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Effects of transglutaminase catalyzed crosslinking on physicochemical characteristics of arachin and conarachin-rich peanut protein fractions

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ABSTRACT

Arachin and conarachin-rich fractions of peanut protein were extracted by using cryoprecipitation followed by centrifugation. These two fractions were individually crosslinked using transglutaminase (TG). The physicochemical characteristics including aggregation due to crosslinking, solubility, thermal denaturation temperature (T_d) and denaturation enthalpy ($\triangle H$), morphology of microstructure and surface hydrophobicity (H_0) of TG-treated and untreated arachin and conarachin-rich fractions were determined. The relative contents of arachin and conarachin in arachin and conarachin-rich fractions were 75% and 65%, respectively. Conarachin-rich fractions were found to more conveniently aggregate than arachin-rich from the results of SDS-PAGE. The solubility of treated arachin and conarachin-rich fractions was decreased by 66.13% and 36.91%, respectively. Only marginal increase in T_d was observed in the case of crosslinked arachin-rich fraction while significant increase in T_d (of the order of 10 °C) was observed in crosslinked conarachin-rich fraction. The H_0 values of both crosslinked arachin and conarachin-rich fractions decreased significantly. The treated arachin and conarachin-rich fractions had more compact microstructure compared to their untreated samples. Hence, the comparison of arachin and conarachin-rich in crosslinking and the mechanism of properties enhancement by TG is the aim of the research.

Introduction

Peanut is one of the most important oilseed crops over the world and most of the harvested peanut is used for extraction of edible oil. The peanut oil extraction process yields a large amount of defatted peanut flour (DPF), which is a protein-rich (47%–55%) and under-utilized by-product of peanut industry (Regena & Chen, 2010). The crude peanut protein mostly comprises globulins (about 96% of total protein) and consists of two major fractions: arachin and conarachin. The arachin and conarachin constitute about 63% and 33% of total protein, respectively (Chun, 2002). The native arachin comprises three acidic subunits (AS) with molecular weights of 42.0, 39.0 and 35.0 kDa and a basic subunit (BS) with a molecular weight of 22.0 kDa (Zhao, Liu, Zhao, Ren, & Yang, 2011). The conarachin contains one major subunit having a molecular weight of 64.0 kDa (Hu, Zhao, Sun, Zhao, & Ren, 2011). The ability of TG to catalyze the crosslinking of the peanut proteins or their individual constituents has been intensively investigated (Clare, Gharst, & Sanders, 2007; Gharst, 2007; Gharst, Clare, Davis, & Sanders, 2007; Hu, 2011). Ammonium sulfate fractionation and cryoprecipitation are the most preferred methods in fractionating arachin from peanut protein (Bhushan & Agarwal, 2006; Neucere, 1969).

TG catalyzes acyl-transfer reactions between γ-carboxamide group of peptide or protein-bound glutaminyl residues and primary amines of lysine (Hu et al., 2011; Truong, Clare, Catignani, & Swaisgood, 2004). The activity of TG depends on temperature and pH and the optimal values for these two parameters are reported to be 40 °C and 7.0, respectively (Chee, Latiff, Cheng, & Azhar, 2009; Pinterits & Arntfield, 2008). TG is commonly used to modify the physicochemical properties of peanut proteins (Clare et al., 2007; Gharst, 2007; Gharst et al., 2007; Hu, 2011; Hu et al., 2011). Through the peanut protein SDS-PAGE profiles it has been shown that polymeric aggregates are formed by the covalent crosslinking catalyzed by TG (Gharst et al., 2007; Hu et al., 2011). Hu et al. (2011) found that the crosslinking of peanut protein isolate (PPI) catalyzed by TG effectively altered the physicochemical and functional properties. For example, this crosslinking significantly reduced the solubility of PPI in water and produced larger aggregates or clumps. Tang et al. (2006) showed that the TG catalyzed crosslinking of soy protein isolate (SPI) increased the thermal T_d of glycinin and β-conglycinin components. It has also been reported that the treatment of glutamine and ε-amino groups with TG lowered the H_0 of these groups due to partial deamidation (Gauche, Vieira, Ogliari, Marilde, & Bordignon, 2008).

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In this study, we wished to test the hypothesis that conarachin and AS of arachin are responsible for the coagulation of peanut protein. It should be made clear which protein mainly participates in crosslinking and the mechanism of property enhancement by TG. For this purpose, we aimed at investigating the TG catalyzed crosslinking process of arachin-rich and conarachin-rich peanut protein fractions and gaining insight regarding the relative importance of arachin and conarachin-rich fractions of PPI on the crosslinking. In addition, the physicochemical properties (molecular weight distribution, solubility, thermal properties, microstructure and H_0) of the TG-crosslinked arachin-rich and conarachin-rich fractions were evaluated. The mechanism of TG catalyzed crosslinking and formation of insoluble in these two protein fractions was also elaborated.

Materials and methods

Materials

A commercial DPF was obtained from Gaotang Lanshan Co., Ltd. (Shandong Province, China). Flour preparation consisted of cleaning, drying, cracking, dehulling, and flaking at low temperature (60 °C). The oil was extracted by using No. 4 solvent (a blend of butane and propane). The oil extracted flour was further dried by cross-ventilation and was finely pulverized by using an altra-mizer (ZhenYuan Powder Engineering Equipment Co., Ltd., Shanghai, China). The oil content of DPF is less than 2.0%. The amount of defatted peanut flour used in the experiment is about 1000 g. The TG having an activity of 1000 units/g was purchased from Solarbio Co. Ltd. (China). All the chemicals used in the experiments were of analytical grade.

