Relationship between pectic substances and strand separation of cooked spaghetti squash

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ABSTRACT

Spaghetti squash is one of the hard-shelled squashes in the cucurbit family. When cooked, the flesh can be pulled apart to form strands that resemble spaghetti, hence its name. The purpose of this paper is to investigate the relationship between pectic substances and the separation into strands during cooking of spaghetti squash. Spaghetti squash flesh separated into strands when boiled or soaked in 0.01N HCl solution of pH 2.0. Pectic substances of raw and cooked flesh were fractionated into three reagents. The galacturonic acid compositions of HCl-soluble pectin (PA), sodium acetate buffer-soluble pectin (PB) and sodium hexametaphosphate-soluble pectin (PC) of raw flesh were 69.0%, 28.9% and 2.1%, respectively. Also, the degree of esterification of pectin was greatest to least: PA > PB > PC, respectively and the DEAE-cellulose column chromatograms of PA and PB showed that they were comparatively in high methoxyl pectin. Therefore, about 50% of pectic substances in flesh were released into a cooking solution during 15~30 min of cooking. High methoxyl pectin was degraded by β-elimination during boiling or extracted by soaking in diluted HCl solution. Consequently, the flesh separated into strands. This suggests that high methoxyl pectin glues cells of strands together in the flesh of spaghetti squash.

Keywords: spaghetti squash; pectin; structure; strand separation; cooking

INTRODUCTION

Spaghetti squash (*Cucurbita pepo L.*) is one of the hard-shelled squashes in the cucurbit family and typically grown as a winter squash. It is an American native vegetable. It is rugby ball sized and oval-shaped and the rind is hard and ivory colored at maturity. Its center contains many large squash seeds. It has a mild taste and crisp texture and may be boiled, steamed, baked or microwaved. When cooked, the flesh can be pulled apart to form strands that resemble spaghetti, hence its name.

Pectin is the main component of the middle lamella. It contributes to adhesion between parenchyma cells of vegetables and mechanical strength of tissues. Maceration of vegetable tissues seems to be brought about mainly by the degradation of pectin [1]. The softening of vegetables during cooking is affected by the properties of pectic substances, especially the degree of esterification (DE) [1] [2] [3]. The vegetables, which were easily softened by thermal treatment, contained more HCl-soluble pectin (high methoxyl pectin, PA) than the sodium acetate buffer soluble pectin (low methoxyl pectin, PB) [1] [4]. The high methoxyl pectin easily broke down in a hot neutral solution and alkaline solutions by β-elimination [5] [6] causing vegetables with a greater amount of PA to decreased intercellular adhesion strength. Conversely, the vegetables which had a larger amount of PB were difficult to soften during cooking [4].

Therefore, the cause of the separation into strands during boiling of spaghetti squash seems to be pectic substances which play a substantial role in the maintenance of intercellular cohesion, especially high methoxyl pectin which breaks down by cooking. Thus, the purpose of this paper is to investigate the relationship of spaghetti squash pectic substances and the separation into strands during cooking.

MATERIALS & METHODS

Sample preparation

Spaghetti squash was cut into 2 cm long pieces, peeled and the seeds discarded. Flesh samples were dropped into boiling distilled water and cooked for 15 min, 30 min or soaked in 0.01N HCl solution (pH 2.0) for 24 hrs at 35˚C.

Extraction of pectin

Pectic substances of raw and boiled samples were successively extracted as follows: 0.01N HCl (at pH 2.0 and 35˚C for 24 hrs × 4 times), 0.1M sodium acetate buffer (at pH 4.0 and 35˚C for 24 hrs × 4 times) and 2% sodium hexametaphosphate solution (at pH 4.0 and 90˚C for 3.5 hrs × 3 times) [1] [2]. Each extraction was repeated until no sugar was detected. Each extract was concentrated at pH 4.0 and dialyzed against distilled water at 5˚C for 2 days. These extracts were designated as PA, PB and PC, respectively. The amount of galacturonic acid was determined by the carbazole method [7].

