INTERACTION BETWEEN MYOFIBRILLAR AND SOY PROTEIN IN RAW STATE

Haruo Negishi and Sumio Yoshikawa, Research Institute, Meiji Milk Products Co. Ltd., 1-21-3, Sakaecho, Higashimurayamashi, Tokyo, 189-Japan

SUMMARY

For the study on the interaction between meat- and soy-proteins, the binding ability of raw meat pieces and the gel formation in raw state of myofibrils and soy proteins were investigated. Isolated soy proteins (ISP) showed the ability to bind the raw meat pieces pretreated by $CaCl_2$ solution. The higher the concentration of $CaCl_2$ (0.30.1 M), the higher the extractability of myofibrillar proteins and also the greater the binding strength of meat pieces. ISP treated with alkali (A-ISP) showed higher binding ability than untreated ISP (U-ISP). Myofibrils did not form non-heating set gels in acidic region at low ionic strength (I=0.16), but did at high ionic strength (I=0.6). By increasing the addition rate of ISP to myofibrils in ISP-myofibril mixtures, the mixtures' gel strength became stronger than the myofibrils'. These results suggested the formation of mutually twisted gels which were induced from the interaction between myofibrils and soy proteins.

INTRODUCTION

A soy protein is one of the most widely used materials in Japanese meat industries because of its excellent functional properties. In the mixture of raw meat and soy proteins, it is important to theoretically elucidate the effects of the interaction between these proteins on product quality. Until now, most research in this field has been mainly concentrated on the characteristics and forming mechanism of heat-induced gels (King 1977; Peng et al. 1982; Lin and Ito 1985). These authors also discussed the low-temperature interaction between meat- and soy-proteins, but their experimental results differed so widely that a clear-cut explanation about the interaction mechanism has not been obtained. As for the ground beef mixtures like raw hamburger patties and raw-bound meat pieces like reconstituted meat, however, the mechanism of the low-temperature interaction between meat- and soy-proteins, especially in raw state and in acidic region like meat pH, should be made clear.

For this purpose, the following subjects are investigated in this study: (1) the effects of soy proteins, used as a meat binder, on the binding ability between raw meat pieces treated with salts, (2) the gel formation mechanism in raw state in case of the addition of soy proteins to myofibrils in acidic region, and (3) the effects of alkali-treatment of soy proteins on their interaction with meat proteins.

MATERIALS AND METHODS

Meat sources and myofibril preparation

Fresh beef rounds aged for 8-9 days at 0-2°C after slaughter and frozen ones imported from John Dee Company, Australia, were used in this study. Myofibrils were prepared from both meat sources according to the procedure of Yang et al. (1970). Myofibrils precipitated by centrifugation at 3000 x G for 7 min were used for the experiments of non-heating set gels.

Soy protein sources and alkali-treated soy protein preparation "New Fujipuro R" (crude protein 91.0%), an isolated soy protein (ISP) commercially produced by Fuji Purina Company, Japan, was used as soy protein sources. An alkali-treated isolated soy protein (A-ISP) was prepared by the reaction of 4% ISP solution with NaOH at pH 12, 20°C for 4 h. The A-ISP solution was adjusted to pH 7.5 and dialysed against deionised water at 5°C for 4-5 days, subsequently powdered by lyophilisation. ISP without alkali-treatment was referred to as untreated ISP (U-ISP).

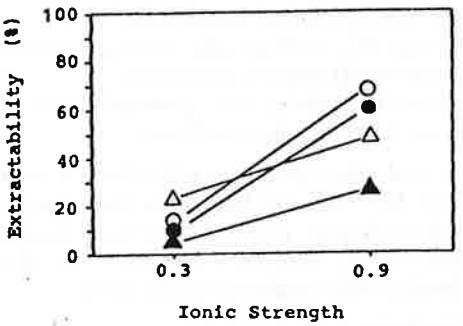


Fig.1. Effects of NaCl and $CaCl_2$ on the extractability of myofibrillar proteins: NaCl-(△-△) and $CaCl_2$-treatment (○-○) of myofibrils from fresh beef, NaCl-(▲-▲) and $CaCl_2$-treatment (●-●) from frozen beef

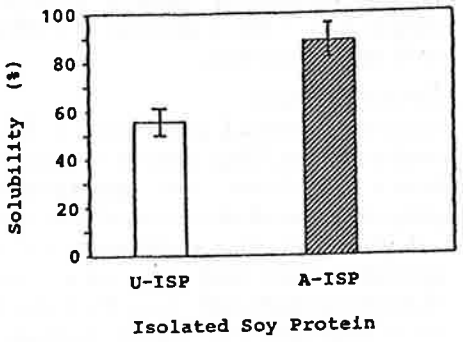


Fig.2. The solubility of untreated (U)- and alkali treated (A)-ISP: Bars indicate means ± standard deviation.

