EFFECT OF MONO- AND DIVALENT METAL IONS ON THE HARDNESS OF PREHEATED VEGETABLES

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1. Introduction

It is well known that such plant foods as vegetables and fruits are hardened by preheating around 60 ℃, and that this preheating suppresses their softening during cooking near the boiling point of water[1-4].

This hardening or firming is useful for maintaining the plant tissue strength. The hardening effect becomes stronger when a calcium salt is added [5]. It has been reported (1) the hardening was controlled by deesterification of the pectins by pectin esterase (PE) activated by liberated intracellular electrolytes, predominantly potassium ions, and (2) that the enzyme reaction increased that the amount of free carboxyl groups in cell-wall pectin which were bridged by calcium and magnesium ions[1]. In the case of Japanese chestnut, Manabe [6] has examined which was more important and concluded that the main contribution was from demethylation by PE and not by binding of the divalent metal ions to free carboxyl groups. Some factors other than (1) and (2) have also been proposed[7,8]. In this work, we investigated how the hardening due to preheating would be controlled by mono- and divalent ions through processes (1), (2) or by something else. There exist in plant tissues mainly the potassium >>sodium ≈ calcium>magnesium ions, and these metal ions need to be systematically investigated for a better understanding of the hardening mechanism.

Van Buren et al.[9] have shown that when sodium and calcium chlorides contained in the substrate pectin increased, the activity of bean PE was maximum against the molar concentration (M=mol/l); 0.2 and 0.05 M, respectively, for NaCl and $CaCl_2$. Thus the PE activity was different even when the molar concentrations of mono- and divalent metal ions had been made equal. The effect of electrostatic interaction between ions in an aqueous solution can be more generally expressed in terms of the ionic strength given by, $I=(1/2)\Sigma m_i z_i^2$, where m_i is the molality of the i-th ion and z_i is the valence of the ion, all of the ions contained in the aqueous solution being summed. We therefore examined here which would be more useful, the molar concentration or ionic strength. There have been a small number of separate studies on the effects of metal ions either on the PE activity[9] or on the hardness during heating.

We examined in this study the effects of mono- and divalent metal ions on the PE activity and simultaneously how these metal ions affected the hardness samples preheated in aqueous solutions containing the sodium, potassium, calcium and magnesium ions individually. We adjusted the ionic strength to 0.15 mol/kg; this value is equal to a 0.85 % (w/w) aqueous solution of NaCl which corresponds to the osmotic pressure in actual plant tissues.

2. Material and methods

2.1 Sample preparation

Japanese radish was bought in a retail store on the day of the experiment. A sample from the middle part of the Japanese radish was cut into cubes of (1cm x 1 cm x 1 cm) by means of a die.

2.2 Heat treatments

The sample was preheated in a water bath at 60 ℃ for 2 h in aqueous solutions of sodium, potassium, calcium and magnesium chlorides. The ionic strength (I) of each chloride salt solution was adjusted to 0.15 mol/kg. Each sample preheated at 60 ℃ for 2 h is referred to as the preheated sample. After preheating, each sample was cooked at 99.5 ℃ for 10 min.

2.3 Measurement of PE activity

Manabe's method[6] was applied to measure the PE activity. To 10 g of sample was added 40 ml of 0.5 M sodium acetate. The mixture was stirred in a vessel while ice-cooling until thoroughly mixed and then left overnight at 5 ℃. The resulting suspension was passed through filter paper. A cellulose tube (43 mm width, 0.02 mm thickness) was filled with the crude enzyme extract obtained. The extract was dialyzed with deionized water by constant stirring at 10 ℃ for 24 h. Sodium, potassium, calcium and magnesium chlorides were added to 0.5 % citrus pectin, the molality of these chloride salts in pectin being in the range of 0.033 to 0.3 mol/kg corresponding to a range of ionic strength of 0.1 to 0.3 mol/kg. A 10-ml portion of the dialyzed or non-dialyzed crude enzyme extract was added to 20 ml of the 0.5 % citrus pectin solution containing the chloride salts. The pH value was rapidly adjusted to 7.5 with 0.02 M NaOH, and the extract was titrated at pH7.5 with 0.02 M NaOH at 50 ℃ for 15 min. The PE activity (min^{-1} g^{-1} of dry matter) was calculated as μmol of galacturonic acid released. Results were obtained from the measure of three to five replicated assays.

