J. Food Sci. Nutr.
Vol. 2, No. 1, p. 77~82(1997)

The Soy Protein Coagulation Phenomenon by Heat- and Enzyme-Treatment

-Minireview-

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Abstract

The comparison of soy protein coagulation by heat- and enzyme-treatment are summarized. The gelation mechanism of glycinin by heating was mainly due to dissociation and aggregation of the basic subunit of 11S globulin. In case of 7S globulin, macro-soluble aggregates may be formed by noncovalent intraction more than 30min at 80°C. Whereas, coagulum occured by the microbial enzyme was more minuter than the other Ca-, HCl-coagulum. Heat treatment attacked the basic subunit of 11S globulin and this results agreed very closely with bromelain treatment, and also it was not changed in the 7S globulin by bromelain. Microbial enzyme, however, preferred acidic subunit to basic subunit of 11S globulin and attacked the 7S globulin, that could produce coagulum products within the 4~5min at 65°C.

Key words: soy protein, microbial enzyme, coagulum, heat-treatment, enzyme-treatment

INTRODUCTION

Authors(1-3) reported in the preceding papers that the bacterial enzyme from *Bacillus* sp. IJ-3 strain is able to coagulate soy protein, and the soy protein curd has a smooth texture and a mild taste. They also reported that the digestion properties of the soy protein are important to understand the enzymatic digestion of soy protein in food usage(4-8). In order that soy protein is spreading over a usage in food manufacture, this paper discusses the coagulation phenomenon of the 7S and 11S globulin by heat- and enzyme-treatment.

SOY PROTEINS

Approximately 90% of the proteins in soy beans, mostly globulins, exist as dehydrated storage proteins. The remaining proteins are composed of intracellular enzymes(lipoxygenase, urease, amlyase), hemagglutinins, protein inhibitors and membrane lipoproteins(9).

Catsimpoolas et al.(10,11) isolated and characterized the storage proteins, 7S(conglycinin) and 11S(glycinin) as the major components of soy protein.

Table 1 shows the typical composition of commercial soy protein preparations(9). Soy flours are used in a wide range of foods, particularly in bakery products and cereals. Concentrates and isolates are used in comminuted meats and dairy foods where emulsifying, gelling properties are of prime importance. The enriched 11S fractions and 7S fractions of soy protein were easily prepared from the several methods(12-15), and the major components are characterized according to their food processing properties(Table 2).

Table 1. Typical composition of soy protein preparation

Component	Soy flours(%)	Concentrates(%)	Isolates(%)
Protein	56.0	72.0	96.0
Carbohydrates	33.5	17.5	0.3
Ash	6.0	5.0	3.5
Fiber	3.5	4.5	0.1
Fat	1.0	1.0	0.1

Table 2. Functional properties of 7S and 11S globulin on food stuffs

Component	Tofu	Cheese-like food
7S	Softness, Adhesion	Water absorption & binding
11S	Gelation, Elasticity	Foaming, Elasticity & gelation

HEAT TREATMENT

Heat treatment at high temperature is one of the processing techniques of soy protein in food industry.

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Heating causes dissociation of 11S into subunits which slowly aggregate up to 70°C but rapidly thereafter, precipitate at 90°C. Upon heating, 11S molecule is initially converted into soluble fractions and insoluble aggregates(16).

Heat-induced aggregation is accelerated in the presence of thiols which enhence the initial dissociation of subunits. The 11S globulins are stabilized against thermal aggregation up to 80°C, and the aggregation occurs rapidly at high ionic strength. On the contrary, the 7S globulins are more stable at low ionic strength, and aggregation is accelerated at high ionic strength(17).

Saio et al.(18,19) reported that the solubility of CIF (cold insoluble fraction)-gels was higher than that of crude 7S-gels at all temperature ranges. It was noted that crude 7S-gels showed the lowest solubility at 105 ~130°C, while the solubility of CIF-gels(11S-gels) increased markedly at temperatures above 110°C.

Soy proteins showed aggregation by heating, but the 7S and 11S globulins differ in gelation behavior. When soy protein was heated at high temperature(90°C) at pH 8.0, high turbidity under these conditions could be ascribed entirely to the aggregation of the basic subunits. The acidic subunits and α, α′ and β subunit remain soluble at this pH.