DEAE-cellulose column chromatography

The DEAE-cellulose column chromatography of PA, PB and PC, extracted from raw and 15 min cooked flesh, was performed by the same method reported previously [1] [3]. The pectic substances (about 10 mg of galacturonic acid) were added to a DEAE-cellulose column (2.0 cm in dia, 5.0 cm long) equilibrated with a 0.02M acetate buffer solution (pH 6.0). The column was first washed with an equilibrating buffer solution (fraction I) and then eluted successively with 0.1 → 1M sodium acetate buffer solution of pH 6.0 (linear gradient, fraction II) and 0.1N NaOH (fraction III). The fractions were monitored by the phenol sulphuric acid method [8] and the carbazole method [7]. The amount of neutral sugar was calculated by the method of [9]. Monosaccharides were analyzed by the gas chromatographic procedure [10].
The degree of esterification of PA, PB and PC were determined using a gas-chromatographic procedure [11]. The dietary fibers (cellulose, hemi-cellulose and lignin) were determined by the method of Van Soest & Wine [12].

Texture and structure measurements

Firmness of raw, cooked flesh (5 mm thick) was measured with a rheometer (NRM-2002J, Fudo Ltd., Tokyo, Japan) by a plunger (cylindrical shape: 2 mm in diameter) using a loadcell of 2 kg. Histological structures of samples were observed using a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo, Japan) [13].

RESULTS & DISCUSSION

Changes in texture and histological structure of spaghetti squash during cooking

Changes in visual appearances and cryo-scanning micrographs of spaghetti squash during cooking are shown in **Figures 1** and **2**, respectively. When spaghetti squash was cooked in boiling water for 15 min or 30 min, flesh was softened (raw, 396×10^4 N/m^2; 15 min cooked, 34.5×10^4 N/m^2; 30min cooked, 26.43×10^4 N/m^2, respectively). When scooped out of cooked flesh (with fork "comb" out the strands), they came out in threads and separated into strands like spaghetti. After soaking in 0.01N HCl (pH 2.0) for 24 hrs, flesh tissues were softened (90.1×10^4 N/m^2) and also separated.
The shape of cells which constituted strands was different from the cells which surrounded strands. The former was round and the latter was elongated (**Figure 2**). When cooked, the shape of the former was maintained, but the latter, which contributed to adhesion between strands, broke down. Thus, flesh separated into strands. Cell walls of flesh were compared. After cooking for 15 min, cell separation in middle lamella, due to solubilizing of pectic substances was observed. After 30 min cooking or soaking in 0.01N HCl solution for 24 hrs, the degree of cell separation in middle lamella and the space among microfibrils increased.

Changes in pectic substances of spaghetti squash during cooking

The composition of pectic substances of raw and cooked spaghetti squash fractionated with three reagents is shown in **Figure 3**. The amount (percentage) of PA, PB and PC in the raw sample was 237.4 mg (69.0%), 99.6 mg (28.9%) and 7.3 mg / 100 g (2.1%), respectively. The degree of esterification (DE) of PA, PB and PC was 67.4%, 61.5% and 55.6%, respectively. The percentage of PA, which was high methoxyl pectin, was greatest. Therefore, when squash was cooked for 15 min, PA decreased greatly, because high methoxyl pectin was broken down by β-elimination when it was heated in a hot neutral solution. Therefore, about 50% pectic substances were released into cooking solution during 15~30 min cooking. The DE of spaghetti squash was higher than DE of the other vegetables such as lotus, burdock and bamboo shoot [4] [3]. Thus, the squash was more easily softened than lotus, burdock and bamboo shoot after cooking. The amount of dietary fiber is shown in **Table 1**. The amounts of cellulose, hemicellulose, lignin and pectin of spaghetti squash were similar to Japanese radish root. It is suggested that cellulose, hemicellulose and pectin remaining in strands after cooking maintained crisp tender strands.

Changes in DEAE-cellulose column chromatogram of pectic substances during cooking

To investigate the properties of pectic polysaccharides in detail, PA, PB and PC were fractionated into neutral and acidic fractions by DEAE-cellulose column chromatography. The results are shown in **Figures 4 ~ 5**. The elution patterns of PA, PB and PC on chromatography were different between raw and 15 min cooked squashes. The neutral polysaccharides, weakly acidic polysaccharides and pectic acid are usually eluted in fractions I, II and III, respectively. Almost all galacturonic acid of PA and PB in raw squash was eluted in fraction II, and only small amounts of them were eluted in fraction III. PA was eluted earlier than PB in fraction II; therefore, DE of PA was higher than DE of PB. On the otherhand, almost all of PC was eluted in fraction III. The percentage of PC in squash was small, so DE of pectin in squash was comparatively high.

