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Changes in Physicochemical Properties of Soybean Protein due to Acetylation during Incubation with Glucose

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Abstract : Native and acetylated soybean protein with acetylation percentage of 25% were incubated with glucose to induce Maillard reaction. Acetylation of ε-amino group of lysine residues changed the conformation of soybean protein. The direct uv spectrum of native and acetylated soybean protein showed conformational changes with accessibility of tyrosine and tryptophan residues increased. Acetylation suppressed Maillard reaction between soybean protein and glucose. Acetylated soybean protein showed improved water sorption, fat binding, foam formation, and emulsion activity of the protein, but depressed brown pigment development and trypsin digestion. Thus aceylation prevented deterioration of certain functional characteristics that occurred during storage, besides causing functional characteristics to be improved on its own.

Keywords : soybean, functionality, physicochemical properties

Introduction

During the past few years much progress has been made in developing commercial soybean products with differing functional characteristics. This has been accomplished through protein enrichment and protein isolation as well as through application of mechanical, thermal, and chemical treatments, and selected combination thereof[1-3]. Consequently, research interest in exploring various chemical and biochemical means for altering the functional properties of soybean protein products is expanding. In order to be useful as food ingredients, soybean protein must possess appropriate functional properties for food applications and comsumer acceptability. These are intrinsic physicochemical characteristics which affect the behaviour of protein in food systems during processing, manufacturing, storage and preparation- for example, sorption, solubility, gelation, surfactancy, storage and ligand -binding, and film formation[4,5].

Studies with food proteins have included succinylation of disrupted yeast cells[6] to facilitate the isolation of yeast protein with a low nucleic acid content. Groninger and Miller[7,8] used bromelin hydrolysis, followed by succinylation of fish myofibrillar protein to improve protein functionality in terms of dispersibility, aeration capacity and foam stability.

Most of foods contain lipids and carbohydrates besides protein, as food components. Lipids and carbohydrates affect functional characteristics of proteins by interaction with them. The nonenzymatic browning, or Maillard reaction, comprises the reaction between reducing sugars and amino groups of amino acids and proteins subsequently resulting in visible browning. This reaction causes deterioration of foods by changing nutritive value due to blockage of amino groups, color, flavor besides causing functionalities of protein to decrease. The results of Maillard reaction, one of the most widely occurring processes in real foods, are polymerization of the system. Since the reaction occur mainly at lysine residues, nutritional value of the food is impaired and the functionalities of the proteins are expected to change due to altered physicochemical properties of the proteins. To inhibit Maillard reaction, and to actually upgrade functional properties of proteins, modification of protein by chemical reagents are being studied by quite a number of researchers around the world[9-11]. Alterations of the chemical, physical, and biological properties of proteins by

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chemically changing their structures have been an objective of protein chemists for many years.

As mentioned above, many researchers have done works on functionalities of some of important proteins and the results of chemical modification, but studies on proteins in actual food system consisting of carbohydrates and lipids, besides proteins, are virtually nonexisting. The purpose of this work was to study the effects of modification on the functionalities of protein isolate during storage with glucose.

Materials and Methods

Materials

Defatted soybean four and soybean oil were purchased from Cheil Sugar Co. (Seoul, Korea). Tris buffer, acetic anhydride, trypsin, α-chymotrypsin, peptidase, and sodium bromide were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sample Preparation

Defatted soy flour was suspended in 10 parts 0.03 M, pH 8.0 Tris-HCl buffer containing 0.01 M β-mercaptoethanol. After stirring for an hour, the slurry was centrifuged for 20 min at 10000 × g to get rid off debris. The whole buffer extract was adjusted to pH 4.2, the isoelectric point of the protein, and recentrifuged at 10000 × g for 20 min. Protein precipitate was washed with the Tris buffer and recentrifuged for at least two times to get rid off β-mercaptoethanol and other unwanted components.

Acetylation

The acetylation procedure used was similar to that of Riordan and Vallee[12]. Acetic anhydride was added slowly to a 2.5% dispersion of soy isolate in water with concurrent addition of sodium hydroxide to maintain the pH at 7.5. Acetic anhydride addition was controlled to obtain lysine residue modification percent of 0%, and 25%. After the reaction, the samples were precipitated by adding absolute ethanol to 50% by volume and centrifuged at 10000 g for 20 min. The samples were washed with 50% ethanol for at least 2 times.

Browning Induction

Modified proteins were mixed with glucose (2:1; w/w) and placed in dessiccators preequilibrated with saturated sodium bromide solution. Incubation was done at 55°C for 0, 2, 4 and 8 days to obtain different degrees of browning for the differentially modified protein samples.

