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Pectic Changes of Bamboo Shoots During Cooking

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When bamboo shoots were cooked at various pH levels, Bamboo shoots cooked at pH 4.0 were the firmest, followed by distilled water, the pH 12.0 cook-water and the pH 1.0 cook-water. The amount of galacturonic acids which were released into cooking solutions was largest at pH 12.0, and least at pH 4.0. Pectic polysaccharides released from bamboo shoots during cooking above pH 6 gave positive results through thiobarbituric acid tests. This suggests that pectic polysaccharides of bamboo shoots degraded through trans-elimination during cooking above pH 6. The pectic polysaccharides of raw and cooked bamboo shoots were fractionated. The content of PC (sodium hexametaphosphate soluble pectin, low methoxyl pectin) in tissues decreased, and that of PA (HCl-soluble pectin, high methoxyl pectin) increased while cooking, but already cooked tissues contained a considerably large amount of xylose and glucose rich PC. One of the reasons why bamboo shoots were difficult to soften was that they contained a large amount of PC. Neutral sugars were largely released into cooking solutions as the pH decreased. Hemicellulose also greatly affected the softening of bamboo shoots especially at low pH level.

Introduction

Bamboo shoots were more difficult to disintegrate during cooking than other vegetables (potato, Japanese radish, carrot, burdock, and East Indian lotus), and never collapsed during cooking.[1] The pectic substances of bamboo shoots were fractionated with three reagents.[2)3)] The uronic acid composition of HCl-soluble pectin (PA), acetate buffer-soluble pectin (PB) and sodium hexametaphosphate-soluble pectin (PC) were about 8～10%, 2～4%, and 88%, respectively.[1] Conversely, the other vegetables contained only a small amount of PC and completely disintegrated after the extraction of PA and PB; bamboo shoots maintained considerable firmness under the same conditions. Acidic polysaccharides in bamboo shoots separated by DEAE-cellulose column chromatography contained large amounts of neutral sugars. This suggested that the glucose and xylose rich PC (low methoxyl pectin) affected the solubilization of pectic polysaccharides and thermal disintegration of bamboo shoots.[1] According to the previous paper,[4] bamboo shoots maintained considerable firmness after being heated with the sodium oxalate solution (pH 4.0) for 1hr 10 times, but they easily macerated when they were soaked successively in 0.1N sodium hydroxide solution at room temperature for 24 hr. The bamboo shoots containing the pectic polysaccharide which was difficult to extract with sodium oxalate solution seemed to be one of the cause of difficulties in thermal

maceration of bamboo shoots.

The objective of the present study was to investigate the resistance to softening of bamboo shoots, focusing on change in texture, release of pectic polysaccharides, and changes in the composition and properties of pectic polysaccharides in tissues, when bamboo shoots were cooked in solutions at various pH levels.

1. Materials and methods

1) Sample preparation. Bamboo shoots *(Phyllostachys pubescens, Mazel ex Houz. de Lehaie;* about 1.4 kg, 30 cm long) were harvested in Okayama in May, peeled and cut into three segments (top, middle and bottom segments). These were decorticated with a cork borer to produce cylinders of the tissue 10.0 mm in diameter and then cut into disks 5.0 mm long.

2) Cooking procedure. Disks (5.0 g) of bamboo shoots were dropped into the following boiling solutions (10 ml) in the test tubes; distilled water, 0.2N HCl-KCl buffer (pH 1.0), 0.1M sodium acetate buffer (pH 4.0), or 0.1N NaOH-sodium phosphate buffer (pH 12.0). The test tubes were sealed to prevent evaporation. After heating for 1 hr in a boiling water bath, the tubes were rapidly cooled in tap water for 10 min, and then the pH of cooking solutions was measured.

3) Hardness testing. Changes in the texture of tissues were measured using the Kiya hardness tester (Kiya Seisakusho Ltd. Tokyo). The experimental results, shown, are the average of twelve measurements.

