Effects of High Pressure on Softening of Japanese Radish and Decomposition of Pectin during Thermal Process

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Received September 1, 1997; Accepted May 6, 1998

The softening of Japanese radishes and the decomposition of pectin (0.5% pectin solution in 1/10 M phosphate buffer, pH 6.5) during the thermal process (100°C–110°C) under atmospheric and high pressures (100 MPa–400 MPa) were investigated. High pressure decreased the rates of softening for Japanese radishes and the decomposition of pectin during the thermal process. The activation volumes for the softening of Japanese radishes were 4.3 cm³/mol at 100°C and 3.1 cm³/mol at 110°C, and those for the decomposition of pectin were 10.4 cm³/mol (100°C) and 8.1 cm³/mol (110°C). Though the values indicated that the pressure lowered the pectin decomposition more than the softening of the radish, the ratios of the activation volume at 110°C to that at 100°C for both phenomena were nearly the same. These results suggested that the resistance to softening of the radish by pressure is closely related to the inhibition of the pectin decomposition in the radish by pressure.

Keywords: high pressure, thermal process, Japanese radish, β -elimination of pectin

In the thermal processing of food, developing a good texture and taste is one of the most important objectives as well as the sterilization of bacteria spores, though processing at a high temperature deteriorates some useful properties of the food. Thus, it is important to develop a new technology that reduces the deterioration of the functional properties of food. For this purpose, we have investigated the effects of the thermal processes combined with high pressure treatment.

Many studies on the utilization of high pressure for food processing have been reported over the past ten years. It was indicated that the pressure-induced egg gels had useful functions superior to the heat-induced egg gels (Hayashi *et al.* 1989), and also that the appearance, odor, taste and texture of many kinds of foods changed by pressurizing at 500 MPa and at room temperature (Shimada *et al.*, 1990). Some of these phenomena due to pressure are useful for new food processing techniques, and a new type food product like a pressure-processed jam was in fact produced (Horie *et al.*, 1991). On the other hand, the combined effects of temperature and pressure on food components have also been investigated. For example, green tea infusion at 700 MPa+80°C retained most of the tea components such as catechines, vitamin C, and amino acids (Kinugawa *et al.*, 1992). The effect of high pressure on the maillard reaction in the moderate temperature range between 40°C and 60°C was reported to significantly weaken the browning reactions (Tamaoka *et al.*, 1991). Although there are some investigations on the effects of high pressure at the moderate temperatures from 40°C to 80°C on the food components, very few reports on the pressure effects at temperatures higher than 80°C have been done.

The object of this study is to clarify the influence of high pressure during the thermal process at temperatures equal or higher than 100°C on food components. The Japanese radish, a common root vegetable in Japan, was used as the sample.

The Japanese radish is easily oversoftened during thermal processing, and this softening phenomenon is closely related to the decomposition (called trans-elimination) of pectin substances in it (Fuchigami, 1987a). Therefore, the influence of high pressure on the pectin decomposition under the same thermal conditions for the Japanese radish was investigated in order to examine the softening phenomenon of the radish as related to the decomposition behavior of pectin.

Materials and Methods

Japanese radishes Japanese radish root (av. weight: 1000 g, av. length: 370 mm) was used as the sample. The middle part of the radish was cut to 30 mm in width and it was hollowed out with a cork borer into a cylindrical shape (7 mm diameter) in the direction of the tissue axis. This sample (O.D:7 mm, L:30 mm) was placed in a pressure vessel with water.

Citrus pectin A pectin derived from citrus (Kanto Cematic Co., Inc., Tokyo), which is almost as high in methoxylpectin as that contained in Japanese radishes, was used for the measurements with purification as follows. One percent (w/v) pectin powder was dissolved in distilled water, and then ethyl alcohol was gradually added to the pectin solution until reaching 70% (v/v). After the pectin precipitate appeared in the solution, it was collected by centrifugation (1400×g). The precipitate was washed successively with a higher concentration of alcohol and finally with 100% ethanol and diethyl ether. After the precipitate was dried at room temperature, it was used as the purified pectin. This purified pectin was redissolved in M/10 phosphate buffer (pH 6.5) so that the pectin concentration become 0.5% (w/v) immediately before the experiment. Two milliliter of the pectin solution was poured into a sample container (4×100-mm silicone rubber tube sealed at one end) without air

bubbles, and the open end of the container was sealed up with a silicon rubber plug.

