Comparison of cell wall pectic polysaccharides in flesh extracted with water and hot water from various fruits.

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Introduction

The cell walls of fruit flesh are composed of a mixture of polysaccharides, e.g. pectic, hemicellulosic and cellulosic polymers. It is important for cell wall structural studies that the cell wall components isolated by enzymatic degradation and chemical extraction reflect the composition of the wall (6). In our laboratory, alcohol insoluble solid (AIS) obtained from fruit flesh have been used extensively to investigate the structures and functions of the polysaccharides from the cell walls. Pectic farctions are normaly extracted with water, chilating reagent and hydrochloric acid, successively (7). Recently, as the chilating reagent, CDTA (10, 11, 4) or EDTA (7, 9) heve been used for the extraction of pectic polymers, followed by Na2CO3. For the extraction of hemicellulose, KOH was used (10, 11, 1, 7,4, 9) in many studies.

In the investigation of the enzymatic degradation and the function of cell wall pectic polymers, the chilating reagent, hydrochloric acid or Na2CO3 remaining in the extracted solutions affected the following reactions, even though dialysis had been carried out to remove them. To avoid such effects, in this study, we attempted to compare the effect of the extraction with water and hot water, followed by the extraction with EDTA and HCl, succesively.

Pectins consist of uronic acids and neutral sugars. In fruit flesh, pentose (arabinose, xylose, rhamnose and fucose) and hexose (galactose, glucose and mannose) are inclused as the components of pectic and hemicellulosic fractions (5). Because in pome fruits, stone fruits, and berries, the total amount of pentoses is higher than that of hexoses (5), in this study, uronic acid and pentose content was measured in pectic fractions.

In addition, in this study, we attempted to determine if the amount and/or type of pectic polymers solubilized from AIS were affected by the extrtaction temperature with water prior to the extarction with EDTA and HCl.

Materials and Methods

Fruit materials

Mature fruits grown at Fujisaki Farm, Hirosaki University; apple (*Malus pumila* Mill. Var. domestica Schneid), Japanese aplicot (*Prunus mume* Seib. E. Zicc.), red raspberry (*Rubus idaeus* L.), gooseberry (*Ribes grossularia* L.), blackcurrant (*Ribus nigrum* L.) and redcurrant (*Ribus vulgare* Lam.); were harvested during the commercial harvest period.

Kiwifruit (*Actinidia chinensis* Planch.), pineapple (*Ananas comosus* Merr.), papaya (*Carica papaya* L.), melon (*Cucumis melo* L.), water melon (*Citrullus lanatus* Matsum. et Nakai) avocado (*Persea americana* Mill.), cherry (*Prunus avium* L.), loquat (*Eriobotrya japonica* Lindl.), peach (*Prunus percica* Batsch var. *vulgalis*

Table 1. Uronic acid and pentose contents in water and hot water soluble fractions Extracted from alcohol insoluble solid in flesh (mg/g AIS)

