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Freezing of soybeans influences the hydrophobicity of soy protein

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Abstract

By using quartz-crystal microbalance (QCM) and cyclic voltammetric (CV) techniques, the effect of freezing on the hydrophobicity of soy protein was investigated. The results were compared to those of a sodium dodecyl sulfate (SDS) binding method. In the QCM studies the highest protein load onto the hydrophobic ethanethiol-monolayer was found with heated soy protein from frozen soybeans (HSFS), followed by heated soy protein from unfrozen soybeans (HSUS), unheated soy protein from frozen soybeans (USFS), and unheated soy protein from unfrozen soybeans (USUS). In the CV studies, it was found that values of an anodic profile decreased with adsorption time: it was the greatest with HSFS, followed by HSUS and USFS, and least wth USUS. Results of SDS binding capacity were found to be in line with those results of a QCM and CV measurements, indicating freeze treatment increased the hydrophobicity of soy protein regardless of heating. In addition, QCM and CV measurements were found to be very convenient to determine the hydrophobicity of soy protein successfully.

Keywords: Soy protein; Freezing; Hydrophobicity; QCM; CV; SDS binding capacity

1. Introduction

The freezing treatment of soybeans has been found to give a better taste than unfrozen soybeans and to reduce the cooking time by half (Lee, Choi, Kim, & Yun, 1992). Freezing has also brought about some changes in the processing characteristics of soybeans. Soymilk obtained from frozen soybeans coagulates faster and produces uniformly structured gels in the presence of coagulants while the soymilk from unfrozen soybeans forms unsatisfactory whey-offed gels (Noh, Park, Pak, Hong, & Yun, 2005). In addition, tofu prepared from frozen soybeans shows a more ordered and dense networking structure than that from unfrozen soybeans, indicating changes in the textural parameters and quality of tofu (Noh et al., 2005). These results suggest that the freezing of soybeans can modify the properties of soy proteins in a positive way. However, only a few studies have been carried out to characterize the effects of freezing on the properties of soy proteins. When the solution of soy protein was frozen, the proteins became partially insoluble due to the polymerization of protein molecules through the formation of intermolecular disulphide bonds (Hashizume, Kakiuchi, Koyama, & Watanabe, 1971). One of the most important functional properties of plant proteins is their ability to form a gel or curd that serves as a matrix to trap water, flavors, and nutrients (Oakenfull, 1987; Ziegler & Foegeding, 1990). Besides the gelling behavior of soy proteins in the manufacturing of Asian foods such as tofu, its gelling property during cooking is also important.

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The hydrophobicity of proteins has gained much attention since it is considered to be closely related to functional properties (Kato, Tsutsui, Kobayashi, & Nakai, 1981; Kinsella, 1979). Hydrophobic interactions of soy proteins are believed to play a major role in tofu manufacturing, since the exposure of hydrophobic regions induced by a heat treatment is a prerequisite for coagulation (Kohyama, Sano, & Doi, 1995). The quantitative analysis of protein hydrophobicity can be the essential step for the accurate prediction of protein functionality (Nakai, 1983). Many techniques to determine the surface hydrophobicity of the proteins have been examined. They include reverse-phase chromatography (van Oss, Absolom, & Neumann, 1979), binding of hydrocarbons to proteins (Mohammadzadeh, Smith, & Feeney, 1969), hydrophobic partition method (Shanbhag & Axelsson, 1975), salting-out effect and surface tension method (Melander & Horvath, 1977), sodium dodecyl sulfate (SDS) binding method (Kato, Matsuda, Matsudomi, & Kobayashi, 1984), and the 1-anilino-8-naphthalene sulfonate (ANS) method (Hayakawa & Nakai, 1985). These methods require laborious and time-consuming procedures, which justify efforts to find simpler methods. Recently, using QCM and CV measurements, the hydrophobic properties of some proteins such as bovine serum albumin, human serum albumin, and immunoglobulin G, were well characterized (Anzai, Guo, & Osa, 1996; Moulton, Barisci, Bath, Stella, & Wallace, 2003).

The present study aims to investigate the effect of the freezing of soybeans on the hydrophobicity of soy proteins. The quartz-crystal microbalance (QCM) and cyclic voltammetric (CV) measurements were employed for the determination of the protein hydrophobicity. These results were compared to those of the SDS binding method (Kato et al., 1984).

2. Materials and methods

2.1. Materials

Soybeans (*Glycine max* Merr., cv Jang-yeob) were purchased from a local grower (Chonbuk province, Korea). All reagent grade chemicals were purchased from Sigma.

2.2. Methods

2.2.1. Soy protein preparations from soybeans

The soybeans (approximately 15 g) were soaked in 80 ml tap water at room temperature for 10 h. The soaked beans were placed in a basket to remove excess water and frozen to −20 °C for 5 h by air-blast freezing. The frozen beans were thawed and ground in a mixer at a high speed. The resulting meal was defatted with *n*-hexane before and after 5 min of boiling, and air-dried, followed by passing through a 325-mesh sieve. The prepared protein bodies were dissolved in a 10 mM phosphate buffer (pH 7.0) to give a desired concentration (wt%) for various measurements. For comparison, soy protein bodies of another batch were prepared by the same procedure described above excluding the freezing treatment.

2.2.2. Quartz-crystal microbalance study

The gold surface (0.2 cm^2) on a 9 MHz quartz crystal resonator was coated with a self-assembled thiol monolayer by contacting the surface to an ethanol solution of 20 mM ethanethiol (C_2H_5SH) for 2 h at room temperature. The modified gold electrode was rinsed with ethanol and water, and then mounted into a cell. After the addition of 0.01% soy proteins, the resonance frequency of the quartz was measured by using a QCM (EQCM 1000 system, SHIN Co., Korea). Measurements were performed at 20 °C.

2.2.3. Cyclic voltammetric study

A clean gold electrode (0.5×0.5 cm^2), to be used as a working electrode, was immersed in a 10 mM ethanethiol solution for 4 h at room temperature to form its self-assembled monolayer. The modified electrode was then exposed to a 0.01% soy protein solution. The conventional three-electrode system with an Ag/AgCl reference and a Pt wire counter-electrode was used. Measurements were performed at 20 °C.

2.2.4. Sodium dodecyl sulfate binding method

Surface hydrophobicity of soy proteins was determined by using the SDS binding method (Kato et al., 1984). SDS-protein solution (0.1% soy protein solution in 0.07 mM SDS) was dialyzed against 0.02 M phosphate buffer (pH 7.0) for 48 h. One milliliter of the dialyzed SDS-protein solution was transferred into a 25-mL screw-capped test tube containing 10 mL chloroform and mixed well. To the mixture, an aliquot of methylene blue solution (2.5 mL of 0.0024%) was added and centrifuged at 800*g* to separate water and insoluble proteins from the chloroform layer. The absorbance of SDS-methylene blue in the chloroform layer was measured at 655 nm. A calibration curve with known amounts of SDS, was used to determine the amount of SDS bound to the protein. SDS binding capacity (μg of SDS bound to 1 mg protein) indicates the measure of the hydrophobicity of protein.

2.2.5. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System program (SAS, 1990). Means comparisons were made by Least Significance Differences (LSD) test. A significant level was defined as a probability of 0.05 or less.