Pressurizing apparatus The apparatus used has been described elsewhere (Okazaki *et al.*, 1996).

Pressurizing procedure The Japanese radish samples or the sample container filled with the pectin solution was inserted into the pressure vessel. After the required pressure was applied to the sample, the pressure vessel was placed for 5 min in a water bath (25°C) to make sure that the initial temperature of each sample was the same. After this procedure, the pressure vessel was kept in an oil bath at 100°C or at 110°C ($\pm 0.2^\circ\text{C}$) from 5 to 60 min. The pressure vessel was then transferred to the water bath again for 5 min to cool the sample. The sample was taken out of the pressure vessel after bringing it to atmospheric pressure. During the initial period of heating, the pressure increased to 20–30 MPa due to the thermal expansion of the water in the vessel. Therefore, the pressure was adjusted by controlling the valve.

Hardness of Japanese radish The treated Japanese radish samples were cut to a 5 mm length and the hardness (N) was measured using a tensipresser (TTP-50BXII, Take-moto Co., Ltd., Tokyo) under the following conditions. The plunger (plane shape, 20 mm ϕ) speed was 120 mm/min, and the highest force recorded was measured as the hardness of the sample. The average hardness was calculated from four measurements.

Degree of softening The degree of softening (DS) of the Japanese radish was defined as the ratio of the hardness of the material treated with or without pressure to that of raw material. Thus, the DS value indicates that the material became hard when the value was larger than unity, and became soft when it was lower than unity.

Measurement of trans-elimination of pectin When pectin solution is heated at above pH 5, 4,5-unsaturated pectic acid is produced by *trans*-elimination (β -elimination) (Albersheim *et al.*, 1960). The 4,5-unsaturated pectic acid reacts with thiobarbituric acid, and the products indicate a peak absorbance at 548 nm. The reaction was carried out according to the following method (Okamoto *et al.*, 1964). After 0.5 ml of sodium periodate (0.025 N in 0.125 N H₂SO₄) was added to

0.4 ml of the pressurized pectin solution in a glass tube with a cup, the pectin solution was heated at 80°C for 15 min. One milliliter of sodium arsenite (2% in 0.5 N HCl) was then added to the pectin solution. After waiting for 2 min, 4 ml of thiobarbituric acid (0.3%) was added to the pectin solution, and the solution was heated at 100°C for 10 min. The absorbance of the solution was measured at 548 nm after it was cooled to room temperature. In this study, the progress of the β -elimination was directly indicated by the change in the absorbance at 548 nm.

Results and Discussion

The hardness of most of the raw Japanese radishes measured in this study was ca. 50 N, and those of the radishes softened at 100°C for 15 min were ca. 10 N. This meant that the DS value was 0.2, and the softened radish was appropriate for eating according to the organoleptic test in our laboratory. Thus, a DS value lower than 0.2 indicates that the radish is in the an “overcooked” state.

The effect of high pressure on the softening of Japanese radishes during the thermal processings at 100°C and 110°C for 15 min is shown in Fig. 1. Under atmospheric pressure, the DS values reached 0.2 or lower. However, under pressurized conditions, the DSs rose with the increase in pressure from the values heated at atmospheric pressure even under the same thermal conditions. Note that the DS values were about 0.8 or higher when the radishes were heated at 100°C at the pressures higher than 300 MPa. The Japanese radishes in these cases retained almost raw hardness. The tissue of the radish heated at 110°C for 15 min under atmospheric pressure was almost destroyed. However, the tissue was not destroyed when the radish was heated at pressures higher than 300 MPa. From these results, it is obvious that high pressure inhibited the softening of the Japanese radishes during the thermal process.