4) Analysis of cooking solutions. The cooking solutions, which were adjusted to pH 7.0, were made alkaline by immersing in 0.05N NaOH for 90 min at 0°C, then adjusted to pH 6.0 with acetic acid and placed on a DEAE-cellulose column (2.0× 5.0 cm) that was previously equilibrated with a 0.02M acetate buffer solution (pH 6.0). The column was first washed with an equilibrating buffer and then eluted with 0.1N NaOH. The first fraction contained neutral sugars (neutral polysaccharides) and the second fraction contained acidic sugars (pectic polysaccharides). The amounts of total sugars and galacturonic acid were determined by the phenol sulfuric acid method[5] and the carbazole method,[6] respectively. The composition of monosaccharides was determined by the gas chromatographic procedure of Kusakabe et al.[7] To investigate the degree of trans-elimination, the thiobarbituric acid test was performed on the cooking solutions by the method of Okamoto et al.[8]

5) Extraction of pectic polysaccharides from bamboo shoots. The pectic polysaccharides were fractionated by successive extraction using the three reagents from raw or cooked bamboo shoots. The pectic polysaccharides in samples homogenized with 0.01N HCl solution were extracted with 0.01N HCl solution (pH 2.0) at 35°C 5 times every 24 hr. The extracts were designated as pectin A (PA). Subsequently, the residues of PA were extracted with 0.1M sodium acetate buffer solution of pH 4.0 at 35°C 3 times every 24 hr. The extracts were

designated as pectin B (PB). The pectic polysaccharides in the residues of PB were extracted with 2% sodium hexametaphosphate solution of pH 4.0 at 90°C 6 times every 24 hr. The extracts were designated as pectin C (PC). Each extracts were concentrated to about 10 ml at pH 4.0 and dialyzed against disilled water (2000 ml) at 5°C 2 times every 24 hr. The size of visking tube was 19.1 mm in diameter. The amount of galacturonic acids of PA, PB and PC was determined by the same procedure as analysis of cooking solution.

6) Fractionation of polysaccharides by DEAE-celluloce column chromatography.

The DEAE-cellulose column chromatography of PA and PC, which were extracted from cooked bamboo shoots, was performed by the same method as the previous papers.[1)4)]

The extracts of polysaccharides were added to a DEAE-cellulose column (2.0×5.0 cm) equilibrated with a 0.02M acetate buffer solution (pH 6.0). The column was first washed with an equilibrating buffer and then eluted successively with 0.1→1M acetate buffer of pH 6.0 (linear gradient) and 0.1N NaOH. The first fraction (neutral polysaccharides: non uronide) was designated as fraction Ⅰ, and the second fraction (weakly acidic pectic polysaccharides) was designated as fraction Ⅱ, and the third fraction (pectic acid) was designated as fraction Ⅲ. The fractions were monitored by the phenol sulfuric acid method[5)] and the carbazole method.[6)] The amount of neutral sugars was calculated by the method of Hatanaka and Ozawa.[9)]

Results and discussion

1. Effect of pH on the release of pectic polysaccharides of bamboo shoots into the cooking solutions

The effect of pH on the softening of bamboo shoots is shown in Fig. 1. The pH of cooking solution changed considerably during cooking, especially from pH 12.0 cook-water to about pH 9, pH 4.0 cook-water to about pH 4.5 and pH 1.0 cook-water to about pH 1.5. Bamboo shoots cooked at pH 4.0 were the firmest, followed by distilled water, the pH 12.0 cook-water and the pH 1.0 cook-water. Hardness of the three segments of bamboo shoots after cooking

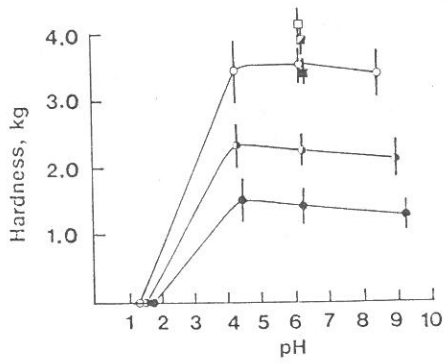

Fig. 1. Effect of pH on the softening of bamboo shoots during cooking
■ Raw top segment
◪ Raw middle segment
□ Raw bottom segment
● Cooked top segment
◑ Cooked middle segment
○ Cooked bottom segment

was different; the bottom > middle > top segment, respectively. Bamboo shoots cooked at pH 1.0 were completely softened.

The amounts of sugars in the cooking solutions separated by DEAE-cellulose column chromatography are shown in Table 1 and Fig. 2. When bamboo shoots were cooked in the various pH solutions, the amounts of neutral sugars eluted in the first fractions were as follows; pH 1.0 > pH 4.0 > water > pH 12.0, respectively (Table 1). Since neutral sugar usually degraded in alkaline solution, the amounts of neutral sugars eluted in pH 12.0 cook-water might be least. Almost all of the neutral sugars eluted in the first fraction were glucose + fructose + sucrose (these sugars gave the same retention time on the gas chromatography). These sugars largely affected the difference in the amount of neutral sugars in the first fraction.

