J. Food Sci. Nutr.
Vol. 1. No. 1, p. 53~58(1996)

Changes of Emulsifying and Foaming Properties of Soy Protein with an Calcium, HCl and Microbial IJ-3 Strain Enzyme

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Abstract

The characteristics of the soy protein curd(enzyme-, HCl- and Ca-curd) were shown by scanning electron micrographs and gel electrophoresis. The emulsion stability of enzyme-curd showed high value in the range of pH 2~10 and a wide range of temperature(20~80°C). While at the isoelectric point(pH 5.0), the emulsion stability of the HCl- and Ca-curd was decreased remarkably, and the emulsion stability of temperature was reduced quickly to the 60% and 40% at the 40°C. The foam stability of enzyme-curd was slightly higher than that of HCl- and Ca-curd in all ranges of pH and temperature. The feature of SEM of enzyme-curd showed the largest coagulum compared with the others. Also PAGE showed that the enzyme-curd produced degradation products faster than that of the HCl- and Ca-curd.

Key words: enzyme-curd, functional properties

INTRODUCTION

The field of food processing has studied the functional properties of soy protein for use in many food products.

Although many workers(1-4) have tried to improve functional properties(emulsion stability, foam stability etc.) by physical(5-7), chemical(8,9) and enzymic reaction(10,11), it has been too difficult for food processing of the new rheological food materials.

Author(12,13) reported in the preceeding papers that bacterial enzyme from *Bacillus* sp. IJ-3 strain is capable of coagulating soy protein, that the soy protein curd has a smooth texture and a mild taste. Although the curd formed as before with protease such as bromelain, ficin and papain etc. could have produced bitterness during ripening, the curd made with the enzyme showed the acceptable organoleptic evaluation(13). This fact indicated that the curd made with an enzyme different from bean-curd by divalent-metals or hydrochloric acid could be new rheological food materials.

The objective of this paper was to obtain basic data for the characteristics of functional properties on soy protein curd with calcium, HCl and IJ-3 strain enzyme. The possesion, therefore, of enzyme-curd has considerably extended the use of soybean proteins in food products.

MATERIALS AND METHODS

Preparation of soy protein curds

The enzyme used was from the *Bacillus* sp. IJ-3 strain which was cultivated for 5 days at 35°C in the lab. "Baegimeal" soymilk produced by Chung's Food Co. Ltd. was used, and the pH value and protein content of the soymilk was 7.0 and 3.0%(w/v), respectively. A mixture of the soymilk protein(500ml adjusted to pH 6.1 with a 1M potassium phosphate buffer solution) and enzyme solution(10ml containing 20 units enzyme activity) was incubated at 65°C for 30min. The coagulum which occured was centrifuged at 10,000rpm for 15min, and separated into supernatant and soy protein curd (Enzyme-curd). A soy protein curd from calcium chloride(Ca-curd) was prepared by adding 20mM of calcium chloride solution, and HCl-curd was prepared by adjusting to the isoelectric point(pH 5.0) of soymilk with potassium phosphate solution.

Chemical analysis

The moisture content was measured by heating at

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105°C until a constant weight was reached. The protein content was measured by the Kjeldahl method(14), and the fat content by the soxhlet method with n-Hexane as a solvent after lyophilizing the soy protein curd. The ash content was measured by heating at 550°C until a constant weight was reached.

Mesurement of the functional properties

Glycine-HCl buffer(pH 2.0), citrate-phosphate buffer (pH 3.0~7.0) and Tris-HCl buffer(pH 8.0~10.0) solutions were used. The effects of pH and temperature on the functional properties were measured at room temperature(20°C) and pH 6.0.

Emulsion stability:

This was determined by using a modified method of Yamauchi et al.(6). A mixture of soyprotein curd (10g) with 20ml of buffer solution was homogenized at 15,000rpm for 3min. in a Waring blender(ACE AM-5 homogenizer, Nihon Seiki Kaisha, Japan), and immediately transferred to a 10ml test tube. After standing for 30min, 5ml of the emulsion from the bottom of the test tube was taken out, and its moisture content was measured. The emulsion stability(E.S.) was calculated from the following equation.

$$\text{Emulsion stability} = \frac{100-M\ \text{test}}{100-M\ \text{original}} \times 100$$

M test : The real moisture content(%) of 5ml of the sample

M original : The initial moisture content(%) of the sample

The solid content in the aqueous solution was negligible because only a small portion was solid.

