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Galacturonic Acid Content and Degree of Esterification of Pectin from Sweet Potato Starch Residue Detected Using ^{13}C CP/MAS Solid State NMR

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Authors' contributions

This work was carried out in collaboration between all authors. Author SN designed the study, performed the laboratory work, the statistical analysis, and wrote the first draft of the manuscript. Author JH was responsible for the acquisition and interpretation of the NMR spectra. Authors JP and Janet P performed critical reviews of the first and draft. All authors read approved the final manuscript.

Research Article

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ABSTRACT

Starch residue samples from two Australian sweet potato varieties (Beauregard and Northern Star) and two Indonesian sweet potato varieties (Bis192 and Bis183), and a commercial sample of sweet potato starch residue, were studied for their pectins. Pectins were extracted using 0.1M HCl, 0.05M NaOH, 0.1M HCl/0.75% SHMP and 0.05M NaOH/0.75% SHMP. Hydrolysis of residual starch in the cell wall of sweet potato using heat stable α-amylase and amyloglucosidase was employed prior to pectin extraction to eliminate starch contamination. Pectins were characterised for yield, galacturonic acid content (GA), and the degree of esterification (DE). Conventionally, pectin is characterized

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by titration, photometry and HPLC. However these methods are cumbersome and time consuming. On the other hand, ^{13}C CP/MAS solid-state NMR, a non-destructive, efficient and direct method, has been found to be well-suited for these purposes since pectin has well-defined ^{13}C NMR spectra. Therefore ^{13}C CP/MAS solid state NMR was used for pectin determination. The pectin characteristics are dependent on variety and extraction process; however, the extraction methods gave variable results. Yields were between 7 and 30% of the cell wall. GA varied from 27 to 80% with the highest found in Bis192 extracted using NaOH/SHMP. DE varied between traceable and 57%. HCl extraction gave higher DE, while NaOH/SHMP caused demethylation. Overall, this study demonstrated that pectin from sweet potato starch residue is mainly low in methoxyl groups.

Keywords: Sweet potato; starch residue; pectin; ^{13}C CPMAS solid state NMR.

1. INTRODUCTION

Pectin has many important functions in plants. It contributes to the structural integrity and mechanical strength of the tissue by forming a hydrated cross-linked three-dimensional network [1,2]. Pectin also plays an important role in the physical and sensory properties of fresh fruit and vegetables (ripeness and texture) and contributes to their processing characteristics in canned products, purees, and juices [3]. Commercially, pectin has broad applications in both the food and pharmaceutical industries, where it acts as gelling and thickening agents [2,4], prevents the formation of cheesy milk layer in gelled milk dessert, and regulates the thickness and mouth-feel of fruit drink powder when the powder is dissolved in cold water [5]. In addition, pectin has proven to have beneficial effects on human health [6,7,8].

To date, citrus peel and apple pomace are the major commercial sources of pectin. Many attempts have been made to prepare pectin from other sources such as tropical fruits [9], sunflower heads[10], beet and potato pulp [11], soy hull [12], and duckweed[13]. However, pectins extracted from those materials have poor gelling ability characteristic as compared to apple and citrus pectin.

The physico-chemical properties of beet pectin has been reported to be influenced by extraction conditions [14,15]. Sugar beet pectin that is not yet utilized fully due to poor gelling ability has been reported to have effective emulsifying properties [16,17]. Recently, Byg et al. [18] reported that industrial potato waste contains appreciable amount of rhamnogalacturonan I (hairy region of pectin). This opens the possibility to investigate the potential use of other crop residue materials, such as sweet potato starch residue, as pectin sources.

Sweet potato (*Ipomoea batatas* (L.) Lam), a fairly drought-tolerant crop, is widely grown throughout the world, primarily in the tropics and subtropics. In Indonesia, although sweet potato production is not as high as that of China, the trend with respect to utilization of sweet potato is changing from domestic consumption to use in various commercial products.