Kinsella and Coworkers(17,20) reported that the basic subunit showed the selective aggregation at pH 8.0 and once dissociated from the acidic subunit in pH 6.5~8.0 these subunit aggregate and that the precipitate contained only the basic subunits and the soluble supernatant contained acidic subunits(16,17,21).

Upon heating, the 11S globulin fraction readily aggregated at 80°C, whereas the 7S and the 7S+11S mixture solutions remained clear and showed very little turbidity even after heating for 30min. The heat-induced gels of the 11S globulin showed higher tensile and shear strength than those obtained from the 7S globulin. These results indicated that the turbidity in heated glycinin solutions is mainly due to dissociation and subsequent aggregation of the basic subunit of 11S or glycinin.

The gelation mechanism of glycinin by heating was shown in Fig. 1. Nakamura et al.(21) suggest that noncovalent interactions are important in the formation of a gel of 7S globulin. The structure of the soluble aggregates does not involve the SH/S-S exchange reaction and these aggregates may be composed of five to seven molecules of 7S globulin.

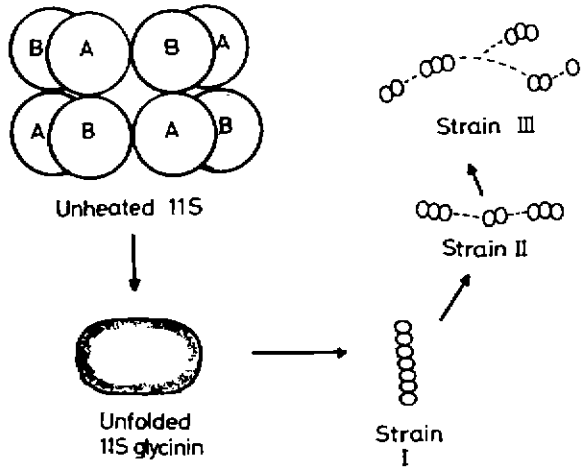


Fig. 1. Scheme of the gelation mechanism of 11S globulin by heat-treatment.

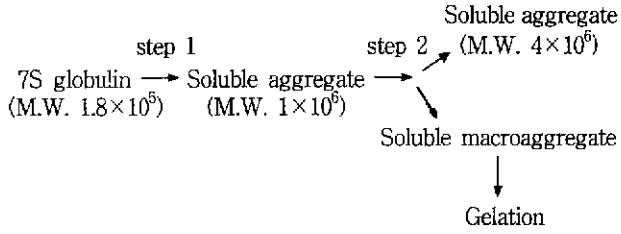


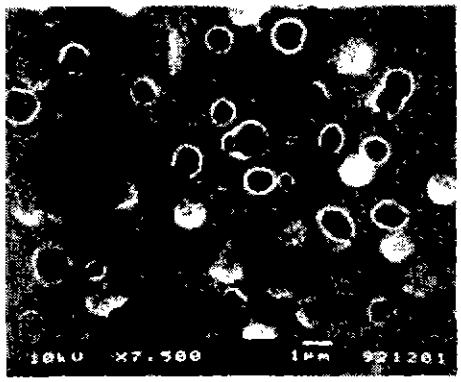
Fig. 2. Scheme of the gelation mechanism of 7S globulin by heat-treatment.

ENZYME TREATMENT

It is well-known that the proteolytic enzymes, e.g., pepsin, ficin, bromelain and microbial enzyme, are able to coagulate the soy protein.

Matsuoka and Fuke(22) have reported that the coagulum produced by treated soy protein with ficin had bitter taste. Fuke et al.(23,24), Mohri and Matsushita (25) have studied the conditions for the coagulation of soy protein treated with stem bromelain. It was found that soy protein coagulum products with slight bitterness could be produced by using stem bromelain as a coagulant of soy protein. But it could be improved if the bitter taste was removed of soy protein coagulum. The other hand, microbial enzyme(1,6-8) could be coagulated the soy protein and the resultant coagulum has a smooth texture and a mild taste without any bitterness.

