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Effect of transglutaminase treatment on the functional properties of native and chymotrypsin-digested soy protein

Elfadil Elfadl Babiker *

Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Shambat, Sudan

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Abstract

Native and chymotrypsin-digested soy protein were polymerized by transglutaminase (TGase). SDS-PAGE pattern of the polymerized proteins showed that TGase treatment resulted in proteins of higher molecular mass above the stacking gel. The free amino groups of the polymerized proteins were greatly reduced. The solubility of the protein polymer was greatly improved at pH 2 and pH 8–12 whereas, at pH 4 and 6, it was least soluble. The solubility of the digest polymer was significantly improved at all pH levels, except pH 4. The protein polymer started to coagulate when the heating temperature exceeded 50°C. The digest polymer, on the other hand, resisted heat-induced aggregation up to 60°C; thereafter, its solubility declined slightly. The emulsifying and foaming properties of the digest polymer were greatly improved compared to the protein polymer. The protein polymer was observed to form a harder gel than the digest polymer. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Soy protein; Transglutaminase; Chymotrypsin; Polymer; Functional properties

1. Introduction

Proteins have unique surface properties due to their large molecular size and their amphiphilic properties. However, the industrial applications of food proteins are limited, because proteins are generally unstable with heating, organic solvents and proteolytic attack. Therefore, if proteins could be converted into stable forms, their applications would be greatly broadened. Modification of food proteins has been investigated to improve their physical functionality, i.e. gelation, viscosity, emulsification and foaming (Sakamoto, Kumazaw & Motoki, 1994). The effect of the chemical and enzymatic deamidation of food proteins on functional properties has recently been of great interest in the food industry (Matsudomi, Sasaki, Kato & Kobayashi, 1985; Matsudomi, Sasaki, Tanaka, Kobayashi & Kato, 1985). Several molecular parameters, such as mass, conformation, flexibility, net charge and hydrophobicity, as well as interactions with other food components have already been shown to play an important part, both in their emulsifying and foaming properties (Nakai & Voutsinas, 1983). Soybean and wheat proteins are usually rich in glutamine and asparagine. These glutamine and asparagine residues can be enzymatically converted into glutamic and aspartic acids, respectively, and the resulting deamidated protein has a lower isoelectric point. Thus, its solubility increases in many mildly acidic food systems (Finley, 1975). It has been reported that deamidation levels as low as 2–6% could enhance the functional properties of proteins (Matsudomi, Sasaki, Kato, et al. 1985). Also, it has been reported that mild acid hydrolysis of gluten caused a significant improvement in the functional properties (Wu, Nakai & Powrie, 1976). Although functionality of proteins has generally been improved by solubility, contradictory results were reported with respect to emulsifying properties (Aoki, Taneyama, Orimo & Kitagawa, 1981). TGase as a polymerizer has been extensively studied (Nio & Motoki, 1983; Nonaka et al., 1989; Sakamoto et al., 1994; Sakamoto, Kumazawa, Toiguchi & Motoki, 1995; Sergo, Kumazawa, Ohtsuka, Toiguchi & Motoki, et al. 1995) and is known to catalyze the transfer reaction between an amide group in a protein-bound glutamine and an ε-amino group in a protein-bound lysine side chain, resulting in cross-links between the protein molecules. TGase was found to be effective in improving

*Present address: Yamaguchi City, Ooaza, Hirai 309-2, Kenei Juutaku, 1tou 105, Yamaguchi 753-0831, Japan.

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the functional properties of gluten (Babiker, Naotoshi, & Akio, 1996a) and soy protein (Babiker, Khan & Akio, 1996b) digests and hydrolysates; also it was found to be very effective in improving gelling of many proteins, such as egg yolk, egg white and caseinate (Sakamoto et al., 1995). In this study, an attempt was made to investigate the effect of transglutaminase treatment on the functional properties of the native and chymotrypsin digested soy protein.