The utilization of sweet potato within the industrial sector, has led to the production of considerable amounts of waste materials such as starch residues all year around. Sweet potato starch residue (non-starch polysaccharide) has an appreciable amount of pectin [7,19,20,21,22]. However, in contrast to pectin from other plant sources, sweet potato pectin has never been studied intensely. Therefore the purpose of this research was to elucidate

some chemical properties of pectin extracted from some varieties of Indonesian and Australian sweet potatoes.

Pectin is embedded between the matrix of starch and cell wall and these have similar solubility in the extraction media examined [23]. Therefore removal of starch from the sweet potato starch residue was attempted before pectin extraction. In this study, the sweet potato starch residue was prepared in the laboratory from the Australian sweet potato varieties. These isolates and the sweet potato starch residue from Indonesian varieties were subjected to starch removal procedures. There are several ways of removing residual starch from the cell wall. However, in order to minimize pectin degradation during starch-free cell wall preparation, the enzymatic hydrolysis method was chosen

2. MATERIALS AND METHODS

2.1 Raw Materials

Beauregard and Northern Star varieties of sweet potato were grown and packed by Kidd Enterprise, Redland Bay Queensland, and ordered via Yep Lum and Co. Stand 281, C Block, Flemington Market, NSW, Australia. BIS 183 and BIS 192 were obtained from Balai Penelitian Kacang Kacangan dan Umbi Umbian (Tuber and Legume Research Center) The ministry of Agriculture, Malang East Java, Indonesia, Australia in the form of dried chips/slices. Sweet potato starch residue was obtained from a local sweet potato manufacturer at South Lampung district, Province of Lampung Indonesia. Brought to Food Science UNSW, Sydney in the form of dried powder. The varieties processed were commercially grown in the surrounding areas, varieties were not known exactly, and consisted of mixed local varieties.

2.2 Cell Wall Materials (CWM) Preparation

CWM was prepared from sweet potato starch residue according to the method of Noda et al. [20] with a slight modification, where the incubation time in boiling water was reduced from 20 min to 5 min. In addition, glucoamylase (synonym amyloglucosidase: exo-l,4-α-glucan glucanohydrolase, EC 3.2. 1. 3) was employed in the second digestion. Ground dried sweet potato starch residue (100 g) was suspended in distilled water (200 mL) and boiled for 5 minutes. The suspension was maintained at 80ºC, and 0.5 mL of heat-stable α-amylase (Termamyl 120 type LS from Novo Nordisk Denmark) was added, and then incubated for 30 min to hydrolyse the residual starch. The enzyme activity was 120 KNU/g (KNU is Novo units α-amylase- that is the amount of enzyme that breaks down 5-26 g of starch per hour at Novo's standard method). The mixture was centrifuged at 3000 rpm for 10 min, supernatant was discarded and digestion of the residue was repeated with 0.5 mL glucoamylase (EC 3.2.1.3 from *Aspergillus niger*, SIGMA, 30-60 units per mg protein). One unit will liberate 1 mg of glucose from starch in 3 min at pH 4.5 at 55ºC. The mixture was filtered using two layers of cheesecloth. The residue was washed with distilled water, methanol and acetone, successively, and air-dried (Fig. 1).