The high pressure also influenced the hardness of the Japanese radish treated without heating treatment. The DS values of the radish pressurized at 25°C under pressures higher than 200 MPa were about 1.1 or higher. This means that the Japanese radish became hard by only applying high pressure. Kasai *et al.* (1995) have previously shown the same phenome-

Fig. 1. Effect of high pressure on softening of Japanese radish during thermal process (■: 25°C, ●: 100°C, ▲: 110°C) for 15 min. *DS; degree of softening (DS=hardness of treated material/that of raw material)

Fig. 2. Changes in hardness of Japanese radish during thermal processes at 100°C under high pressure (▼: untreated, ●: 0.1 MPa, ■: 200 MPa, ▲: 400 MPa). *DS is the same as that of Fig. 1.

non, and discussed the effect of high pressure on the activities of some enzymes which affect pectic substances in the tissue.

The influence of time on the DS value of the Japanese radish heated at 100°C under atmospheric pressure, 200 MPa and 400 MPa is shown in Fig. 2. At atmospheric pressure, the DS value reached 0.2 during heating for only 15 min. However, it took about 36 min to reach the same DS value at 200 MPa, and the radish did not soften to the DS value required to eat the radish even when the sample was treated for more than 50 min at 400 MPa. These results showed that the high pressure significantly delayed the softening rate, and the effects of higher pressure were greater.

The results shown in Fig. 2 indicated that the softening of the Japanese radish during the thermal process at high pressure could be analyzed by a first order kinetic model as well as that during the thermal process at atmospheric pressure (Okazaki *et al.*, 1997). The softening rate constant and the regression coefficient at each pressure level evaluated by the linear regression are shown in Table 1 (the data at 100 MPa and 300 MPa, and those at 110°C are not shown in Fig. 2). The obtained regression coefficients were higher than 0.9 except for the results at 300 MPa and 400 MPa at 100°C and 400 MPa at 110°C.

Fuchigami (1987b) has already studied the relationship between the maceration of vegetables and β -elimination of pectins in vegetables during cooking. If the softening of a vegetable is closely related to the β -elimination of pectin, the high pressure might influence the β -elimination rate of pectin during the thermal process. The effects of high pressure on the β -elimination of the citrus pectin, which is almost as high in methoxylpectin as that contained in Japanese radish, are shown in Fig. 3, where the experimental conditions were the same as those shown in Fig. 1. The high pressure significantly inhibited the β -elimination of the pectin. The higher pressure delayed the β -elimination more effectively at both 100°C and 110°C. Furthermore, the effect of the pressure on the β -elimination as shown in Fig. 3 indicated nearly the same tendency as the effect of the softening of the Japanese radish shown in Fig. 1.

The progress of β -elimination of pectin at 100°C under normal and high pressures (200 MPa and 400 MPa) are shown in Fig. 4. The β -elimination rapidly progressed at atmospheric pressure compared with that at high pressures. The high pressure significantly inhibited the progresses of β -elimination. At 400 MPa, the β -elimination changed only one-third of the equilibrium value for atmospheric pressure,

even after the pectin solution was treated for 180 min. The relationship between the progress of β -elimination and pressure at 110°C (figure not shown) was almost the same as that at 100°C shown in Fig. 4.

The kinetic study of the β -elimination of pectin during the thermal process at atmospheric pressure was discussed in a previous paper (Okazaki *et al.*, 1997), in which the decomposition ratio of pectin (X_p) is defined by the following equation;

$$X_p = (A - A_0) / (A_e - A_0) \quad (1)$$

where A is the absorbance (at 548 nm) of the treated pectin solution, A_0 is the initial absorbance of the pectin solution (0.02) and A_e is the equilibrium value of β -elimination (0.243) that was determined as the absorbance of pectin solution heated at 110°C for 8 h. If the β -elimination process obeys a first order kinetic model, the next equations are available;

$$dX_p/dt = k_p(1 - X_p) \quad (2)$$

$$\ln(1 - X_p) = -k_p \cdot t \quad (3)$$

Fig. 3. Effect of high pressure on β -elimination of citrus pectin during thermal processes (■: 25°C, ●: 100°C, ▲: 110°C) for 15 min. Pectin solution is 0.5% (w/v) with 1/10 M phosphate buffer (pH 6.5).

Fig. 4. Progress in β -elimination of pectin at 100°C under high pressure (▼: untreated, ●: 0.1 MPa, ■: 200 MPa, ▲: 400 Ma).