Family	Fruit	Water fraction				Hot water fraction			
		Uronic	Pentose	Total	P/U+P(%)	Uronic	Pentose	Total	P/U+P(%)
Actinidiaseae	Kiwifruit	117.8	41.0	158.8	25.8	155.6	60.7	216.4	28.1
Anacardiaceae	Mango	225.8	64.1	289.9	22.1	263.7	57.5	321.2	17.9
Bromeliaseae	Pineapple	14.2	10.8	25.0	43.2	37.6	48.4	86.0	56.3
Bombacaceae	Durain	74.6	37.7	112.3	33.6	125.8	36.1	161.9	22.3
Caricaseae	Papaya	233.9	77.9	311.8	25.0	272.6	70.9	343.5	20.6
Cucurbitaceae	Melon	107	54.8	161.8	33.9	175.5	79.1	254.6	31.1
	Watermelon	48.2	69.0	117.2	58.9	97.4	62.5	159.9	39.1
Cuttiferae	Mangosteen	86.5	34.5	121.0	28.5	177.2	68.1	245.4	27.8
Lauraceae	Avocado	61.6	53.6	115.2	46.5	76.3	54.7	131.0	41.8
Rosaseae	Apple (Fuji)	86.1	60.8	146.9	41.4	210.7	105.1	315.9	33.3
	Cherry	105.1	68.0	173.1	39.3	169.0	107.2	276.2	38.8
	Japanese apricot	77.9	47.1	125.0	37.7	220.4	122.8	343.2	35.8
	Loquat	80.1	42.7	122.8	34.8	128.6	57.7	186.3	31.0
	Peach	145.8	49.9	195.7	25.5	234.1	74.1	308.3	24.0
	Plum	101.5	64.73	166.3	38.9	247.6	111.9	359.5	31.1
	Raspberry	38.6	17.0	55.6	30.6	60.6	27.2	87.8	31.0
	Strawberry	114.6	61.8	176.3	35.0	252.5	90.0	342.5	26.3
Rutaceae	Orange	156.7	50.1	206.8	24.2	276.0	74.2	350.2	21.2
Sapindaceae	Lychee	92.1	30.8	123.0	25.1	197.9	67.1	265.0	25.3
	Rambutan	59.0	41.5	100.4	41.3	128.4	66.2	194.5	34.0
Saxifragaseae	Blackcurrant	90.6	22.7	113.3	20.1	118.8	33.6	152.5	22.1
	Gooseberyy	140.2	64.1	204.3	31.4	252.7	85.4	338.1	25.3
	Redcurrant	100.6	30.5	131.2	23.3	202.6	52.6	255.1	20.6
Vitaceae	Grape (Kyoho)	65.5	24.8	90.3	27.5	113.1	31.6	144.8	21.9
	Grape (Delawar)	84.6	30.9	115.5	26.8	136.5	37.0	173.5	21.3

P/P+U=(Pentose/Uronic acid+Pentose)×100

Maxim.), plum (*Prunus salicina* Lindl.), strawberry (*Fragaria x ananassa* Duch.), orange (*Citrus sinensis* osbeck), lychee (*Litchi chinensis* Sonn.), and garpes (*Vitis labrusca* L.×*Vitis vinifera* L. cv. Delaware and Kyoho) were purchased through a market.

Mango (*Mangifera indica* L.), durian (*Durio zibethinus* Murr.), papaya (*Carica papaya* L.), mangosteen (*Garcinia mangostana* L.) and rambutan (*Nephelium lappaceum* L.), were collected and extarcted in Chiang Mai University of Thailand. These fruits were classified into thirteen families depending on plant taxonomy (Table 1). Five replicates of each fruit were sampled and analysed individually, except small fruits, for which about 30 g of flesh were collected as one sample and five replicates were prepared.

Extraction and analysis of pectic substances

Peel, seeds and core of the fruit were removed and flesh was stored −20℃ until the extraction was carried out. Frozen flesh was extracted several times with 80% methanol at 70℃. Alcohol extract and residue was separated by filtration. In avocado and durian, the residue were extracted with acetone to remove lipid (2). The residue was lyophilized and pulverized after the dry weight (alcohol insoluble solid: AIS) was weighed. The cell wall pectic substances in AIS of flesh were extracted with water (20℃, 2 H×3 times) or hot water (100℃, 2 h×3 times). Uronic acid and pentose contents in water and hot water fractions were determined with the m-hydroxydiphenyl method (3), and orcinol method (8), respectively.

Six kinds of fruit (mango, durian, papaya, apple, Japanese apricot, and strawberry) were selected from these 25 fruits and the residues of water or hot water fractions were extracted successively with EDTA

(20℃, 2h×3 times) and 0.05M HCl (85℃, 1h×3 times). Uronic acid and pentose contents in these fractions were determined as above.

Gel permeation chromatography

Water or hot water fractions from AIS was applied to a Cellulofine GCL-2000-m column (18×400mm) equibrated with 0.1% NaCl. Elution was caried out with 0.1% NaCl, at the flow rate of 0.3ml/min. The eluate was fractionated every 3ml. Uronic acid and pentose contents in each eluted fraction were determined as above.