Free fructose, glucose and sucrose were also major constituents of hot 70% ethanol soluble sugar in bamboo shoots.[10] Therefore, free sugars in bamboo shoots also released into cooking solutions. As for the other neutral sugar, a comparatively large amount of arabinose was released into the cooking solution when the bamboo shoot was cooked at pH 1.0 and pH 12.0. Arabinose was extracted easily by acid treatment as usual. The large amounts of xylose and galactose were released into pH 1.0 cook-water, those of mannose and galactose were released into pH 12.0 cook-water solution.

Table 1. The amounts* of monosaccharides released from the middle segment of bamboo shoots during cooking

Fraction by DEAE**	pH of cooking solution	Uronic acid	Neutral sugar	Monosaccharides					
				Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose***
Neutral sugar fraction	1	0	1409.3	6.2	188.7	56.7	11.4	59.5	1087.0
	4	0	789.4	0	11.7	2.4	7.4	7.4	759.1
	water	0	579.6	7.2	3.7	1.2	2.5	2.5	557.6
	12	0	519.8	11.7	120.4	trace	87.7	87.7	297.3
Acidic sugar fraction	1	39.9	50.6	1.8	2.3	8.7	1.0	6.0	30.8
	4	8.3	14.2	0.2	2.0	1.0	0.5	6.1	4.4
	water	39.3	23.7	0.9	6.0	1.1	0.2	4.3	11.7
	12	42.3	34.2	5.9	3.6	4.2	4.6	5.0	10.2

* Fresh weight basis (mg/ 100g).

** Neutral sugar and acidic sugar fractions: 0.02M acetate buffer fraction and 0.1N NaOH fraction separated by DEAE-cellulose column chromatography, respectively.

*** Glucose + fructose + sucrose.

Bamboo shoots contained a large amount of hemicellulose.[1] Hemicellulose was usually eluted in the first fraction on DEAE-cellulose column chromatography. The amount of neutral sugar in the first fraction released during cooking at pH 1.0 was larger than when cooked at pH 12.0. The causes for these results seemed to be that neutral polysaccharides more easily

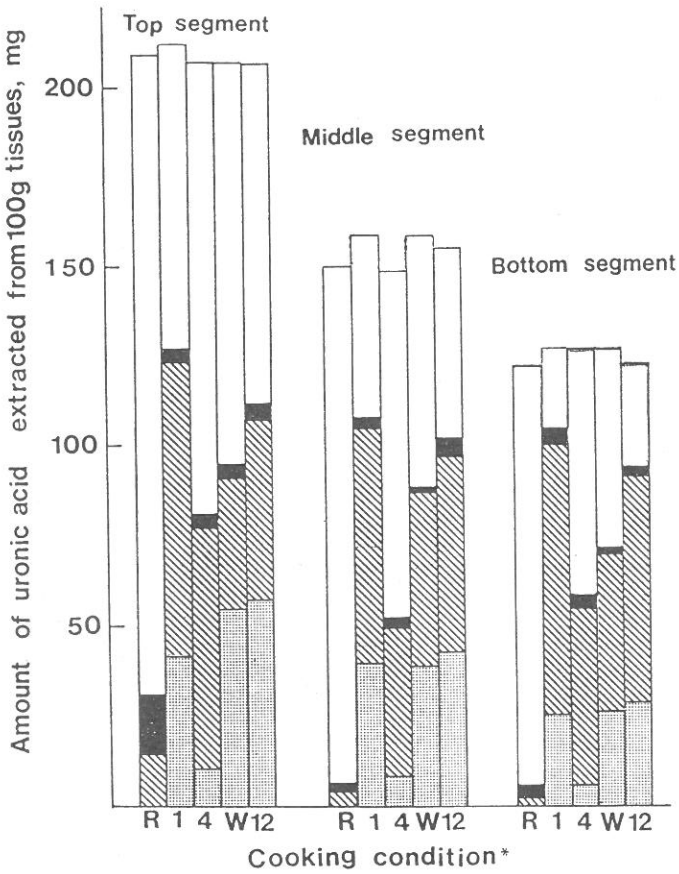

Fig. 2. Change in uronic acid composition of pectic polysaccharides extracted from bamboo shoots cooked at various pH levels

* Cooking condition R, 1, 4, W, 12: raw bamboo shoots cooked in pH 1, pH 4, distilled water, and pH 12 cook-water, respectively.