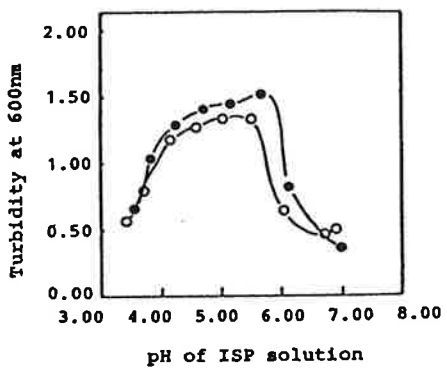


Fig.3. The comparison of turbidity changes between U-(○-○) and A-ISP (●-●).

Salt-soluble protein extractability of myofibrils

Myofibrils were fully suspended with NaCl (0.3 or 0.9 M) and $CaCl_2$ (0.2 or 0.3 M) solutions, kept for 30 min at ice cold temperature and centrifuged at 15,000 x G for 20 min. Myofibrillar protein extractability was expressed as a percentage of the protein contents in the supernatent to those in the suspension.

Chemical properties of ISP: Solubility and Turbidity

ISP solution (5 mg/ml) was kept overnight at 0-2°C and centrifuged at 28,000 x G for 15 min. The solubility of ISP was expressed as a percentage of the protein contents in the supernatent to those in the solution. The turbidity of ISP solution (1 mg/ml) formed after changing the pH value of the solution from 3.0 to 7.0 with 50 mM acetate buffer was measured at 600 nm.

The protein concentration of myofibrils and ISP solution was determined by biuret procedure using bovine serum albumin as a standard.

Binding ability

Raw meat pieces of approximately 40 x 40 x 20 mm were soaked in 0.1 or 0.3 M $CaCl_2$ solution for 30 min at ice cold temperature. Subsequently ISP was attached to the surfaces of the meat pieces as a binder; then two pieces of meat were stuffed in round shaped plastic moulds (44 mm diameter, 25 mm height) and held overnight under the pressure (10 g/cm2) at 0-2°C. The bound meat masses prepared in these ways were sliced in 25 x 44 x 5 mm. The binding strength between two meat pieces was measured by using a swing test. The subjective evaluation of the swing test was carried out with the following scores on 8-point scale: 7 = very strong bind (swinging frequency required to separate bound meat pieces; more than 7 times), 6 = strong bind (6 times), 5 = moderately strong bind (5 times), 4 = slightly strong bind (4 times), 3 = slightly weak bind (3 times), 2 = moderately weak bind (2 times), 1 = weak bind (1 time), 0 = virtually no bind.

The pH value on the interface of bound meat pieces was directly measured with a surface pH meter.

Experiments of gel formation in raw state

Non-heating set gels of myofibrils (56 mg/ml), ISP (150 mg/ml) and myofibril-ISP mixtures (56 mg/ml) were prepared mainly according to the procedure of Fretheim et al. (1985). The strength of the gels formed at 0-2°C from these proteins and their mixtures was measured with Rheo Meter and expressed as breaking strength (g/cm^2).

RESULTS

Effects of salts on the extractability of myofibrillar proteins

Figure 1 shows the effects of NaCl and $CaCl_2$ on the protein extractability of myofibrils isolated from fresh and frozen beef were not significantly different at low ionic strength (I = 0.3). At high ionic strength (I = 0.9), however, the $CaCl_2$ treatment showed significantly higher extractability than NaCl treatment. Fresh beef invariably showed higher extractability than frozen beef in either treatment; the difference was greater in NaCl treatment.

Chemical properties of ISP

The solubility of ISP increased by alkali-treatment (Fig.2). The turbidity of both untreated- and alkali-treated ISP, as shown in Fig.3, increased extremely in the pH range 4.0-6.0. Alkali-treatment of soy proteins changed the turbidity pattern, raising the value slightly upward.

