Relationship between Pectic Substances and Strand Separation of Cooked Spaghetti Squash

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博士論文

金糸瓜の加熱による糸状剥離とペクチン質の関係

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CONTENTS

Introduction

In this chapter a short review is given of pectic substances which are of major importance in intercellular cohesion of plant tissues, because of their presence in the middle lamella.

Kertesz stated that fruit jelly making was practiced long before pectin was discovered, in his book “The pectic substances” [1]. The first information on water-soluble substances with a strong jellying power, occurring in fruits, was presented by Vauquelin in l790, and the next well-known scientific publication on these substances was written by Braconnot in 1825 [2]. In this publication Braconnot related the name of these substances to their jellying properties, when he derived it from the Greek word “πηκτος” meaning to congeal or solidify [2].

Pectic substances are a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plants. They are deposited mainly in the early stages of growth when the area of the wall is increasing. Meristematic and parenchymous tissues are therefore particularly rich in pectic substances [3] [4].

Van Buren described as follows in “The Chemistry and Technology of Pectin” [5]. Pectic substances are complex mixtures of polysaccharides that make up about one third of the cell-wall dry substance of dicotyledonous and some monocotyledonous plants [6] [7]. Much smaller proportions of these substances are found in the cell walls of grasses [8]. The location of pectin in the cell-wall-middle lamella complex has been known since the earliest work on this material [1]. Highest concentrations are seen in the middle lamella, with a gradual decrease as one passes through the primary wall toward the plasma membrane [9]. Digestion of tissues with pectolytic enzymes leads to dissolution of the middle lamella and cell separation [10].

The pectic substances contribute both to the adhesion between the cells and to the mechanical strength of the cell wall, behaving in the manner of stabilized gels [11]. They are brought into solution more easily than other cell-wall polymers, although their extractability varies widely from species to species. They have a higher degree of chemical reactivity than do other polymeric wall components. Physical changes, such as softening, are frequently accompanied by changes in the properties of the pectic substances [5].

From studies on pectins from many sources, it has become clear that pectin is not a homopolysaccharide. The pectic substances are heteropolysaccharides, built up from a galacturonan main chain α-1, 4-glycosidically linked (**Fig. 1**), interrupted by 2-linked rhamnosyl residues (**Fig. 2**). Galactan-arabinan side chains are probably bound to C4 of rhamnose (**Fig. 4**). Rhamnose introduces a kink into the otherwise straight chain. The

mole-percent of rhamnose in potato chelator-soluble pectin was much lower than that found in potato protopectin [12]

Other constituent sugars are attached in side chains, the most common being D-galactose, arabinose, and D-xylose. D-glucose, D-mannose, L-fucose, and D-g1ucuronic acid are found less frequently [4]. The major sugars D-galactose and L-arabinose are present in more complex chains with structures similar to those of arabinans and arabinogalactans and with chain lengths that can be considerable [4]. Often, arabinan, galactan, or arabinogalactan side-chains are linked (1→4) to the rhamnose. In the side-chains, the arabinose units have (1→5) linkages while galactoses are mutually joined mainly by (1→4) linkages, but (1→3) and (1→6) linkages also occur.

The formula of a part of a polygalacturonic acid molecule, partly esterified with methanol, is presented in **Fig. 1**.

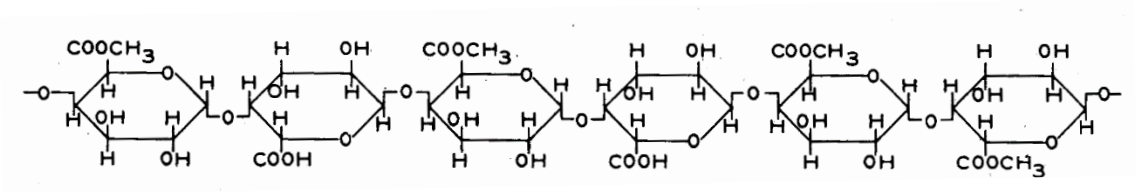

Fig. 1. Part of polygalacturonic acid molecule, partly esterified with methanol. [2]

The measure of esterification of pure galacturonic acids may be indicated by the methoxyl (CH_3O) content or by the degree of esterification which represents the number of esterified carboxyl groups calculated as the percentage of the total number galacturonic acid units. When the carboxyl groups in pure polygalacturonic acids are all esterified the methoxyl content is 16.32% and the degree of esterification 100 % [2].

The Talmadge model [13] for rhamnogalacturonan from sycamore cell walls has a zigzag shape (**Fig. 2**) [14]. It is likely that interspersement of 2-linked rhamnosyl residues in an otherwise linear galacturonan chain, although a feature of primary structure, influence secondary and passively tertiary structure [14] [15]

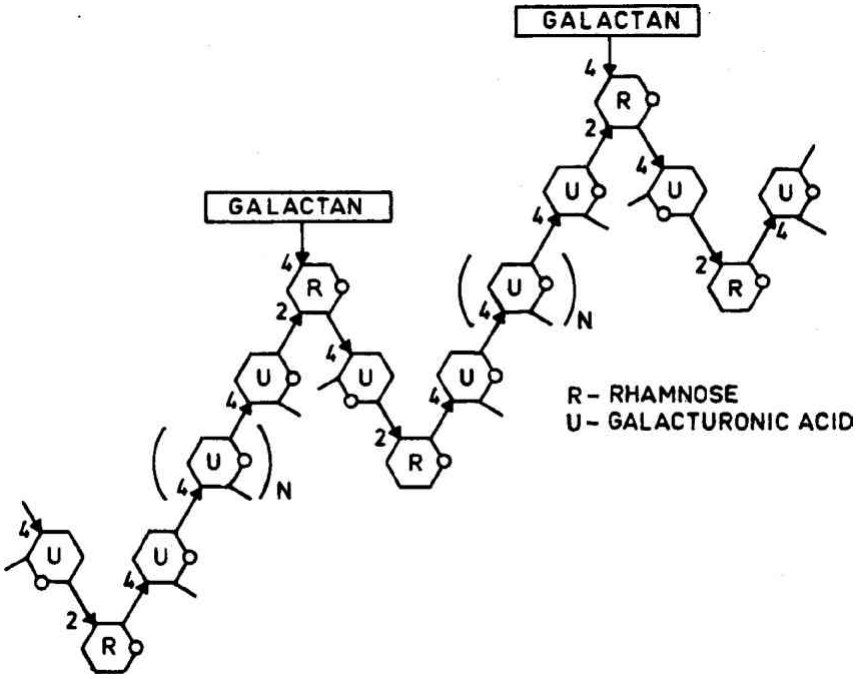

Fig. 2. Proposed structure for the rhamnogalacturonan part of pectic substances of cell wall of suspension-cultured sycamore cells. [13] [14] R = rhamnose; U = galacturonic acid; N = undetermined number, probably between 4 and 10.

Besides the primary structure, the secondary and tertiary structures of pectin were considered. Furthermore it was pointed out that esterification of the carboxyls of galacturonan with methanol and molecular weight are important characteristics [14].

Schematic structure of pectin molecules and a model of primary cell wall of flowering plants are shown in **Figs. 3~5.** The primary cell wall is composed of interwoven cellulose fibrils embedded in an amorphous polysaccharide matrix. Its strength is related to its thickness [5].

Fig. 4 showed that there is an intramolecular distribution in which the neutral sugars are concentrated in blocks of more highly substituted rhamnogalacturonan regions (“hairy”), separated by unsubstituted (“smooth”) regions containing almost exclusively D-galactosyluronic residues. This concept explains the isolation from cell walls of pectic fragments rich in neutral sugars, such as rhamnogalacturonan I, by the use of pectolytic enzymes [4].

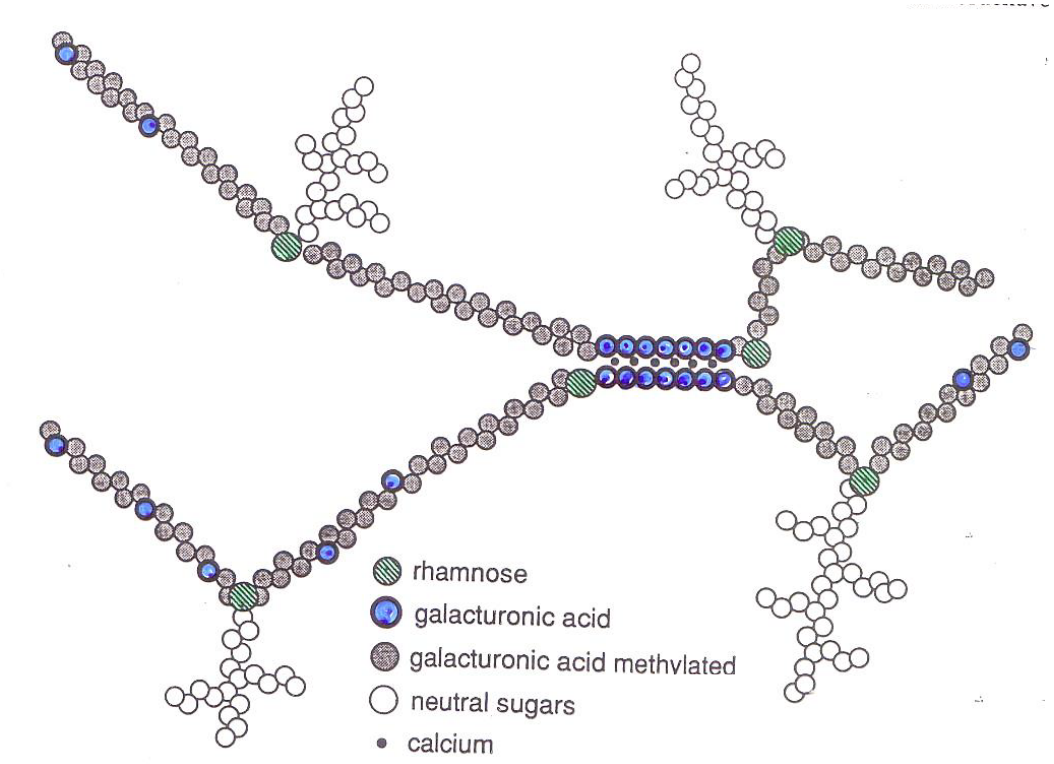

Fig. 3. Structure of pectins. (Courtesy of Dr. Roger Prat) [15]