As shown in Fig. 3, the coagulum made with the microbial enzyme(6) was more elaborate than the other Ca-, HCl- coagulum. Enzyme were found to give a coagulating time of 4~5min(Fig. 4). In SDS-PAGE, acidic subunit A_3(M.W.45,000) almost completely disappeared



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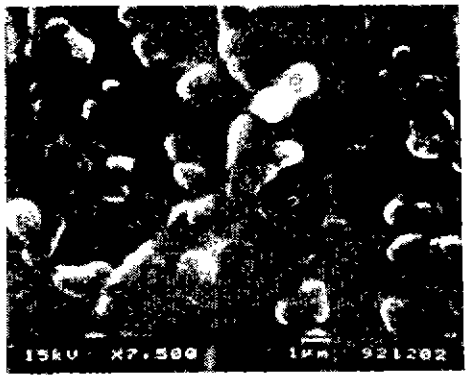

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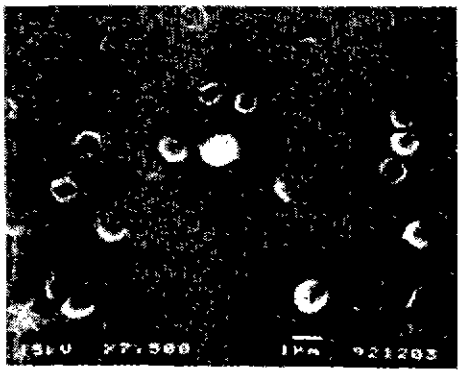

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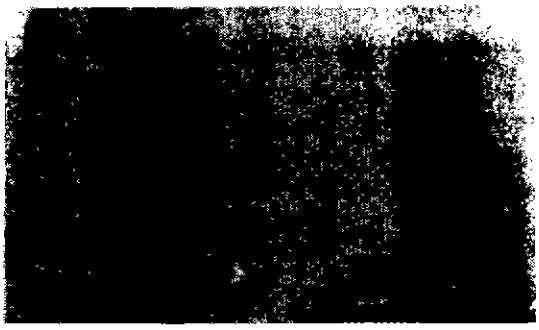
Fig. 3. Scanning electron micrographs of acid-, microbial enzyme- and metal ion-induced aggregates produced from the soy protein.
1: SEM of acid-induced aggregate
2: SEM of microbial enzyme-induced aggregate
3: SEM of metal-ion induced aggregate

within 2 min by the actions of enzyme.

Mohri and Matsushita(25) reported that the basic subunits may be easily attacked by bromelain because they contain more basic amino acid residues than acidic subunits. This results agreed very closely with that obtained by the heat-induced formation of the basic subunit of 11S globulin. By comparing its degradation feature of 7S grobulin in the SDS-PAGE, Fuke et al. (23,24) reported that the patterns of 7S globulin did not change in the 7S protein by bromelain treatment. On the contrary, Fig. 5 showed 7S globulin was aggregates by microbial enzyme and the obtained coagulum was minuter than the other Ca- or HCl-induced coagulum.

Also as shown in Fig. 6, the degradative patterns of Ca- and HCl-induced curds were similar to those of the native 7S globulin. The microbial enzymes, however, attacked the 7S globulin, 7S protein almost disappeared and that could be produced as a coagulum products. This fact indicated that the characteristics on the soy protein of microbial enzyme differ from that of the bromelain and heat treatment.



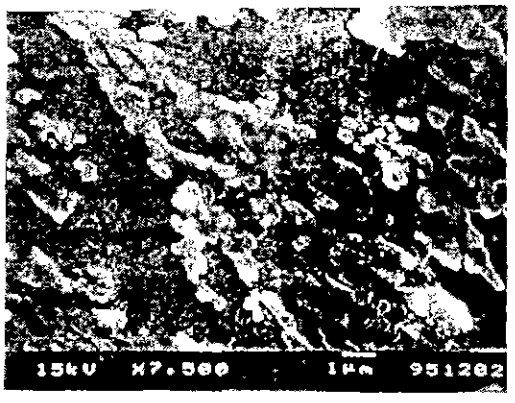
0 1 2 3 5 6 8 10 30

Reaction time(min)

(A)

(B)

Fig. 4. Profile of the degradation patterns(A) and aggregation(B) of acidic subunit(A_3) and by microbial enzyme.