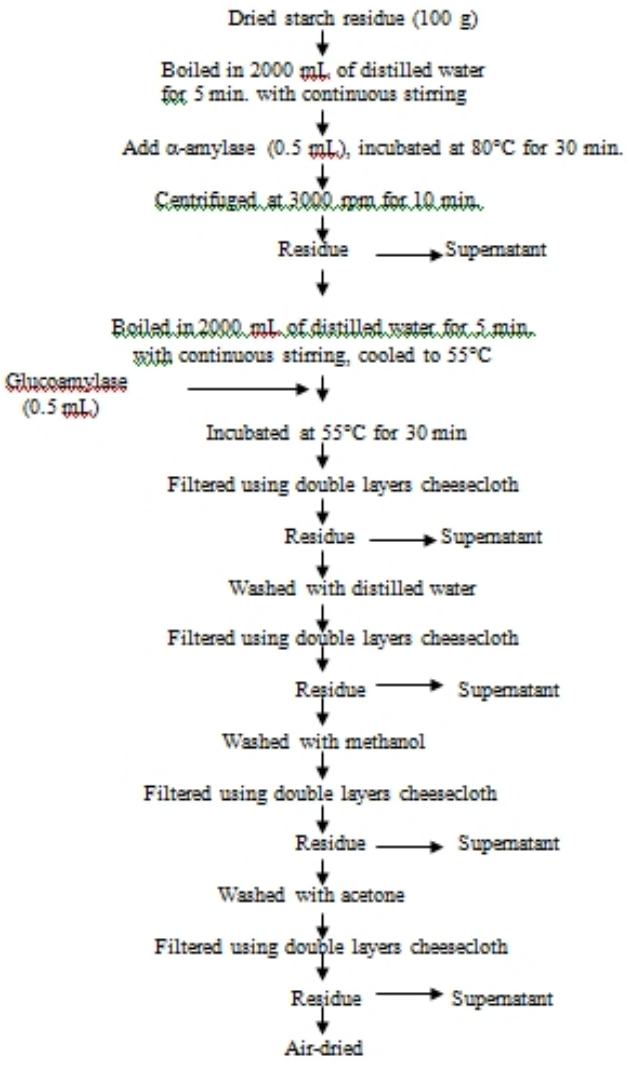

Fig. 1. Flow chart of cell wall preparation from starch residue

2.3 Pectin Extraction

Pectins were extracted from the cell wall by using solutions, namely 0.1 M hydrochloric acid, 0.05 M sodium hydroxide, 0.1 M HCL containing 0.75% sodium hexametaphosphate, and 0.05 M sodium hydroxide containing 0.75% sodium hexametaphosphate. These pectin extraction methods were slightly modified from Turquois et al. (1997) where the pectin was not extracted directly from the sweet potato pulp, instead, the pulp was previously freed from residual starch, using procedure described by Noda *et al*. (1994) with slight modification as described in Section 2.2. This non-starch residue refers to alcohol insoluble residue [24,25].

2.3.1 0.1 M HCl extraction

Samples (10 g) of dried ground cell wall materials were dispersed in 250 mL 0.1 M HCl. The dispersion was stirred and kept at 90ºC for 1 hour. After incubation, the suspensions were centrifuged at 10ºC for 15 min at 10000 rpm. The liquid fraction containing extracted pectin materials was neutralised with 32% NaOH (Laboratory UNILAB Reagent AJAX), then the same volume of 95% ethanol was added, the mixture was stirred for 5 minutes and then stored at 4ºC for 12 hours. The mixture was then centrifuged at 10000 rpm for 15 min and the pectin residue washed with 70, 80, 90% ethanol, successively. Finally the extracted pectin was dried in a freeze dryer for 18 hours, ground and then stored in a desiccator (Fig. 2) prior to analysis for its galacturonic acid content, degree of esterification and starch content

