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Effects of limited proteolysis and high-pressure homogenisation on structural and functional characteristics of glycinin

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ABSTRACT

Glycinin was treated by limited proteolysis and/or high-pressure homogenisation in this work. The combined treatments significantly improved the solubility of glycinin, especially in acidic aqueous solution. Moreover, the modified glycinin had better emulsification capability and lower surface hydrophobicity. Electrophoresis analysis indicated more aggregates were formed after above treatments. But the laser scanning test showed the particle size of glycinin decreased. Compared with limited proteolysis, the combined treatments further increased the storage modulus and postponed the onset of glycinin gelation. The results of scanning electron microscopy showed that a fibrous curly structure was present in native glycinin. It was not found in modified glycinins. High-pressure homogenisation slightly shortened the onset of glycinin gelation, while limited proteolysis apparently postponed this behaviour. All the treatments formed weaker gels than native glycinin.

1. Introduction

Glycinin known as 11S fraction on the basis of sedimentation coefficient, accounts for approximate one third of the water extractable proteins of soybean (Iwabuchi & Yamauchi, 1987). It consists of an acidic (ca. 38 kDa) and a basic polypeptide fraction (ca. 20 kDa), linked by a disulphide bridge. Each pair of acidic and basic polypeptide fractions is encoded by a single gene and cleaved post translationally (Lakemond et al., 2003). Compared with soy β-conglycinin, native glycinin has limited functional properties due to its impact globular conformation and low molecular flexibility (Wagner & Guéguen, 1995).

Alteration of protein structure by physical and enzymatic methods is a well-accepted safe method for protein modification (Wang, Yang, Wang, & Du, 2008). Enzymatic hydrolysis and high-pressure homogenisation are two useful techniques due to the safety and efficiency. Limited proteolysis of soy proteins with enzymes has been used to alter protein native state and size-to-charge ratio, then to change functional properties, such as solubility and emulsification capability (Govindaraju & Srinivas, 2007; Rickert, Johnson, & Murphy, 2004; Tsumura et al., 2005). A common problem with extensively hydrolysed proteins is formation of bitterness due to the accumulation of low molecular-weight peptides with hydrophobic amino acids (Panyam & Kilara, 1996). In order to avoid the production of bitter peptides, limited proteolysis of proteins is often chosen to modify the functional properties. High-pressure homogenisation is a novel technique for macro-molecule dissociation or denaturation (Yang, Jiang, Wang, Zhao, & Sun, 2009). It can affect the protein structure and functionality (Bouaouina, Desrumaux, & Loise, 2006). The effect of high-pressure homogenisation over 100 MPa on soybean proteins has been documented (Puppo et al., 2005). It has been proven that high-pressure homogenisation has a disruptive effect on the tertiary and quaternary structures of most globular proteins and has little impact on the secondary structure (Subirade, Loupil, Allain, & Paquin, 1998), whereas limited proteolysis can change secondary structure to a certain extent (Panyam & Kilara, 1996). A combination of limited proteolysis and high-pressure homogenisation might result in a more prominent effect on structure and functional properties of proteins. Therefore, the objective of this work was to investigate the effects of limited proteolysis and/or high-pressure homogenisation on the structural changes of glycinin by electrophoresis, surface hydrophobicity and microstructure analysis. The solubility, emulsification and gelling properties of glycinin were also measured.

2. Materials and methods

2.1. Materials and chemicals

Defatted soy flour was purchased from Yuwang Soy Company (Shandong, China). The protease used was Alcalase 2.4 l (Novo

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Nordisk, Denmark). It had a specific activity of 2.4 Anson units per gram. Phenylmethanesulfonyl fluoride was obtained from Sigma Chemical Co. (Shanghai, China). All the other reagents were of analytical grade.

2.2. Preparation of soy glycinin samples

Various glycinins treated with limited proteolysis, high-pressure homogenisation and their combination were prepared according to the method of Deak, Murphy, and Johnson (2007) with a slight modification. The preparation scheme is shown in Fig. 1. The untreated glycinin (UC-glycinin) was prepared as follows: defatted soy flour was extracted with 15-fold (w/v) deionized water and the pH was adjusted to 8.0 with 10 ml of 1 M NaOH. The slurry was stirred for 1 h and centrifuged at 10,000*g* for 30 min (25 °C). The extracted proteins (Protein extract I, as shown in Fig. 1) were combined with 5 mM of $NaHSO_3$ and $CaCl_2$. The pH was adjusted to 6.4 with 1 M HCl. After storage at 4 °C for 14 h, the slurry was centrifuged at 9000*g* for 30 min at 4 °C. A glycinin fraction was obtained as the precipitated curd, which was redissolved in deionized water and adjusted to pH 7.5 with 2 M NaOH. Finally, the suspension was dialysed against deionized water and freeze-dried.

