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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.

3. Results

3.1. QCM measurements

The adsorption of soy proteins onto the hydrophobic surface of ethanethiol-monolayer-modified gold electrodes was monitored using a QCM technique. The frequency changes (ΔF) of a 9 MHz quartz crystal with time are shown in Fig. 1. The resonance frequency (F) decreases upon the additions of soy protein into the buffer solution, implying that the mass increases as the protein molecules are adsorbed on the surface of the thiol monolayer. The frequency change (ΔF) was the greatest in the solution of heated soy protein prepared 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). The degree of the ΔF values was clearly depended on the treatment of soy protein.

The mass change (ΔM) due to the adsorption of soy protein can be calculated from the frequency change (ΔF) using the Sauerbrey equation (Eq. 1) (Buttry, 1991; Ward, 1995)

$$\Delta F\ (\mathrm{Hz}) = -0.91\Delta M\ (\mathrm{ng/cm^2}). \quad (1)$$

As shown in Table 1, differences in protein load are clear such that heating or freezing treatment gave more loading of the soy proteins onto the monolayer surface. Using Eq. (1), the proteins load after 10 min of exposure were estimated to be 768 ng cm^{-2} for the HSFS, 678 ng cm^{-2} for the HSUS, 559 ng cm^{-2} for the USFS, and 501 ng cm^{-2} for the USUS.

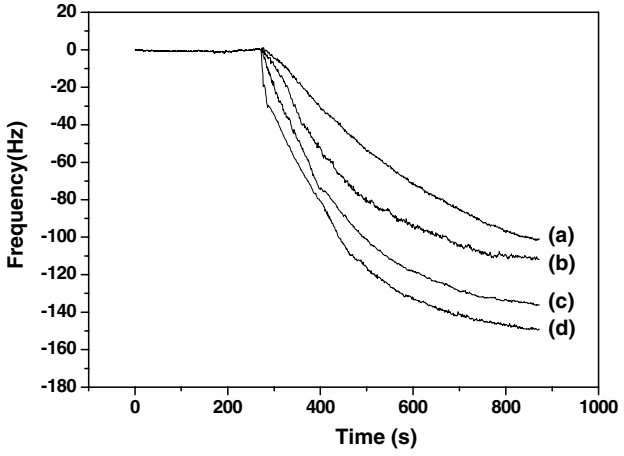

Fig. 1. Frequency changes in an ethanethiol-monolayer-coated QCM upon additions of the unheated soy proteins prepared from unfrozen soybeans (USUS, a), the unheated soy proteins from frozen soybeans (USFS, b), the heated soy proteins from unfrozen soybeans (HSUS, c), or the heated soy proteins from frozen soybeans (HSFS, d).

3.2. Electrochemical CV measurements

A gold electrode coated with the hydrophobic ethanethiol-monolayer was used to monitor the adsorption behavior of soy proteins by measuring CV curves in a solution containing $Fe(CN)_6^{4-}$. In previous studies, bovine serum albumin (Anzai, Guo, & Osa, 1994), human serum albumin, and immunoglobulin (Moulton et al., 2003) were bound to the bare Pt and Au surfaces spontaneously, and the electron transfer between the electrodes and $Fe(CN)_6^{3-/4-}$ ions in solution was considerably suppressed by the protein adsorption on the surface of the electrode.

Fig. 2 shows CV curves changing with time. The magnitude of the anodic and cathodic peaks gradually decreased with increasing-peak separation. The decreasing rate of the peak currents were different depending on different soy proteins. It is now thought that the changes in the CV arise from the decrease in the electron transfer rate (Bard & Faulkner, 2001) as the electrode surface is coated with a protein layer. This layer acts as a barrier interrupting the electron transfer between the ferricyanide ions and the electrode surface.