2.4 Measurement of hardness

The hardness of each sample was measured by a texturometer under the following conditions: the plunger used was a V-type, the clearance was 1 mm, and the bite speed was 6 times/min. The average hardness (N) was determined from seven to ten runs.

2.5 Measurement of the metal ions

The contents of the potassium, sodium, calcium and magnesium ions in the dialyzed and non-dialyzed crude enzyme extracts were determined by atomic absorption spectrophotometry. Lanthanum chloride was added to suppress interference from anions like phosphoric ions; the final concentration was 5 mM. The wavelengths used were 589, 766.5, 422.7, 285.2 nm for the potassium, sodium, calcium and magnesium, respectively.

3. Results and discussion

3.1 Effect of metal ions on the PE activity

It is important to investigate systematically how each mono- and divalent metal ion in the plant tissue can affect the PE activity. We examined the effects of the metal ions contained in plant tissue on the PE activity by using crude enzyme and non-crude enzyme extract. Before investigating the ion effects, we removed the metal ions contained in the crude enzyme extract by dialyzing against deionized water.

Table 1 shows the compositions of metal ions in the crude enzyme extracts before and after dialysis and their PE activities. In the case of the extract before dialyzing, the amount of the Na^+ ion was much greater than the other ions due to the sodium acetate used for extracting the crude enzyme. The metal ions were decreased to less than 3 % by the dialysis except for the calcium ions. As a result, the PE activity only decreased to 25 %. This indicates that the metal ions non-linearly affected the PE activity. The exceptional behavior of the calcium ions seems to indicate a stronger binding affinity to some tissue components.

Table1 Composition of metal ions in the crude enzyme extract of Japanese radish before and after dialyzing

Crude enzyme extract	Metal ions (mM)				PE activity
	K^+	Na^+	Ca^{2+}	Mg^{2+}	(min^{-1} g^{-1} of DM)
Before dialyzing	5.3	22.3	0.5	0.3	16.7±3.2
After dialyzing	0.04	0.7	0.1	0.004	4.0±0.3

The crude enzyme extract was dialyzed by deionized water.

In order to examine the effects of individual metal ions on the PE activity, the mono- and divalent ions were added to a pectin solution which served as the substrate for PE.

The PE activity was increased by the addition of each kind of mono- and divalent metal ions, although it depended more strongly on the divalent metal ions than on the monovalent metal ions. The molality dependence of the PE activity could not be represented by one but two separate curves. When the ionic strength was used instead of the molality, the dependence of the PE activity could be ascribed more simply.

Table2 Effect of the ionic strength of the salt solutions on the PE activity of Japanese radish

	PE activity (min^{-1} g^{-1} of DM)		
	I=0.1	I=0.2	I=0.3
KCl	10.7±2.3	20.1±2.7	18.5±3.3
NaCl	10.5±2.0	21.7±1.2	20.0±1.1
$CaCl_2$	19.0±2.9	22.1±3.1	26.0±4.2
$MgCl_2$	20.3±2.5	24.1±2.4	29.1±5.7

I: ionic strength (mol/kg)

The difference in the PE activity between the mono- and divalent metal ions was relatively small at around I=0.2 mol/kg. At the other ionic strength of 0.1 and 0.3 mol/kg, however, the divalent metal ions had a greater effect on the PE activity than the monovalent metal ions. The unique ionic strength value (0.2 mol/kg) is close to that (0.15 mol/kg) of the actual plant tissue. The solute concentration of the cellular tissue corresponds to 0.15 M NaCl, and its ionic strength is equal to 0.15 mol/kg, quite close to 0.2 mol/kg. To understand the effect of the metal ions on hardening, the ionic strength in the plant tissue should be considered. As already mentioned, the effect of the mono- and divalent ions on the PE activity can be regarded as being almost the same at an ionic strength corresponding to that of the plant tissue. In view of this finding, we adjusted the solute concentrations of the various chlorides.

