Bull. Mukogawa Women's Univ. Nat. Sci., **46**, 105－112(1998)
武庫川女子大紀要（自然科学）

Factors Affecting Water Permeability of Aleurone Layer in Soybean Seeds

Hiroko Noda and Mitsuru Fukuda

Department of Food Science and Nutrition,
School of Human Environmental Sciences,
Mukogawa Women's University, Nishinomiya 663-8558, Japan

The effect of the immersion condition of soybean seeds on the water permeability in aleurone layer was investigated to clarify the water permeability at the initial stage of water sorption. The amounts of water absorbed in seeds coated with only aleurone layer(embryos uncovered with seed coat)and untreated seeds(embryos covered with seed coat and aleurone layer; intact seeds)were compared under several conditions of temperature, pH, ion species, and salt concentration. The relative weight in both types of seeds increased with the rise of soaking temperature, but it decreased after 180min at 50℃. Whereas both water uptakes were inclined to be little affected by soaking pH, the remarkable increase in the water uptake of seeds coated with only aleurone layer was observed only by soaking in sodium acetate buffer(pH 4.0). Although a lower concentration of KCl(0.01M)stimulated both water uptakes compared with water, a higher concentration of KCl(0.1M and 0.2M)depressed inversely them. The rates of water uptake in both types of seeds changed depending upon ionic strength rather than ion species. From these results, it was found that the state of water sorption in soybean seeds having the aleurone layer scarcely changed even if the seed coat was removed. It is suggested that the water permeability of soybean seeds is mainly due to the characteristics of aleurone layer.

Introduction

The water sorption of soybean seed is a very important treatment on the cooking and processing. It is known that the soybean seed having a low original water content (below 10%) is inclined to suffer imbibitional damage[1]. McDonald *et al.* reported that the water uptake of soybean seed is regulated by the seed coat[2]. However, Matsui *et al.* suggested that the aleurone layer in the inside of seed coat regulates the water sorption of soybean seeds[3]. The soybean seed has a more developed aleurone layer than that of other bean seeds, and the aleurone layer exhibits a very thick and strong structure[4]. Hill and West investigated the tissue structure of soybean seed contaminated with fungi[5]. They described that the mycelia entered pores on the seed coat surface was possible to reach easily the hourglass layer in the outside of spongy parenchyma and aleurone layers. From these facts, it is suggested that the aleurone layer physically protects the embryo. Moreover, it was reported that glycine-rich proteins(GRPs)exist in cell walls of the aleurone layer[6)-7)], and GRPs would participate in the water permeability of aleurone layer[3]. Several workers have reported on the water uptake of soybean seeds[8)-12)]. However, the role of soybean aleurone layer on water sorption has still been unknown. We recently reported the permeability of several cations into the soybean aleurone layer in a previous paper[13]. The present paper describes the factors affecting the water sorption of soybean seeds at the initial stage and the characteristics of the water permeability through aleurone layer into embryos. The final object of this study is to search major factors raising the rate of water sorption of soybeans.

Materials and Methods

1. Soybean

Soybean seeds (*Glycine max* L. cv. Enrei) produced in Fukui prefecture in 1996 were kindly provided by Tamura Co., Ltd. (Osaka, Japan).

2. Observation of the seed coat and aleurone layer of soybeans immersed in water by scanning electron microscope

Soybean seeds were immersed in distilled water at 25℃ for 0, 60, 180 and 300min. After the lyophilization of seeds, those vertical sections were observed by a Hitachi scanning electron microscope (type S–530) at 20kV.

On the other hand, soybean seeds with or without seed coat were immersed in distilled water at 10 or 50℃ for 180min to clarify the effect of temperature on water uptake. To see the influence of salt on water uptake, soybean seeds with or without seed coat were immersed in distilled water or 0.2M KCl solution at 25℃ for 180min. Swollen seeds were lyophilized and were observed by a scanning electron microscope, as described above.