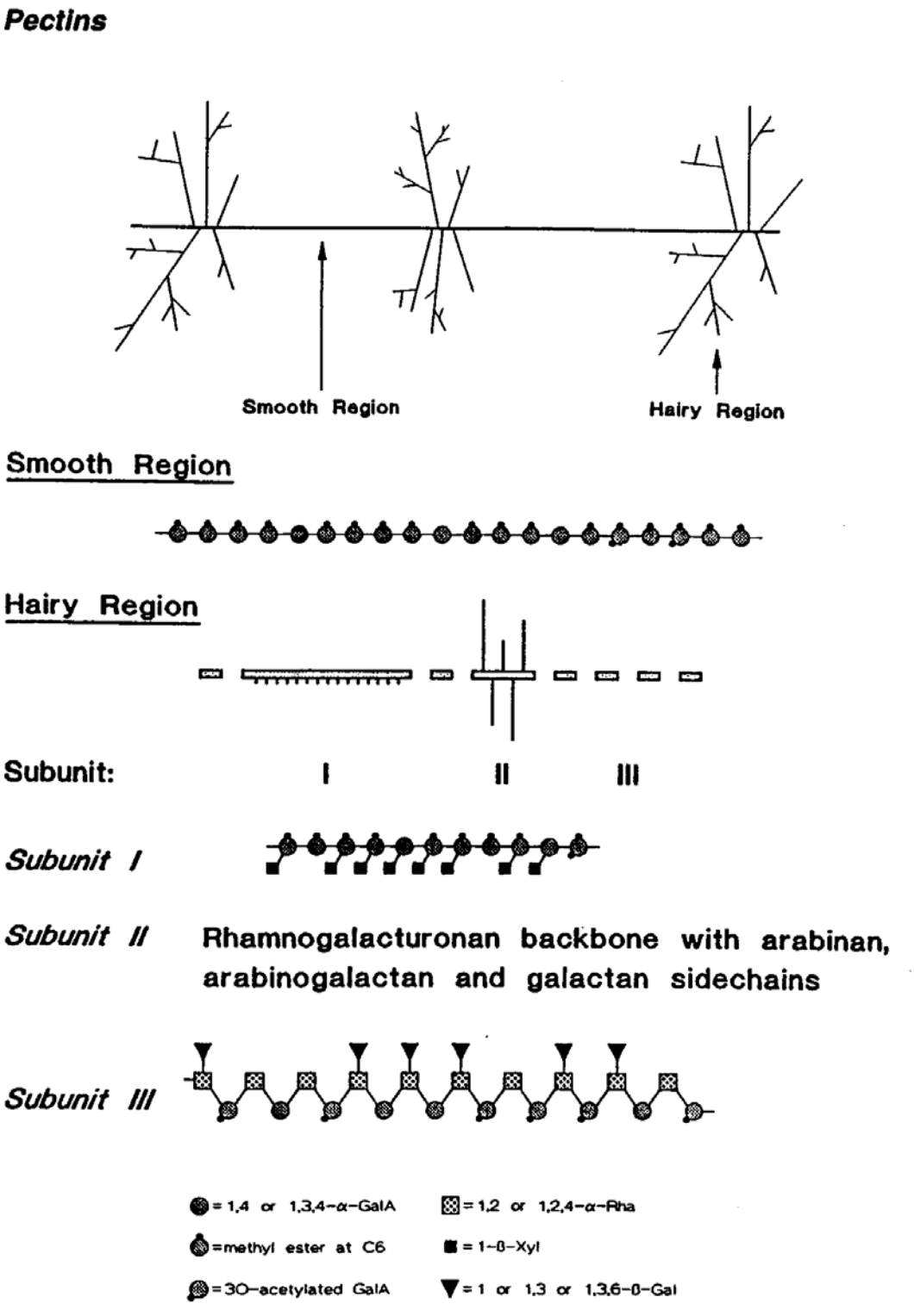

Fig. 4. Schematic structure of pectin molecules. [4]

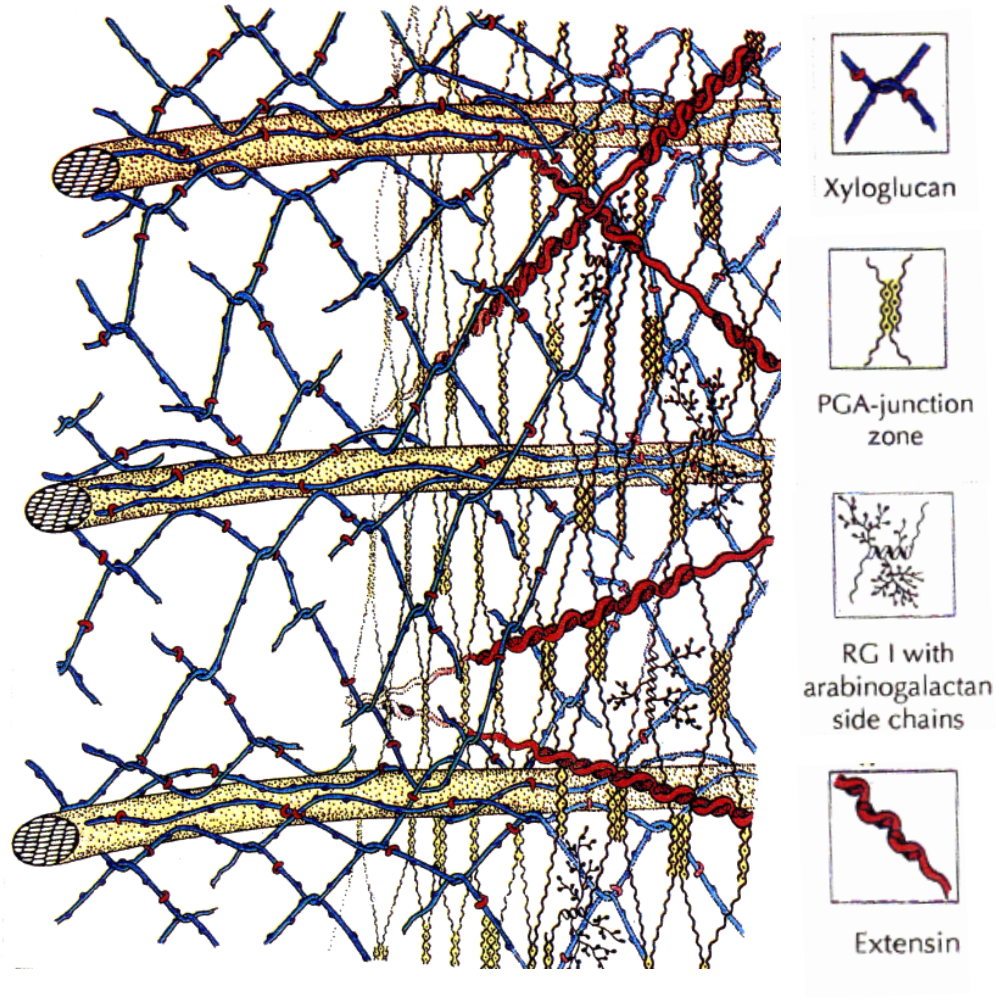

Fig. 5. A model of the expanding primary cell wall of flowering plants except grasses. [16]

A single layer is represented: several such layers condense to form the wall. Three thick cellulosic microfibrils arc aligned in parallel but in a helical formation around elongating cells. They are cross-linked with hemicellulosic xyloglucan polymers that have been partially cleaved to permit microfibril separation. This domain is embedded in a second consisting of matrix of pectic polygalacturonic acid (PGA) which forms junction zones in the presence of Ca^{2+} and rhamnogalacturonan I (RG1) with attendant arabinogalactan side chains. A third domain contains extensin molecules, which are inserted radially to stabilize the separated microfibrils and limit further stretching upon the cessation of growth. Source: *Carpita and Gibeaut*, 1993. [16]

The dominant feature of pectins is a linear chain of α-(1→4)-1inked D-galacturonic acid units in which varying proportions of the acid groups are present as methoxyl (methyl) esters. An important factor characterizing pectin chains is the degree of esterification (DE) of the uronide carboxyl groups with methyl alcohol. Pectins might be formed initially in a highly esterified form, undergoing some deesterification after they have been inserted into the cell wall or middle lamella [5]. There can be a wide range of DEs dependent on species, tissue, and maturity. In general, tissue pectins range from 60 to 90% DE [5]. Water-soluble pectins and protopectins have slightly higher DEs than do chelator-soluble pectins [5]. It seems that the distribution of free carboxyl groups along the pectin chains is somewhat regular, and the free carboxyl groups are largely isolated from one another [17] (DeVries et al., 1986).

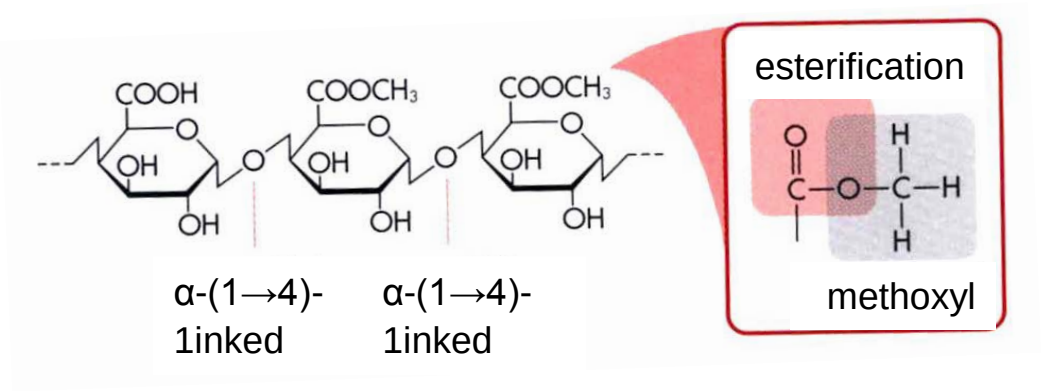

Fig. 6. Structure of pectinic acid.

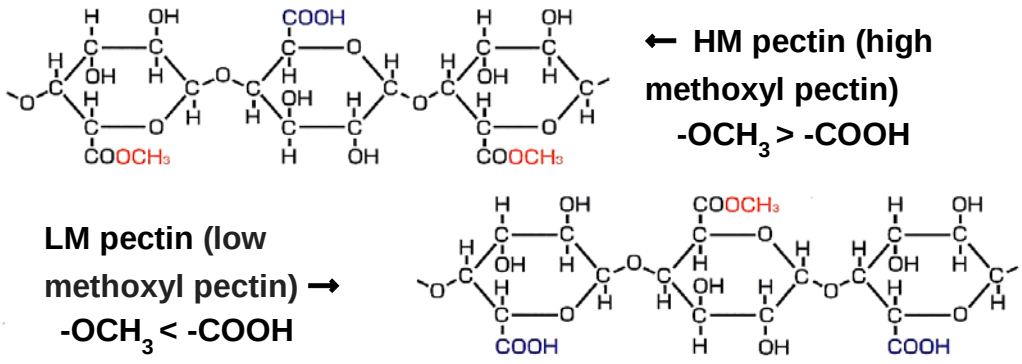

Fig. 7. Structure of HM pectin (High methoxyl pectin) and LM pectin (low methoxyl pectin). [18]

The term pectic substances is commonly used to encompass the methoxyl ester, pectin, the deesterified pectic acid, and its salts, pectates, and certain neutral polysaccharides (arabinans, arabinogalactans, galactans) lacking the galacturonan backbone often found in association with pectin [4] [19]. The unspecific term protopectin is often used to designate the native pectin fractions in cell walls that can not be extracted by nondegradative methods [4]. HM pectin and LM pectin are terms used for commercial pectins of high and low methoxyl content, respectively, applied mainly in the food industry as gelling agents [4] [20].

A schematic survey of the interrelationship of pectic substances is presented in **Fig. 8** and **Fig. 9**.