Extraction of arachin and conarachin-rich peanut protein fractions

The DPF was mixed with phosphate buffer (0.3 M, pH 7.5) at 4/10 (w/v) ratio at an ambient temperature. The mixture was homogenized at room temperature for 1 h and then centrifuged at 6000 *g* for 30 min. The precipitate was discarded and the supernatant was cooled down to 2 °C and was kept at this temperature for 4 h. This chilled supernatant was further centrifuged at 6000 *g* for 30 min at 2 °C. The precipitate obtained from the second centrifugation was rich in arachin. The supernatant obtained from the second centrifugation was further precipitated by acidification and by lowering the pH to 4.5. The conarachin-rich fraction was obtained by centrifuging the precipitate at 2000 *g* for 20 min. These arachin-rich and conarachin-rich fractions of peanut protein were dried using a Buchi Mini spray dryer (B-290, BUCHI Laboratory Equipment Trading Ltd., Switzerland) using inlet and outlet temperatures of 150 °C and 70 °C, respectively. These spray dried powders were stored in desiccator for further tests.

TG catalyzed crosslinking

All the polymerization or crosslinking reactions (catalyzed by TG) were carried out at 40 °C in deionized water (pH 7.0) containing 15% (w/v) protein (arachin or conarachin-rich fractions). The concentration of TG was varied between 0 (no TG) and 21 units per gram (U/g) of protein substrate. The reacting mixtures were incubated at 40 °C for 90 min. Once the polymerization (crosslinking) reaction was completed, the TG was inactivated by heating to 95 °C and maintaining this temperature for 5 min. The fully reacted (crosslinked) solution was freeze-dried using a LGJ-25 freeze dryer (Sihuan Science Instrument Factory, Beijing). These freeze dried samples were stored in desiccator for further analysis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE experiments were performed accordingly to Ramırez-Suarez, Xiong, and Wang (2001). The discontinuous system consisted of 5% (w/v) acrylamide stacking gel (pH 8.8) and 13% (w/v) acrylamide resolving gel (pH 6.8). Two milligram (2.0 mg) sample was dissolved in 0.5 ml buffer and then heated for 5 min in boiling water. This boiled sample was centrifuged at 4000 *g* for 10 min before electrophoresis. Four microliter (4.0 μL) sample was loaded into each well. Finally, the gel was stained by 0.1% (w/v) coomassie brilliant blue in 10% acetic acid, 45% fermentation alcohol and destained in 10% (v/v) acetic acid (fermentation alcohol:acetic acid:water ratio = 2:1:17).

Determination of protein solubility

The solubility of protein was determined according to Wu, Wang, Ma, and Ren's (2009) method with slight modification. The arachin and conarachin-rich peanut protein fractions were separately mixed with deionized water at the protein to water ratio of 1:100 (w/v). The pH value of these suspensions was adjusted to 7.0 with 0.5 M HCl or NaOH. These suspensions were stirred at ambient temperature for 30 min and the pH was readjusted. After continuously stirring for 60 min at room temperature, the suspensions were centrifuged at 8000 *g* for 20 min. The protein content in the supernatant was determined according to Lowry method (Lowry et al., 1951) using BSA as the standard. Triplicate tests were carried out and the protein solubility was determined using Eq. (1).

$$\text{Protein solubility}\,(\%) = \frac{W_1}{W_0} \times 100\% \quad (1)$$

where, W_1 is the mass of protein in the supernatant (g), and W_0 is the mass of protein in the powder sample (g).

Determination of thermal stability

The thermal stability of protein fractions crosslinked with TG was determined using a NANO DSC (TA Instruments, Inc., New Castle, USA) according to Damodaran and Agyare's (2013) method. Approximately two (2.0) milligrams of crosslinked sample was weighed in an aluminum pan and 10 μL of 0.1 M phosphate buffer (pH 7.0) was added. The sample pan along with the sample was hermetically sealed using an aluminum cap. Thermal scanning was carried out from 20 °C to 125 °C at a heating rate of 10 °C/min. The temperature at maximum heat flow or the T_d and the amount of (peak) heat flow (enthalpy of transition, $\triangle H$) were determined from the thermograms by the accompanied software (Universal Analysis 2000, Version 4.1 D, TA Instruments — Waters LLC). All experiments were conducted in triplicate for each sample and the average values are reported.

Determination of morphology

A Hitachi S-570 electron microscope was used for capturing the morphology of crosslinked samples as suggested by Han, Bourgeois, and Lacroix (2009). The dried sample was mounted on a copper stub with double sided carbon tabs and coated with gold. The scanning electron microscopic (SEM) images were taken at accelerating voltage of 20 kV.

Surface hydrophobicity (H_0)

H_0 was determined according to Hu's (2011) method with slight modification. The 1-anilino-naphthalene-8-sulfonate (ANS) was used as fluorescence probe in the absence of SDS. Protein suspensions were diluted (0.15, 0.075, 0.038, 0.019 mg/mL) in phosphate buffer (0.01 M, pH 7.0). The ANS solution (8.0 mM) was also prepared in the same phosphate buffer. Twenty microliter (20 μL) ANS solution was added to 4 mL of each (diluted) suspension and the fluorescence intensity (FI) was immediately measured at 470 nm (emission) and 390 nm