3.2 Effects of the mono- and divalent metal ions on the hardness

We examined the difference in the hardening effect between the mono- and divalent metal ions at a fixed ionic strength (0.15 mol/kg) for the reason already given.

Table 3 shows the hardness of the samples preheated in individual aqueous solutions of potassium, sodium, calcium and magnesium chlorides before and after cooking at 99.5 ℃. There was a significant difference in the

hardness between the non-preheated and preheated samples before and after cooking. The hardening took place to a similar extent with or without metal ions added. This suggests that comparable amounts of metal ions were already contained in the tissues. This means that the deesterification of pectin by PE was the main cause of hardening and that the effect of binding between the divalent metal ions and pectin was not a major factor as elucidated by another study[6]. It is well known that the effect of a pretreatment on the hardness of samples after cooking was greater than that before cooking. This can be explained by the softening with cooking mainly being caused by the βelimination of pectin. The lower the degree of the esterification of pectin, the less the βelimination.

Table3 Hardness of Japanese radish preheated in the salt solutions

	Hardness (N)	
	(1)Before cooking	(2)After cooking
Control	26.0±2.1	3.8±1.2
Deionized water	38.0±1.8	34.0±1.8
KCl	36.9±3.6	30.9±3.6
NaCl	34.7±3.1	30.7±3.1
$CaCl_2$	37.1±2.5	40.1±2.5
$MgCl_2$	35.6±1.7	36.6±1.7

The ionic strength of each salt solution was adjusted to 0.15 mol/kg.
(1) Samples other than the control were preheated at 60 ℃ for 2 h.
(2) Samples other than the control were cooked at 99.5 ℃ for 10 min.

Compared to the difference between the non-preheated and preheated samples after cooking, there was only a small difference in hardness between the mono- and divalent metal ions added. The hardness of the sample treated with the divalent metal ions was slightly greater than that treated with monovalent metal ion. This small difference could be ascribed to a difference in the affinity of these ions to pectin. An experiment on a pectin was in the order of $Na^{+}>K^{+}>Ca^{2+}>Mg^{2+}$ [10]. When the monovalent ions were replaced by divalent ions, the tissue strength became stronger, as mentioned in the introduction. We found that, however, that the effect of replacing the monovalent ions by the divalent was much smaller than we expected.

3.3 Decrease in pH value by preheating

We measured the pH value of each salt solution before and after preheating because the deesterification of pectin by PE was expected to cause a decrease in the pH value due to the production of the acid form of pectin.

As shown in Table 4, the pH values measured for the various solutions were decreased by preheating with or without metal ions. The difference in pH value before and after preheating the aqueous solutions containing the metal ions was slightly greater than that of deionized water. This indicates that binding between the metal ions and the free carboxyl groups of pectin had followed deesterification. It has been reported that the reduction in pH of a calcium chloride solution used for cooking potatoes was possibly due to the binding of calcium ions to the potato tissue, and that this calcium binding resulted in an increase in the hydrogen chloride concentration in the aqueous solution[11]. Binding has been reported between calcium and pectin.

Table4 Change in pH value of the salt solutions during the preheating Japanese radish			
		pH value	
	Before preheating	After preheating	Difference
Deionized water	6.34±0.10	5.87±0.20	0.47
KCl	6.23±0.13	5.5±0.24	0.73
NaCl	6.09±0.08	5.95±0.13	0.64
$CaCl_2$	5.95±0.12	5.25±0.04	0.7
$MgCl_2$	5.92±0.05	4.97±0.16	0.95
The ionic strength of each salt solution was adjusted to 0.15 mol/kg.			

The effect of calcium chloride on the reduction in pH values is expected to have occurred in the cases of the other salt chlorides studied here. In the aqueous solution containing the magnesium ions, the decrease in pH value was slightly greater than that of each other metal ion. It is suggested that the effect of magnesium ions on PE was different from that of the calcium ions, despite their same valence.

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