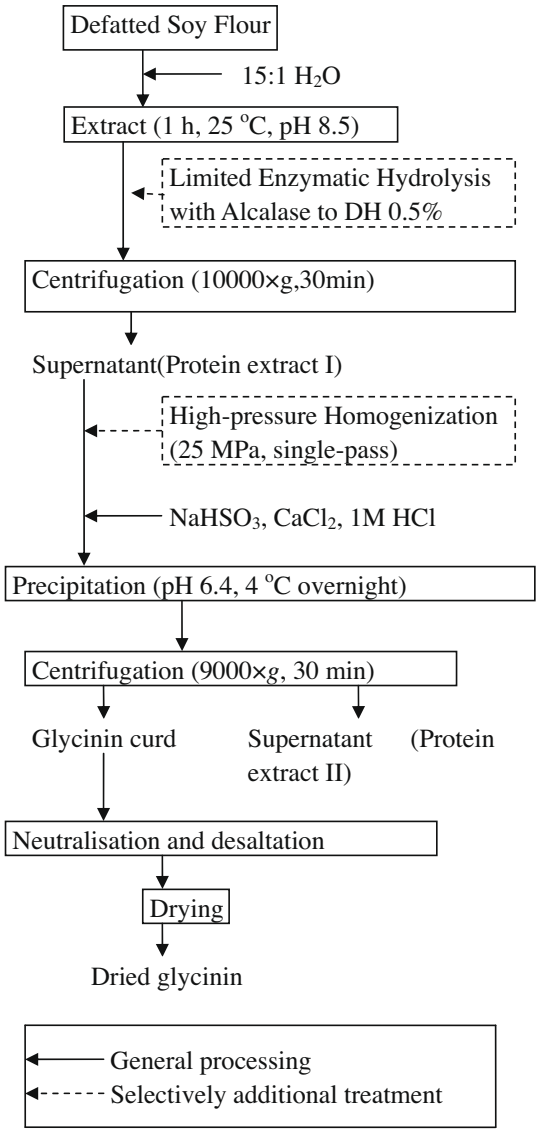

Fig. 1. Scheme of sample preparation.

The glycinin treated with limited proteolysis is referred to as SLP-glycinin. Defatted soy flour solution was hydrolysed to a DH of 0.5% with Alcalase 2.4 l at 50 °C for 16 min. The enzyme/substrate ratio was 0.0058 AU/g of protein. The pH was maintained constantly at 8.0 by 0.5 M NaOH. The enzymatic hydrolysis was stopped by addition of 7.5 ml of 100 mM phenylmethanesulfonyl fluoride stock solution in 2-propanol to a final concentration of 1 mM when the desired DH was reached. The DH was calculated according to the method of Adler-Nissen (1979). The total number of amino groups was determined in a sample that had been 100% hydrolysed at 110 °C for 24 h in 6 M HCl.

The glycinin treated with high-pressure homogenisation is referred to as SHPH-glycinin. Protein extract I was homogenised (25 MPa, one pass) before adding $NaHSO_3$ and $CaCl_2$, and all the other procedures were carried out as UC-glycinin. Moreover, the referred LPCHPH-glycinin was obtained by the combined treatments of limited proteolysis and high-pressure homogenisation sequentially. This treatment was conducted under the conditions used for limited proteolysis and high-pressure homogenisation alone.

2.3. Sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and native-PAGE

SDS–PAGE was performed on two buffer systems using 12% separating gel (pH 8.8) and 5% stacking gel (pH 6.8) according to the method of Tari, Dogan, and Gogus (2008). The protein samples were dissolved in 0.06 M Tris–HCl buffer, containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 25% (v/v) glycerol and 0.1% (w/v) bromophenol blue, and heated for 5 min in boiling water before electrophoresis. Fifteen microlitres of supernatant were loaded into each lane. After the electrophoresis, the gel was dyed by 0.25% Coomassie blue in 50% trichloroacetic acid and then destained in methanol/acetic acid/water (1:1:8, v/v/v). The procedure of native-PAGE was similar to that of SDS–PAGE, except that there was no SDS and 2-mercaptoethanol in the Tris–HCl buffer and no thermal treatment before electrophoresis.

2.4. Microstructure

The mirostructure was determined by the method of Yang, Jiang, Zhao, Shi, and Wang (2008). The sample was deposited on a silicon wafer and coated with a conductive material (gold) to ensure sufficient electron refraction. They were then mounted onto an aluminium stub with epoxy and coated with a gold platinum alloy in a sputter coating device. The samples were observed with a JSM-6360LV scanning electron microscope (JEOL, Tokyo, Japan) at an operating voltage of 20 kV and a vacuum of 15 Pa.

2.5. Surface hydrophobicity

Surface hydrophobicity was determined by the hydrophobicity fluorescence probe l-anilino-8-naphthalenesulfonate according to the method of Kato and Nakai (1980), in the absence of SDS. Protein dispersions (1 mg/ml) in 0.01 M phosphate buffer (pH 7.0) were stirred for 2 h at 20 °C and centrifuged at 8000*g* for 20 min. Protein concentration in the supernatants was determined according to the method of Lowry, Rosebrough, Farr, and Randall (1951). Each supernatant was serially diluted with the same buffer to obtain protein concentrations ranging from 0.005 to 0.1 mg/ml. Then 20 μl of l-anilino-8-naphthalenesulfonate (8.0 mM in the same buffer) was added to 4 ml of sample. Fluorescence intensity was measured with a Perkin–Elmer 2000 fluorescence spectrometer at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of fluorescence intensity vs. protein concentration plot (calculated by linear regression analysis) was used as the index of protein hydrophobicity.