2. Materials and methods

2.1. Materials

Commercially available soy protein was obtained from Japan Flour Milling Co. Chymotrypsin (52 unit(mg) was purchased from the Sigma Chemical Co. Unless otherwise stated, all reagents used in this study were reagent grade.

2.2. Methods

2.2.1. Acid precipitated soy protein (APP) preparation

Acid precipitated soy protein was prepared by the method of Iwabuchi and Yamauchi (1987). A sample of defatted meal (100 g) was extracted once with 2 l of 0.03 M Tris–HCl buffer (pH 8) containing 10 mM 2-mercaptoethanol (2-ME) at 20°C. After centrifugation, the supernatant was acidified to pH 4.8 with 2 N HCl and then centrifuged. The precipitated protein was dissolved in water at 4°C and the pH adjusted to 8. After centrifugation (8000 rpm), the clear supernatant was dialyzed against distilled water for 24 h at 4°C and then freeze-dried.

2.2.2. Preparation of chymotrypsin-digested soy protein

A freeze-dried sample (4 g) of soy protein was suspended in 400 ml of 0.05M Tris-HCI (pH 8.0) containing 0.05% sodium azide, and then 40 mg of chymotrypsin was added (Babiker et al., 1996b). The mixture was incubated at 37°C for 18 h. After incubation, the enzyme was inactivated by heating at 100°C for 3 min. The digested mixture was centrifuged (8000 rpm for 10 min) to remove a small amount of undigested protein, and then the supernatant was dialyzed (3000–4000, molecular weight cutoff) against distilled water at 4°C and then freeze-dried.

2.2.3. Preparation of microbial transglutaminase (TGase)

TGase was prepared as described previously (Babiker, et al., 1996b).

2.2.4. Transglutaminase treatment

Soy protein or chymotrypsin digest were dissolved in 0.1 M phosphate buffer (pH 7.5;10 mg/ml) and then reacted with TGase (0.5 mg/ml). The mixture was incubated at 55°C for 60 min. The enzyme was inactivated by *N*-ethylmaleimide (0.1 ml; 0.1%) (Kato, Shimokawa & Kobayashi, 1991). The treated samples were dialyzed against distilled water and then freeze-dried.

2.2.5. Changes in free amino groups

Changes in free amino groups of 0.1% protein solutions were determined by spectrophotometric assay (OD340) using *o*-phthaldiadehyde as described by Church, Swaisgood, Porter and Catignani (1983).

2.2.6. SDS-Polyacrylamide gel electrophoresis

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method of Laemmli (1970) with a 15% acrylamide separating gel and a 3% acrylamide stacking gel containing 0.1% SDS. Samples (20 µl, 0.2%) were prepared in a Tris–glycine buffer at pH 8.8 containing 1% SDS. Electrophoresis was done at a current of 10 mA for 5 h in electrophoretic Tris–glycine buffer containing 0.1% SDS. After electrophoresis, the gel sheets were stained for proteins with 0.2% Coomassie brilliant blue-R250 and then destained with 10% acetic acid containing 20% methanol.

2.2.7. Measurement of solubility

Freeze-dried samples of soy protein and chymotrysin digest with and without TGase treatment (0.2%) were used for the determination of solubility at various pHs: pH 2–3, 0.05 M citrate buffer; pH 4–5, 0.05 M acetate buffer; pH 6–8, 0.05 M phosphate buffer; pH 9–11, 0.05 M carbonate buffer and pH 12, 0.05 M NaOH slightly adjusted with 0.05 M HCl. Samples were dissolved in the buffer and shaken with a vortex mixer (Scientific Industries, adjusted on digit 4 to work on touch) for 10 s, and the turbidity was measured at 500 nm. Values obtained are means of triplicate samples.

2.2.8. Heat stability

Heat stability was determined by the method described by Kato et al. (1995). The samples were dissolved at a protein concentration of 2 mg/ml in 50 mM Tris-HCl buffer (pH 7.0) and heated at 50–90°C for 20 min. Protein turbidity was measured at 500 nm.