Foam stability:

This was determined by using a compromised method of Sosulski and McCurdy(7), Kuwata et al.(8) and Kato et al.(15). A mixture of 10g of soy protein curd and 20ml of buffer solution(0.04M potassium phosphate buffer, pH 6.1) was homogenized at 15,000rpm for 30min, and immediately transferred to a 50ml measuring cylinder. After standing for 30min, the volume of the water phase was measured. The foam stability(F.S.) was calculated from the following equation :

$$\text{Foam stability} = (Vt - Vd)/20\text{ml}$$

Where Vt was total volume(ml) after homogenizing, Vd was volume(ml) drained after 30min.

Microscopic observation

Micrographs of the soy protein coagulum were taken using an scanning electron microscope.

Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed in 12.5% disc gel by the method of Laemmli(16).

RESULTS AND DISCUSSION

Coagulum of soy protein by SEM

The protein concentration was taken at 1.0% and the sample solution for soy protein curd was incubated at 65°C for 30min. The results for Enzyme-, HCl- and Ca-curd are shown in Fig. 1. As shown in the figure, the coagulum of the curds were different from that of the other. HCl-curd showed a smaller coagulum than the others, and the HCl- and Ca-curd gave almost the lowest coagulum. The enzyme-curd produced the largest coagulum at 65°C for 30min incubation. Moreover the coagulum with IJ-3 strain enzyme had a more elaborated structure than that of Ca- and HCl-curd.

The feature of degradation for enzyme-, Ca- and HCl-curd

Fig. 2 shows the degradation patterns of enzyme-, Ca- and HCl-curd. The native soy protein gave general PAGE patterns(Fig. 2, lane A) at a protein concentration of 1%, where the electrophoretic patterns showed the most authentic figure among all the soy proteins. As shown in Fig. 2, the degradative pattern of the Ca- and HCl-curd(Fig. 2, lane B, C) was similar to that of the native soy protein. However, during the entire course of the reaction, enzyme-curd(Fig. 2, lane D) produced low molecular weight products faster than that of the others. This fact indicated that enzyme-curd was produced by the enzymic reaction, and was different from that of the Ca- and HCl-curd formation.

Chemical analysis

Table 1 shows that the chemical composition of three types of soy protein curd.