3. Soaking solutions

The effect of soaking solutions on the water permeation of soybean seeds were examined as follows. Distilled waters at different temperatures of 10, 20, 30, 40, and 50℃ were used for the effect of temperature on soaking. Several kinds of buffer of 0.05M sodium acetate buffer (pH 4.0 and 5.5), 0.05M sodium phosphate buffer (pH 5.5, 7.0, and 8.0), 0.05M Tris–HCl buffer (pH 8.0) were used for the effect of pH. Different concentrations of KCl solution (0, 0.01, 0.1, and 0.2M) were used for the effect of salt concentration. Several kinds of salt solution of 0.1M KCl, 0.1M NaCl, 0.1M $MgCl_2$, and 0.1M $CaCl_2$ were used for the effect of ion species, respectively. Ten soybean seeds were used in 40 mℓ of each soaking solution.

4. Preparation of soybean seeds with or without seed coat and their immersion

Soybean seeds were previously immersed in distilled water at 30℃ for 30min. Swollen seed coats were carefully removed from the half of immersed soybean seeds to get embryos uncovered with seed coat. Remaining seeds were used as intact ones. Both types of seeds, intact ones and embryos without seed coat, were put in a moistured chamber until use, and then they were immersed in soaking solution at 25℃.

5. Measurement of water sorption in soybean seeds

Soybean seeds were picked up from soaking solution and were wiped with a filter paper to remove excess water from the surface of seeds. Their weights were immediately measured with an electronic balance. The measurements were carried out after 30, 60, 90, 120, 150, 180, 240, and 300min during soaking. The relative weight was expressed by evaluating the weight before immersion (0min) as 100. The values were determined from the mean of duplicates.