Modified Percentage Assay

The number of free amino groups present in the protein before and after modification was monitored by reaction with ninhydrin solution. Briefly, 1 m*l* of 1% protein solution was mixed with 1 m*l* of ninhydrin solution made from 30 m*l* of pH 5 citrate buffer of 0.2 M, 30 m*l* of 4% ninhydrin in ethyl cellosolve, and 1 m*l* of stannous chloride suspension containing 50 mg of it, and heated at 100°C for 5 min. The extent of reaction was followed by adding 5 m*l* of distilled water of the reactant and reading absorbancy at 580 nm.

Degree of Browning Detection

Degree of browning of the incubated samples were detected using protease. Briely, 1 g of the each samples was dispersed in 20 m*l* distilled water and stirred for 15 min in 37°C water bath. After fixing pH to 8, 3 m*l* enzyme solution of pH 8 containing 0.6 mg trypsin/m*l*, 3 mg α-chymotrypsin/m*l* and 1.3 mg peptidase/m*l* were added to each samples and incubated for 1 hr to extract brown pigments. Three m*l* of 50% trichloriacetic acid were added and the mixture were filtered with grass-wool gravimetrically. The eluted solutions were detected at 420 nm.

Water Sorption

In the controlled humidity chambers set at 57% relative humudity, 0.5 g of each samples was equilibrated in duplicate for at least 48 hr. The water held by each sample was measured as the difference in wet and dry weights and was recorded as percent of dry weight[13].

Oil Adsorption

As described by Fleming *et al.*,[14] 0.5 g of each samples was mixed with 3.0 m*l* soybean oil and stirred with brass wire for 1 min. After standing for 30 min at room temperature the contents were centrifuged at 1610×g for 25 min. The free oil was drawn off and weighed. Oil adsorption was calculated as grams of oil

adsorbed by one gram of samples.

Foam Formation

Protein solutions (0.2 g protein in 20 m*l* pH7, 0.02 M Tris buffer) were prepared in duplicate in 25×100 mm cylinders. Mouths were sealed and shaken rigorously for one minute at room temperature to allow foams to form. After standing for 30 min, large foams and unstable foams were destroyed by tapping volumes of each sample was determined.

Emulsion Activity

To 0.02 g of each samples was blended with 10 m*l* of 0.05 M pH 7 phosphate buffer and 1.1 m*l* soybean oil using Warring blender for 60 sec. 0.5 m*l* aliquotes of emulsions produced was diluted with 20 m*l* of 1% sodium dodecyl sulfate. Turbidity was detected at 600 nm[15].

Enzyme Digestion

The enzymes used in the in vitro digestion study was the porcine pancreatic trypsin. 0.02 g samples were dispersed in 20 m*l* borate buffer of pH 8.2, 0.02 M and blended for 60 sec in Warring blender. One m*l* solution containing 0.02 g trypsin were added to each samples and incubated for 30 min at 38°C. Amino acid released were detected using ninhydrin reaction.

Results and Discussion

UV Absorption Spectra of Native and Acetylated Soybean Protein

To detect the changes in the three-dimensional structure of protein, various spectrophotometric techniques can be employed. One of such method is the change in ultraviolet spectra of protein. Fig. 1 shows the direct UV absorption spectra of 0.1% native and acetylated soybean protein samples. UV spectra of the samples revealed a shift of λ_{max} (maximum absorbance) to a shorter wavelength with modification; the blue shift of 95% modified protein was substantial, while that of 25% modified protein was marginal. The blue shift and the decrease in absorbance indicate that the hydrophobic chromophores shifted to a more polar environment. According to

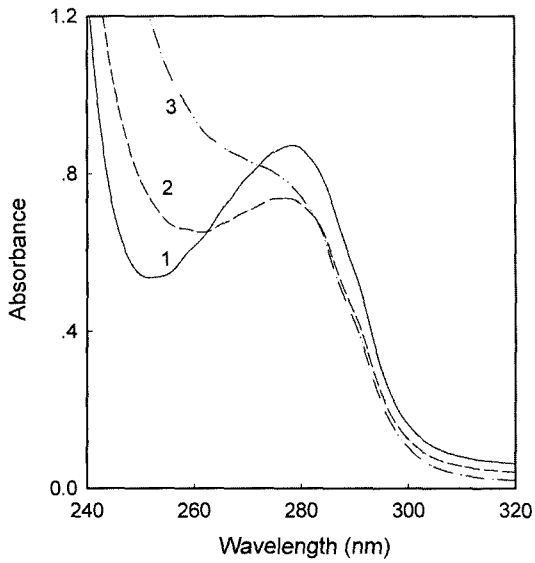

Fig. 1. Absorbance spectra of native and acetylated soybean protein. 1: native soybean protein: 2: 25% acetylated soybean protein; 3: 85% acetylated soybean protein.