Results and Discussions

1. Uronic acid and pentose contents in water and hot water soluble fractions.

Uronic acid content in hot water fractions were higher than those in water fraction in all 24 kinds of fruits (Table 1). The differences in the uronic acid content between water and hot water fractions were apparently bigger in apple, Japanese aplicot, plum, strawberry, lychee and gooseberry.

Pentose contents in the hot water fractions were comparatively higher than those in the water fractions in most fruits, with the exception of kiwifruit, pineapple and blackcurrant. The differences in the pentose contents between water and hot water fractions were comparatively bigger in pineapple, mangosteen, apple, Japanese aplicot, plum and gooseberry. No relationship was found between the taxonomical classification and uronic or pentoses contents in water and hot water fractions.

These results showed that hot water was more effective than water insolubilizing pectic polysaccharides.

2. Uronic acid and pentose contents in water or hot water, EDTA and HCl fractions.

As hot water fraction included much more pectic polymers than water fraction (Table 1), uronic acid and pentoses contents in EDTA and HCl fractions were determined, using six kinds of fruit (Table 2). The data of uronic acid and pentose contents in water (C-WSP) and hot water (H-WSP) farctions in Table 2 were same as those in Table 1.

In EDTA fraction after the extraction with water (C-ESP), uronic acid content in most fruits except Japanese aplicot was higher than after the extraction with hot water (H-ESP). But pentose content in

Table 2. Uronic acid and pentose contents in water or hot water, EDTA and HCl soluble fractions from alcohol insoluble solid (mg/g AIS)

Content	Fruit	Water fraction				Hot water fraction			
		C-WSP	C-ESP	C-HCP	Total	H-WSP	H-ESP	H-HCP	Total
Uronic acid	Mango	225.8	42.2	30.7	298.6	263.7	24.7	7.4	295.8
	Durian	74.6	17.7	33.1	125.4	125.8	13.6	11.1	150.5
	Papaya	233.9	103.8	20.6	358.4	272.6	71.5	5.0	349.2
	Avocado	61.6	10.8	6.7	79.1	76.3	8.7	2.7	87.7
	Apple	86.1	57.3	84.0	227.5	210.7	44.4	30.7	285.9
	Japanese apricot	77.9	24.4	179.3	281.6	220.4	40.0	23.3	283.7
	Strawberry	114.6	81.6	68.4	264.6	252.5	58.6	20.6	331.7
Pentose	Mango	64.1	13.2	17.0	84.3	57.5	6.2	7.2	70.9
	Durian	37.7	1.5	14.7	53.9	36.1	1.7	6.1	44.0
	Papaya	77.9	8.5	9.8	96.2	70.9	9.3	4.1	84.2
	Avocado	53.6	5.9	33.4	92.9	54.7	7.9	16.1	78.8
	Apple	60.8	4.0	92.8	157.7	105.1	7.4	21.0	133.6
	Japanese apricot	47.1	9.5	129.4	186.0	122.8	19.4	17.7	159.9
	Strawberry	61.8	11.5	35.7	109.1	90.0	6.0	11.6	107.6

Table 3. Percent distribution of uronic acd and pentoses to water or hot water, EDTA and HCl soluble fractions (%)

Component	Fruit	Water fraction			Hot water fraction		
		C-WSP	C-ESP	C-HCP	H-WSP	H-ESP	H-HCP
Uronic acid	Mango	75.6	14.1	10.3	89.1	8.4	2.5
	Durian	59.5	14.1	26.4	83.6	9.0	7.4
	Papaya	65.3	29.0	5.8	78.1	20.5	1.4
	Apple	37.8	25.2	36.9	73.7	15.5	10.7
	Japanese apricot	27.7	8.7	63.7	77.7	14.1	8.2
	Strawberry	43.3	30.9	25.9	76.1	17.7	6.2
Pentose	Mango	68.0	14.0	18.0	81.1	8.8	10.1
	Durian	70.0	2.8	27.2	82.1	3.9	14.0
	Papaya	81.0	8.8	10.2	84.2	11.0	4.8
	Apple	38.6	2.5	58.9	78.7	5.6	15.7
	Japanese apricot	25.3	5.1	69.6	76.8	12.1	11.1
	Strawberry	56.7	10.6	32.8	83.6	5.6	10.8

C-ESP was higher than in H-ESP only in strawberry and mango.