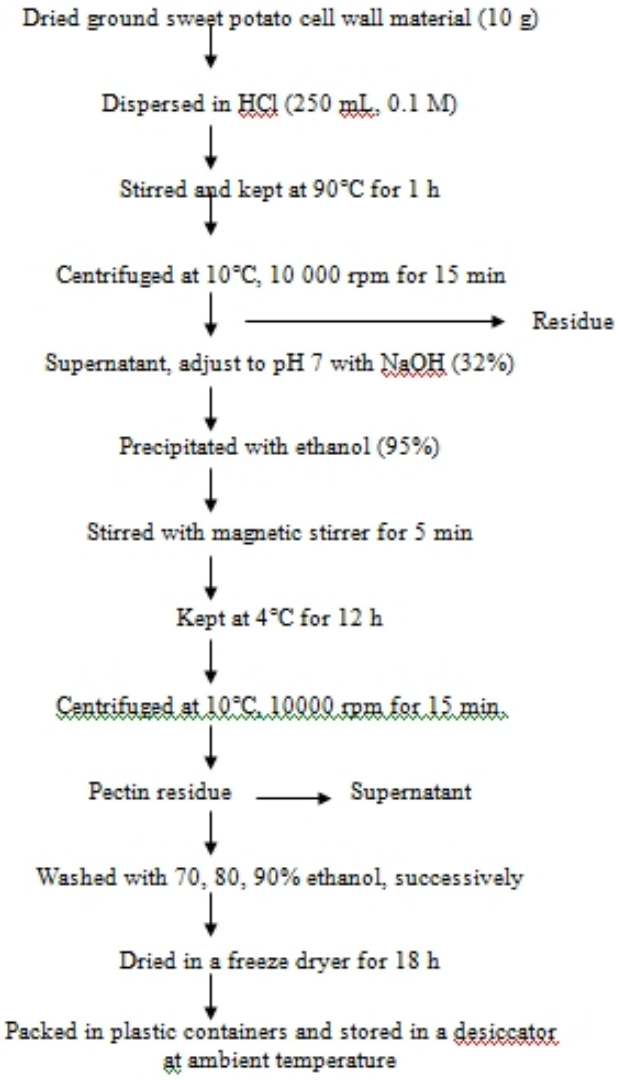

Fig. 2. Flow chart of pectin extraction using 0.1M HCl

2.3.2 Extraction using 0.1 M HCl and 0.75% sodium hexaxmetaphosphate

Pectin was extracted by using 0.1M HCl containing 0.75% sodium hexametaphosphate, using the procedure described in Section 2.3.1.

2.3.3 0.05 M NaOH extraction

Samples (10 g) of dried ground cell wall materials were dispersed in 250 mL 0.05M NaOH. The mixture was kept for 2 hours at 25ºC, then centrifuged at 10ºC, 10000 rpm for 15 minutes. The liquid fraction was neutralised with 5M HCl, and the same volume of 95% ethanol was added, stirred for 5 minutes then stored at 4ºC for 12 hours. The mixture was centrifuged at 10ºC, 10000 rpm for 15 min to separate the precipitated pectin from the ethanol solution. The precipitated pectin was washed successively with 70, 80, and 90% ethanol. The mixture was centrifuged at 10ºC, 10000 rpm for 15 min, then dried in a freeze dryer for 18 hours. The dried pectin was ground, packed and stored in a desiccator until further analysis. A flow chart of this process is shown in Fig. 3.