Binding of raw meat pieces

Since $CaCl_2$ treatment of raw meat pieces turned out to be more effective to extract myofibrillar proteins than NaCl treatment, binding tests were carried out by using the raw meat treated with 0.1 or 0.3 M $CaCl_2$ solution. Fig.4 shows the results of a swing test of bound meat pieces; ISP, sandwiched between raw meat pieces which were pretreated with $CaCl_2$ solution, worked effectively to bind them together. The higher concentration of $CaCl_2$ solution (0.3 M) seemed to be more effective on meat binding than the lower one (0.1 M); A-ISP possessed greater binding ability than U-ISP.

Formation of non-heating set gels

Since the interaction between ISP and $CaCl_2$-soluble meat proteins caused the formation of the non-heating set gels which was considered to play a significant role for meat binding, the formation mechanisms and characteristics of these gels were investigated.

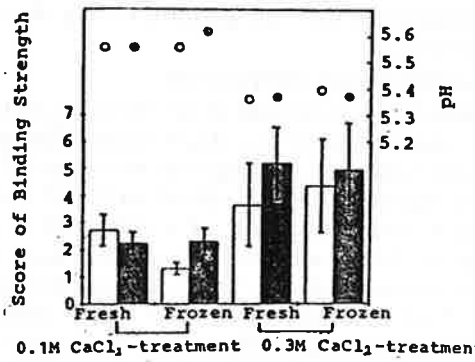
Figure 5 and 6 show the effect of U- and A-ISP, respectively, on the gel strength at low ionic strength (I = 0.16). Although myofibrils formed no non-heating set gel by themselves, these types of gel were gradually formed with the addition of 4 or 8% ISP to them. The addition of 8% ISP made the mixtures form non-heating set gels with stronger gel strength than myofibrils or ISP alone. The gel strength of myofibril-U-ISP mixture was greater than that of the mixture with A-ISP.

Figure 7 and 8 show the effect of U- and A-ISP, respectively, on the gel strength at high ionic strength (I = 0.6). In these cases, even myofibrils made non-heating set gels by themselves. Increasing the addition rate of either type of ISP to myofibrils from 2 to 8%, however, the gel strength of the mixtures became remarkably stronger than myofibrils or ISP alone. The addition of U-ISP formed stronger gel than A-ISP.

DISCUSSION

The results in Fig.4 - the higher the concentration of $CaCl_2$, the greater the binding strength - were parallel to the results in Fig.1 - the higher the ionic strength, the higher the extractability of myofibrillar proteins. Therefore, salt-soluble proteins were considered to have a great influence on raw-meat binding. The binding mechanism was supposedly brought about by the interaction between $CaCl_2$-soluble muscle proteins and the ISP added as a binder.

ISP treated with alkali (A-ISP) showed higher binding ability than U-ISP. This alkali-treatment probably caused the conformational changes of soy-protein molecules which were estimated from the increase of solubility and the turbidity of ISP by this treatment. Ishino and Okamoto (1975) reported that the conformational changes of soy proteins in alkaline region occurred as follows: below pH 11.0 the reversible changes of protein molecules, and in the range of pH 11.0-12.8 the irreversible changes followed by the interaction among unfolding protein molecules. A-ISP used in this study was treated with alkali at pH 12.0, so the irreversible conformational changes of soy protein molecules must have occurred.



Sources of raw meat and concentration of $CaCl_2$ solution

Fig.4. The effects of meat sources, $CaCl_2$ concentration and ISP(□U-ISP, ▨A-ISP) on binding strength: Bars indicate means ± standard deviation. circle symbols illustrate the pH value on the interface of bound meat pieces.

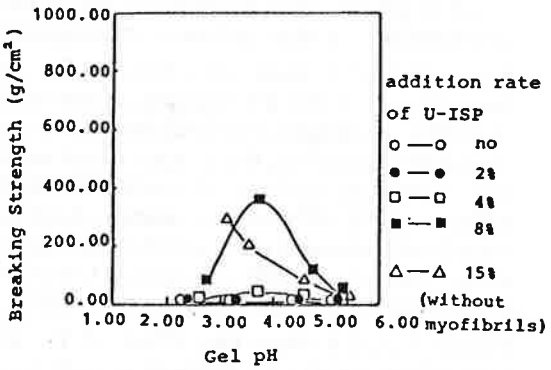


Fig.5. Effects of U-ISP on the gel strength formed from myofibril-U-ISP mixtures in raw state at low ionic strength (I=0.16).