2.2.9. Gel preparation

An aqueous dispersion (10% protein, w/v) of the protein was prepared in deionized water and adjusted to pH 6.5 with 1 M NaOH. The mixture of the protein was stirred gently for 10 min at room temperature and readjusted to pH 6.5; then the homogenized mixture (1.0 ml) was put into a glass tube (6.0 mm in diameter) previously treated with Sigmacote (Sigma Chemical Co.). The contents of each tube were deaerated by placing in a sharp sonicator (model UT-205, Tokyo) under vacuum for 1 min. The tubes were heat-sealed and

heated for 30 min in a water bath at 80°C. After heating, tubes were removed from the water bath and held overnight at 4°C.

2.2.10. Determination of gel hardness

The tubes were tempered at room temperature for 20 min, and the gel was removed from each tube without disrupting the gel surface. Each gel was cut into uniformly flat 5.0 mm thick sections and compressed to 50% of its original height by a tensile tester (Tension TM-II, Toyo Baldwin Co., Tokyo) as described by Matsudomi, Oshita and Kobayashi (1994). The force required to compress the gel to 50% was expressed as gel hardness.

2.2.11. Measurement of emulsifying properties

The emulsifying properties of the samples were determined by the method of Pearce and Kinsella (1978). To prepare emulsions, 1.0 ml of corn oil and 3.0 ml of protein solution (0.2%) in 0.1M phosphate buffer (pH 7.0) were shaken together and homogenized in an Uitra Turrax instrument (Hansen & Co., West Germany) at 12 000 *g* for 1 min at 20°C. A 50 μl sample of the emulsion was taken from the bottom of the container at different times and diluted with 5 ml of a 0.1% sodium dodecylsulfate solution. The absorbance of the diluted emulsion was then determined at 500 nm. The emulsifying activity was determined from the absorbance meansured immediately after the emulsion formation. The emulsion stability was estimated by measuring the half-time of the initial turbidity of the emulsion.

2.2.12. Measurement of foaming properties

The foaming properties of freeze-dried sample solutions were determined using the conductivity method (Kato, Takahashi, Matsudomi & Kotayashi 1983). Electric conductivity of foams was measured when air was introduced into 5 ml of a 0.2% protein solution in 0.02 M phosphate buffer (pH 7) in a glass filter (G-4) at a constant flow rate (90 cm^3/min). The conductivity reading was recorded automatically using a recorder connected with conductivity curves. The foaming power was expressed as the maximum conductivity during aeration. The foam stability was indicated as the time for the disappearance of the foams, i.e. the absence of conductivity. Values obtained are means of triplicate samples.

3. Results and discussion

3.1. Effect of transglutaminase (TGase) treatment on the molecular mass and free amino group of the protein

The native and chymotrypsin-digested soy protein polymerized by TGase showed high-molecular mass bands in the top of the stacking gel of the SDS-PAGE with a decrease in the monomeric fraction of the intact protein and a novel polymer fraction was formed (Fig. 1, lanes 4 and 5). The result indicated that TGase may have catalyzed the transfer reaction between an amide group in a protein-bound glutamine and an ε-amino group in a protein-bound lysine side chain, resulting in cross-links between the protein molecules (Nonaka et al., 1989; Sakamoto et al., 1994, 1995; Sergo et al., 1995). Similar results were obtained when gluten digests and hydrolysates were polymerized by TGase (Babiker et al., 1996a). Changes in free amino groups of the protein before and after TGase treatment are shown in Fig. 2. The free amino groups of soy protein ($OD_{340} = 0.20$) were greatly increased after chymotrypsin digestion ($OD_{340} = 0.32$). Polymerization of both proteins greatly reduced the free amino groups and was found to be 0.10 (OD_{340}) for both protein polymers. Results revealed that the protein molecules of each soybean and its digest were cross-linked by TGase.