After cooking, the elution patterns of PA and PB changed. Since the low methoxyl pectin was usually eluted later in fraction II, DE of PB especially decreased during cooking. The high methoxyl pectin was released into the cooking solution, thus comparatively low methoxyl pectin remained in cooked squash.

The monosaccharide composition of pectic substances (fractions II and III) in spaghetti squash separated by DEAE-cellulose column chromatography is shown in **Table 2**. The percentage of galactose / neutral sugar in raw samples was highest, and the percentages of arabinose and rhamnose were comparatively high. Thus, it is suggested that galactan and arabinan are attached with rhamnose residues, the maior branch point of the main chain of pectin (rhamnogalacturonan).

CONCLUSION

Spaghetti squash separated into strands when boiled or soaked in HCl solution of pH 2.0. High methoxyl pectin was degraded by β-elimination during boiling and extracted by soaking; consequently, the flesh separated into strands. This suggests that high methoxyl pectin glues cells together in the flesh of spaghetti squash.

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Table 1. The amount of dietary fiber and the percentage of PA, PB and PC.

Vegetables	**Dietary fiber (mg% on a fresh weight basis)**				**Pectin (% of PA, PB and PC)**		
	Cellulose	**Hemicellulose**	**Lignin**	**Pectin**	**PA**	**PB**	**PC**
Spaghetti squash	691	209	48	344	69.0	28.9	2.1
Bamboo shoot*	1003	1677	72	133	8.2	2.2	88.6
East Indian lotus*	544	126	82	217	30.9	55.7	13.4
Edible burdock*	2249	88	301	985	38.4	53.9	6.7
Carrot*	1036	9	101	849	63.5	33.9	2.6
Japanese radish*	501	252	48	410	45.0	47.3	7.7
Potato*	981	655	155	287	76.8	15.6	7.6

* Fuchigami, M., Journal of Food Science, 55 (3) 739-745, 1990.

PA, PB and PC: see Figure 3.

Table 2. Monosaccharide composition of pectic substances in spaghetti squash separated by DEAE-cellulose column chromatography.

Types of pectin	**Raw or cooked**	**Fraction**	**Composition of monosaccharides (%)**					
			Rhamnose	**Arabinose**	**Xylose**	**Mannose**	**Galactose**	**Glucose**
PA	Raw	II	12.3	24.3	8.2	5.6	49.0	trace
		III	5.4	7.0	14.3	18.2	32.1	23.1
	Cooked for 15 min	II	17.6	21.9	2.2	3.3	55.0	trace
		III	3.3	12.7	0.5	18.7	49.3	15.5
PB	Raw	II	21.5	13.1	10.8	9.3	28.1	17.3
		III	7.9	11.2	7.4	23.8	18.1	34.7
	Cooked for 15 min	II	21.0	7.4	5.5	4.2	36.5	25.4
		III	18.8	2.2	0.0	10.5	15.2	10.6

