Note

Coagulation of Proteins in Leguminous Milk by Commercial Proteinases[†]

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We reported in the preceding papers^{1~3)} that acidic, neutral and alkaline proteinases originating from microorganisms, plants and animals are capable of coagulating soymilk protein. Recently, we found that some of the proteinases also have a clotting activity towards various proteins in leguminous milk. This paper investigates the coagulation of the protein by a variety of commercial acidic, neutral and alkaline proteinases.

The legumes used in this experiment were broad bean (*Vicia faba*), adzuki bean (*Vigna angularis*), soy bean (*Glycine max* Merr.), black-colored soy bean (*Glycine max* Merr.), kidney bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*) and garden pea (*Pisum sativum*). They were in air-dried form except for broad bean and garden pea (both frozen), and peanut (defatted flour). The commercial proteinases (Fig. 1) were used in this experiment without any additional purification. Leguminous milk samples were prepared by soaking each legume in water at room temperature for 15 hr, before adding a 10-fold weight of deionized water (based on its original weight) containing 0.1% sodium bicarbonate to each swollen legume. The legumes were then separately homogenized for 10 min by a kitchen mixer. The slurry thus obtained was centrifuged at $5000 \times g$ for 30 min to separate the protein solution and solid materials, and followed by filtration of the supernatant liquid through a bleached cotton cloth. The filtrate was used as the leguminous milk after heating at 100°C for 10 min. Both the broad bean and garden pea milk, and the peanut milk were prepared by adding 6- and 20-fold weights of deionized water containing 0.1% sodium bicarbonate, respectively, according to the conditions already described.

The coagulability of proteinases for proteins in leguminous milk was measured by the method described in our previous paper.²⁾ Prior to the measurement, the concentration of each enzyme was adjusted to 1 unit of soymilk-clotting activity/ml. The enzyme solution (0.3 ml) was added to each leguminous milk (3 ml adjusted to pH 6.1 with sodium phosphate buffer solution and pre-incubated at 65°C), only the coagulability in peanut milk being measured at pH 6.7 because the protein in the milk coagulated without adding the enzyme solution below pH 6.5. Each reaction mixture was incubated at the same temperature for 3 min, and the resultant coagulum was centrifuged at $1600 \times g$ for 10 min to separate the whey and curd. In this experiment, the curd refers to the precipitate after centrifuging the coagulum. The protein recovery based on the protein in each leguminous milk and the moisture content of the curd were measured according to methods quoted in the preceding paper.²⁾

Figure 1 shows the coagulability of proteinases for the protein in each leguminous milk. All the proteinases used in this experiment coagulated the protein in leguminous milk except for the garden pea milk (data not shown). Each of the resultant coagula separated into whey and curd after centrifugation under the conditions already mentioned. The pH value of each coagulum was maintained in the range from 6.0 to 6.1. On the other hand, coagulation of the protein in leguminous milk was not observed with all of the proteinases after treating at 100°C for 10 min (data not shown). Thus, the results show that the coagulation was not due to the reduction of pH value, but to the action of the enzyme. Table I shows the protein recovery, and it can be seen that the recovery varied according to the proteinase and legume. This may have depended on the difference in proteinase specificity to the leguminous proteins. The moisture content of the curds was 95% for broad bean, 95% for adzuki bean, 81% for soy bean, 78% for black-colored soy bean, 66% for kid-

TABLE I. PERCENTAGE RECOVERY OF PROTEIN FROM THAT AVAILABLE IN LEGUMINOUS MILK

Enzyme source	Broad bean	Adzuki bean	Soy bean	Black-colored bean	Kidney bean	Peanut
1	68.7	84.4	73.1	72.8	82.5	51.9
2	60.1	81.7	79.7	80.6	82.8	57.6
3	60.2	68.5	69.4	63.4	77.5	48.0
4	63.7	80.6	78.9	87.2	82.2	58.7
5	73.6	82.7	82.0	86.8	83.3	74.3
6	65.3	81.9	83.5	77.9	84.2	76.4
7	62.3	77.8	83.6	78.2	79.2	55.3

The numbers for the enzyme source correspond to those in Fig. 1.

[†] Offprints should be requested from I. Kusakabe.

ney bean and 54% for peanut. There was no remarkable difference in the moisture content among the proteinases (data not shown), but there was among the legumes.

It has been reported by Fuke *et al.*⁴⁻⁶⁾ that some plant proteinases such as bromelain and ficin coagulate soymilk protein. However, it was not known whether or not a proteinase was capable of coagulating the various proteins in leguminous milk. Therefore, we studied the coagulability of proteinases for the various proteins in leguminous milk. From the results of this experiment, it became clear that a variety of acidic, neutral and alkaline proteinases possess not only coagulability for soymilk protein, but also for the various proteins in leguminous milk. This is the first report dealing with the ability of proteinases to coagulate proteins in leguminous milk. The mechanism for coagulation is not clear at present, but may be similar to that of soymilk protein by proteinases. Anyhow, it is particularly interesting, for a variety of proteinases coagulated various kinds of proteins in leguminous milk.

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FIG. 1. Coagulability of Various Proteinases for Proteins in Leguminous Milk.

1, *Bacillus subtilis* (alkaline proteinase, Sigma Chemical, U.S.A.); 2, *Aspergillus oryzae* (alkaline proteinase, ditto); 3, *Bacillus thermoproteolyticus* (neutral proteinase, ditto); 4, *Bacillus polymyxa* (neutral proteinase, ditto); 5, *Endothia parasitica* (acidic proteinase, Pfizer, U.S.A.); 6, *Mucor miehei* (acidic proteinase, Rapidase, France); 7, Bromelain (Nakarai Chemical, Japan); 8, deionized water (as a control).