In hydrochloric acid soluble fraction after the extraction with hot water and EDTA (H-HCP), both uronic acid and pentose contents were remarkably lower than in the fraction after the extraction with water and EDTA (C-HCP).

Total amounts of uronic acid (H-Total) in hot water (H-WSP), EDTA (H-ESP) and HCl (H-HCP) farctions were distinctly higher than the total amount (C-Total) of water (C-WSP), EDTA (C-ESP) and HCl (C-HCP) soluble fractions, in all 6 fruits. Similarly, total amount of pentoses (H-Total) in hot water (H-WSP), EDTA (H-ESP) and HCl (HCP) fractions were lower than the total amount of them (C-Total) in water, (C-WSP), EDTA (C-ECP) and HCl (C-HCP) fractions.

These results showed that by the extraction with water at high temperature, the polymers including more uronate in C-HCP could be solubilized, resulting in the increase of pectic polymers in hot water fraction (H-WSP).

3. Percent distributions of uronic acid and pentose in each extracted fraction.

Among the extracted components, percentages of uronic acid and pentoses in H-WSP were remarkably higher than in C-WSP, while the percentage of hydrochloric acid soluble component in H-HCP was lower than that in C-HCP (Table 3).

In usual procedures for the extraction of pectic substances, polymers soluble in hydrochloric acid are extacted by heating at 85℃, after the extraction with water and EDTA. From the results shown in Tables 2 and 3, it is conceivable that the polymers in HCl fraction (C-HCP) could include two groups of polymers, which are hot water soluble fraction and hot HCl soluble fraction.

4. Relative ratio of uronic acid and pentose in each fraction.

Pectic compounds were constituted with uronate polymers as the main chain, rhamnose are inserted in the uronate polymers, and neutral sugars attached to the rhamnose residue, forming the side chain in the pectin molecules. Every pectic polymer includes uronic acid (and/or its derivatives) and neutral sugars in different ratio, by fruit species, stage of growth, maturation and ripening. The ratio of uronic acid and neutral sugars in each pectic fraction could be a clue to investigate the type of pectic polymers. Based on this idea, the percentage of pentose in the amount of uronic acid and pentose was calculated and expressed as the pentose ratio (Table 4).

Table 4. Relative ratio of pentose to the amount of uronic acid and pentose in each fraction (%)

Fruit	Water fraction				Hot water fraction			
	C-WSP	C-ESP	C-HCP	Total	H-WSP	H-ESP	H-HCP	Total
Mango	22.1	23.8	35.7	24.0	17.9	20.1	49.3	19.3
Durian	33.6	7.9	30.7	30.1	22.3	11.3	35.7	22.6
Papaya	25.0	7.6	32.1	21.2	20.6	11.5	44.8	19.4
Apple	41.4	6.5	52.5	40.9	33.3	14.3	40.6	31.8
Japanese apricot	37.7	28.0	41.9	39.8	35.8	32.7	43.1	36.0
Strawberry	35.0	12.4	34.3	29.2	26.3	9.3	36.1	24.5

Pentose ratio in C-WSP and H-HCP were higher than in H-WSP and in C-HCP, respectively, in most fruits. Among C-ESP and H-ESP, no difference was detected.

Compared to Table 1, pentose ratios of C-WSP and H-WSP from 24 kinds of fruits showed similar tendencies. Between the pentose ratio in C-WSP and H-WSP, significant (0.01%) difference in the statistical analysis was detected (Table 1).