3.2. Effect of TGase treatment on solubility and heat stability of the proteins

The pH dependence of the solubility of soy protein and chymotrypsin digest with and without TGase treatment was investigated (Fig. 3). The results showed that soy protein and chymotrypsin digest were markedly insoluble at pH 4 and 6. However, after being polymerized by TGase, soy protein was observed to be insoluble at pH 4 and 6, while at pH 2 and alkaline pH

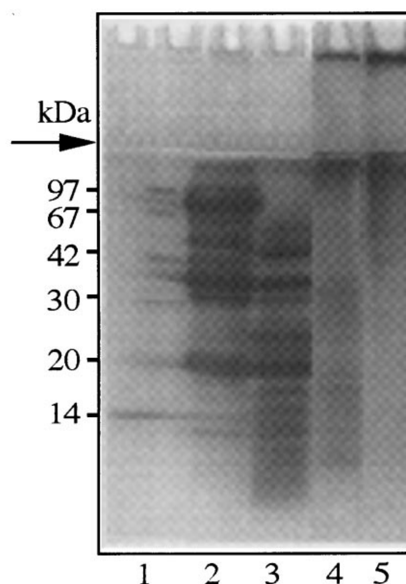

Fig. 1. SDS-PAGE pattern of acid-precipitated soy protein (APP), chymotrypsin digest of (APP) and transglutaminase (TGase) polymers of (APP) and digest. Lane 1, molecular marker; lane 2, APP; lane 3, chymotrypsin digest; lane 4, APP + TGase and lane 5, chymotrypsin digest + TGase. Arrow indicates the boundary between the stacking and separating gel.

(pH 8–12) it was completely soluble. Chymotrypsin digestion of the protein was found to have no effect on the protein solubility but, after polymerization, the protein was completely soluble over wide ranges of pH, except at pH 4. In addition, shift of maximum insolubility at pH 6 to the lower pH 4 was observed in chymotrypsin digest polymer, which indicates that deamidation of the digests may have occurred by TGase treatment (Motoki, Seguro, Nio & Takinami, 1986). It is probable that TGase can deamidate the glutamine residues without cross-linkage between the amide group in glutamine and ε-amino group in lysine residues (Motoki et al., 1986). Therefore, the effect of polymerization on the solubility of the protein may be small and is mainly due to deamidation. The improvement in the solubility due to TGase treatment of the protein digests at various acidic and alkaline pH values is mainly due to the fact that chymotrypsin digestion creates recognizable sites for TGase reaction and the resultant polymer, as shown in Fig. 1, had a higher molecular mass. Also, TGase treatment decreases the surface hydrophobicity of the proteins molecules and increases the electrostatic repulsion as a result of partial deamidation of glutamine and asparagine (Babiker et al., 1996a). The solubility (OD_{500nm}) of both soy protein and chymotrypsin digest decreased as the heating temperature increased (Fig. 4) and was observed to reach 0.38 and 0.20 at 90°C for the proteins, respectively. Soy protein polymer started to coagulate when the heating temperature exceeded 50°C and its solubility (OD 500nm) greatly decreased and reached 0.36 at 90°C (Fig. 4). On the other hand, the digest polymer was least soluble after being heated at 90°C for 20 min ($OD_{500}=0.13$). Results indicated that TGase treatment was found to be effective in improving heat stability of soy protein digest compared to the native protein.