Table 1. Kinetic parameters for softening of Japanese radish by thermal process under high pressure.

Temp. (K)	Pressure (MPa)	Rate constant ($\times 10^{-3} \text{ s}^{-1}$)	r^2	Apparent activation volume (ml/mol)	r^2
373.2	0.1	0.577	0.946	4.25 ± 0.59	0.946
	100	0.365	0.949		
	200	0.339	0.916		
	300	0.186	0.715		
	400	0.167	0.813		
383.2	0.1	1.817	0.953	3.13 ± 0.56	0.969
	200	1.005	0.917		
	400	0.735	0.960		

Table 2. Kinetic parameters for decomposition of pectin by thermal process under high pressure.

Temp. (K)	Pressure (MPa)	Rate constant ($\times 10^{-4} \text{ s}^{-1}$)	r^2	Apparent activation volume (ml/mol)	r^2
373.2	0.1	1.467	0.995	10.35 ± 1.70	0.925
	100	0.922	0.998		
	200	0.690	0.990		
	300	0.131	0.930		
	400	0.084	0.855		
383.2	0.1	3.290	0.991	8.14 ± 0.69	0.986
	100	1.795	0.983		
	200	1.182	0.991		
	300	0.533	0.955		
	400	0.531	0.969		

where k_p is the decomposition rate constant (1/s) and t is the time (s).

From the relationship between $(1-X_p)$ and the treatment time, the decomposition rate constant at each pressure is determined by the linear regression. The decomposition rate constants and the regression coefficients are presented in Table 2. The regression coefficients of the rate constants were obtained in the range from 0.93 to 0.99 except for the case at 100°C+400 MPa. This result indicates that the decomposition of pectin by β -elimination could be represented by a first order kinetic model even at high pressures.

By plotting the logarithmic values of the softening rate constants or the decomposition rate constants as a function of pressure at a constant temperature, activation volumes (ΔV , cm³/mol) could be calculated from the slopes of the linear regression curves, $\Delta V/(RT)$, where R is a gas constant (8.314 cm³·MPa/(K·mole)) and T is the absolute temperature (K). The activation volumes for the softening of Japanese radish were 4.3 cm³/mol at 100°C and 3.1 cm³/mol at 110°C (Table 1), and those for the decomposition of pectin were 10.4 cm³/mol at 100°C and 8.1 cm³/mol at 110°C (Table 2). Although both values are positive, the latter values were approximately twice as large as the former ones. These results indicated that the pressure lowered the pectin decomposition more than the softening of the radish. Also, nearly the same ratios for the activation volume at 110°C as that at 100°C for both phenomena (1.4 for the softening of Japanese radish and 1.3 for the decomposition of pectin) suggest that the problem of radish softening by pressure is closely related to the inhibition of the decomposition of pectin in the radish by pressure.

In general, when bond cleavage in a chemical reaction is suppressed by pressure, the activation volume has a positive value (Asano & Noble, 1978). The activation volumes evaluated in this study were also positive values, and this indicates that the softening of the Japanese radish is related to bond cleavages or decomposition of some components such as pectin (Okazaki *et al.*, 1997), which is suppressed by pressure. The result that the activation volumes for softening of the Japanese radish are approximately half those for the decomposition of pectin suggests that the softening of the Japanese radish during the thermal process under high pressure may not solely caused by decomposition of pectin by β -elimination. Other physico-chemical changes and/or enzymatic reactions except for β -elimination *in vivo* may also

possibly affect the softening of the Japanese radish during the thermal process. Although both activation volumes for the softening and the decomposition became smaller at higher temperature, the limits of this study preclude time for further discussion on this interesting phenomenon.

The hardness or tenderness of food is one of the key elements in its deliciousness. Usually, plant substances exposed to a thermal process for a long time are often too soft. For instance, root vegetables such as Japanese radish and carrot, fruits such as apples, cherries and peaches are easily over-softened by heating, and therefore, it is relatively difficult to use these substances on food products processed by heating at temperatures higher than 100°C. However, if the thermal process is combined with high pressure, the over-softening of such food substances may be avoided, since the softening of vegetables and fruits during heating depends on the decomposition of pectic substances by β -elimination, as shown in this Japanese radish study.

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