Results and Discussion

1. Structural change in soybean seed by water uptake

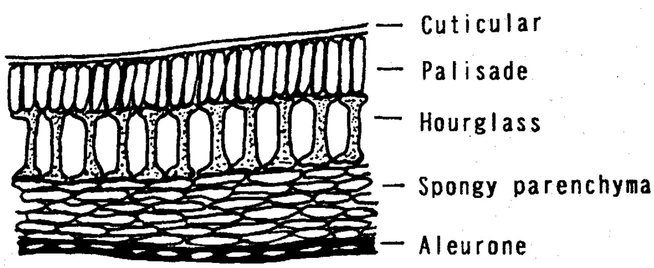

Fig. 1. The model structure of soybean seed coat and aleurone layer

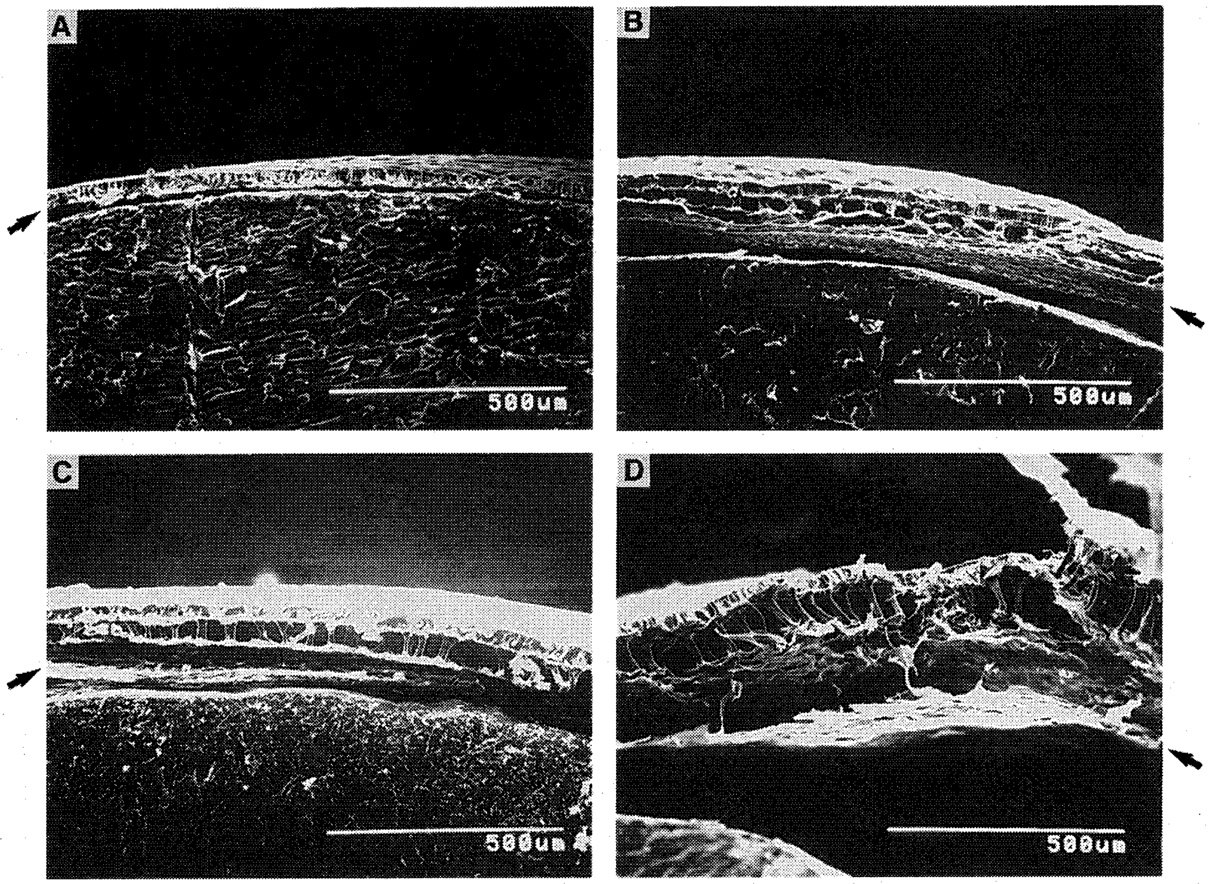

Fig. 2. Change in cross-sectional structure of soybean seed during soaking in water(× 100)
A, 0min; B, after 60min; C, after 180min; D, after 300min.
Arrow shows the aleurone layer.

The model structure of soybean seed coat and aleurone layer was shown in Fig. 1. The structural change of soybean seed by water uptake was observed by a scanning electron microscope (Fig. 2). The seed coat was observed to be composed of cuticular, palisade, hourglass, and spongy parenchyma layers. The aleurone layer occured under the seed coat. As shown in Fig. 2-A, the hourglass layer was easily confirmed before immersion(0min). However, it was impossible to distinguish the cuticular and the palisade layers. The aleurone layer closely adhered to cotyledon. The palisade and hourglass layers were extended with passage of immersion time. The aleurone layer was also considerably expanded in 180min after immersion(C). The space between the seed coat and the aleurone layer of seeds immersed for 180min(C)was larger than that of seeds immersed for 60min(B). In addition, this phenomenon was more remarkable in seeds soaked for 300min(D). The thickness of aleurone layer was increased with the extension of immersion time. Though the cotyledon tissue was considerably shrinked by freeze-drying treatment, the tissues of seed coat and aleurone layer were scarcely affected. It was proved that the immersion for 300min fairly expanded the cotyledon as well as the seed coat and aleurone layer. The seed immersed for 300min was fragile(D).

2. Effect of temperature

Intact seeds and embryos without seed coat were incubated in distilled water for 300min at 10, 20, 30, 40 and 50℃, respectively. Figure 3 shows the time course of the water sorption at different temperatures. The water sorption rates of both types of seeds were increased with the rise of soaking temperature. They were markedly influenced by the soaking temperature. Similar phenomenon on the other kind of soybean seeds was presented by Nakamura *et al.*[8], Hsu *et al.*[9], and Singh and Kulshrestha[10]. The relative weight during soaking was decreased after 180min at 50℃. It is assumed that the decrease of relative weight mainly depends on the exudation of internal components from seeds because the destruction of seed structure was not observed at 50℃ (Fig. 4). In fact, the soaking solution at 50℃ showed slightly a turbidity after 300min. Asano *et al.* reported that exuding components such as proteins and carbohydrates from soybean seeds increased at a higher soaking temperature[14]. It is also suggested that the function of seed coat and aleurone layer on water permeation might be lost due to protein denaturation by heat.

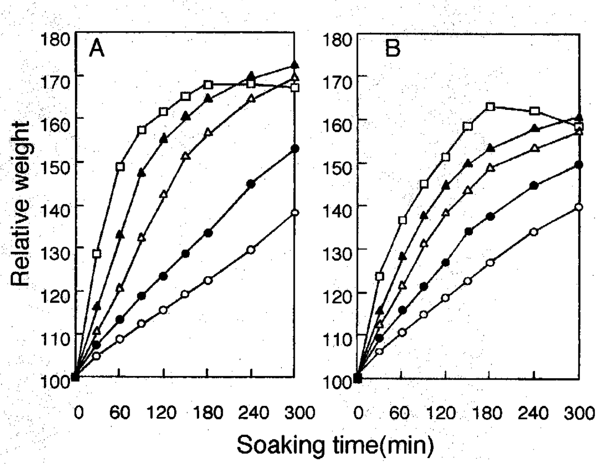

Fig. 3. Effect of soaking temperature on the water uptake of soybean seeds
A, intact seeds; B, embryos without seed coat; ○, 10℃; ●, 20℃; △, 30℃; ▲, 40℃; □, 50℃.