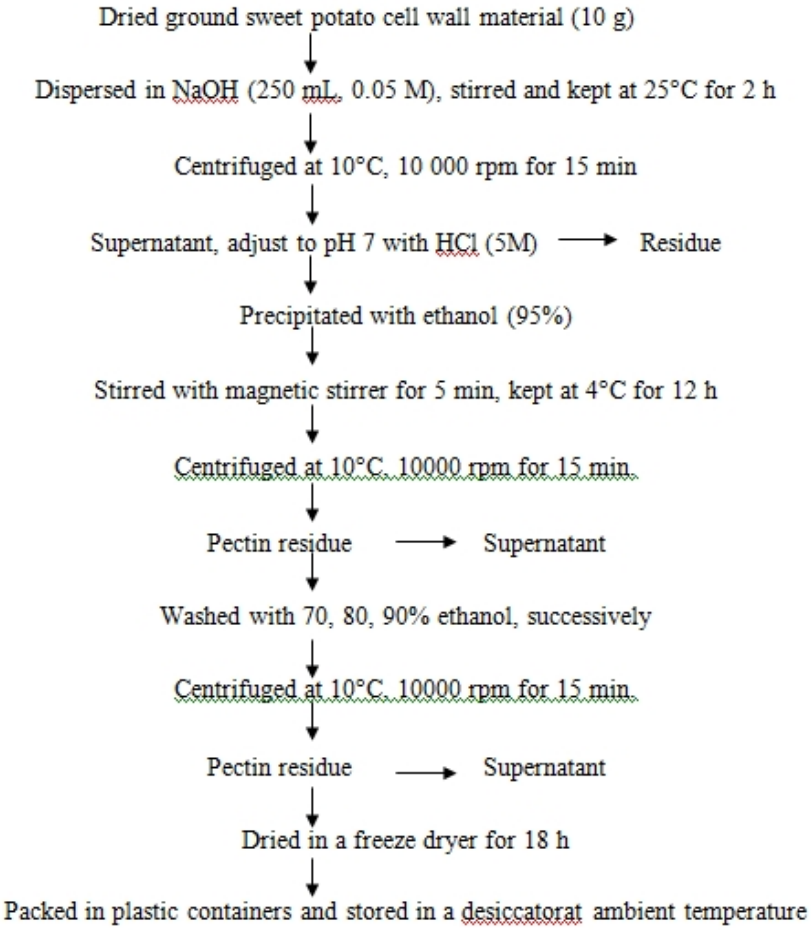

Fig. 3. Flow chart of pectin extraction using 0.05 M NaOH

2.3.4 0.05M NaOH and 0.75% sodium hexametaphosphate extraction

Pectin was extracted by using 0.05M NaOH containing 0.75% sodium hexametaphosphate, using the procedure described in Section 2.3.2.

2.4 Pectin Yield

Pectin yield was calculated as the ratio of dried pectin extracted to dried cell wall materials.

2.5 Galacturonic Acid and Degree of Esterification

The content of galacturonic acid and the degree of esterification were determined by using ^{13}C solid-state NMR method described as follows: ~300 mg of powdered samples were packed into partially-stabilsed zirconia rotors, sealed with fluted caps, then inserted into 7.5 mm CP/MAS Chemagnetics probe and spun at the magic angle (spinning at 54.7º between the direction of the static magnetic field and the rotor axis to reduce the chemical shift anisotropy/CSA pattern to its isotropic) at 4 kHz. Spectra were acquired using a Varian Inova 300 wide-bore solid state NMR spectrometer (Varian Associates, USA). Typical conditions were: observe frequency, 75.4 MHz, recycle delay 5 s, contact time 1 ms, sweep width of 50 kHz. The chemical shifts were adjusted using secondary referencing to hexamethylbenzene (HMB, methyl groups set to 17.3 ppm).

The ^{13}C CP/MAS spectra of non-methylated polygalacturonic acid and methylated citrus pectin (Sigma, Australia) were used as the basis for the interpretation pectin spectra. The signal at 172.7 ppm was assigned to the C-6 carbon of the COOH group and the intense signal at 101.9 ppm was assigned to the C-1 carbon. The peaks between 60 and 90 ppm are carbons of pyranoid rings (C-2,3,5), and the signal at 80.3 is from the C-4 carbon. The signal at 53.7 ppm is assigned to the methyl carbon of the methyl ester groups ($COO\underline{C}H_3$), and the resonances between 18 and 20 ppm are assigned to acetyl ester groups ($OC\underline{C}H_3$). The formulas recommended by Sullivan [26] and Sinitsya [27] were used for GA and DE calculation as follows: Galacturonic Acid (%) = (Area C-6/average area of C1-C5) X 100. Degree of Esterification (%)=($COO\underline{C}H_3$/average area of C1-C5) X 100. The area of resonance of C-1 is converted to 100 and the areas of other resonances are relatively based on that of C-1. The calculation of peak area was performed using SOLARIS 2.7 software program.

2.6 Statistical Analysis

The experiment for cell wall material extraction was constructed as a complete randomized design with 3 replications, whereas the experiment for pectin extraction was constructed as a factorial in a complete randomised design with 3 replications. The first factor was sweet potato variety and the second factor was method of extraction. Analysis of variance (ANOVA) was used to analyse the data, and the comparison of means was carried out at the 5% significance level using the least significant different (LSD) test according to Steel and Torrie[28].

3. RESULTS AND DISCUSSION

3.1 Cell Wall Material of Sweet Potato

The content of cell wall material from the different sweet potato varieties varied from 35 to 52% of dry starch residue (Table 1). These results are higher than those obtained for different sweet potato varieties by Noda et al. [20] where the cell wall material was 33% of dried starch residue. This difference was attributed to method of starch extraction and different varieties. Noda et al. [20] extracted starch from sweet potato flesh by sieving, leading to a higher residual starch content in the residue and lower cell wall material content.