Kim and Rhee[17], the most useful side-chain optical properties are those that occur at wavelengths longer than 230 nm, where the peptide absorption is reduced to negligible values. Between 230 and 300 nm, effects of the aromatic amino acids such as Phe, Tyr, and Trp become predominant. Absorption spectra of aromatic amino acids showing blue shift and the decrease in absorptivity following transfer to a more polar environment are indications for conformation change of protein containing these amino acids.

Degree of Browning

Browning is the reaction between amino groups and carbonyl groups. As the model food system consists of proteins and glucose, it is expected that browning reaction occur rigorously when incubated at 55°C with relative humidity of 58%. Degree of modification versus browning graph (Fig. 2) indicate such results. Degree of browning increases with incubation time and decreases with modification. 58% relative humidity and 55°C temperature conditions were employed so that Maillard reaction occurred maximally as it is reported that browning accelerate at intermediate moisture level and high temperature.

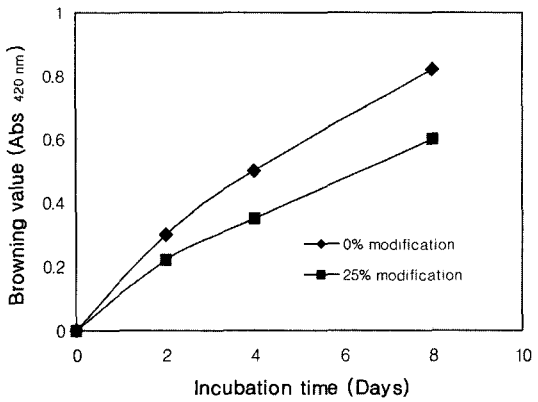

Fig. 2. Effect of acetylation on browning of soybean protein during incubation with glucose.

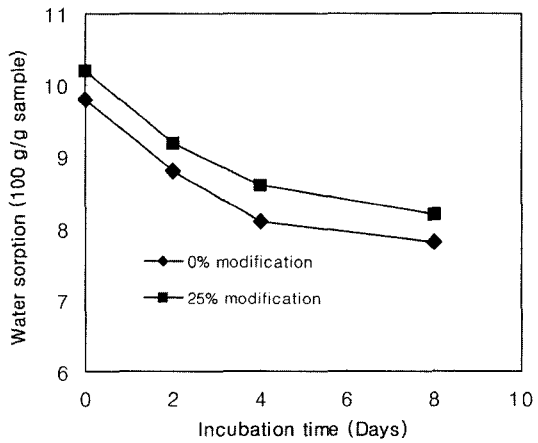

Fig. 3. Effect of browning induction time on water sorption of acetylated and native soybean protein.

Water Sorption

As with Childs and Park[18], who reported increases in water holding capacity of glandless cottonseed flour when acylated with acetic anhydride, water sorption characteristic of the model food system improved with modification (Fig. 3). This is probably due to general unfolding and expansion of protein molecules, so that more water molecules could bond to them. However, water sorption ability of the system decreased as browning progressed. Browning results in polymerization and aggregation of protein molecules, as well as increases in hydrophobicity of protein molecules. Such factors as mentioned above might be accounted for the decrease in sorption characteristic of browned samples.

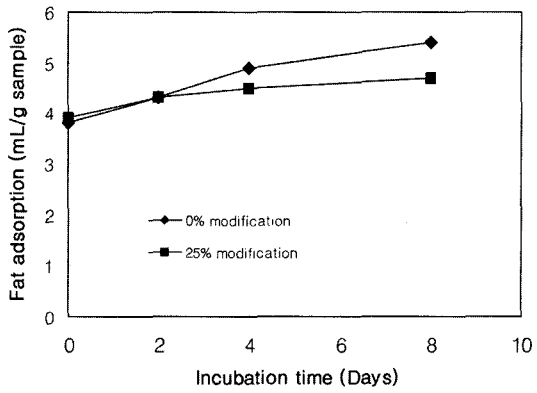

Fig. 4. Effect of browning induction time on fat adsorption of acetylated and native soybean protein.

Fat Adsorption

The oil retention properties of the samples are influenced by modification and browning, as shown in the Fig. 4. in the case of zero day incubation, it can be seen that acetylation influenced the amount of oil absorbed by the protein-glucose system. Acetylation results in unfolding and expansion of protein molecules, exposing hydrophobic interior to surrounding, thus more sited for oil bonding. Also acetylation of lysine residues result in replacement of positive charges with neutral acetyl groups, which provide additional oil bonding sites. Fat adsorption was also enhanced by browning. Although the reasons are not so clear, as in the case of water sorption, it might be due to increase in hydrophobicity as the result of Maillard reaction and also cross linkage could have somehow helped adsorption of lipid to protein molecules.