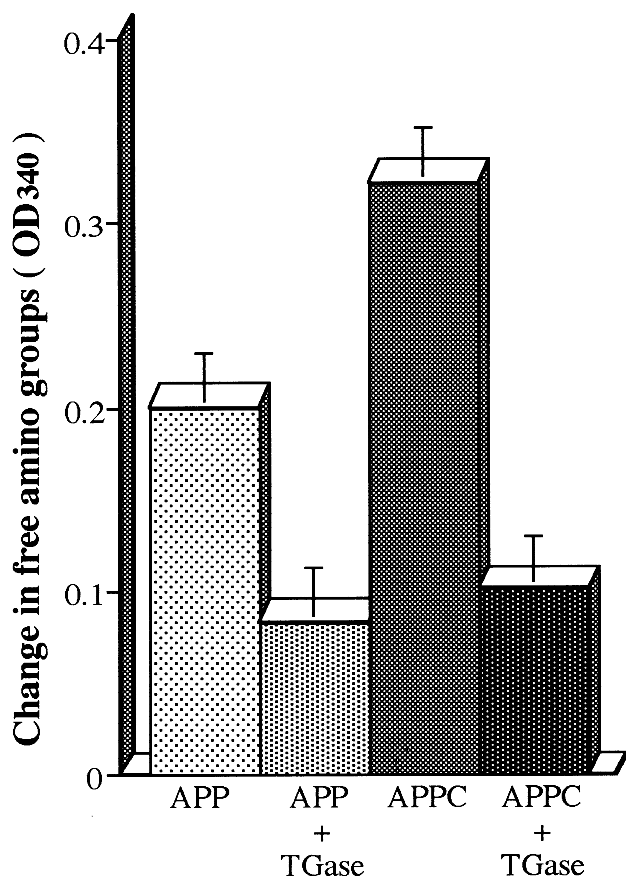

Fig. 2. Changes is free amino groups of acid-precipitated soy protein (APP) and chymotrypsin digest (APPC) with and without transglutminase (TGase) treatment. Error bars indicate the standard deviation of three replicates.

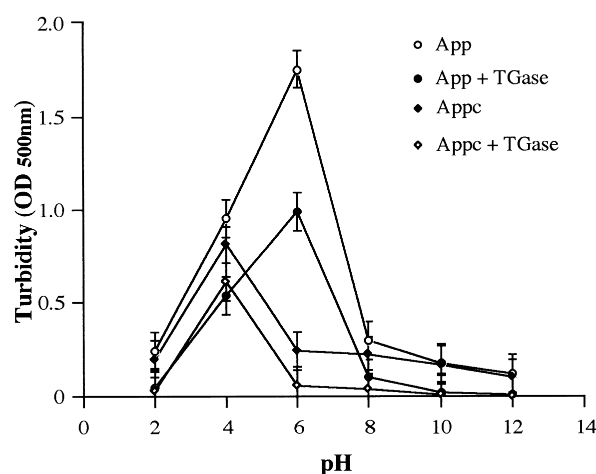

Fig. 3. Effect of pH on solubility of acid-precipitated soy protein (APP) and chymotrypsin digest (APPC) with and without transglutaminase (TGase) treatment. Error bars indicate the standard deviation of three replicates.

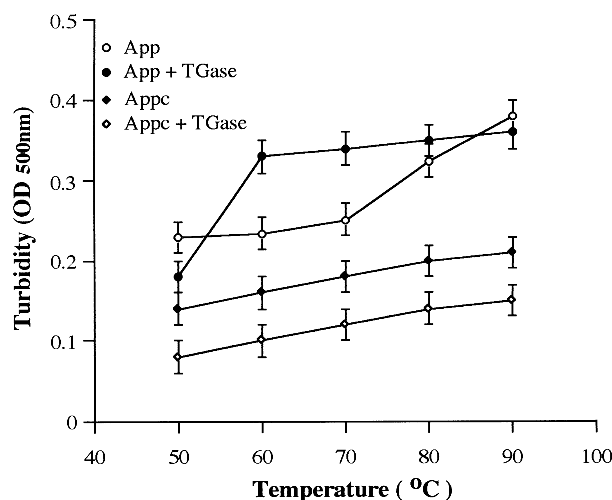

Fig. 4. Effect of temperature on solubility of acid-precipitated soy protein (APP) and chymotrypsin digest (APPC) with and without transgluaminase (TGase) treatment. Error bars indicate the standard deviation of three replicates.