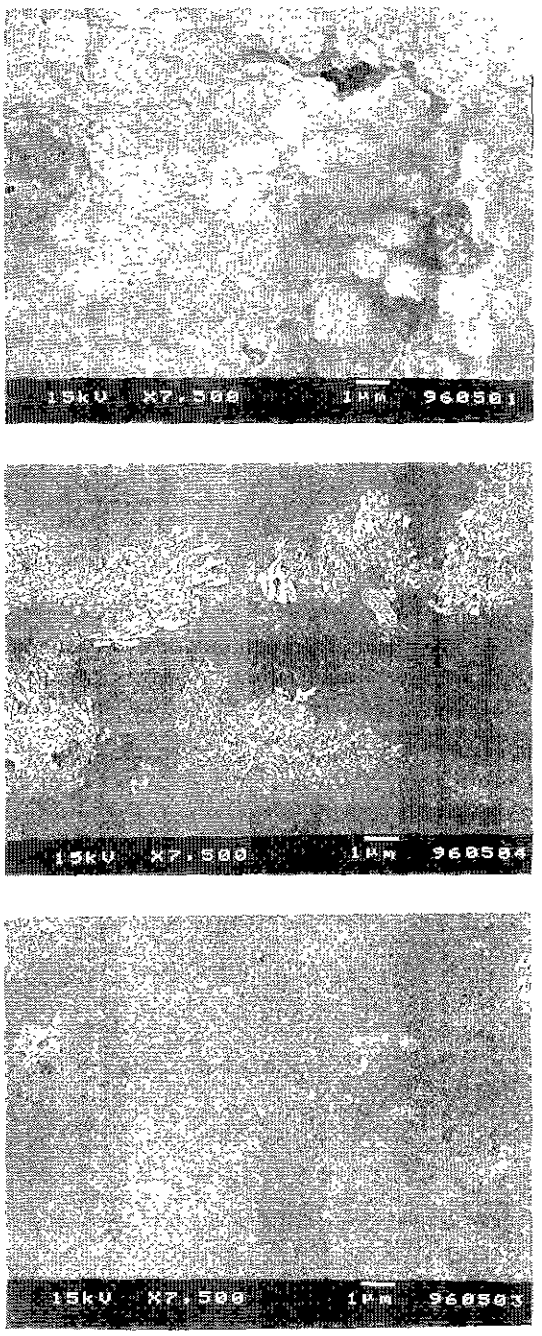

Fig. 1. Scanning electron micrographs of the soy protein.
1: Enzyme-curd, 2: Ca-curd, 3: HCl-curd

The moisture content of the enzyme-, Ca- and HCl-curd were 65.3, 59.7 and 62.5%, respectively. Their protein content were 5.8, 5.9 and 5.8%, respectively. The soy protein curds used in this experiment contained 3.8, 3.9 and 3.9% fat. The ash content of soy protein curd were in the range of 0.5~0.55%.

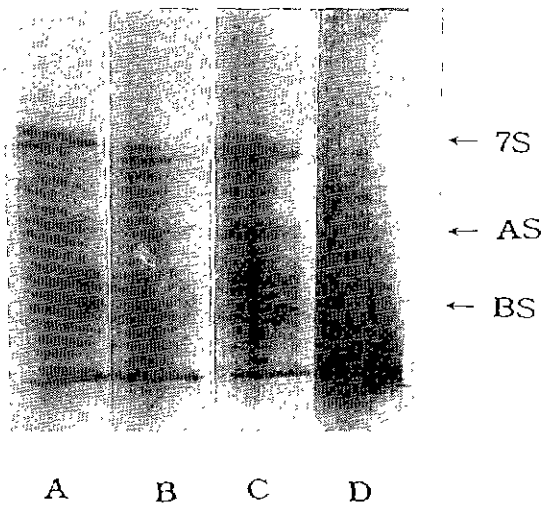

Fig. 2. The degradation patterns of the enzyme-, Ca- and HCl-curd
7S: 7S protein, AS: Acidic subunit, BS: Basic subunit
A: Native soybean protein, B: Ca-curd, C: HCl-curd, D: Enzyme-curd

Table 1. Composition of the soy protein curds

(%)

Components	Enzyme-curd	Ca-curd	HCl-curd
Moisture	63.5	59.7	62.7
Protein	5.8	5.9	5.8
Fat	3.8	3.9	3.9
Ash	0.5	0.55	0.5

Effect of pH on the emulsion stability

Fig. 3 shows the effect of pH on the stability of the soy protein curds. The stability of the enzyme-curd had a high value in the range of pH 2~10, while the stability of the Ca- and HCl-curd had a variation on the pH range which decreased remarkably at pH 5.0. Yamauchi et al.(5,6) reported that the emulsion stability for soy protein was sharply decreased at the isoelectric point of the soy protein. Aoki and Nagano(1) reported that the emusifying properties of soy protein were affected by pH. Although the report showed that the no emulsion stability had the characteristic of soybean protein by enzyme treament at pH 7.0, emulsion stability increased when soy protein was hydrolyzed by enzyme(2) at pH 4.5.

Enzymic degradation probably creates some amphiphilic fragments. These fragments at the interface substance(oil : water) lower the surface tension and make