Anodic current profiles of the different protein solutions were compared. The anodic peak current $(i_p)_t$ at a time after the adsorption was divided by the initial anodic peak current $(i_p)_0$ and then the $(i_p)_t/(i_p)_0$ values were plotted in Fig. 3. The decreasing rate of the $(i_p)_t/(i_p)_0$ value was the highest for the HSFS (0.63 at 10 min) and it was followed by HSUS (0.68 at 10 min), the USFS (0.75 at 10 min), and the USUS (0.74 at 10 min). This trend from the CV measurements

Table 1
Some results from the QCM, CV, and SDS binding measurements

Samples	Mass loading[A]	$(i_p)_t/(i_p)_0$[B]	SDS binding[C]
Unheated soy proteins from unfrozen soybeans (USUS)	501[aD]	0.74[a]	6.5[a]
Unheated soy proteins from frozen soybeans (USFS)	559[b]	0.75[a]	7.3[a]
Heated soy proteins from unfrozen soybeans (HSUS)	678[c]	0.68[b]	10.6[b]
Heated soy proteins from frozen soybeans (HSFS)	768[d]	0.63[c]	13.2[c]

[A] The loading (ng/cm^2) of soy proteins onto the hydrophobic surface of ethanethiol-monolayer-modified gold electrode.
[B] $(i_p)_t$ and $(i_p)_0$ denote the peak currents at adsorption times at t = 10 min and zero, respectively.
[C] μg of SDS/mg of protein.
[D] Mean scores within the column by the same letter are not significantly different (<0.05).

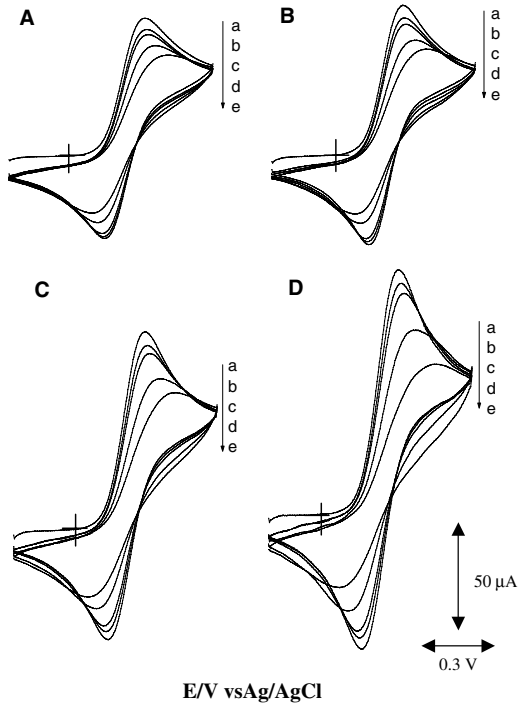

Fig. 2. Cyclic voltammograms with a gold electrode coated with ethanethiol monolayer in a solution of 1.5 mM $Fe(CN)_6^{4-}$. The exposure times are 0 (a), 1 (b), 2 (c), 5 (d), and 10 min (e). Scan rate, 25 mV s^{-1}. Supporting electrolyte, 10 mM phosphate buffer (pH 7.0). (A: unheated soy proteins from unfrozen soybeans (USUS); B: unheated soy proteins from frozen soybeans (USFS); C: heated soy proteins from unfrozen soybeans (HSUS); D: heated soy proteins from frozen soybeans (HSFS)).

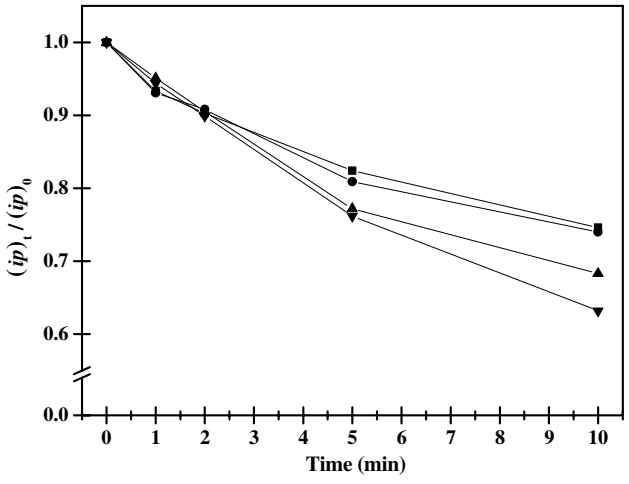

Fig. 3. Anodic current profile from the cyclic voltammograms in Fig. 2. The $(i_p)_t$ and $(i_p)_0$ denote the peak currents at adsorption times at $t = 10$ mins and zero, respectively. Symbols: ■, unheated soy proteins from unfrozen soybeans (USUS); ●, unheated soy proteins from frozen soybeans (USFS); ▲, heated soy proteins from unfrozen soybeans (HSUS); ▼, heated soy proteins from frozen soybeans

shows a good agreement with that observed in the QCM measurements. Both the CV and QCM data suggest that the HSFS was adsorbed on the hydrophobic surface in the largest amount among the protein bodies tested and the electron transfer was blocked the most significantly.