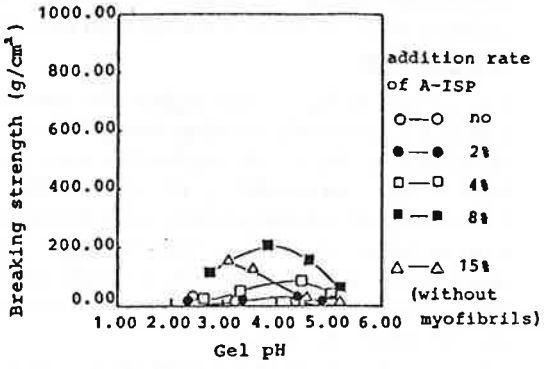


Fig.6. Effects of A-ISP on the gel strength formed from myofibril-A-ISP mixtures in raw state at low ionic strength (I=0.16).

The phenomenon of gel formation in raw state was shown by the myofibrils and myofibril-ISP mixtures which formed non-heating set gels by making use of salt-soluble myofibrillar proteins extracted at high ionic strength. The fact that myofibril-ISP mixtures formed the gels with greater gel strength than myofibrils alone suggested the formation of mutually twisted gels by the interaction between these proteins. We want to call this type of gels "eutectic gels".

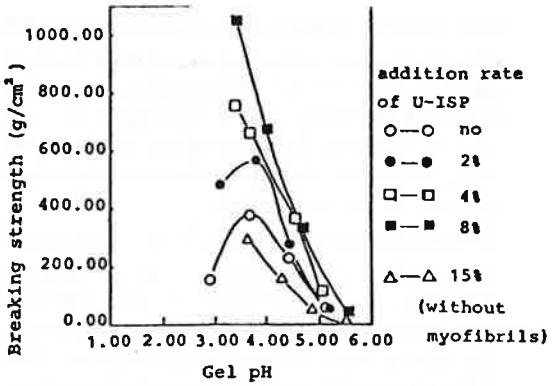


Fig.7. Effects of U-ISP on the gel strength formed from myofibril -U-ISP mixtures in raw state at high ionic strength (I=0.6).

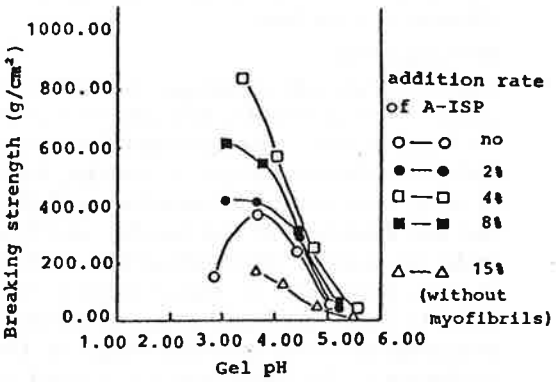


Fig.8. Effects of A-ISP on the gel strength formed from myofibril-A-ISP mixtures in raw state at high ionic strength (I=0.6).

CONCLUSIONS

Binding of raw meat pieces occurs by the action of the eutectic gels induced from the interaction between salt-soluble muscle proteins and ISP in acidic region. A-ISP works as a more effective raw-meat binder than U-ISP, probably due to its high reactivity with salt-extractable muscle proteins. In regard to the gel strength of non-heating set gels, however, U-ISP made stronger gels with myofibrils than A-ISP. This suggests that in addition to solubility and gel strength some other properties of ISP have influences on binding of meat pieces.

REFERENCES

Fretheim, K., Egelandsdal, B., Harbitz, O. and Samejima, K. (1985). *Food Chemistry* **18**:169.

Ishino, K. and Okamoto, S. (1975). *American Association of Cereal Chemists* **52**:9.

King, N.L. (1977). *Journal of Agricultural and Food Chemistry* **25**(1):166.

Lin, L.C. and Ito, T. (1985). *Journal of Food Technology* **20**:219.

Peng, I.C., Dayton, W.R., Quass, D.W. and Allen, C.E. (1982). *Journal of Food Science* **47**:1984.

Yang, R., Okitani, A. and Fujimaki, M. (1970). *Agricultural and Biological Chemistry* **34**(12):1765.