Table 1. Cell wall material content of sweet potato starch residue

Variety	Weight of dry starch residue (g)	Weight of cell wall material (g)	Percentage of cell wall material from dry starch residue*
Beauregard	100	35.3	35.3 a
Northern Star	100	40.6	40.6 b
Bis 192	100	41.6	41.6 b
Bis 183	100	43.3	43.3 b
Starch residue	100	52.1	52.1 c

Means within columns followed by the same letter are not significantly different (P>=0.05)

Beauregard sweet potato had the lowest cell wall material content which explains why this variety is famous for its texture for being less fibrous, soft and moist, with good eating quality (Kidd Enterprise, Queensland, Australia, Personal communication). The factory sample of sweet potato starch residue had the lowest starch content (33%, data not shown) but a higher cell wall material content than the starch residue sample prepared in the laboratory, which was also reported by Salvador et al. [21].

The separation of residual starch from the cell wall is very important because it gives rise to significant contamination during pectin extraction. In general, there are two different methods for starch hydrolysis: enzyme treatment, such as the use of α-amylase, and chemically, such as the use of dimethyl sulphoxide (DMSO). Extraction of starch with DMSO has disadvantages such as the loss of about 6% of the cell wall, mainly pectin [29], incomplete removal of starch (Noda *et al*. 1994), and safety problems because it is a skin, eye and respiratory irritant [30]. Therefore, a combination of heat stable α-amylase and glucoamylase was employed consecutively in this experiment.

Alpha-amylase (1,4-α-D-glucan glucanohydrolase) is an endo-enzyme that catalyses the hydrolysis of 1,4-α-D glucosidic bonds in a random fashion along the polysaccharide chains, whereas glucoamylase catalyses the hydrolysis of terminal 1,4-linkaged α-D-glucose residues successively from the non-reducing ends of malto-oligo and polysaccharides with release of β-D-glucose. Most forms of the enzyme can rapidly hydrolyse 1,6-α-D-glucosidic bonds when the next bond in the sequence is 1,4. α-Amylase alone was not sufficient to hydrolyse all the residual starch in the cell wall, as indicated by a positive reaction with I_2KI (Lugol's solution). Therefore, glucoamylase was employed for subsequent starch removal, mainly the branched polymer amylopectin.

3.1 Pectin yield

The yields of pectin extracted from cell wall materials of sweet potato using various conditions were between 7.2 and 29.3% of dry CWM, or between 0.3 and 1.2 % of sweet potato fresh weight. There are significant differences among varieties and treatments. Northern Star gave the highest yield, followed by Beauregard, Bis 192, Bis 183, and starch residue (Table 2).

Table 2. The yield of sweet potato pectin extracted using different conditions

Varieties	Extraction methods	Pectin yield (g)	Pectin yield (% of cell wall material)*
Beauregard	0.1M HCl	1.47	14.7 fg
	0.1M HCl cont. 0.75%SHMP	1.60	16.0 e
	0.05M NaOH	0.98	9.8 ij
	0.05M NaOH cont.0.75% SHMP	2.88	28.8 b
Northen Star	0.1M HCl	1.53	15.3 ef
	0.1N HCl cont. 0.75%SHMP	1.77	17.7 d
	0.05M NaOH	1.11	11.1 h
	0.05M NaOH cont.0.75% SHMP	3.00	30.0 a
Bis192	0.1M HCl	1.04	10.4 hi
	0.1M HCl cont. 0.75%SHMP	1.37	13.7 g
	0.05M NaOH	0.74	7.4 m
	0.05M NaOH cont.0.75% SHMP	2.78	27.8 b
Bis 183	0.1M HCl	0.93	9.3 jk
	0.1M HCl cont. 0.75%SHMP	1.11	11.1 h
	0.05M NaOH	0.86	8.6 kl
	0.05M NaOH cont.0.75% SHMP	2.86	28.6 b
Starch residue	0.1M HCl	0.89	8.9 jkl
	0.1M HCl cont. 0.75%SHMP	0.81	8.1 lm
	0.05M NaOH	0.72	7.2 m
	0.05M NaOH cont.0.75% SHMP	2.47	24.7 c

Means within columns followed by the same letter are not significantly different (P= 0.05).