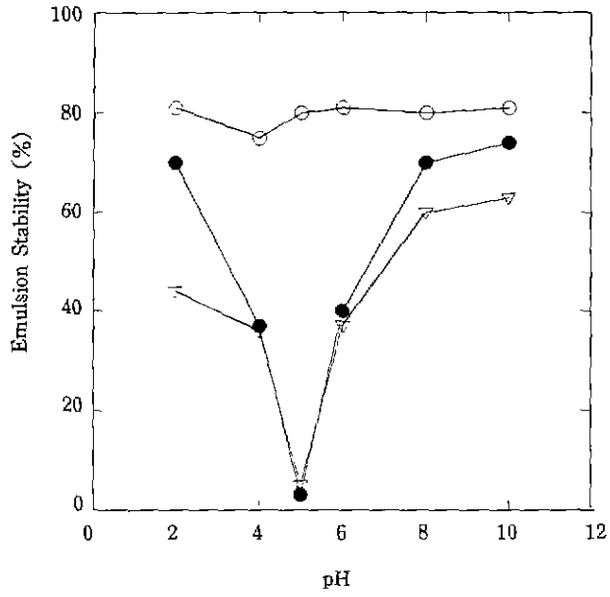

Fig. 3. The effect of pH on the emulsion stability of soy protein curd.
This experiment was carried out at 20°C.
○-○: Enzyme-curd, ●-●: HCl-curd
△-△: Ca-curd

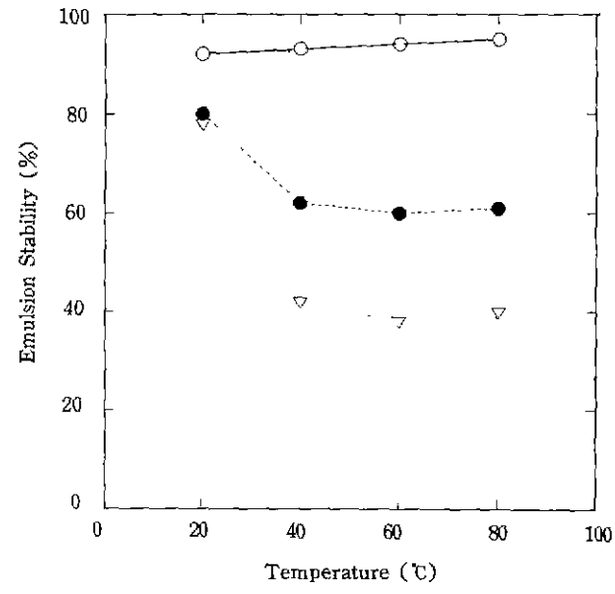

Fig. 4. The effect of temperature on emulsion stability of soy protein curd.
This experiment was carried out at pH 6.0.
Symbols are the same as in Fig. 3.

it possible for emulsion to form(17). Once the emulsion is formed, these fragments at the surface of the oil or water droplets act as barriers(18) that prevent the droplets from coalescing and thus they stabilize the emulsion.

Effect of temperature on emulsion stability of soy protein curd

Fig. 4 shows the effect of temperature on stability. The enzyme-curd was a higher value than that of the HCl- and the Ca-curd in the range 40~80°C.

It is well-known that proteins are susceptible to denaturation at oil-water interface. If proteins undergo surface denaturation, the surface hydrophobicity(19) increases and this results in good emulsifying and foaming properties. Thus, the susceptability of surface denaturation at the oil-water interface may be involved in the functional properties of proteins. It can be assumed that flexible protein(amphiphilic protein, protein of enzyme-curd) molecules are susceptible to denaturation at the interface, while the rigid protein (protein of Ca- and HCl-curd) molecules are not.

The results of Fig. 3 and 4 show, obviously, that the emulsion stability of enzyme-curd was higher than that of Ca- and HCl-curd, and the results were consistent with the feature of the degradation patterns and scanning electron micrographs of enzyme-curd. This means that the high emulsion stability of enzyme-curd was due to adequate degradation of soy protein by an enzymic action, and the ability to change their properties by an enzyme.

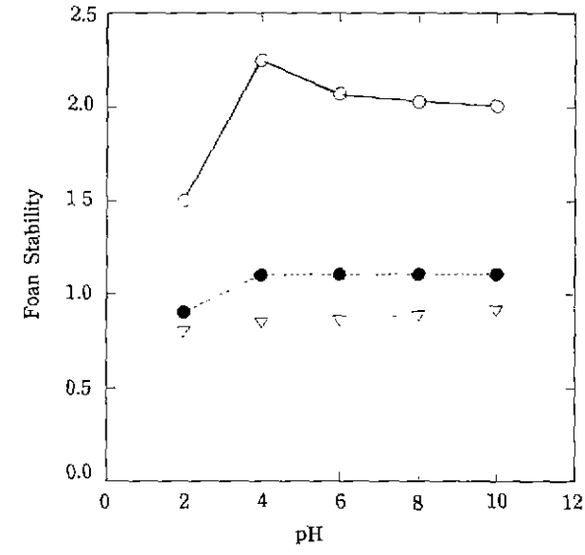

Fig. 5. The effect of pH on the foam stability of soy protein curd.
This experiment was carried out at 20°C.
Symbols are the same as in Fig. 3.