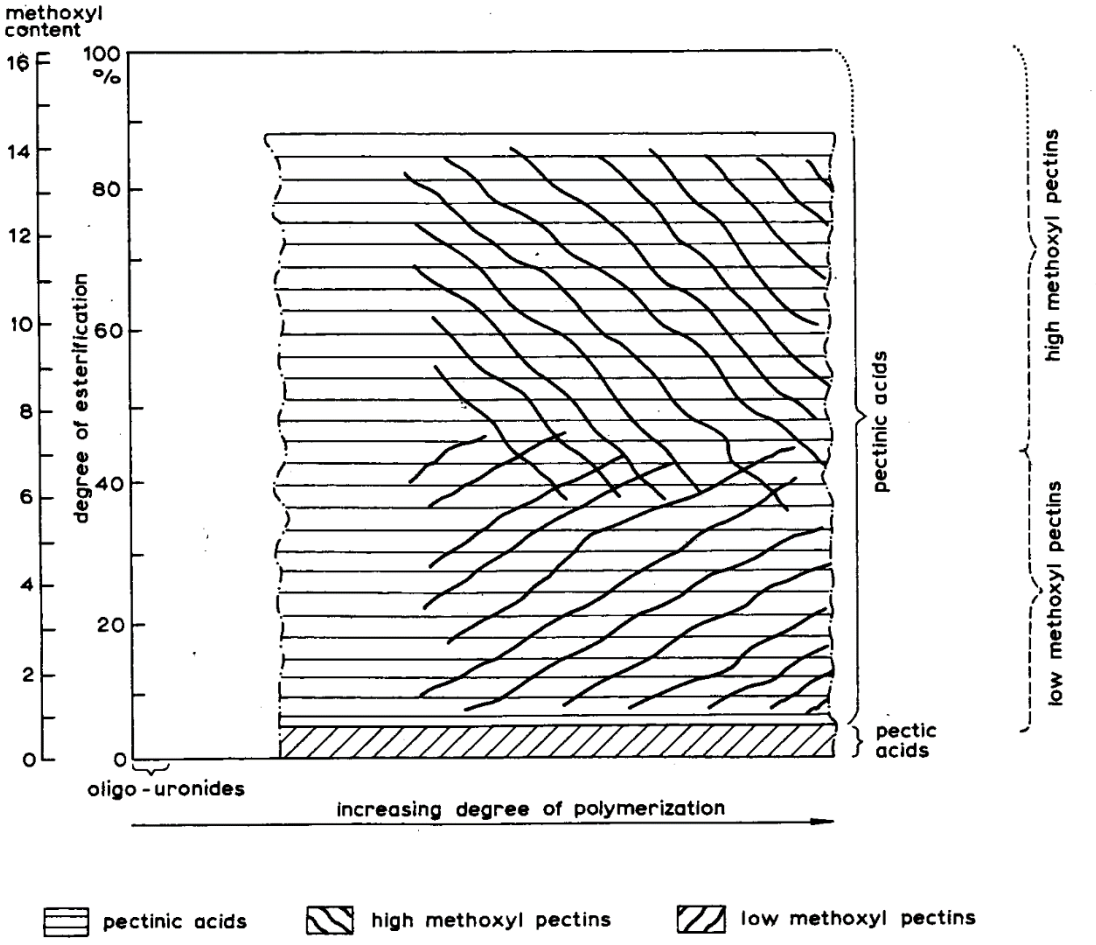

Fig. 8. Schematic illustration of the interrelationship of pectic substances (modification of illustration by Kertesz [1]). [2]

The limit of colloidal behavior is passed at an unknown degree of polymerization. The jellying properties of a high-and low-methoxyl pectins occur at a higher degree of polymerization. Polygalacturonic acids containing 2-4 anhydrogalacturonic acid units are mentioned oligo-uronides. [2]

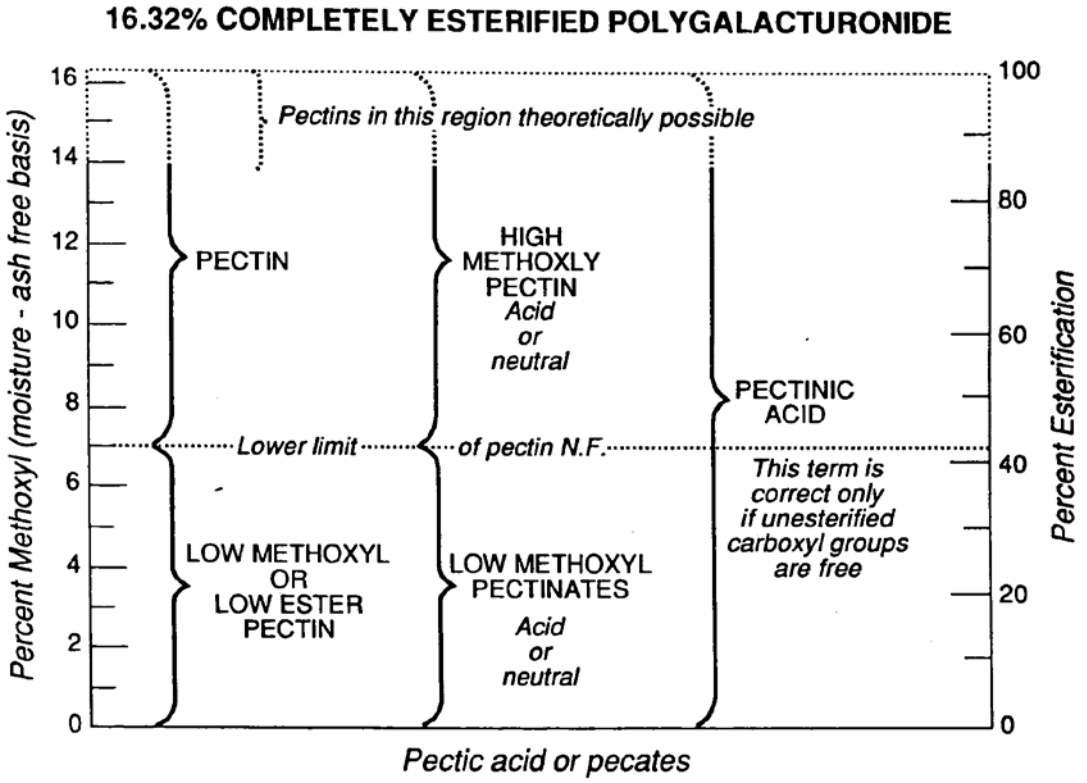

Fig. 9. Classification of pectins on the basis of methylester content. Reproduced with permission from Joseph (1953). [21]

Maceration of vegetable tissues seems to be brought about mainly by the degradation of pectin [2] [14] [22]. Softening of vegetables during cooking is affected by the properties of pectic substances, especially the degree of esterification (DE) [23] [24]. The low methoxyl pectins are difficult to break down in hot neutral solutions by trans-elimination (β-elimination) [25] [26] (**Fig. 10, Fig. 11**). Therefore, the vegetables which have a comparatively larger amount of low methoxyl pectin are difficult to soften during cooking [23] [24]. In β-elimination, the glycosidic bond is split in conjunction with the formation of a double bond between the C_5 and C_4 of newly formed non-reducing end group [22] [25] [26] (**Fig. 10, Fig. 11**). The reaction requires that carboxyl group of the residue undergoing β-elimination be esterified since this enhances the electron deficit at the C_5 position. Demethoxylated pectic substances do not under go β-elimination reaction and are relatively stable when heated under mild alkaline conditions [22].

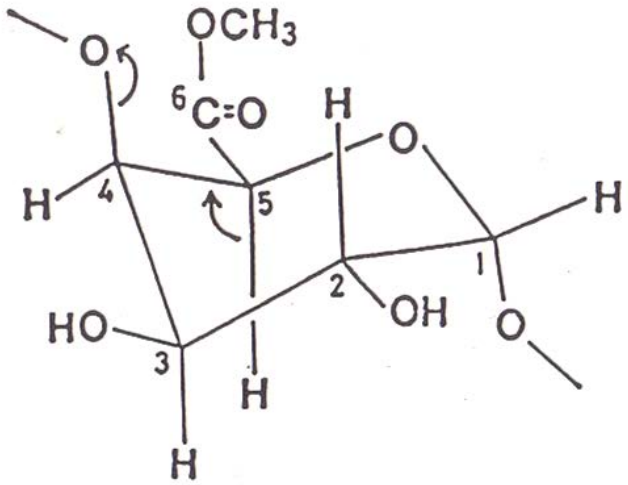

Fig. 10. Trans-elimination (β-elimination). [25][26]

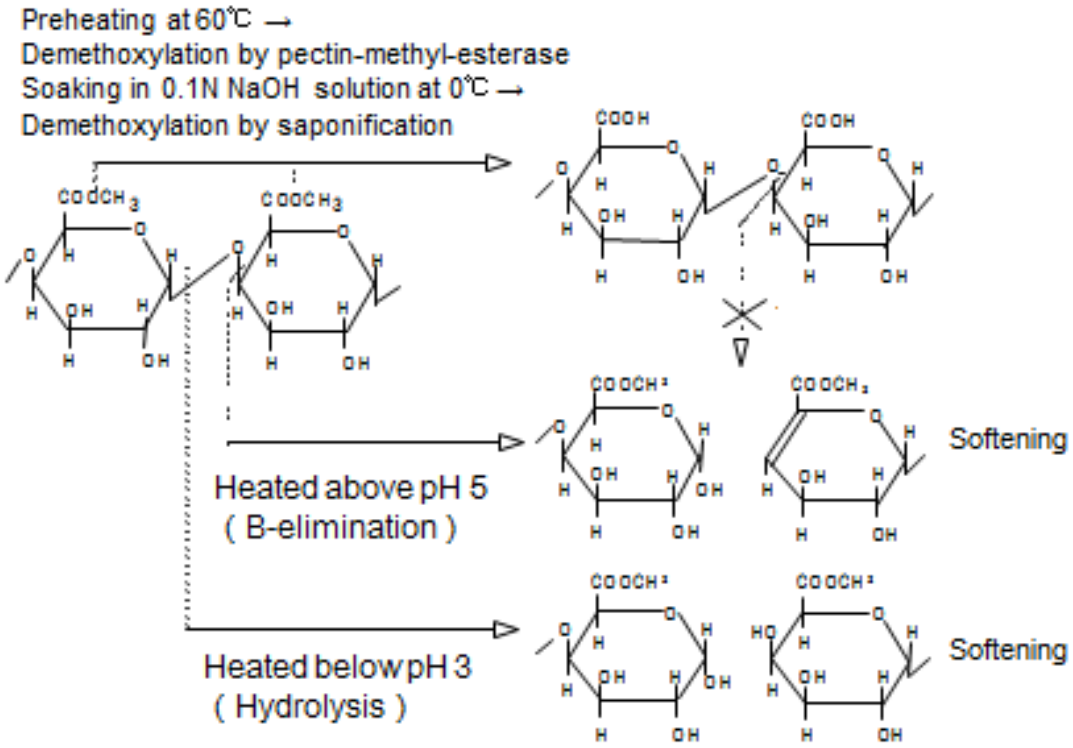

Fig. 11. Splitting of glycoside bond by β-elimination and hydrolysis.

Thus the DE has a bearing on the firmness and cohesion of plant tissues. Reductions in DE result in greater cohesion, which is particularly apparent in heated tissues. The pectin methylesterase enzyme, present in most tissues, can slowly bring about demethoxylation. This enzyme has a rather low activity in normal tissue, but it becomes much more active when tissue is damaged by procedures such as heating to 50 to 80℃, bruising, chilling, or freezing [5] [27] [28]. These conditions are often experienced during processing. The effect of preheating vegetables on the pectin DE and softness and after cooking was investigated [27] [28]. Low-temperature alkaline demethoxylation results in firmer heated tissue [29]. The firming effect involves two separate phenomena. In fresh tissue, the formation of free carboxyl groups increases the possibilities and the strength of calcium binding between pectin polymers. In heated tissue, there is a combination of increased calcium binding and a decrease in the susceptibility of the pectin to depolymerization by β-elimination [5].

The low methoxyl pectins are difficult to break down in hot neutral solution byβ-elimination. Therefore, the vegetables which have a comparatively larger amount of low methoxyl pectin are difficult to soften during cooking [23] [30] [31].

Vegetable tissues are usually kept firm when they are cooked at pH 4 and are rapidly softened by cooking either above pH 5 or below pH 3 [32]. Pectic substances released from vegetables during cooking were the lowest at pH 4 and increased both above the pH 5 level and below the pH 3 level. Pectic substances released from vegetables during cooking (above pH 6) gave positive results by the thiobarbituric acid test [32]. The β-elimination of pectins increased with rising pH and methoxylation when they were heated above pH 5 [33]. These results suggested that enhanced softening above pH 5 was due to degradation of pectin by the β-elimination mechanism.