3.3. SDS binding capacity of soy proteins

Surface hydrophobicity of soy proteins was determined by using the SDS binding method. Table 1 shows the SDS binding capacity of soy proteins (μg of SDS/mg protein), with the mass loading and the anodic current profile for comparison. It was found that the HSFS had the highest SDS binding capacity (13.2 μg of SDS/mg of protein), followed by HSUS (10.6 μg of SDS/mg of protein), the USFS (7.3 μg of SDS/mg of protein), and USUS (6.5 μg of SDS/mg of protein). This tendency is exactly consistent with the results of CV study but agrees in part with the results of QCM study: the difference between unheated soy protein from unfrozen soybeans (USUS) and unheated soy protein from frozen soybeans (USFS) is not significant in both the SDS-binding method and CV study unlike the QCM study. This may be ascribed to the uncertainty of each measurement in our experimental conditions, although the exact reason is not known.

4. Discussion

Considering the hydrophobicity of the end group of the ethanethiol-monolayer in the QCM and CV studies, the increased hydrophobicity of the soy protein from frozen soybeans was observed. The results of the SDS binding measurements also supported this tendency. From these results, it appears that the freezing treatment increases the hydrophobicity of soy proteins, regardless of the heating treatment. In addition, their hydrophobicity was even more increased when both treatments were combined. This may indicate that the freezing of soy proteins causes the conformation of soy proteins to be changed, thereby making the proteins more susceptible to heat denaturation.

Interactions during the gelation of soy proteins such as hydrogen bonding, hydrophobic interactions, electrostatic interactions, and disulfide bonds have been documented (Kinsella, 1979; Utsumi & Kinsella, 1985a). While many factors influence the properties of soy proteins, hydrophobic interactions are likely to play a major role in enhancing the heat-induced gelation of soy proteins. The storage proteins, 7S (conglycinin) and 11S (glycinin), are known to be the principal components of soy protein (Kinsella, 1979). The 11S component is involved in disulphide bonding (Utsumi & Kinsella, 1985b), which has been considered to be negligible within the gel network (Kohyama et al., 1995). The 7S component is mainly involved in hydrogen bonding and hydrophobic interactions with no disulphide bonding

(Nakamura, Utsumi, & Mori, 1986). A gel of 7S has shown to be harder than that of 11S from soy protein (Utsumi & Kinsella, 1985a). These results suggest that hydrophobic interactions as well as hydrogen bonding play an important role in gelation, making the protein gel matrix denser. However, the formation of intermolecular disulphide linkages was observed when a solution of acid-precipitated soybean or 11S protein was frozen (Hashizume et al., 1971). Such a conformational change of the soy proteins also could induce enhanced aggregation of the protein particles upon heating.

Within these contexts, the reason why freezing can manipulate the coagulating process of soymilk in a positive way in our previous study (Noh et al., 2005) may be explained. The coagulation of soymilk has been known to involve a two-step process: protein denaturation by heating, followed by hydrophobic coagulation promoted by coagulants (Kohyama et al., 1995). As described above, it was proved that the hydrophobicity of soy proteins was increased by the freezing treatment. Therefore, a proposal of the previous study (Noh et al., 2005) that a modification in the coagulation process of soymilk was induced by freezing, is reasonable.

In addition, we demonstrated successfully that QCM and CV measurements can be used to estimate the hydrophobicity of soy proteins conveniently. With gold electrodes which are covered by hydrophilic, positively charged and negatively charged self-assembled monolayers, such measurements may be possibly applied to the determination of other physical properties of protein with simplicity.

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