It is conceivable that by heating water with AIS at the first step of the extarction, pectic polymers with comparatively higher amount of uronate can be solubilized.

5. Distribution of molecular size in water and hot water soluble fractions.

The profile of gel permeation chromatography construcrted by uronic acid content (Fig. 1) was roughly similar. One peak was detected in both of C-WSP and H-WSP, in all fruit. In both of C-WSP and H-WSP soluble fractions, most of the polymers were eluted in the fractions 12-30, but the elution profile of H-WSP seemed to sift to low molecules compared with C-WSP.

In pentose, the elution profiles were different shape by fruits species. This result showed that the neutral sugar side chains of pectic polymers were different by fruit. The elution profiles of H-WSP indicated that the polymers with smaller size were included compered with those in C-WSP. In all fruits, the profiles seemed to include two peakes more or less, and the retantion volume of the former peak was corresponded to that of uronic acid. However, we could not know whether the pentoses were linked to the uronate polymer or not.

From these results, H-WSP can be used for the investigtion on the enzymatic degradation and on the function of the pectic polymers from plant cell wall, because it has the advantage of not requiring removal of the chilating reagent and HCl from the extracted solutions.

Summary

Using 24 kinds of fruits, in the procedure for the extraction of pectic polymers from alcohol insoluble solid (AIS) in fruit flesh, amount and type of pectic substances in water and hot water extracts were compared. Amount of uronate and pentose contents in hot water fraction were apparently higher than those in water fraction, in all fruits analyzed in this study. Using six kinds of fruit, after the extraction with water or hot water, pectic polymers were extarcted with EDTA and HCl successively. In HCl fraction after hot water extraction, amounts of uronate and pentose contents were apparently lower than in the fraction after the extraction with water. The percentage of pentose in the amount of uronic acid and pentose were higher in the water fractions than in the hot water fractions. From these results, by heating with water in the first step of extraction, polymers included much more uronate were solubilized compared with water extraction. A part of pectic polymers in HCl fraction (after the extraction with water and EDTA), seemes to be solubilized by heating during extraction. Hot water fraction from AIS can be used for the investigations