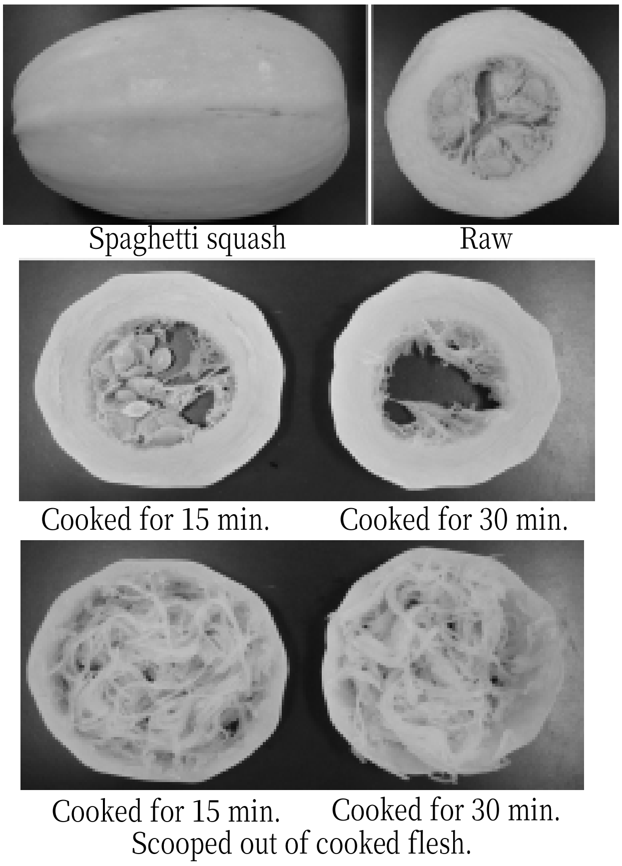

Figure 1. Changes in visual appearances of spaghetti squash during cooking.

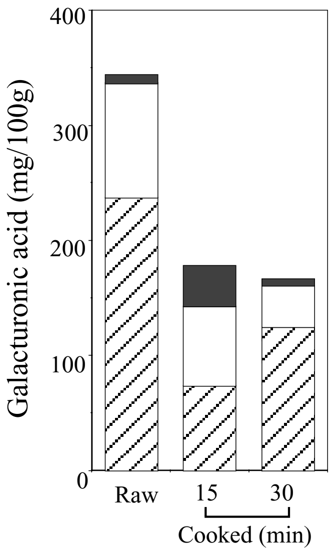

Figure 3. Changes in pectic substances of spaghetti squash during cooking.

PA: 0.01N HCl soluble pectin

PB: 0.1M acetate buffer soluble pectin

PC: 2% sodium hexametaphosphate soluble pectin

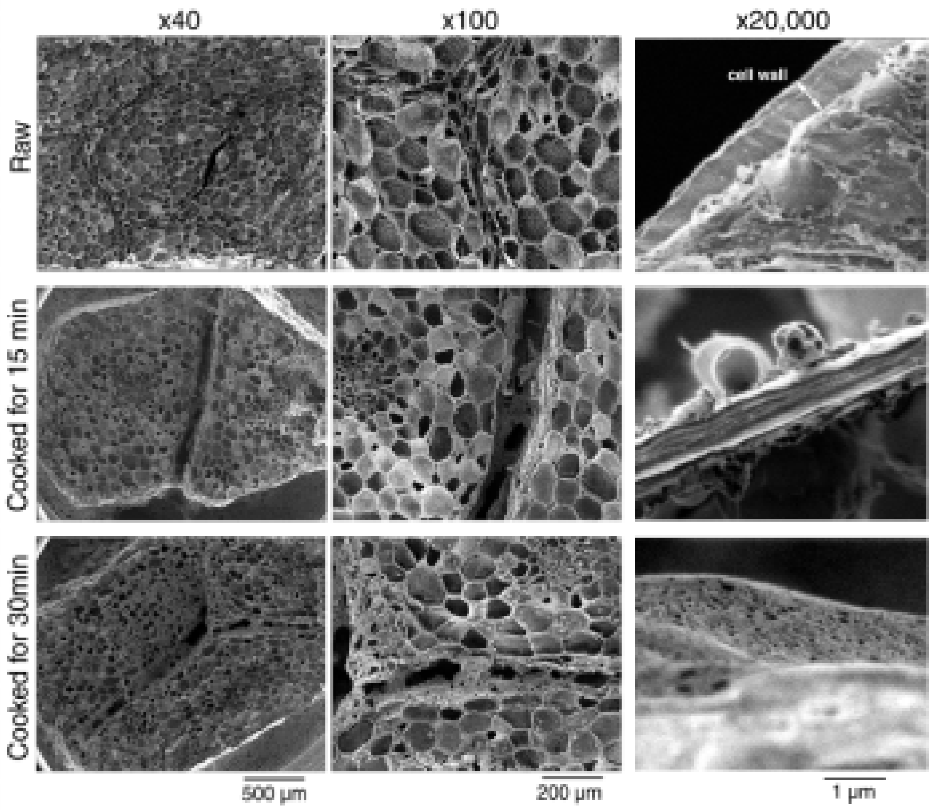

Figure 2. Changes in cryo-scanning electron micrographs of spaghetti squash during cooking.