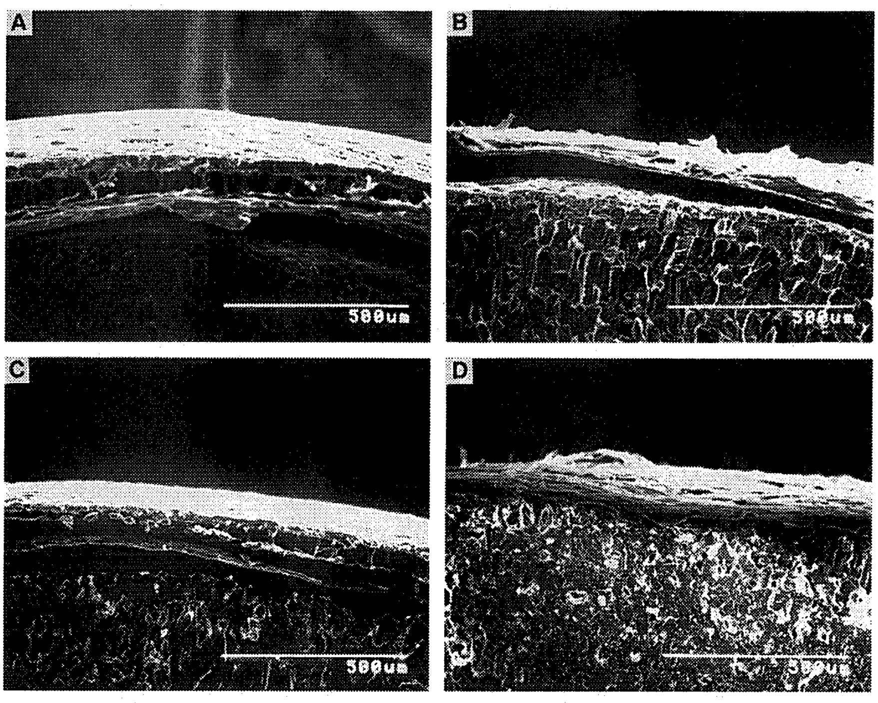

Fig. 4. Structure of soybean seed soaked at different temperature for 180min (×100)
A, intact seed at 10℃; B, intact seed at 50℃; C, embryo without seed coat at 10℃; D, embryo without seed coat at 50℃.

3. Effect of pH

Intact seeds and embryos without seed coat were incubated in 0.05M sodium acetate buffer (pH 4.0 and 5.5), 0.05M sodium phosphate buffer (pH 5.5, 7.0 and 8.0), and 0.05M Tris-HCl buffer (pH 8.0) for 300min at 25℃. As shown in Fig. 5, the water uptake of intact seed was influenced by the kind of buffer rather than that of pH. Sodium acetate buffer disturbed the normal water uptake. There was no significant difference in the water uptake of soybean seeds uncovered with seed coat between sodium phosphate buffer and Tris-HCl buffer. The water uptake of embryos without seed coat in sodium acetate buffer was the lowest at pH 5.5, but it was remarkably increased in the same kind of buffer at pH 4.0. Fuchigami and Okamoto extracted pectic substances from several vegetable tissues with dilute hydrochloric acid (pH 2.0) and acetate buffer (pH 4.0)[15]. O'Neill *et al.* showed that the borate ester cross-linked with rhamnogalacturonan Ⅱ (RG-Ⅱ) is partially hydrolyzed at room temperature within 30min between pH 2 and 4[16]. RG-Ⅱ is structurally complex pectic substance in plant cell walls[17]. The rate of water uptake in soybean embryos without seed coat immersed in 0.05M sodium citrate buffer (pH 4.0) was increased as same as that in 0.05M sodium acetate buffer (pH 4.0). Moreover, the relative weight of embryos without seed coat immersed in 0.05M acetic acid increased more rapidly than that in sodium acetate buffer (pH 4.0). Therefore, it is suggested that the acetate buffer or acetic acid accelerated the solubilization of pectic substances and the loosened cell wall enabled the active water permeation. It is assumed that the barrier on the water passage through symplast in the aleurone layer was lost by elution of pectic substances from cell wall.