▒ Uronic acid released into cooking solution. Uronic acid in polysaccharides extracted from cooked 100 g tissues.

▨ PA, ■ PB, □ PC. (PA, PB, PC: see Table 2)

hydrolyzed as the pH decreased. The amount of hemicellulose and cellulose in bamboo shoots were greater in the bottom > middle > top segments, respectively.[1] A remarkable feature of bamboo shoots was that they contained larger amount of hemicellulose and a smaller amount of pectic polysaccharides (uronic acids) than other vegetables. Hemicellulose was more quantitatively important in bamboo shoots than in other vegetables.[1] These results suggested that hemicellulose also greatly affected the softening of bamboo shoots especially at low pH level (especially at pH 1.0).

The amount of galacturonic acid released into cooking solution eluted in 0.1N NaOH on DEAE-cellulose column chromatography was least when bamboo shoots were cooked in pH 4.0

cook- water, and largest from the top segment > middle segment > bottom segment, respectively (Fig. 2). The amount of neutral sugars linked with acidic sugars eluted with 0.1N NaOH solution on DEAE-cellulose column chromatography was largest when bamboo shoots were cooked in solutions of pH 1.0 > pH 12.0 > water > pH 4.0, respectively (Table 1).

Bamboo shoots contained a large percentage of neutral sugars in pectic polysaccharides. The PC contained especially large amounts of glucose and xylose.[1] When bamboo shoots were cooked at pH 1.0 and pH 12, the glucose and xylose in acidic polysaccharides released into cooking solution, was greater at pH 1.0 than at pH 12.0 (Table 1). These neutral sugars in pectic polysaccharides seemed to also affect the softening of bamboo shoots.

Pectic polysaccharides released from bamboo shoots during cooking above pH 6.0 gave positive results using the thiobarbituric acid tests (Fig. 3). These results suggest that the enhanced softening at neutral and alkaline pH may be due to the degradation of methoxylated pectins through the trans-elimination mechanism. At low pH it may be caused by both the hydrolytic cleavage of polysaccharides and removal of divalent cations from the cell walls of bamboo shoots.[2]

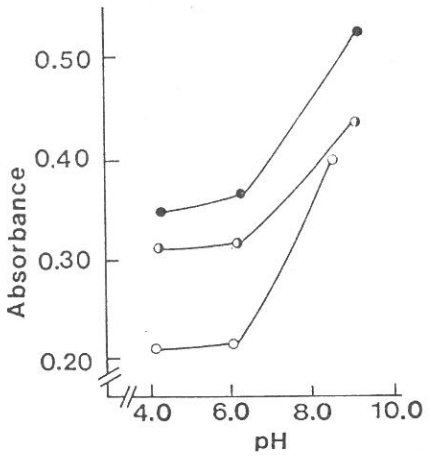

Fig. 3. Thiobarbituric acid test of the cooking solutions of bamboo shoots

● Top segment
◑ Middle segment
○ Bottom segment

2. Change in the uronic acid composition of pectic polysaccharides during cooking

Change in the uronic acid composition of pectic polysaccharides in bamboo shoots after cooking for 1 hr is shown in Fig. 2. The amount of uronic acids of raw bamboo shoots were greater in the top > middle > bottom segments, respectively. The raw bamboo shoots had a large amount of PC. After cooking, the amount of PA increased; on the other hand, that of PC decreased. A comparatively large amount of PC remained in cooked tissues, however. The amount of PB did not change greatly during cooking. The increase of PA seems to be due to the depolymerization of PC by cooking. Bamboo shoots contained a small amount of PA

which was high-methoxyl pectin (the degree of esterification, DE, 50.3%)[1] but PB and PC 1-10[1] (extracted for 3.5 hr×10 times with sodium hexametaphosphate solution), which were relatively low methoxyl pectin (DE of PC1-10, 42.1%), were degraded slightly through the trans-elimination mechanism during cooking (1 hr). Therefore, the amount of pectin released into the cooking solution, and that of PA increased. The DE of PC 11-20[1] (extracted from the residues of PC1-10 for 24 hr×10 times with sodium hexametaphosphate solution) was about 10%,[1] so PC 11-20 was difficult to degrade through the trans-elimination mechanism during cooking and remained in the cooked tissues.