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Chapter Ⅰ. Changes in Pectic Substances during Cooking

1. Introduction

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Spaghetti squash (*Cucurbita pepo* var. *fastigata*) 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.

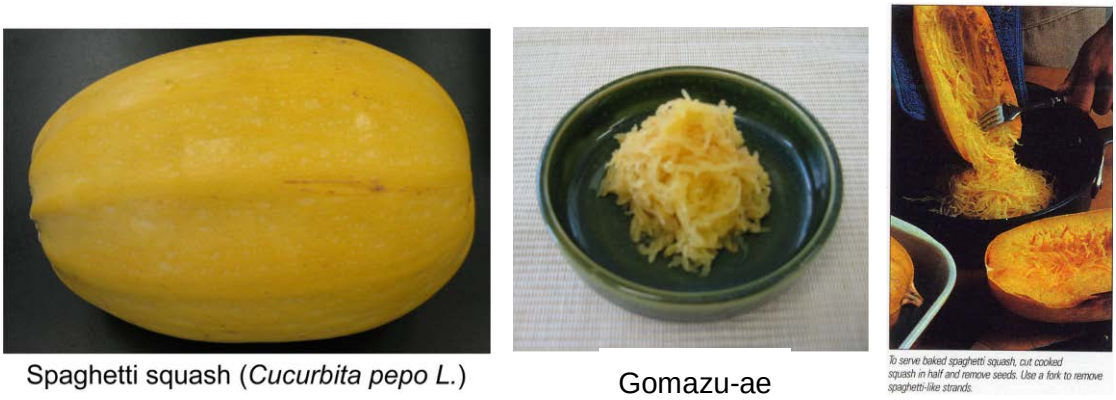

Fig. 1. Spaghetti squash.

The spaghetti squash is also called vegetable spaghetti, noodle squash, vegetable marrow, spaghetti marrow in English and kinshi-uri, somen-uri, somen-kabocha in Jananese. It is an oblong seed-bearing variety of winter squash. The fruit can range either from ivory to yellow or orange in color. The orange varieties have a higher carotene content. Its center contains many large seeds. Its flesh is bright yellow or orange. When raw, the flesh is solid and similar to other raw squash; when cooked, the flesh falls away from the fruit in ribbons or strands like spaghetti.

Spaghetti squash can be baked, boiled, steamed, and/or microwaved. It can be served with or without sauce, as a substitute for pasta. The seeds can be roasted, similar to pumpkin seeds. Spaghetti squash contains many nutrients, including folic acid, potassium, vitamin A, and beta carotene. It is low in calories.

Spaghetti squash are relatively easy to grow, thriving in gardens or in containers. The plants are monoecious, with male and female flowers on the same plant. Male flowers have long, thin stems that extend upwards from the vine. Female flowers are

shorter, with a small round growth underneath the petals. This round growth turns into the squash if the flower is successfully pollinated.

Female flower Male flower

Fig. 2. Flowers of spaghetti squash. (From HP)

Fig. 3. Dishes of spaghetti squash. (From HP)

Fig. 4. The separation into strands of cooked spaghetti squash.
(From HP)

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.

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) [2-5].

A method of fractional extraction of pectic substances under a mild condition has been reported [2]. Extraction was done successively with three reagents: HCl, sodium acetate buffer and sodium hexametaphosphate solutions. While dipping tissues in 0.01N HCl (pH 2.0) solution at 35˚C, high methoxyl pectin was extracted due to removal of polyvalent cations such as Ca^{2+}, because low methoxyl pectin is usually precipitated at pH 2.0. After repeating the extraction with 0.01N HCl solution, the residue was extracted with of 0.1M acetate buffer solution (pH 4.0) at 35˚C. The last extraction was done with 2% sodium hexametaphosphate solution (pH 4.0) at 90˚C for 3.5 h.

The vegetables, which were easily softened by thermal treatment, contained more HCl-soluble pectin (high methoxyl pectin, pectin A, PA) than the sodium acetate buffer soluble pectin (low methoxyl pectin, pectin B, PB) [2-5]. The high methoxyl pectin easily broke down in a hot neutral solution and alkaline solutions by β-elimination [6-7]; therefore, vegetables with a greater amount of PA had decreased intercellular adhesion strength. Conversely, the vegetables which had a larger amount of PB were difficult to soften during cooking [2-5].

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.

2. Materials and Methods

2.1 Sample Preparation and Cooking Procedure

Spaghetti squash (1,392 ± 432 g, harvested in Okayama, Japan, obtained in July) was cut into 2 cm long crosswise pieces, peeled and the seeds discarded. Flesh samples (100 g) were dropped into boiling distilled water (1,000 mL) in a 1,000 mL beaker and cooked for 15 min or 30 min, stirring at short intervals. Then they were strained and weighed.

2.2 Structure Measurements

Histological structures of raw and cooked (for 15 min or 30 min) samples were observed using a light microscope. Samples were cut in blocks of 1 mm^3. Tissue blocks were fixed in a 3% glutaraldehyde solution buffered at pH 7.4 with a 0.01M phosphate buffer for 17 h at 4˚C, and then post-fixed in 1% OsO_4 solution buffered with the same buffer for 2 h. After dehydration through graded concentration of ethanol, specimens were embedded in Epok 812. All specimens were cut at 1 μm with an ultramicrotome and stained with toluidine blue for observation with a light microscope (LM) [8].

2.3 Extraction of Pectin

Pectic substances of raw and cooked (for 15 min and 30 min) samples (10 g) were successively extracted as follows: 0.01N HCl (at pH 2.0 and 35˚C for 24 h × 4 times), 0.1M sodium acetate buffer (at pH 4.0 and 35˚C for 24 h × 4 times) and 2% sodium hexametaphosphate solution (at pH 4.0 and 90˚C for 3.5 h × 3 times) [2-5]. 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 (pectin A), PB (pectin B) and PC (pectin C), respectively. The amount of galacturonic acid was determined by the carbazole method [9].

2.4 Determinations of Esterification of Pectin and Dietary Fiber

The degree of esterification of PA, PB and PC were determined using a gas-chromatographic procedure [10]. The dietary fibers (cellulose, hemi-cellulose and lignin) were determined by the method of Van Soest & Wine [11].

2.5 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 [4-5]. The pectic substances (about 10 mg of galacturonic acid) were added to a DEAE-cellulose column (2 cm in diameter, 5 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 [12] and the carbazole method [9]. The amount of neutral sugar was calculated by the method of Hatanaka and Ozawa [13]. Monosaccharides were analyzed by the gas chromatographic procedure [14].

2.6 Gel-filtration

The three fractions separated by DEAE-cellulose column chromatography were dialyzed against distilled water after concentrating at pH 4.0, and were then added to a Sepharose CL-6B column (2 cm in diameter, 80 cm long) equilibrated with a 0.02M acetate buffer solution (pH 4.0). The column was eluted with the same solution, the fractions being monitored by the same method as that just described. Blue dextran (average molecular weight (MW, 2×10^6), Dextran T500 (MW, 539,000), Dextran T40 (MW, 39,400) and Dextran T10 (MW, 9,500) were used as standard [15].

3 Results and Discussion

3.1 Changes in Visual Appearances of Spaghetti Squash during Cooking

Changes in visual appearances of spaghetti squash during cooking are shown in **Fig. 5**. Appearance of a whole fruit is a cylindrical fruit with hard rind (Fig. 5a). When it was cut into 2 cm long pieces in crosswise (Fig. 5b), an off-white flesh such as a long thread

adhered in the shape of an eddy. There were many large seeds in the center of squash.

The squash was peeled and the seeds were discarded, then flesh samples were cooked for 15 or 30 min in a beaker, stirring at short intervals. The pH values of cooking solution were 5.80 ± 0.16 and 5.83 ± 0.13 after cooking for 15 min and 30 min, respectively. After cooking for 15 min (Fig. 5c) or 30 min (Fig. 5d), flesh came out in threads and separated into strands like spaghetti. When boiled for 30 min, broth (cooking solution) became muddy. After cooking, they were strained. The strands cooked for 15 min (Fig. 5e) or for 30 min (Fig. 5f) were crunchy and watery. When spaghetti squash was halved in lengthwise, the full spaghetti-like length of strands was provided.

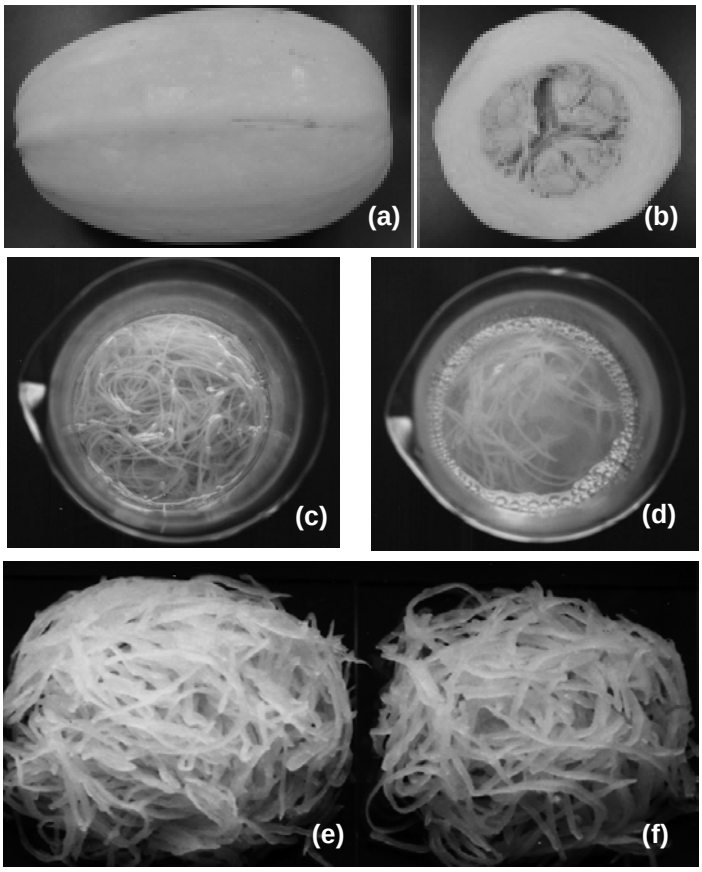

Fig. 5 Changes in visual appearances of spaghetti squash during cooking.
(a) Appearance of a whole fruit, (b) the squash cut in crosswise, (c) squash cooked for 15 min in a beaker, (d) squash cooked for 30 min in a beaker, (e) the strands cooked for 15 min, (f) the strands cooked for 30 min. Flesh was stirred during cooking. The pH values of cooking solution were 5.80 ± 0.16 and 5.83 ± 0.13 after cooking for 15 min and 30 min, respectively.

3.2 Changes in Histological Structure of Spaghetti Squash during Cooking

Changes in light micrographs of spaghetti squash during cooking are shown in **Fig. 6**. The micrographs of raw flesh are shown in Figs. 6a~6c. When the tissue was cut in crosswise, a vascular bundle (the mass of small cells) was observed in the center of the strand (Figs. 6a and 6c). The shape of cells which constituted the strand differed from that of cells which surrounded the strand. The former cells were round and the latter cells were elongated (Fig. 6a). When cooked, the shape of the former cells was maintained, but the latter cells, which contributed to adhesion between strands, broke down. Thus, flesh separated into strands. When cooked for 15 min (Fig. 6d) or 30 min (Figs. 6e and 6f), the parenchyma maintained the same structure as the raw parenchyma (Fig. 6a), although they were dyed somewhat thinly. This is regarded as a cause of crisp texture.