3.3. Effect of TGase treatment on physical functionality of the proteins.

The physical functionalities such as the emulsification, foaming and gelling properties of soy protein peptides, are poor (Aoki et al., 1980). In order to improve these physical properties, the effect of TGase treatment was investigated. As shown in Fig. 5, the emulsifying properties of the protein polymers were improved. The emulsifying activity of soy protein, which is estimated by the turbidity of emulsion, measured immediately (0 min) after emulsion formation, was 0.39, while that of the digests was 0.42, and that of the protein polymer was increased to 0.65 and the digest polymer increased to 0.70. The emulsion stability (the half time of the initial turbidity) of soy protein was 1.5 min, while that of the digest was 10.0 min, and after polymerization it increased for both soy protein and its digest and was found to be more than 15 min for both polymers. The improvement in the emulsifying properties is likely due to an increase in the negative charges which result from the hydrolysis of the amide groups in glutamine and asparagine, as reported for gluten treated by proteases (Babiker et al., 1996a; Matsudomi, Tanaka, Kato & Kobayashi, 1986) and mild acid treatment of ovalbumin (Matsudomi, Sasaki, Tanaka, et al., 1985). The results obtained show that polymerization of soy protein and its digest was very effective in the improvement of the emulsifying properties. The foaming properties of soy protein and its digest were also improved after polymerization by TGase (Fig. 6).

The foaming properties of soy protein were low. The foaming power (maximum conductivity during aeration) of the protein was 450 μv/cm, while that of the digest was 550 μv/cm and, after polymerization, was further increased to 500 and 590 μv/cm for the protein and digest polymers, respectively. The foam stability (the time for the disappearance of the foam) of the protein was 18.0 min, while that of the digest was 35.0 min; after polymerization there were further increased to 43.0 and 45.0 min for the polymers, respectively. The improvement of the foaming properties of the protein polymers reflects the importance of protein association or polymerization as a structural factor governing the foaming property (Kato et al., 1983). The effect of TGase treatment on gel-forming properties of the protein and its digest was investigated as shown in Fig. 7. The gel-forming property of the protein was examined at different protein concentrations, pH and temperatures and for different time intervals (data not shown). The best conditions to perform the gel were 10% (w/v) protein, pH 6.5, and heating at 80°C, for the 30 min, at which gel forming property of the proteins was investigated. Soy protein and chymotrypsin digest were observed to form very weak gels (Fig. 7). Polymerization of the digest was found to have no effect on ge-forming properties whereas soy protein polymerization had a profound effect on the gel-forming ability of the protein which was found to form a firmer gel, with a hardness of 33 g than the digest polymer. This observation indicates that electrostatic attractive and repulsive forces between the protein molecules play an important role in gel network structure (Schmidt, 1981). The amount of cross-links at pH 6.5 were higher than those at other pHs and the glutaminyl and lysyl residues of the protein polymer were probably more accessible; therefore, a strong gel was formed (Sakamoto et al., 1994). Also, the ability of a protein solution to gel is influenced by the extent of heat-induced protein denaturation (Kitabatake, Tani & Doi, 1989) and the number of free amino

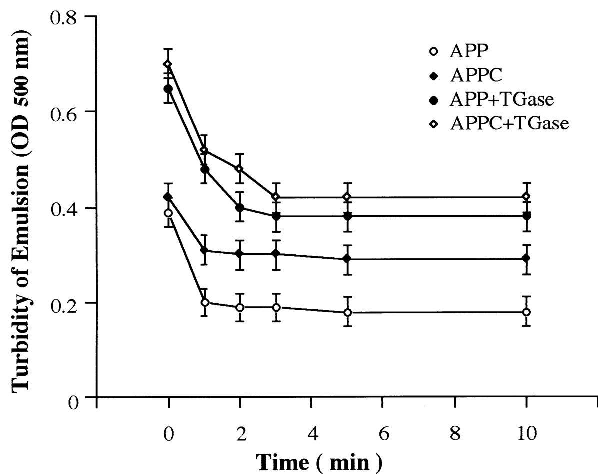

Fig. 5. Emulsifying properties of acid-precipitated soy protein (APP) and chymotrypsin digest (APPC) with and without transglutminase (TGase) treatment. Error bars indicate the standard deviation of three replicates.