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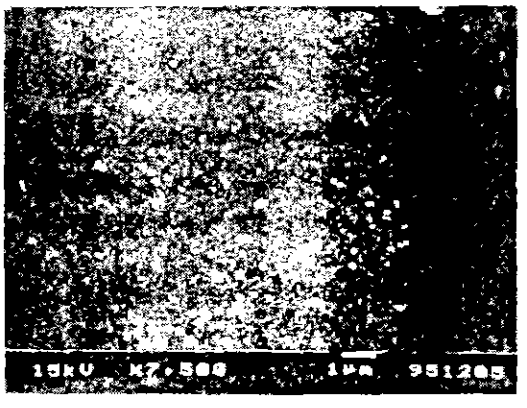

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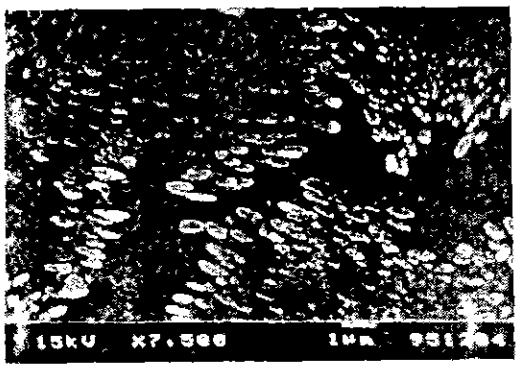

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Fig. 5. Scanning electron micrographs(SEM) of microbial enzyme-, acid- and metal ion-induced aggregates from the 7S globulin.
1: SEM of micro-bial enzyme-induced aggregate
2: SEM of acid-induced aggregate
3: SEM of metal ion-induced aggregate.

In preceding papers, Park et al.(3,4) reported that the microbial enzyme cleavged the Glu-Ala peptide bond of oxidized insulin B chain, while Murachi et al. (26) reported that the bromelain hydrolyzed the peptide bond of Arg-Gly of oxidized insulin B chain. Thus

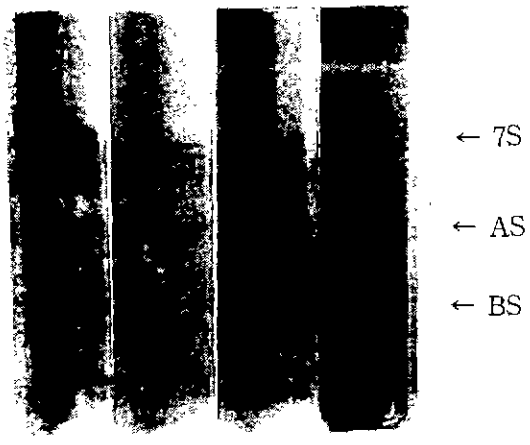

Fig. 6. The degradation patterns of 7S globulin by the enzyme-, Ca- and HCl.
A: Native soy protein, B: Ca-curd,
C: HCl-curd, D: enzyme-curd

bromelain shows specificity for basic amino acid as well as trypsin.

Recently, Park and Kim(8) reported that protein of enzyme curd, flexible protein(amphiphilic protein) are susceptible to denaturation at the interface compared to the protein of Ca- and HCl-curd.

These facts let us consider that the curd occured with the microbial enzyme treatment was more effective than that of Ca-, HCl- and bromelain-induced curd in their food properties.

GENERAL OBSERVATION

Heat-induced gel from 11S globulin indicated better functional properties than that from 7S globulin for foods when gels were prepared by heating at more than 80°C. However, thermally degraded phenolic acids account for some of the objectionable cooked odors of soy products that have been subjected to high temperature treatment(27). To achive its potential as a food, the organoleptic and funtional characteristics of soy protein must be defined in detail, and it should be applied with each individual characteristic, such as gelation, water absorption, flavor etc., for soy protein products.

Although their complete amino acid sequence of each subunit in major globulin was determined(28-31), the influence on the gelation and association-dissociation interaction of soy protein(32-36), particulary in the two major globulins, is more important factor than amino acid sequence determination in food processing and

manufacture. Park et al.(6-8) reported that the curd made with enzyme treatment has mild taste and desirable organoleptic characteristics of soy protein, also the enzyme curd shows variable functional properties such as emulsifying and foaming properties as a new rheological food item.

At present time, although the soy proteins are considered as substitutes for almost protein ingredients, the improvement of their taste and functional properties with enzyme treatment will be promising for soy protein foods. The authors are still working on this fields.

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(Received December 3, 1996)