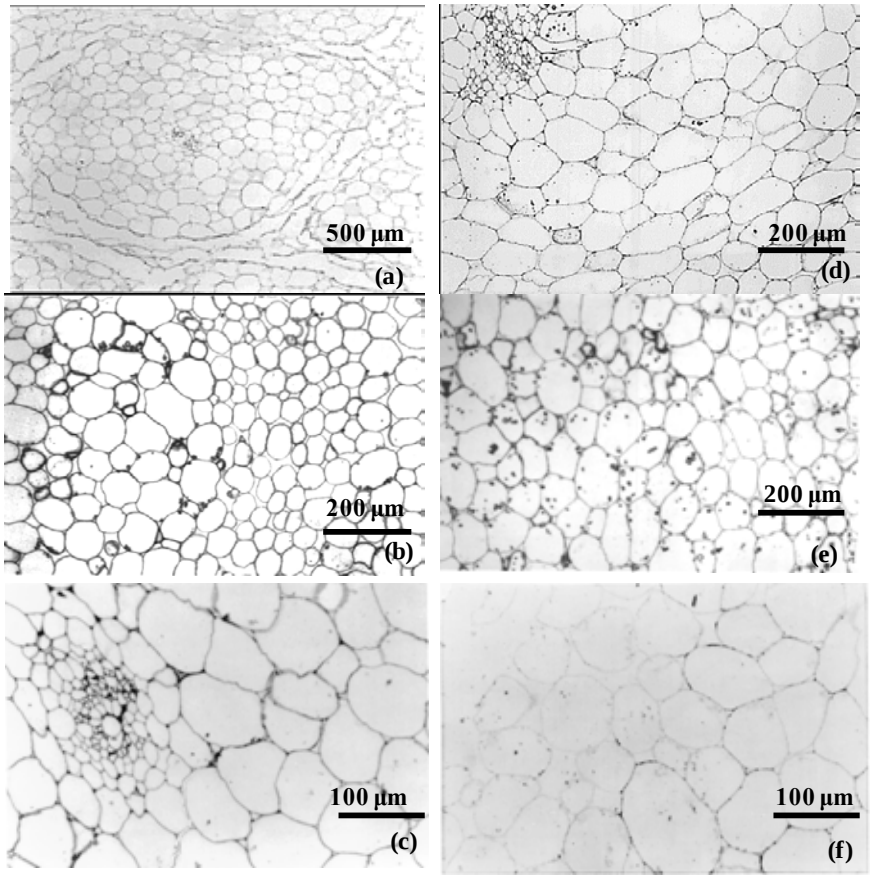

Fig. 6 Changes in light micrographs of spaghetti squash during cooking.
(a, b and c) raw, (d) cooked for 15 min, (e and f) cooked for 30 min.

3.3 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 **Fig. 7** and **Table 1**. 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 (2.1%) 100 g^{-1}, respectively. The percentage of PA was greatest. After cooking for 15 min, about 50% of pectic substances were released into cooking solution. The decrease of PA in flesh was extremely (from 237.4 mg to 73.8 mg/ 100/g). However, release of pectin during cooking for 15 ~ 30 min was slight, but the percentage of PA in flesh increased after 30 min cooking.

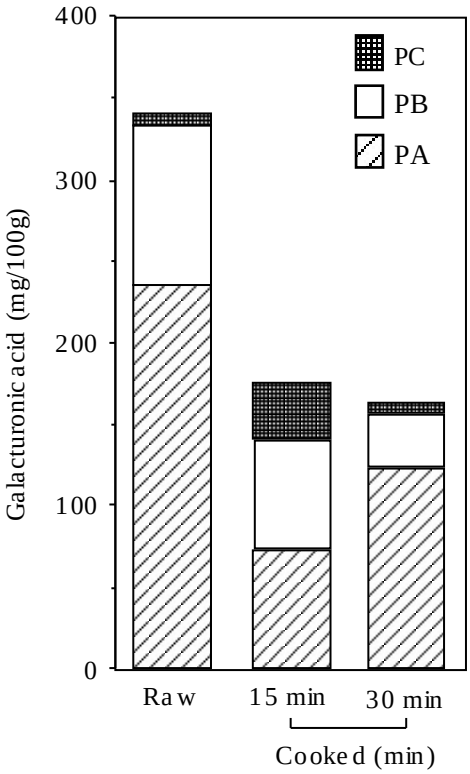

Fig. 7 Changes in pectic substances of spaghetti squash during cooking.
PA: 0.01N HCl soluble pectin, PB: 0.1M acetate buffer soluble pectin and PC: 2% sodium hexametaphosphate soluble pectin.

Table 1 The amount of galacturonic acid and the degree of esterification of pectic substances extracted from spaghetti squash.

	Types of pectin	Amount of galacturonic acid (mg/100 g)	Degree of esterification (%)
Raw	PA	237.4	67.4
	PB	99.6	61.5
	PC	7.3	55.6
	Total	344.3	
Cooked in distilled water for 15 min	PA	73.8	69.4
	PB	68.6	51.0
	PC	35.8	50.8
	Total	178.2	

PA, PB and PC: see Fig. 7.

The amount of galacturonic acid and the degree of esterification (DE) of pectic substances extracted from spaghetti squash are shown in **Table 1**. The 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 broke down by β-elimination when it was boiled in a hot neutral solution [6]. Therefore, about 50% of pectic substances were released into cooking solution during 15 ~ 30 min cooking.

The amount of dietary fiber and the percentage of PA, PB and PC of spaghetti squash and various vegetables are shown in **Table 2**. The percentage of PA of spaghetti squash was higher than lotus, burdock and bamboo shoot. Also, the DE of pectin in spaghetti squash was higher than DE of the other vegetables such as lotus [2] [3], burdock [15] and bamboo shoot [5]. Thus, the squash was more easily softened than lotus, burdock and bamboo shoot after cooking.

The amounts of cellulose, hemicellulose, lignin and pectin of spaghetti squash were similar to those of Japanese radish root. It is suggested that cellulose, hemicellulose and pectin remaining in strands after cooking maintained crisp tender strands.

Table 2 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*	1,003	1,677	72	133	8.2	2.2	88.6
East Indian lotus*	544	126	82	217	30.9	55.7	13.4
Edible burdock*	2,249	88	301	985	38.4	53.9	6.7
Carrot*	1,036	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

*[5]

PA: 0.01 N HCl soluble pectin; PB: 0.1 M acetate buffer soluble pectin; PC: 2% sodium hexametaphosphate soluble pectin.

3.4 Changes in DEAE-cellulose Column Chromatogram and Gel-filtration Profiles of Pectic Substances during Cooking

To investigate the properties of pectic polysaccharides in detail, PA, PB and PC were fractionated into neutral and acidic polysaccharide fractions by DEAE-cellulose column chromatography. The results are shown in **Fig. 8**. The elution patterns of PA, PB and PC on chromatography were different between raw and 15 min cooked squashes. The neutral polysaccharides (100% neutral sugar), weakly acidic polysaccharides and pectic acid are usually eluted in fractions I, II and III, respectively. High-methoxyl pectin was eluted earlier and low-methoxyl pectin was eluted more slowly in fraction II [4]. The pectic substances having no metoxyl group (pectic acid) were eluted in fraction III [4].

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. Also, the percentage of PC in squash was small, therefore, 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.

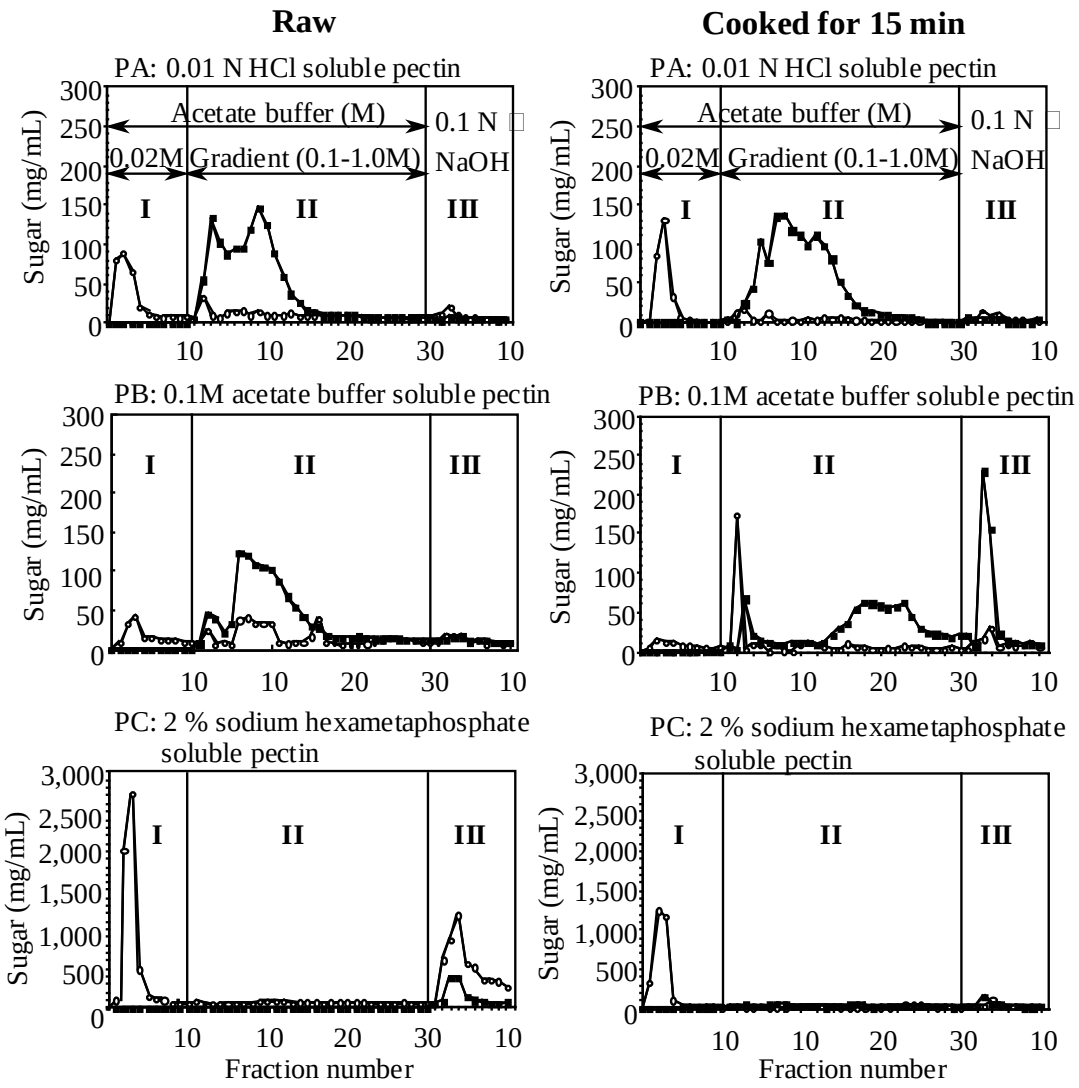

Fig. 8 DEAE-cellulose column chromatograms of pectic substances in raw and cooked spaghetti squash.

■: Galacturonic acid, ○: neutral sugar.

The eluate was divided into 10 mL fraction.