Effect of pH on the foam stability

Fig. 5 shows the effect of pH on the foam stability of soy protein curd. Foam stability was sharply increased at the isoelectric point of the enzyme-curd. However, that of the Ca- and the HCl-curd had a low value in the whole pH range.

Protein foams consist of gas droplets encapsulated by a liquid film containing the soluble surfactant protein. The presence of lipid in soy preparations is detrimental to foaming because it destabilizes protein films (15). Modified protein degraded by enzymatic hydrolysis further improves foaming properties.

Effect of temperature on the foam stability

Fig. 6 shows the foam stability of the enzyme-curd was higher than that of Ca- and HCl-curd in the range of 20~80°C.

Larger particles of protein gave foam stability(5). This is due to the fact that large particles make a thick and strong membrane, and it requires a lot of energy to get back into the solution. As shown in Fig. 2, however, it can be seen that the foam stability of the enzyme-curd, in spite of a comparatively low molecular weight, was higher than that of HCl- and Ca-curd. Kato et al.(15) reported that the flexibility of protein is consistent with that of surface hydrophobicity as structural factors of the functional properties of protein,

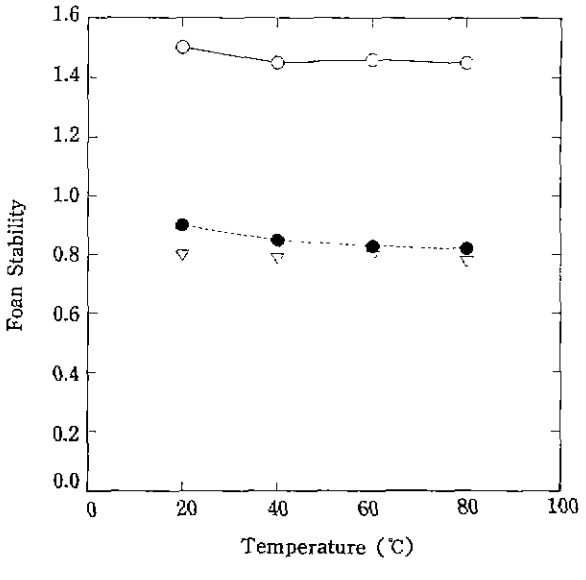

Fig. 6. The effect of temperature on the foam stability of soy protein curd.
This experiment was carried out at pH 6.0. Symbols are the same as in Fig. 3.

and the foaming properties seem to corelate with the flexibility of protein. This is the reason why the foam stability of enzyme-curd was higher than the others.

Although the soy protein emulsion with $CaCl_2$ and HCl produced the coagulum, the feature of these was different from that of enzyme-curd(Fig. 1). Also, Fig. 2 shows that enzyme-curd resulted in the formation of the largest coagulum which played an important role in the emulsifying and foaming properties.

The emulsion and foaming stability of enzyme-curd may be explained by inhibition of flocculation, for example, the effect of hydration of the surface of the particles. This means, as shown in the figures, that a protein is changed by enzymic action. These properties, thus, showed that the enzyme-curd could be widely used in the field of food processing and the manufacture of soy protein.

The IJ-3 strain enzyme exchanges the characteristics of the soybean protein, and is susceptible to denaturation in all ranges of pH, suggesting the flexibility (14) of structure.

Good correlations were observed between the foaming and emulsifying properties of soybean proteins determined by IJ-3 strain enzyme(Figs. 1 and 2). Since the surface hydrophobicity of soybean protein increases with denaturation at the interface, the emulsifying and foaming properties may be improved.

Further investigation on the identification of the functional properties of 7S and 11S globulin by this enzyme is necessary, and is now in progress.

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(Received May 25, 1996)