsibility, foamability, gelling and antioxidant activities(Li et al., 2013; Xue, Li, Zhu, Wang, & Pan, 2013). Unfortunately, potential adverse effects of the Maillard-type glycosylation cannot be ignored, such as the formation of toxic and mutagenic compounds (Brands, Alink, Van-Boekel, & Jongen, 2000), undesired browning and flavour products (Guerra-Hernandez, Gomez, Garcia-Villanova, Sanchez, & Gomez, 2002) as well as difficulty to control the degree of glycosylation. Enzymatic glycosylation of protein catalyzed by transglutaminase (TGase, EC 2.3.2.13) is an alternative protocol because it increases the safety and occurs under mild reaction conditions.

TGase, i.e. protein-glutamine γ-glutamyltransferase, is an enzyme involved in post-translational modification of proteins (Trespalacios & Pla, 2007). The TGase derived from eukaryotic and prokaryotic sources, which does not require calcium ions, is popular in dairy and meat processing sectors in food industry. TGase catalyses the formation of an isopeptide bond between the group of γ-carboxamides of glutamine residues, as acyl donors, with the first order ε-amine groups of different compounds, as acyl acceptors. If lysine is an acceptor of acyl, the transfer of acyl onto a

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lysine residue bound in the polypeptide chain induces formation of inter- and intra-molecular cross-linkages (Kieliszek & Misiewicz, 2014). Moreover, reactive lysine may be substituted by several compounds containing primary amino groups in cross-linking reaction of proteins or peptides. Thus, saccharides containing primary amino groups or aminated saccharides could serve as an amine donor that allows TGase to bind its ε-amino group to a glutamine-containing peptide/protein to modify the functionality and bioactivity of some food proteins. Jiang and Zhao (2010–2012) studied glycosylation between glucosamine and soybean protein isolates (SPI) as well as with casein catalyzed by TGase and found that the modified product exhibited lower surface hydrophobicity, better interfacial properties, markedly increased apparent viscosity, and higher enzymatic digestibility *in vitro*. Song and Zhao (2013) and Song and Zhao (2014) reported that oligochitosan was incorporated into caseinate and SPI by TGase, resulting in a modified product with higher apparent viscosity. On the other hand, TGase was also applied to modify fish gelatin peptides and wheat gluten peptides at mild temperatures (25 °C or 37 °C), resulting in the synthesis of glycopeptides with higher antioxidant and antimicrobial properties (Gottardi, Hong, Ndagijimana, & Betti, 2014; Hong et al., 2014).

Since corn protein contains high proportion of glutamine (21.4%) and lacks lysine, the enzymatic glycosylation reaction can occur mainly between corn protein and saccharides with reactive primary amino group with low probability of intra-molecular cross-linkage between lysine and glutamine. Therefore, corn protein could be a good substrate in enzymatic glycosylation for preparation of glycoprotein. In the literature available, the conjugation of saccharides with primary amines to zein by TGase has not been reported so far. In the present study, zein was modified by chitosan using microbial TGase as biocatalyst. Partial functional properties of the modified products were evaluated, including solubility, surface hydrophobicity, and antioxidant activities, and effect of the glycosylated zein on inhibiting lipid oxidation of ground pork was also investigated.

2. Materials and methods

2.1. Material and chemicals

CGM was purchased from Longfeng Corn Development Co., Ltd. (Heilongjiang, China), with a total protein content of 61.25%. Microbial TGase (1000 U/g) was purchased from Yiming Biological Products Co., Ltd. (Taixing, Jiangsu, China). Chitosan, with a declared deacetylation degree of about 90% and an average molecular weight of 1500 Da by the supplier, was purchased from Shandong Bozhihuili Biochemical Co. (Qingdao, China). D-(+)-glucosamine hydrochloride of >99% purity was purchased from Sangon Biological Engineering Co., Ltd. (Shanghai, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 8-anilino-1-naphthalene sulfonic acid (ANS), 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox) and 1,1,3,3-Tetraethoxypropane (TEP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All reagents in the HPLC analysis were HPLC grade. Other chemicals and reagents used were of analytical grade.