The monosaccharide composition of pectic substances (fractions II and III) in spaghetti squash separated by DEAE (diethylaminoethyl)-cellulose column chromatography is shown in **Table 3**. 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). When cooked for 15 min, the percentage of galactose in fraction II of PA and PB increased, on the other hand, that of arabinose, xylose and mannose decreased. This suggests that low methoxyl pectin with galactose remained in strands after cooking and maintained the firmness of strands. The pectic substances cementing strands might be high-methoxyl pectin with arabinose, xylose and mannose. High methoxyl pectin easily broke down by β-elimination during boiling [2~5]. Consequently, the flesh separated into strands.

Table 3 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

PA: 0.01 N HCl soluble pectin; PB: 0.1 M acetate buffer soluble pectin.

Changes in gel-filtration profiles (Sepharose CL-6B) of pectic substances in spaghetti squash during cooking for 15 min are shown in **Fig. 9**. The molecular weight (MW) of PA ($1\times10^6 \sim 5\times10^5$) in raw tissue was higher **(a)** than MW of PB (peak: 5×10^5, the range of MW distribution: from 5×10^5 to 1×10^4) **(b)**. After cooking for 15 min, MW of PA remaining in tissue did not change **(c)**, but PB with low MW ($5\times10^5 \sim 1\times10^4$) eluted into cooking solution and PB with only high MW (2×10^6) was remained **(d)**. The MW of pectin eluted in cooking solution was comparatively low ($< 1\times10^4$) **(e)**.

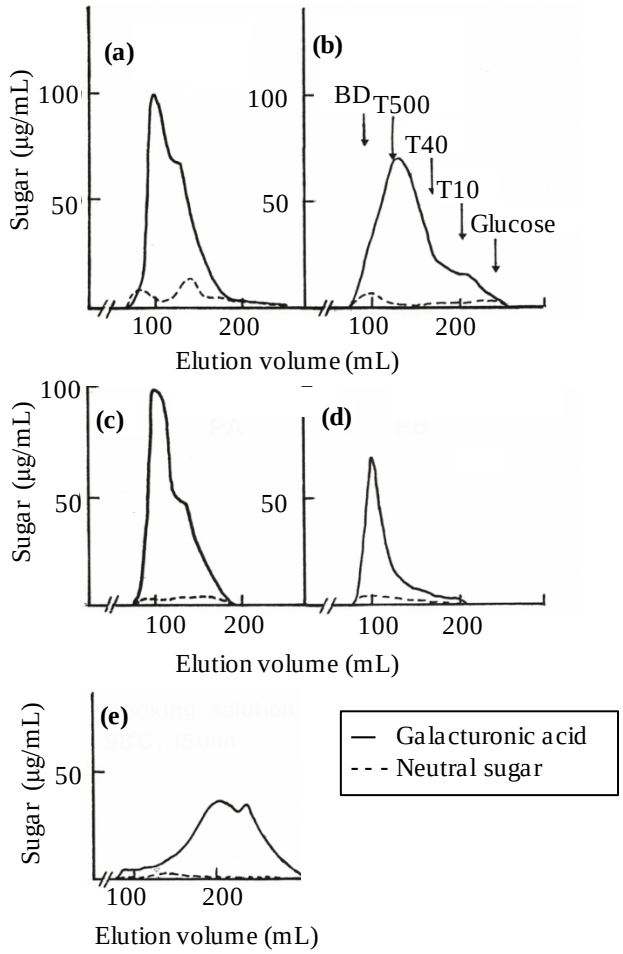

Fig. 9 Changes in gel-filtration profiles (Sepharose CL-6B) of pectic substances in spaghetti squash during cooking for 15 min.

(a) PA extracted from raw flesh, (b) PB extracted from raw flesh, (c) PA extracted from flesh cooked for 15 min, (d) PB extracted from flesh cooked for 15 min, (e) cooking solution after cooking for 15 min.

Summary

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. 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 (DE) of PA, PB and PC was 67.4%,

61.5% and 55.6%, respectively. The DE 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. Consequently, the flesh separated into strands. This suggests that high methoxyl pectin glues cells of strands together in the flesh of spaghetti squash.

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Chapter Ⅱ. Changes in Firmness, Histological Structure and Pectic Substances during Soaking in Pectin Extractants

1. Introduction

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Spaghetti squash (*Cucurbita pepo L.*) is one of the hard-shelled squashes in the cucurbit family. It has a mild taste and crisp texture. When boiled the spaghetti squash flesh separated into strands. [1].

Pectin, which is a main component of middle lamella, contributes to adhesion between the parenchyma cells of vegetables and the mechanical strength of tissues. The softening of vegetables during cooking is affected by the degree of esterification of pectin [2-5]. A method of fractional extraction of pectic substances under a mild condition has been reported previously [2-5]. Extraction was done successively with three reagents: HCl, sodium acetate buffer, and sodium hexametaphosphate solutions. 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) [2-5].

In a previous paper [1], the relationship between pectic substances and the separation into strands during cooking of spaghetti squash was investigated. Pectic substances of raw and cooked flesh were fractionated into three reagents [1]. The galacturonic acid compositions of PA, 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. About 50% of pectic substances in flesh were released into a cooking solution when cooked while stirring for 15~30 min. Also, the shape of cells which constituted the strand differed from that of cells which surrounded the strand. The former shape was round and the latter was elongated. When cooked, the former shape was maintained, but the latter, which contributed to adhesion between strands, broke down.

High methoxyl pectin is degraded by β-elimination during boiling [6-8], so the flesh separated into strands when boiled. This suggests that high methoxyl pectin glues cells of strands together in the flesh of spaghetti squash. However, it was difficult to distinguish where high-methoxyl pectin existed in the round cells-in the strand, or the elongated cells which surrounded the strand. Therefore, in this paper, when flesh is soaked in 0.01N HCl (pH 2.0), ammonium oxalate (pH 4.0) or 2% sodium hexametaphosphate (pH 4.0) solutions which were used for the extraction of pectin,

whether the flesh separates or not is investigated. While dipping tissues in 0.01N HCl solution at 35˚C, high methoxyl pectin was extracted due to removal of polyvalent cations such as Ca^{2+}, because low methoxyl pectin is usually precipitated at pH 2.0 [2-5]. This suggests that if flesh separates during soaking in 0.01N HCl solution, the elongated cells which surrounded the strand may contain high methoxyl pectin.

Also, in a previous paper, flesh was boiled while stirring; therefore, the flesh separated into strands during cooking. Thus change in firmness of flesh could be not measured.

The purpose of this paper is to investigate changes in firmness, histological structure by cryo-scanning electron microscopy, pectic substances of spaghetti squash and the extraction of calcium from tissue during soaking without stirring in 0.01N HCl (pH 2.0), ammonium oxalate (pH 4.0) and 2% sodium hexametaphosphate (pH 4.0) solutions.

2. Materials and Methods

2.1 Sample Preparation

Spaghetti squash (1,533 ± 417 g, harvested in Okayama, Japan, obtained in July and August) was cut into 2 cm long crosswise pieces, peeled, and the seeds discarded. The exocarp, endocarp and seeds were removed, while the mesocarp (sarcocarp) was used in this experiment.

2. 2 Cooking and Soaking Procedure

Flesh samples (100 g) were dropped into boiling distilled water (1,000 mL) in a 1,000 mL beaker and cooked for 15 min or 30 min without stirring. Flesh samples (100 g) were also soaked for 24 h at 35˚C in the three solutions (1,000 mL): 0.01N HCl (pH 2.0) solution, 0.035M ammonium oxalate solution adjusted to pH 4.0 with oxalic acid, and 2% sodium hexametaphosphate solution (pH 4.0). These agents were designated as HCl, AO (Ammonium Oxalate) and HMP (Sodium Hexa-Meta-Phosphate), respectively.

After cooking or soaking, samples were separated into flesh and cooking/soaking solutions through glass filters (26G1). The appearances of flesh were observed. The properties of pectin and the amount of minerals extracted into cooking or soaking solutions were determined.

2.3 Texture Measurement

Raw, cooked and soaked fleshes were cut into 5 mm thick. Rupture stress of these fleshes 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 [4].

2.4 Analysis of Extracts Eluted into Cooking or Soaking Solutions

2.4.1 Determination of pectin

Cooking or soaking solutions were adjusted to pH 4.0, and then concentrated using an evaporator. To avoid the neutral sugars in the determination of pectin, the extracts were separated into neutral and acidic polysaccharide fractions by a DEAE-cellulose column chromatography. The sample was saponified in 0.05N NaOH for 90 min at 0˚C and neutralized with acetic acid, then loaded into a column (2.0 cm in diameter and 5.0 cm long). The column was first washed with an equilibrating buffer (0.02M acetate buffer solution) and then eluted with a 0.1N NaOH solution. The amount of galacturonic acid in the second fraction was determined by the carbazole method [9].

2.4.2 Determination of the degree of esterification of pectin

The degree of esterification of pectin was determined using a gas-chromatographic procedure [10].

2.4.3 Measurement of minerals

The amounts of minerals (Ca and Mg) in raw flesh and extracts were determined by atomic absorption spectrophotometry [11].

2.5 Measurement of the Firmness of Flesh after Eextraction of Pectic Substances

The soaking at 35˚C for 24 h in the three solutions (HCl, AO or HMP) was repeated seven times. The firmness of the outside and inside of samples soaked for 24 h, 48 h and 1 week was measured. The solutions containing pectin were discarded. After soaking for 1 week, the flesh soaked in AO or HMP solutions disintegrated, while the flesh soaked in HCl solution maintained its firmness. Therefore, this flesh was successively soaked in 0.1M acetate buffer solution (pH 4.0) at 35˚C for 24 h. This treatment was repeated 6 times. A final extraction of pectin was done with 2% sodium

hexametaphosphate solution at 90˚C for 3.5 h × 4 times, until flesh was completely disintegrated.

2.6 Structure Measurement

Histological structures of cooked or soaked samples were observed using a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo, Japan) [12]. Samples were cut into 6 mm × 1 mm × 1 mm, and dehydrated with 40% and 50% ethanol. The specimen was contained in a metal holder and quickly frozen by immersing in liquid nitrogen. A frozen specimen was transferred to the cold stage of a cryo-SEM and then cut with a knife (-150˚C). After etching at -85˚C and further cooling, the surface was observed (at about -120˚C) under low acceleration voltage (1 kV). The magnification used to observe cells was ×40 and ×100 and that for cell walls was ×20,000, respectively.

2.7 Extraction and Determination of Pectin from AIS

An alcohol insoluble solid (AIS) was prepared from raw, cooked (for 15 min and 30 min) or soaked (for 24 h in three solutions) samples. Samples were cut into tiny pieces manually with a knife and blended with ethanol (4 times sample amount) using an excel auto homogenizer (Nissei Ltd., Tokyo). The precipitates were then successively washed with 80% ethanol until sugars could not be detected in the wash by the phenol sulphuric acid method, then washed with 99% ethanol, followed by an acetone wash before drying at room temperature.

Pectic substances were extracted from AIS by successive extraction with distilled water (20℃, 24 h), 0.01N HCl (at pH 2.0 and 35˚C for 24 h × 2-5 times), 0.1M sodium acetate buffer (at pH 4.0 and 35˚C for 24 h × 1-2 times), 2% sodium hexametaphosphate solution (at pH 4.0 and 90˚C for 3.5 h × 2-6 times), and 0.05N HCl (at 90˚C for 3.5 h × 3-8 times). Each extraction was repeated until no sugar was detected. These extracts were designated as WSP, PA, PB, PC and PD, respectively. The amount of galacturonic acid was determined by the carbazole method.

3. Results and Discussion

3.1 Changes in Visual Aappearances of Spaghetti Squash during Cooking or Soaking

Changes in visual appearances of spaghetti squash during cooking are shown in **Fig. 1**. When raw flesh (Fig. 1a) was cooked for 30 min without stirring, the flesh did not separate into strands (Fig. 1b). However, when scooped out of cooked flesh, they came out in threads and separated into strands completely like spaghetti (Figs. 1c and 1d).