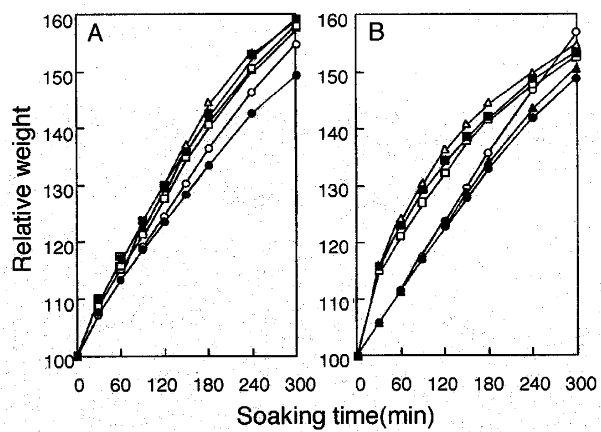

Fig. 5. Effect of soaking pH on the water uptake of soybean seeds
A, intact seeds; B, embryos without seed coat; ○, pH 4.5 (0.05M sodium acetate buffer); ●, pH 5.5 (0.05M sodium acetate buffer); △, pH 5.5 (0.05M sodium phosphate buffer); ▲, pH 7.0 (0.05M sodium phosphate buffer); □, pH 8.0 (0.05M sodium phosphate buffer); ■, pH 8.0 (0.05M Tris-HCL buffer).

4. Effect of salt concentration

Intact seeds and embryos without seed coat were incubated in 0, 0.01, 0.1 and 0.2M KCl solutions at 25℃ for 300min. Figure 6 indicates the time course of the water sorption at various concentrations of KCl. The water uptake of intact seeds was depressed in presence of a high concentration of KCl. It seems that the aleurone layer in 0.2M KCl was not enough expanded compared with that in distilled water (Fig. 7). On the other hand, soaking solution containing 0.01M KCl stimulated the water uptake. The similar result was obtained from embryos without seed coat. Hsu *et al.* reported that

$NaHCO_3$ concentration more than 1%(approximately 0.1M)depressed the water uptake of soybean seeds[9]. They also described that $NaHCO_3$ concentration less than 0.5% showed neither depression nor stimulation on the water sorption. It is supposed that the stimulatory effect of 0.01M KCl on the water sorption of seeds is due to the active transport of potassium in seed coat and aleurone layer. On the other hand, a higher concentration of KCl solution depressed the water uptake by the decrease in water potential between the outside and inside of the aleurone layer cell.

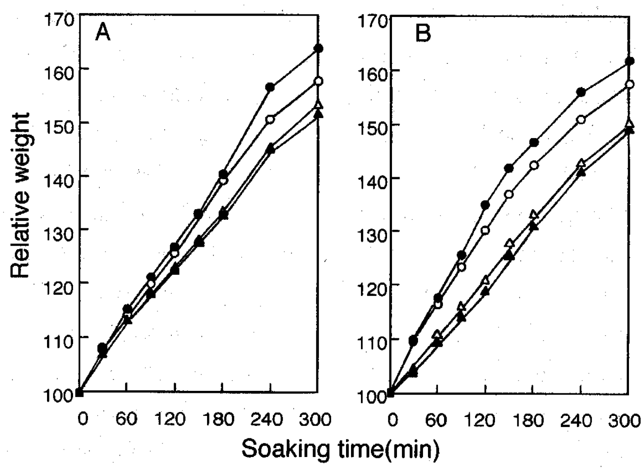

Fig. 6. Effect of various concentrations of KCl on the water uptake of soybean seeds
A, intact seeds; B, embryos without seed coat; ○, 0M; ●, 0.01M; △, 0.1M; ▲, 0.2M.