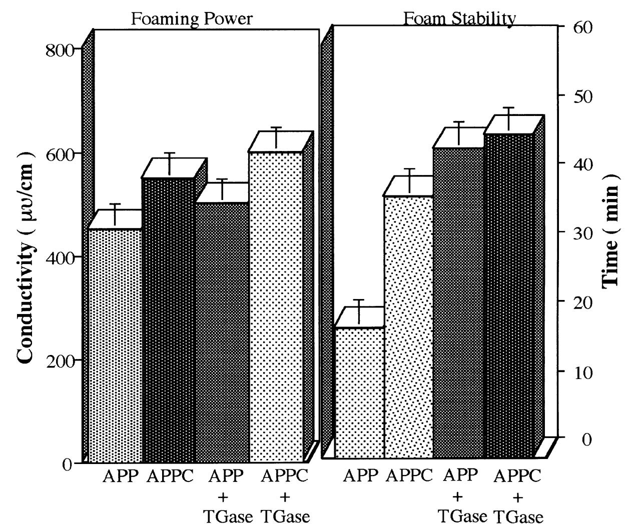

Fig. 6. Foaming properties of acid-precipitated soy protein (APP) and chymotrypsin digest (APPC) with and without transglutaminase (TGase) treatment. Error bars indicate the standard deviation of three replicates.

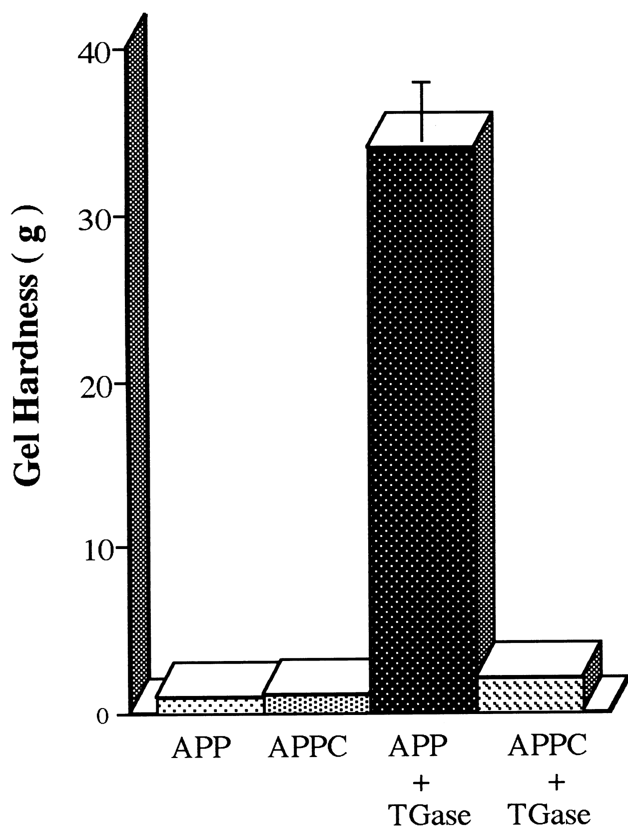

Fig. 7. Gel-forming property of acid-precipitated soy protein (APP) and chymotrypsin digest (APPC) with and without transglutaminase (TGase) treatment. Error bars indicate the standard deviation of three samples.

groups of the protein. Despite being polymerized by TGase, chymotrypsin digests formed very weak gels. This may be due to the fact that, when the negative forces exceed a critical number, protein:protein interactions are reduced and a weaker gel is obtained (Hillier, Lyster & Cheeseman, 1980).

In conclusion, digestion of soy protein followed by polymerization was found to be more effective in improving the protein solubility, heat stability, foaming and emulsifying properties compared to the native protein polymer. Polymerization of the native protein was found to improve the protein physical properties especially gelling.

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