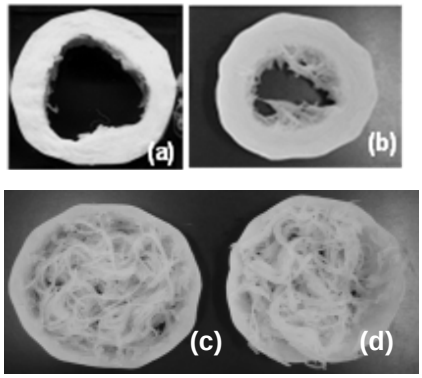

Fig. 1 Visual appearances of spaghetti squash cooked without stirring.
(a) Raw, (b) cooked for 30 min, (c) scooped out of 15 min cooked flesh, (d) scooped out of 30 min cooked flesh.

Changes in visual appearances of spaghetti squash during soaking for 24 h are shown in **Fig. 2.** When flesh was soaked in HCl solution then scooped out, flesh separated into strands (Fig. 2a). However, when soaked in AO solution, the separation was slight (Fig. 2b). When soaked in HMP solution, flesh did not separate and surface of flesh melted slightly (Fig. 2c).

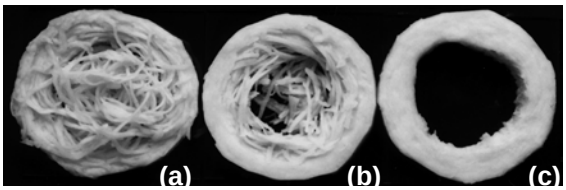

Fig. 2 Visual appearances of spaghetti squash soaked for 24 h at 35˚C in the three pectin extractants.
Flesh was soaked in a, b or c. (a) 0.01N HCl (pH 2.0), (b) 0.035M ammonium oxalate (pH 4.0), (c) 2% sodium hexametaphosphate (pH 4.0) solutions.

3.2 Changes in Texture of Spaghetti Squash during Cooking and Soaking

Changes in rupture stress of spaghetti squash during cooking or soaking are shown in **Fig. 3**. Rapture stress of raw flesh was 396×10^4 N/m^2 and that of flesh cooked for 15 min or 30 min was 34.5×10^4 N/m^2 or 26.43×10^4 N/m^2, respectively. When flesh was cooked in boiling water, flesh was extremely softened.

Soaking at 35˚C for 24 h in the three solutions (0.01N HCl, AO or HMP solutions) was repeated seven times. When soaked in HCl solution, flesh softened most rapidly to 48 h, while the same firmness was maintained to 1 week. On the other hand, the flesh soaked in HMP solution softened most slowly. Flesh soaked in AO solution softened at middle speed compared to the former and the latter. However, after soaking for 1 week, fleshes soaked in HMP or AO were disintegrated.

The flesh soaked in HCl solution for 1 week was successively soaked in 0.1M acetate buffer solution (pH 4.0) at 35˚C for 24 h. The flesh softened due to extraction of low-methoxyl-pectin. However, the shape of flesh was maintained after soaking for 6 days. By the final extraction with HMP solution at 90˚C for 3.5 h × 4 times, the flesh was completely disintegrated.

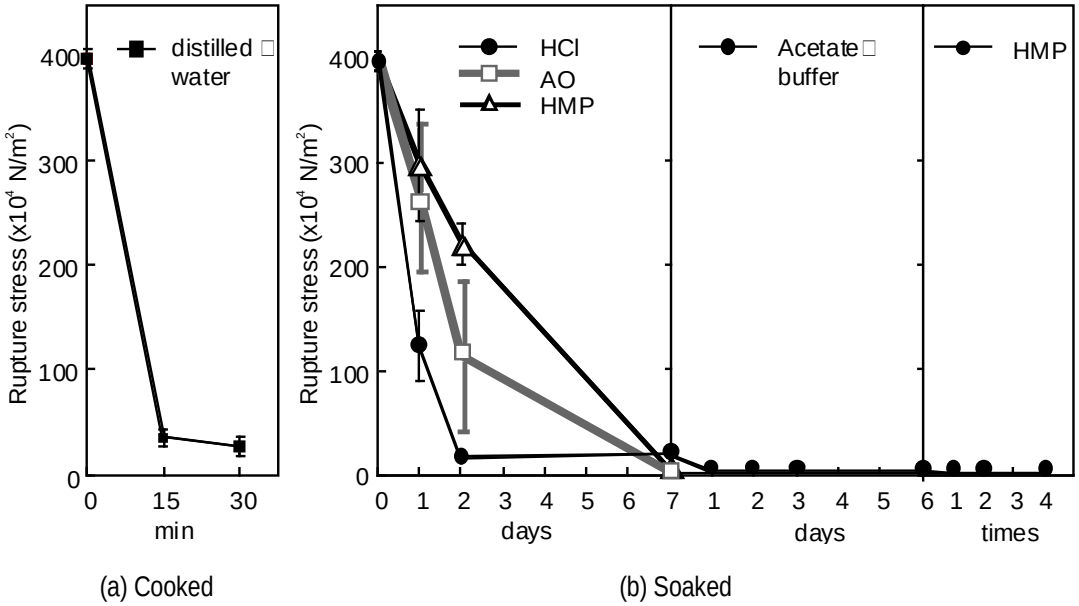

Fig. 3 Changes in texture of spaghetti squash during cooking or soaking.

(a) Flesh was cooked in boiling water. (b) Flesh was soaked at 35˚C in 0.01N HCl, AO (0.035M ammonium oxalate) or HMP (2% sodium hexametaphosphate) solutions for 7 days. Flesh soaked in HCl solution was subsequently soaked in 0.1M acetate buffer solution (pH 4.0) at 35˚C**,** then heated in HMP at 90˚C.

3.3 Changes in Histological Structure of Spaghetti Squash during Cooking

Changes in cryo-scanning micrographs of spaghetti squash during cooking are shown in **Fig. 4**. The shape of cells which constituted strands was different from that of the cells which surrounded strands. The former was round and the latter was elongated (Figs. 4a-1 and 4a-2). When cooked for 15 min, the shape of the former was maintained, but the latter, which contributed to adhesion between strands, broke down (Figs. 4b-1 and 4b-2). Thus, the flesh separated into strands.

Cell walls of raw and cooked flesh were compared. Raw cell wall adhered well (Fig. 4a-3). After cooking for 15 min, cell separation in middle lamella was observed, due to solubilizing of pectic substances (Fig. 4b-3). After 30 min cooking, the degree of cell separation in middle lamella and the space among microfibrils increased (Fig. 4c-3).

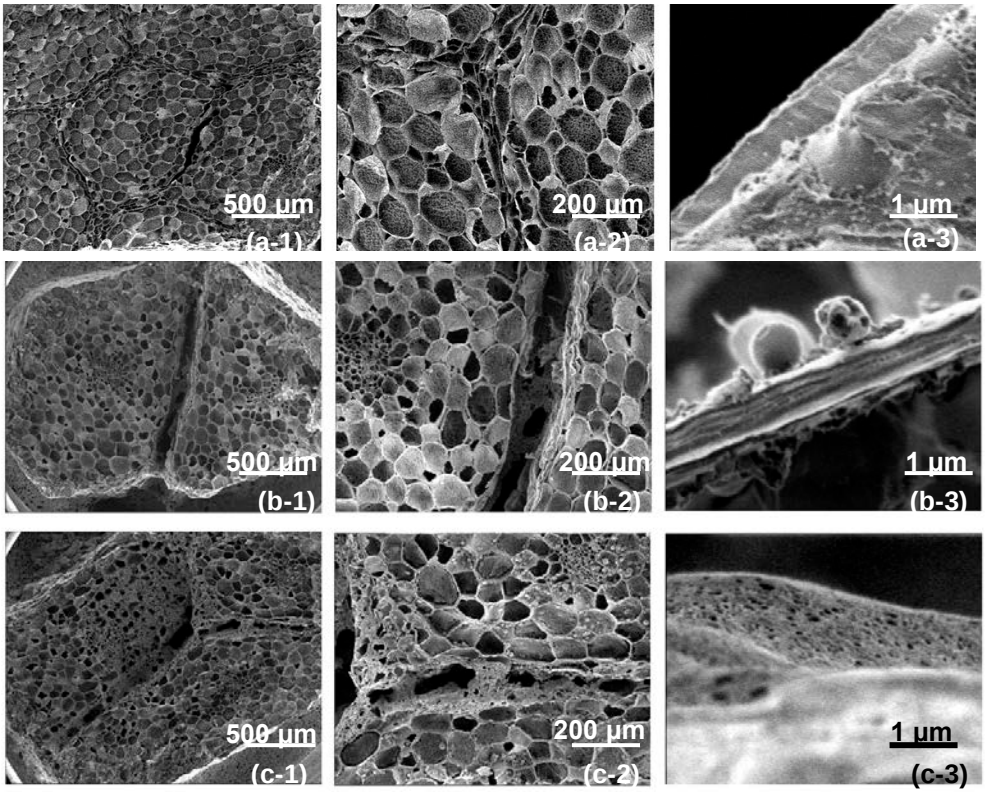

Fig. 4 Changes in cryo-scanning electron micrographs of spaghetti squash during cooking.
(a) raw flesh, (b) cooked for 15 min, (c) cooked for 30 min. (1)(2)(3) showed strands, cemented strands and cell wall, respectively. The magnifications were (1) ×40, (2) ×100 and (3) ×20,000, respectively.

3.5 Changes in Histological Structure of Spaghetti Squash during Soaking

The histological structures of flesh after soaking for 24 h in HCl, AO or HMP solutions are shown in **Fig. 5**. When soaked in HCl solution, the cells which glued threads together were missing (Figs. 5a-1 and 5a-2) and the cell wall of threads became loose (Fig. 5a-3). However, when soaked in AO or HMP solutions, the elongated cells which surrounded strands were maintained (Figs. 5b-1, 5b-2, 5c-1 and 5c-2) and both cell walls adhered well (Figs. 5b-3, 5c-3). When the fleshes were successively soaked in HCl solutions for 7 d at 35˚C, acetate buffer solution for 6 d at 35˚C and 2% sodium hexametaphosphate solution for 3.5 h at 90˚C (**Fig. 6**), the flesh separated into strands, and also the middle lamella and primary cell walls separated (Figs. 6a-3, 6b-3 and 6c-3).