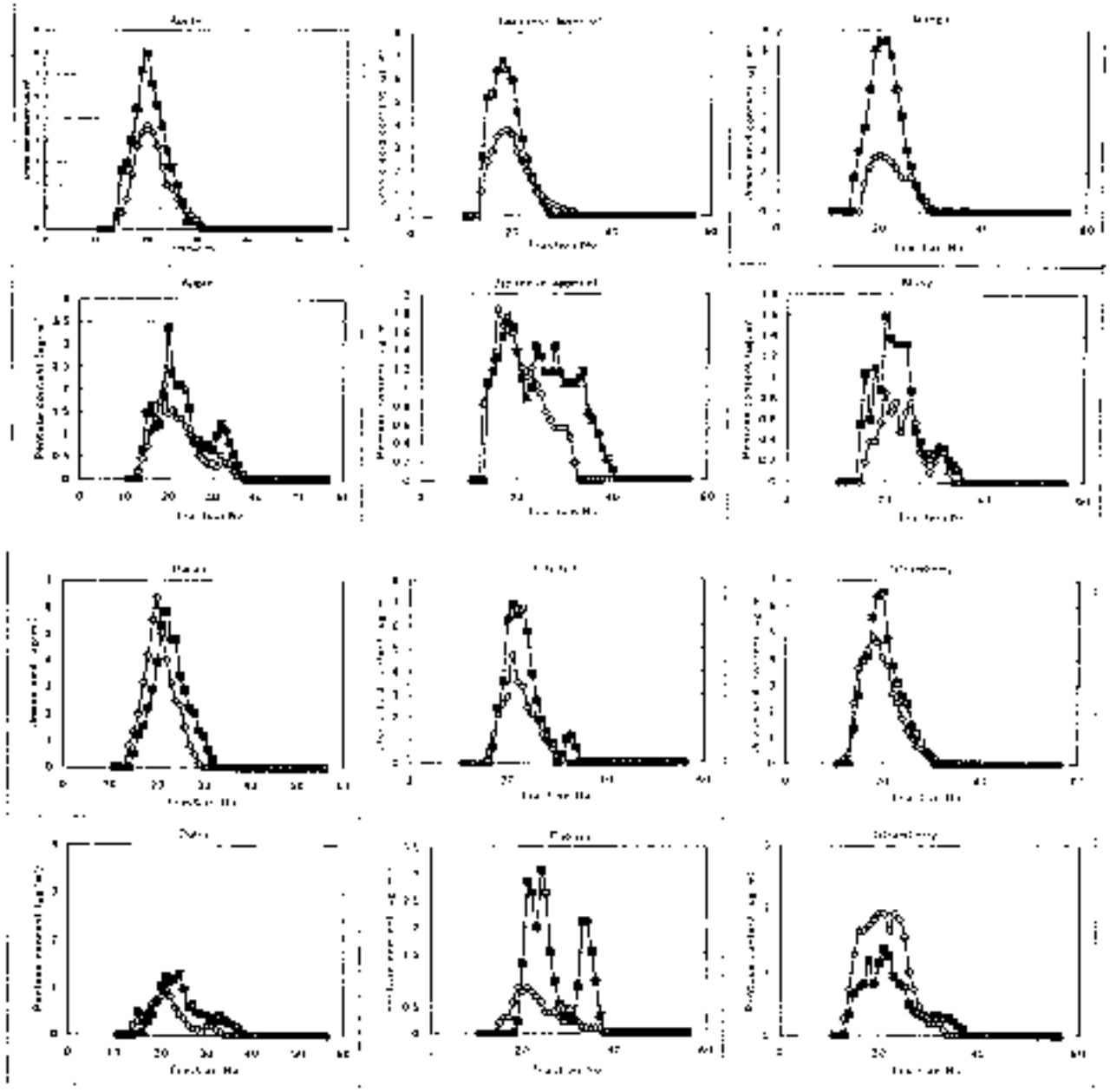

Fig. 1 Profiles gel permiation chromatography of water and hot water fractions, determined by uronic acid and pentose contents.
—◇— Water fraction, —⊠— Hot water fraction.

of the enzymatic degradation and the function of the pectic polymers with the advantage of not requiring to removal of the chilating reagent and HCl from the extracted solutions.

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多種類の果実細胞壁から水または熱水で抽出したペクチン性多糖類の比較

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果肉からペクチン性多糖類を水で抽出するにあたって，抽出温度の違いが抽出される多糖類の構成に及ぼす影響の有無を検索することを目的として，植物分類上，13科に属する24種の果実の果肉を用いて，アルコール不溶性固形物を水または熱水で抽出し，得られた多糖の量，それぞれの画分に含まれるウロン酸およびペントースの量的な比率の比較を行った。用いたすべての果実で，熱水画分のウロン酸含量は水画分のウロン酸よりも明らかに多く，両画分の差が特に大きかったのは，リンゴ，ウメ，スモモ，イチゴ，オレンジ，レイシ，およびグズベリーであった。熱水画分のペントース含量は水画分よりやや多く，両画分の差が比較的大きかったのはパイナップル，マンゴスチン，リンゴ，ウメ，スモモ，およびレイシであった。

水または熱水抽出後の不溶性残さをEDTA，HClで順次抽出したところ，水抽出後の塩酸画分に比べて，熱水抽出後の塩酸可溶性画分が著しく少なかった。水抽出後に塩酸で抽出される画分の一部が熱水で抽出されることから，水抽出後の塩酸画分には，熱水可溶性画分と熱塩酸可溶性画分が含まれていると推定された。熱水で抽出される多糖類は水で抽出される多糖類よりもペントースに対するウロン酸の割合が高かった。また，ゲルろ過の結果から，熱水で抽出される多糖類と，水で抽出される多糖類とは，中性糖の分子量分布に違いがあることが明らかにされた。

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