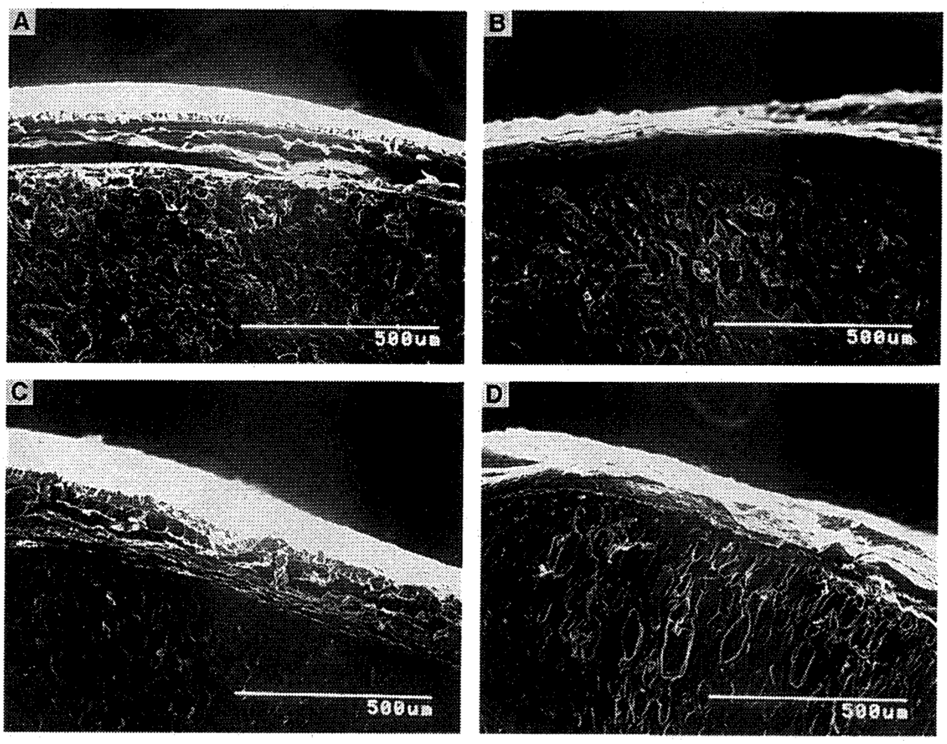

Fig. 7. Structure of soybean seeds soaked with and without KCl for 180min(×100)
A, intact seed in distilled water; B, intact seed in 0.2M KCl; C, embryo without seed coat in distilled water; D, embryo without seed coat in 0.2M KCl.

5. Effect of ion species

Intact seeds and embryos without seed coat were incubated in distilled water, 0.1M KCl, 0.1M NaCl, 0.1M $MgCl_2$, and 0.1M $CaCl_2$ for 300min at 25℃. As shown in Fig. 8, the increase of relative weight in both types of seeds was depressed in the presence of ion than in the case of the absence. The depression of water sorption with divalent cation was greater than that with monovalent cation. When the ionic strength was same, the difference of water sorption was not found between monovalent cation and divalent cation. From these results, it is presumed that the water uptake of seeds is influenced by ionic strength rather than ion species.

6. Water permeability of aleurone layer

The increase in relative weight of intact seeds often seemed to be somewhat more rapidly compared with that of embryos without seed coat as illustrated above(Fig. 3, 5, 6 and 8). It is supposed that such a difference is due to the faster swelling of seed coat during water uptake.

In general, it is said that the water uptake of seeds at the initial stage arises from a physical action depending on the difference of water potential between seeds and the environment around them. It is suggested from the result shown in Fig. 6 that the rate of water sorption in saline solution was depressed by raising the concentration because the difference of the water potential between seeds and soaking solution decreased as the ionic strength of the solution increased. Although the stimulatory effect of a lower concentration of KCl on the water uptake of seed may be due to the other reason, the reason is not obvious in detail.