3. Change in DEAE-cellulose column chromatogram of pectic polysaccharides during cooking

DEAE-cellulose column chromatography of PA and PC, which were extracted from the middle segments after cooking in hot water, was performed. The amounts of uronic acid, neutral sugar, and monosaccharides in three fractions separated by DEAE-cellulose column chromatography, are shown in Table 2. The fraction Ⅰ, Ⅱ and Ⅲ were neutral polysaccharides, weakly acidic polysaccharides and pectic acid, respectively.

Table 2. The amounts* and monosaccharide composition of polysaccharides in cooked bamboo shoots** separated by DEAE-cellulose column chromatography[1)4)]

Types of pectin	Fraction by DEAE	Total sugar (T)mg	Uronic Acid mg	Neutral sugar (N)mg	N/T ×100 %	Composition of monosaccharide, mg					
						Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
PA	Ⅰ	102.2	0	102.2	(100)	trace	35.6	8.2	trace	trace	58.6
	Ⅱ	161.9	33.8	128.1	(79.1)	3.3	119.3	1.3	2.8	0.9	0.5
	Ⅲ	65.6	13.1	52.5	(80.0)	1.6	12.1	2.8	8.2	13.5	15.5
PB			2.2								
PC	Ⅰ	335.3	0	335.3	(100)	trace	98.5	185.5	3.7	trace	47.6
	Ⅱ	193.2	42.5	150.7	(78.0)	5.2	13.7	55.7	3.2	72.8	0
	Ⅲ	451.9	26.2	425.7	(94.2)	trace	17.7	68.8	trace	trace	339.3

* Fresh weight basis (mg / 100 g).

** The middle segment of bamboo shoots was cooked in distilled water for 1 hr.

PA (pectin A): Extraction with 0.01N HCl (pH 2.0) at 35°C for 24 hr × 5 times.

PB (pectin B): Residues of PA were extracted with 0.1M soduim acetate buffer solution (pH 4.0) at 35°C for 24 hr × 3 times.

PC (pectin C): Residues of PB were extracted with 2% soduim hexametaphosphate solution (pH 4.0) at 90°C for 24 hr × 6 times.

The neutral polysaccharides fraction (Ⅰ) of PA contained glucose (58.6 mg), arabinose (35.6 mg), and xylose (8.2 mg). That of PC contained xylose (185.5 mg), arabinose (98.5 mg), glucose (47.6 mg), and mannose (3.7 mg).

Fraction Ⅱ of PA and PC was a neutral sugar rich pectin (neutral sugar %/ total sugar,

78～79%). Almost all of the neutral sugar in fraction II of PA was arabinose (119.3 mg). Conversely, fraction II of PC contained a lot of galactose (72.9 mg) and xylose (55.7 mg).

Pectic substances eluted in fraction III contained a large percentage of neutral sugar (PA; 80%, PC; 94.2%). The fraction III of PC contained large amounts of glucose (339.3 mg) and xylose (68.8 mg).

It seemed that PA in raw tissues was released into the cooking solution due to degradation by the trans-elimination mechanism, and arabinose rich PC moved to PA. Even after 1 hr of cooking, a large amount of PC, especially xylose and glucose rich pectin, still remained in the tissues. It appears that xylose and glucose rich PC is difficult to be extracted into hot water, and affects the difficulty in thermal disintegration of bamboo shoots.

References

1) Fuchigami, M.: *J. Food Sci.* **55**, 739 (1990)
2) Fuchigami, M. and Okamoto, K.: *Nippon Eiyo Syokuryo Gakkaishi (J. Jpn. Soc. Nutr. Food Sci.)*, **37**, 57 (1984)
3) Fuchigami, M.: *J. Food Sci.* **52**, 1317 (1987)
4) Fuchigami, M.: *Nihon Kasei Gakkaishi (J. Home Econ. Jpn.)*, **42**, 231 (1991)
5) Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F.: *Anal. Chem.*, **28**, 350 (1956)
6) Galambos, J. T.: *Anal. Chem.*, **20**, 262 (1969)
7) Kusakabe, I., Kamiyama, Y., and Yasui, T.: *J. Agric. Chems. Soc. Japan*, **51**, 167 (1977)
8) Okamoto, K., Hatanaka, C., and Ozawa, J.: *Nogaku Kenkyu*, **50**, 61 (1964)
9) Hatanaka, C. and Ozawa, J.: *J. Agric. Chem. Soc. Japan*, **42**, 645 (1968)
10) Kozukue, E., Kozukue, N., Kurosaki, T.: *J. Food Sci.*, **48**, 935 (1983)

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