Foam Formation

Foaming, the capacity of proteins to form stable foams with gas by forming impervious protein films, depend upon physiochemical properties of them. The protein facilely locates at the interface and unfolds, exposing hydrophobic segments to interior and hydrophilic portions to exterior. Thus, the increase in foam volume with modification (Fig. 5) could be explained by the ease in protein to unfold at the film interface and the increase in hydrophobicity. However, foaming capacity decreased with browning,

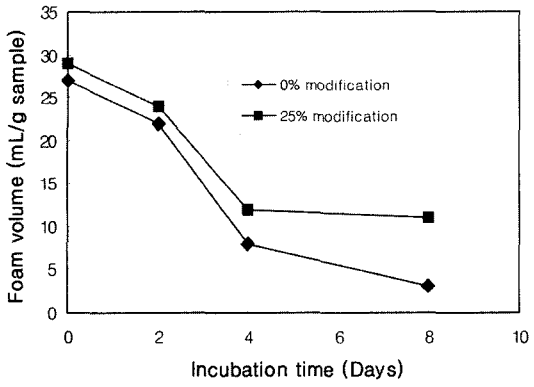

Fig. 5. Effect of browning induction time on foam volume of acetylated and native soybean protein.

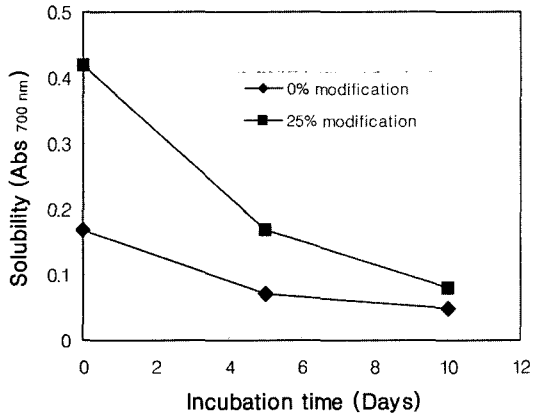

Fig. 6. Effect of browning induction time on solubility of acetylated and native soybean protein.

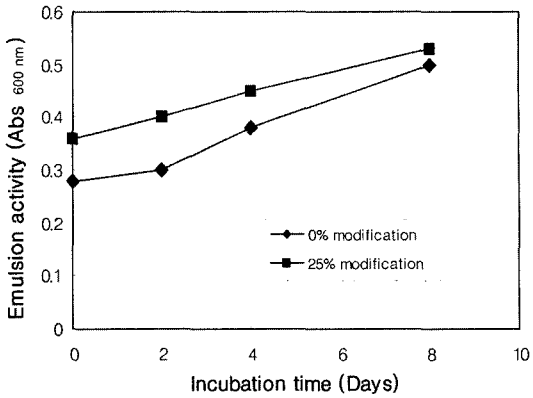

Fig. 7. Effect of browning induction time on emulsion activity of acetylated and native soybean protein.

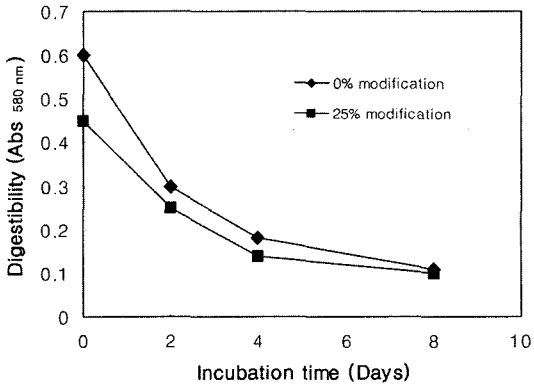

Fig. 8. Effect of browning induction time on digestibility of acetylated and native soybean protein.

as cross linking between proteins, the result of Maillard reaction, could have inhibited the protein to denature at the foam interface as required in proper foam formation. Also decrease in solibility for browned protein could be accounted for such result (Fig. 6).

Emulsion Activity

As in foam formation, the protein's conformational equilibrium shifts at the interface, exposing hydrophobic segments to the lipid interface and the polar segments of the polypeptide to the aqueous phase. As with other researchers[19,20], the samples showed increase in emulsifying activity with modification (Fig. 7). This may be due to ease of protein to undergo denaturation, and other positive effects such as increase in hydrophobicity. Also, the experiment showed that the emulsifying activity increased with browning induction time.

Digestion

As shown in the Fig. 8, the modification was accompanied by a corresponding decrease in the hydrolysis rate by trypsin. This is expected as the modification at lysine residues inhibits the action of trypsin whose sited of reaction includes the lysine residues. Browning also affected digestibility by trypsin, as browning occur between ε-amino groups of lysines and the glucose in the food system. As expected, digestibility decreased to largest extent for the unmodified sample, with incubation.

As discussed above, modification of protein improves certain functionalities of proteins, such

as emulsifying capacity, foam formation, fat binding, water sorption, and inhibited browning reaction.

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