The pectin yield from the industrial starch residue was considerably lower than that from laboratory-prepared starch residue possibly because there was a delay of one hour at around 35ºC involved in the transportation of the sample from the starch-processing centre to the laboratory in the case of the former. Activity of endogenous pectinases during this delay may have led to degradation of some pectic substances.

Conditions of extraction also significantly affected pectin yields. In all varieties, the alkali/SHMP combination provided the most efficient procedure, followed by acid containing SHMP, and acid extraction. The alkali without SHMP gave the lowest yield. The low pectin yield in acid extraction was in contrast to other reports, which reported that generally, the highest pectin yields were obtained by hot acid extraction [31,32,33].

The effectiveness of alkali and chelating agents such as SHMP as extraction media for pectin involves two mechanisms, chelation of Ca^{2+} by SHMP and destruction of the alkali-labile linkages such as esters, some glycosidic linkages between methoxylated galacturonic residues and hydrogen bonds [34]. This indicates that pectins in sweet potato cell walls are

mainly calcium-bound low methoxyl pectin that is not extractable with mild acid or alkaline [35].

3.3 Galacturonic Acid, Degree of Esterification and Acetylation of Pectin

The ^{13}C CP/MAS NMR spectra of polygalacturonic acid and citrus pectin (Sigma, Australia) shown in Figs. 4 and 5 were used as the basis for the interpretation of related pectin spectra presented in Table 3. The intense signal at 172.7 ppm was assigned to the C-6 carbon of COOH group and the intense signal at 101.9 ppm was assigned to the C-1 carbon. The peaks between 60 and 90 ppm are from the carbons of the pyranoid rings (C-2,3,5), and the signal at 80.3 is from the C-4 carbon. The intense signal at 53.7 ppm is assigned to the methyl carbon of the methyl ester groups ($COO\underline{C}H_3$).