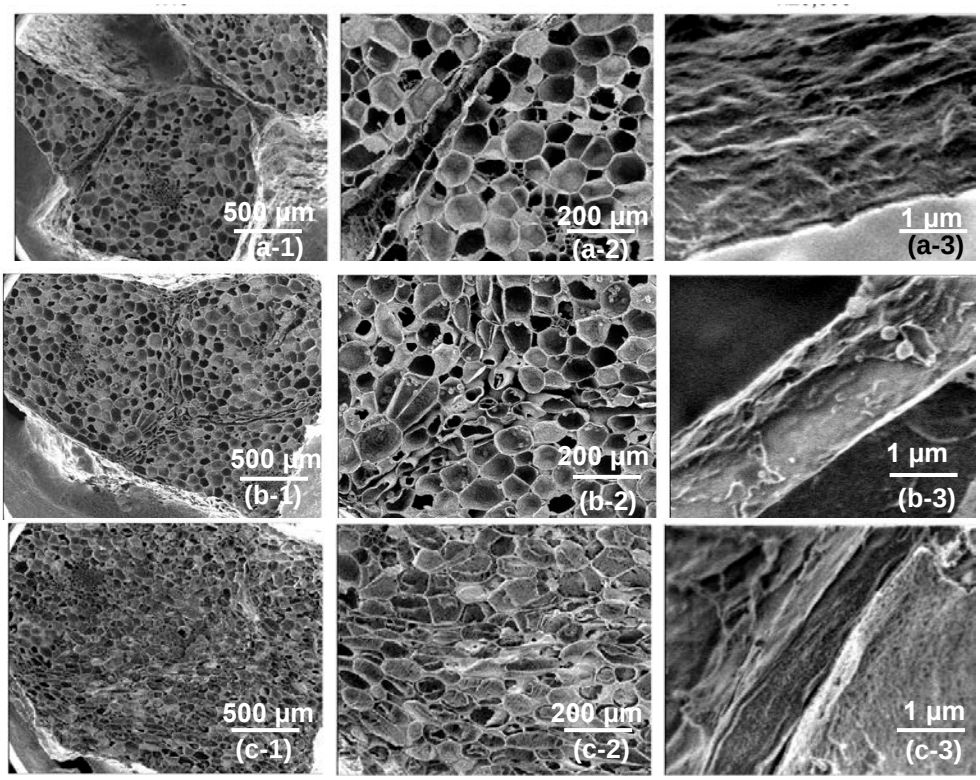

Fig. 5 Cryo-scanning electron micrographs of spaghetti squash soaked for 24 h at 35˚C in the three pectin extractants.

Flesh was soaked in (a) 0.01N HCl solution (pH 2.0), (b) 0.035M ammonium oxalate solution (pH 4.0) or in (c) 2% sodium hexametaphosphate solution (pH 4.0).

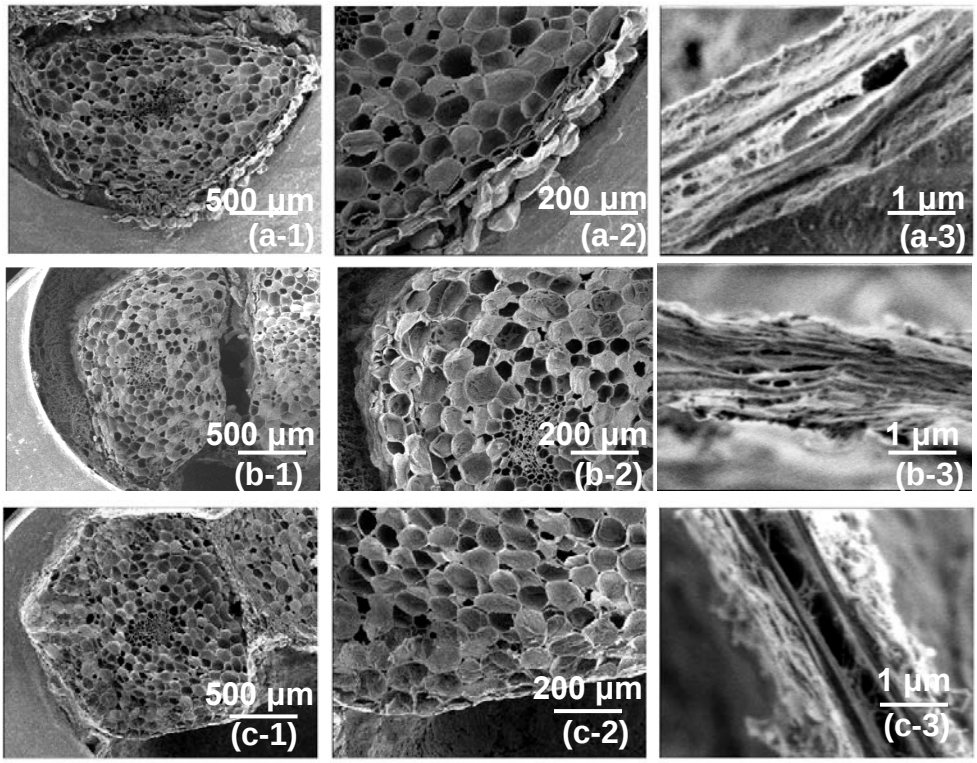

Fig. 6 Cryo-electron micrographs of spaghetti squash after successive extraction of pectic substances by soaking in HCl, sodium acetate buffer and sodium hexametaphosphate solutions.

(a) soaked in 0.01N HCl solution (pH 2.0) for 7 d at 35˚C, (b) 0.1M acetate buffer solution (pH 4.0) for 6 d at 35˚C or (c) 2% sodium hexametaphosphate solution (pH 4.0) at 90˚C for 3.5 h.

3.5 Analysis of Extracts Eluted into Cooking or Soaking Solutions

Amounts of calcium and magnesium extracted from flesh are shown in **Table 1**. Raw flesh contains 15.72 mg of calcium and 14.38 mg of magnesium. When cooked for 15 min, 4.16 mg of calcium and 7.64 mg of magnesium were eluted into the cooking solution. However, when soaked in HCl solution for 24 h, 15.40 mg of calcium and 10.55 mg of magnesium were extracted while when soaked in AO and HMP solutions, about 2 mg of calcium and 5~8 mg of magnesium were eluted and separation into strands was slight (**Fig. 2**). HCl was the most effective calcium and magnesium sequestering agent. Therefore, when soaked in HCl solution, flesh separated into strands. When vegetables were soaked in 0.01N HCl solution (pH 2.0), high-methoxyl-pectin was extracted from the tissues due to removal of polyvalent cations [2-5]. Therefore, it is suggested that a substance gluing threads of flesh together should be high-methoxyl-pectin.

Table 1 Amounts of calcium and magnesium extracted from spaghetti squash

Cooking or soaking condition	Ca	Mg
Raw squash	15.72	14.38
Cooked in distilled water for 15 min	4.16	7.64
Soaked in 0.01N HCl solution for 24 h at 35℃	15.40	10.55
0.035M ammonium oxalate solution for 24 h at 35℃	2.47	8.09
2% sodium hexametaphosphate solution for 24 h at 35℃	2.13	4.83

*The amounts of calcium and magnesium were determined by fresh weight basis (mg/100g).

3.6 Changes in Pectic Substances of Spaghetti Squash during Cooking

The composition of pectic substances of raw, cooked and soaked spaghetti squash fractionated with five reagents is shown in **Fig. 7**. Pectic substances were extracted from AIS for this paper.

Pectic composition of spaghetti squash used in this experiment differed from that reported previously [1]. In the previous report [1], the percentage of PA, PB and PC in the raw sample was 69.0%, 28.9% and 2.1%, respectively. However, the amounts of PB and PC in raw tissue were larger for this paper than those in the previous report [1]. The percentage of WSP in raw flesh was least.

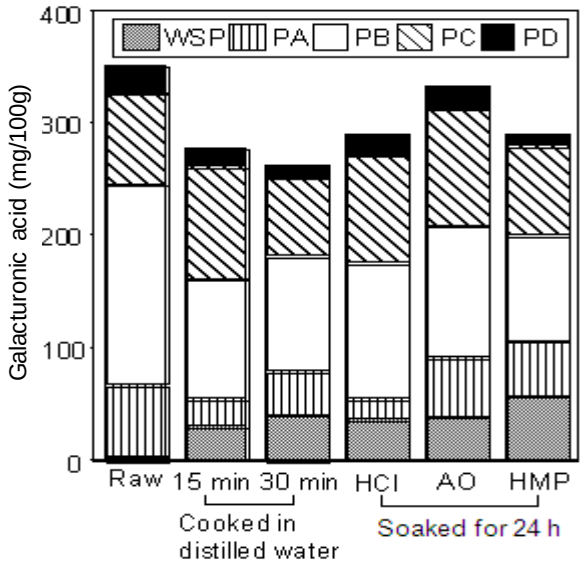

Fig. 7 Changes in pectic substances of spaghetti squash during cooking in distilled water and soaking for 24 h at 35˚C in three pectin extractants.
HCl: 0.01N HCl solution (pH 2.0), AO: 0.035M ammonium oxalate solution (pH 4.0), HMP: 2% sodium hexametaphosphate solution (pH 4.0), WSP: water soluble pectin, PA: 0.01N HCl soluble pectin, PB: 0.1M acetate buffer soluble pectin, PC: 2% sodium hexametaphosphate soluble pectin, and PD: 0.05N HCl soluble pectin.

When cooked for 15 or 30 min without stirring, cooking method differed; flesh was stirred during cooking for the previous report [1], but was not stirred for this report. About 50% pectin was released into cooking solution during cooking for 15~30 min. However, the total pectin remaining after cooking was larger for this paper than those in the previous report [1]. Therefore, the amount of pectin eluted into cooking water in this report might be smaller than in the previous report [1].

When flesh was soaked in 0.01N HCl solution for 24 h, pectin remaining in tissue was smallest, the amount of PA decreased, and WSP in tissue increased. This suggests that high-methoxyl-pectin was eluted into 0.01N HCl solution and PB became WSP during soaking. The pectin remaining in tissue was larger when soaked in AO solution than that soaked in HMP solution. When soaked in HMP solution, only pectin in the surface of tissue might be eluted, but the pectin in the center of tissue might remain, thus the center of tissue was found to be the firmest **(Fig. 3)**.

4. Summary

The flesh of spaghetti squash separates into strands when cooked. The purpose of this paper is to investigate the cause of strand separation (during cooking) by soaking for 24 h at 35˚C in solutions with three kinds of pectin extractant. The changes in strand separation, firmness, histological structure and the pectin of flesh during soaking in 0.01N HCl solution (pH 2.0), 0.035M ammonium oxalate solution (pH 4.0), or 2% sodium hexametaphosphate solution (pH 4.0) were investigated. When flesh was soaked in the HCl solution, the separation into strands and removal of calcium and magnesium were greater than that soaked in other agents. High-methoxyl-pectin was extracted by soaking in HCl solution (pH 2.0) due to removal of polyvalent cations. This result shows that high-methoxyl-pectin glues cells together in the flesh of spaghetti squash. The shape of the cells which constituted strands was round; on the other hand, that of cells surrounded strands was elongated. When cooked in boiling water or soaked at pH 2.0, the shape of the former cells was maintained, but the latter cells, which contributed to adhesion between strands, broke down. Thus, the flesh separated into strands. When flesh was boiled for 15-30 min, pectin degraded and dissolved in the cooking solution; consequently, the flesh separated into strands and also the middle lamella of cell walls of strands separated. However, pectin remaining in strands maintained their crispness.

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Conclusion

Chapter I

Spaghetti squash separated into strands when boiled with stirring. After cooking for 15 min, the shape of round cells which contributed the strands was maintained, but the elongated cells which surrounded the strands broke down. 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 (DE) of PA, PB and PC was 67.4%, 61.5% and 55.6%, respectively. 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 cooking. High methoxyl pectin was degraded by β-elimination during boiling; consequently, the flesh separated into strands. This suggests that high methoxyl pectin glues cells together in the flesh of spaghetti squash.

Chapter II

When spaghetti squash was boiled for 15-30 min, high-methoxyl-pectin was degraded and dissolved in cooking water. Consequently, the flesh separated into strands and the middle lamella of cell walls was separated. When flesh was soaked in 0.01N HCl solution of pH 2.0 for 24 h at 35˚C, the separation into strands and the extraction of calcium were greater than that soaked in the other solutions (AO and HMP). Soaking in 0.01N HCl solution promoted the extraction of high-methoxyl-pectin due to the removal of calcium; consequently, the flesh separated into strands. This suggests that high methoxyl pectin glues cells together in the flesh of spaghetti squash. The pectin of cells, which the surface of the thread composes, might be high-methoxyl-pectin.

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