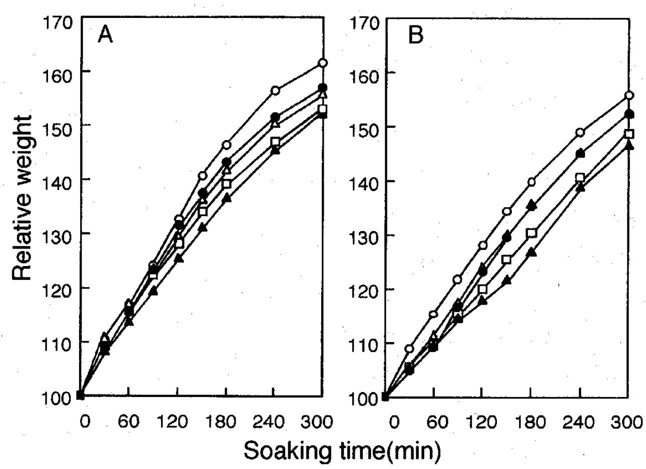

Fig. 8. Effect of various kinds of ion on the water uptake of soybean seeds
A, intact seeds; B, embryos without seed coat; ○, distilled water; ●, 0.1M KCl; △, 0.1M NaCl; ▲, 0.1M $MgCl_2$; □, 0.1M $CaCl_2$.

In the previous paper[13], it was proved that a great amount of Ca ion entered seed coats and aleurone layers of soybean seeds. However, $CaCl_2$ solution did not accelerate the water uptake of seeds compared with KCl solution having same ionic strength. It seems that the route of water transportation differs from that of ion. The water permeability of seeds having only aleurone layer was almost same as that of intact seeds under several conditions affecting the water uptake. Therefore, it is obvious that the water permeability of soybean seeds mainly depends on the characteristics of aleurone layer. Moreover, the aleurone layer was found to be sensitive for the change of temperature because the elevation of temperature was able to alter vigorously the rate of water sorption as shown in Fig. 3. These informations related to factors affecting the water uptake of soybean seeds will be useful for controlling the water uptake. When the shortening of the water sorption time of soybeans will be required for the cooking and processing, the establishment of method controlling the water permeability of aleurone layer would be greatly available.

References

1) Ishida, N., Kano, H., Kobayashi, T., Hamaguchi, H. and Yoshida, T., *Agric. Biol. Chem.*, **52**, 2771-2775(1988).
2) McDonald, M. B., Jr., Vertucci, C. W. and Roos, E. E., *Crop Sci.*, **28**, 987-992(1988).
3) Matsui, M. Uenaka, T., Toyosawa, I. and Fukuda, M., *Nippon Nogeikagaku Kaishi*(in Japanese), **70**, 663-669(1996).
4) Matsui, M., Nakai, R., Toyosawa, I. and Fukuda, M., *Nippon Eiyo Shyokuryo Gakkaishi*(in Japanese), **48**, 477-483(1995).
5) Hill, H. J. and West, S. H., *Crop Sci.*, **22**, 602-605(1982).
6) Matsui, M., Toyosawa, I. and Fukuda, M. *Biosci. Biotech. Biochem.*, **59**, 2231-2234(1995).
7) Fukuda, M., Noda, H. and Toyosawa, I., *Biosci. Biotech. Biochem.*, **61**, 1184-1186. (1997).
8) Nakamura, I., Kurusu, K. and Nakagawa, M., *Nippon Shokuhin Kogyo Gakkaishi*, **26**, 362-364(1979).
9) Hsu, K. H., Kim, C. J. and Wilson, L. A., *Cereal Chem.*, **60**, 208-211(1983).
10) Singh, B. P. N. and Kulshrestha, S. P., *J. Food Sci.*, **52**, 1538-1541, 1544(1987).
11) Kuroda, S., Masago, Y. and Ooshika, J., *Nippon Kaseigaku Kaishi*(in Japanese), **44**, 97-101(1993).
12) Rie, J., Miura, M. and Taneya, S. *Nippon Shokuhin Kagaku Kogaku Kaishi*(in Japanese), **43**, 288-298(1996).
13) Noda, H. and Fukuda, M., *Bull. Mukogawa Women's Univ. Nat. Sci.*, **45**, 101-106(1997).
14) Asano, M., Okubo, K. and Yamauchi, F., *Nippon Shokuhin Kogyo Gakkaishi*(in Japanese), **36**, 636-642(1989).
15) Fuchigami, M. and Okamoto, K., *Nippon Eiyo Shokuryo Gakkaishi*(in Japanese), **37**, 57-64(1984).
16) O'Neill, M. A., Warrenfeltz, D., Kates, K., Pellerin, P., Doco, T., Darvill, A. G. and Albersheim, P., *J. Biol. Chem.*, **271**, 22923-22930(1996).
17) Kobayashi, M., Matoh, T. and Azuma, J., *Plant Physiol.*, **110**, 1017-1020(1996).