2.2. Preparation of zein

Zein was extracted according to the method described by Zheng et al. (2014) with some modifications. Extrusion and starch removal of CGM were used as pretreatment procedures before extraction. Briefly, the processed CGM powder was mixed with acetone (acetone: CGM = 10:1, v/w) for 30 min, and the mixture was centrifuged at 4000*g* for 15 min and the supernatant containing most of the pigment in CGM was discarded. The sediment was collected and dispersed with 70% ethanol (ethanol: CGM = 10:1, v/w) at 60 °C for 2 h, followed by centrifugation at 4000*g* for 15 min. The supernatant was collected and the sediment was further processed once using the same method as above. Then, the supernatants were pooled, and concentrated to allow precipitation under rotary evaporation at 60 °C. Finally, zein powder was obtained by freeze-drying.

2.3. Glycosylation of zein

Glycosylation of zein was performed in a water bath with constant agitation. Briefly, zein was suspended in distilled water, and chitosan was added and mixed well, heated to appropriate temperature and adjusted to appropriate pH. Then, TGase was added to initiate glycosylation reaction. The following parameters were varied in the glycosylation: pH at 0.2–0.3 intervals from 7.0 to 8.0, temperature at 3 °C intervals from 31 to 43 °C, zein concentration at 1% intervals from 1% to 5%, molar ratio of acyl donor and acyl acceptor (i.e. zein and chitosan) of 1:1, 1:2, 1:3, 1:4 and 1:5, the E/S ratio at 10 U/g zein intervals from 40 to 80 U/g zein, and reaction time at 1 h intervals from 6 to 10 h. After glycosylation, the TGase in the samples was inactivated immediately by heat treatment at 85 °C for 5 min. Finally, excess of chitosan was removed by dialysis using 2000 Da cut off membrane against distilled water for two days at 4 °C. The control of cross-linked zein was prepared without chitosan. All prepared samples, i.e. the glycosylated zein and cross-linked zein, were lyophilized and stored in a desiccator for further study.

2.4. HPLC analysis of the glycosylated zein

Analysis of chitosan conjugated to zein was measured according to the method described by Jiang and Zhao (2010) with some modifications. Briefly, a RP-HPLC method using pre-column derivatization with anthranilic acid (AA) was applied to analyze the chitosan content, which was expressed on the basis of glucosamine. The analysis was performed on a liquid chromatograph 1200 series (Agilent Technologies Inc., Wilmington, DE, USA) with a C18-reversed phase column (Waters ODS 4.6 mm × 250 mm i.d.5 μm, Waters Corporation, Milford, MA, US) with a flow rate of 1.0 mL/min. Solvent A consisted of 0.4% *n*-butylamine, 0.5% phosphoric acid and 1.0% tetrahydrofuran in water, and solvent B consisted of 50% solvent A and 50% acetonitrile. After sample application, the column was eluted with 96% solvent A and 4% solvent B for 30 min. Absorbance was monitored at 230 nm.

Hydrolysis and derivatization of the glycosylated zein products and standard glucosamine were conducted by the method of Jiang and Zhao (2010) with some modifications. 20 mg of the glycosylated zein was hydrolyzed with 5 mL of 6 mol/L HCl at 100 °C for 8.5 h, then 100 μL of the obtained hydrolysates were dried using pressure blowing concentrator at 40 °C. A methanol-acetate-borate solution was prepared by dissolving 4.0 g sodium acetate and 2.0 g boric acid in 100 mL methanol. The AA-reagent was prepared by dissolving 30 mg of anthranilic acid and 20 mg of sodium cyanoborohydride in 1 mL of methanol-acetate-borate solution. 100 μL of freshly prepared sodium acetate solution (1%, w/v) and 100 μL of AA-reagent were added to the dried hydrolysates or 100 μL of standard glucosamine solutions followed by derivatizing at 80 °C for 1 h, cooled to ambient temperature, diluted to 1 mL with HPLC solvent A, and then filtered through 0.22 μm membranes. 10 μL of the supernatant was loaded onto C18 column for analysis. The chitosan content conjugated to zein was expressed as mg of glucosamine per g of zein.

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