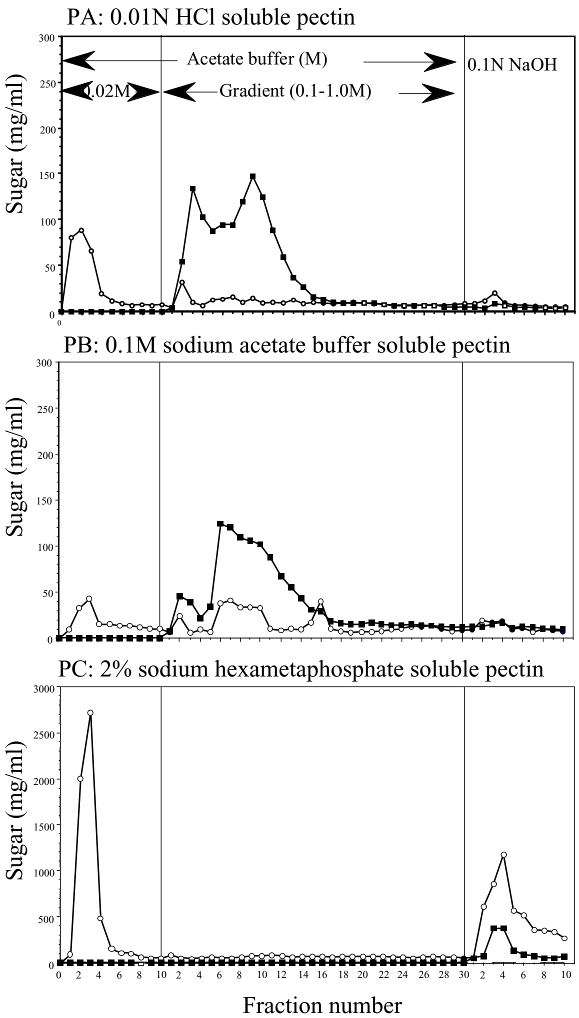

Figure 4. DEAE-cellulose column chromatograms of pectic substances of raw spaghetti squash.

—○— Neutral sugar

—■— Galacturonic acid

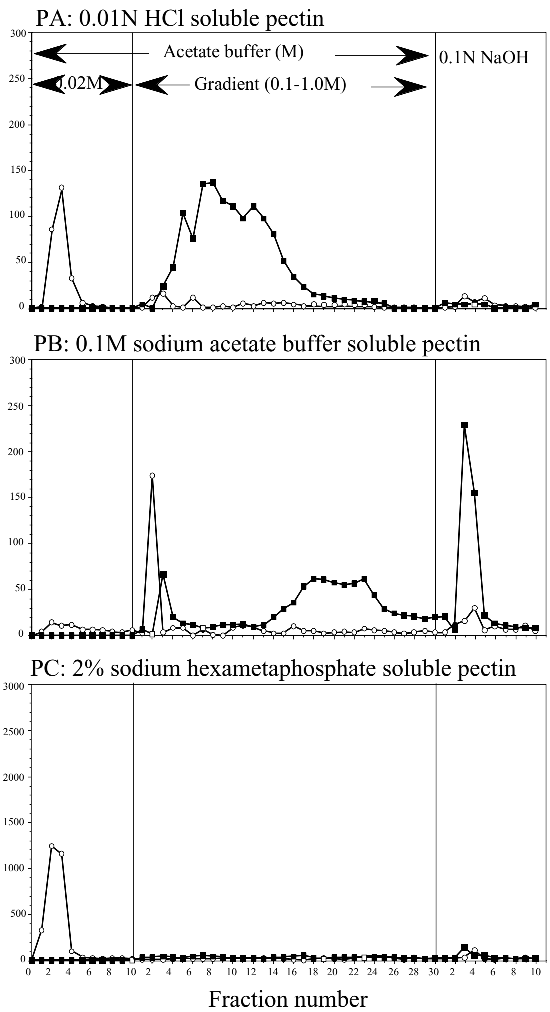

Figure 5. DEAE-cellulose column chromatograms of pectic substances of spaghetti squash cooked for 15 min.

—○— Neutral sugar

—■— Galacturonic acid