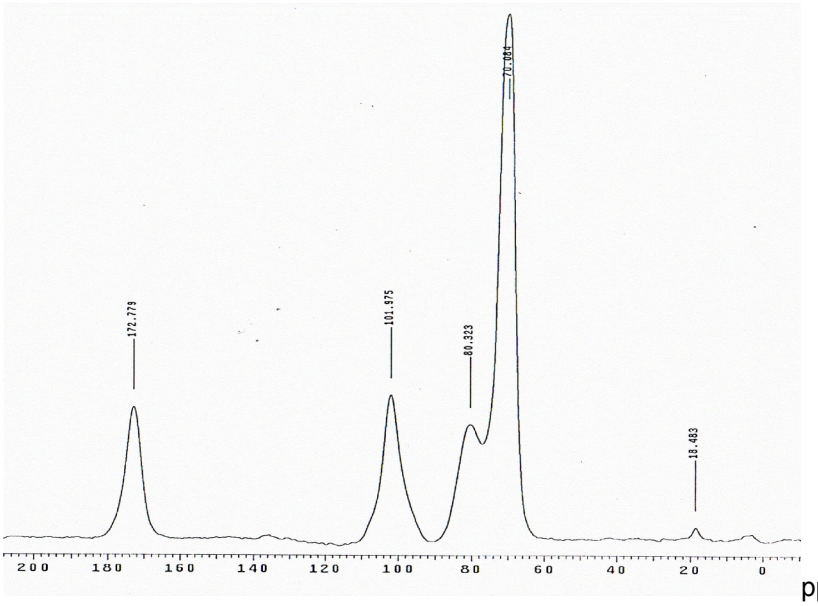

Fig. 4. ^{13}C CP/MAS solid state NMR spectrum of polygalacturonic acid

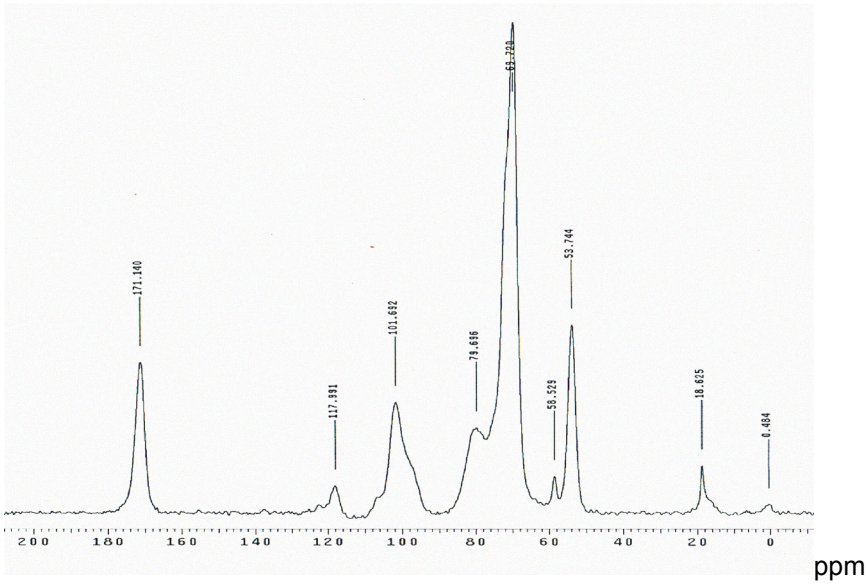

Fig. 5. ^{13}C CP/MAS solid state NMR spectrum of esterified citrus pectin

The spectra of Beauregard sweet potato pectin are shown in Figs. 6-9. It was noticed that the signal of C-6 carbons of galacturonic units of pectin extracted using HCl containing SHMP shifted downfield to 177.25 ppm (Fig. 7), indicating that the galacturonic is in the form of carboxylate anion (COO^-) [27,36]. The use of NaOH and NaOH containing SHMP for pectin extraction led to the disappearance of the peaks at ~ 53 ppm (Figs. 8 and 9) and this was attributed to saponification of the methyl ester.

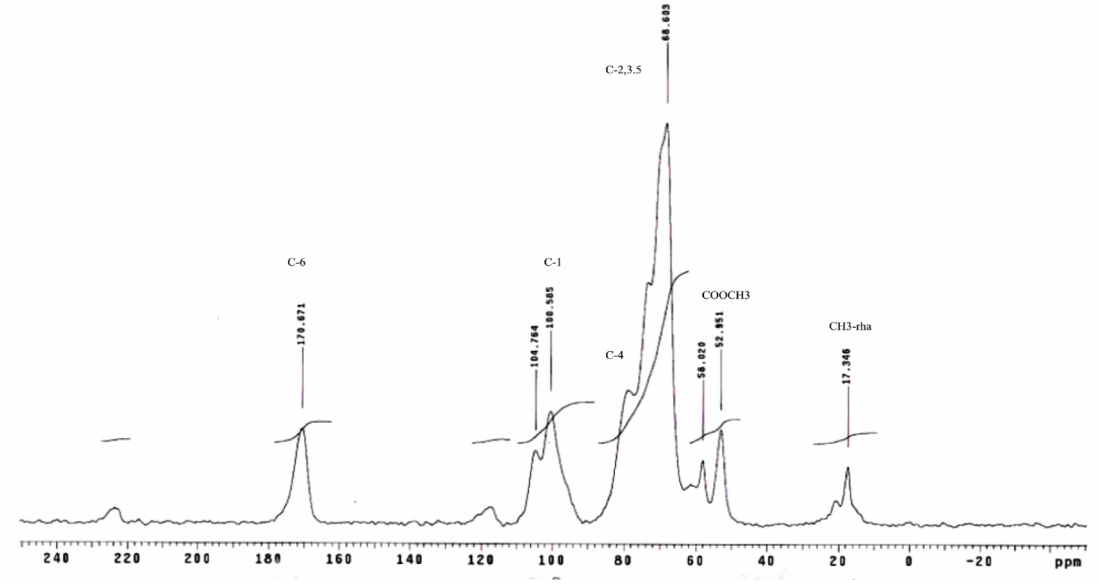

Fig. 6. ^{13} C CP/MAS solid-state NMR spectra of HCl-extracted Beauregard sweet potato pectin

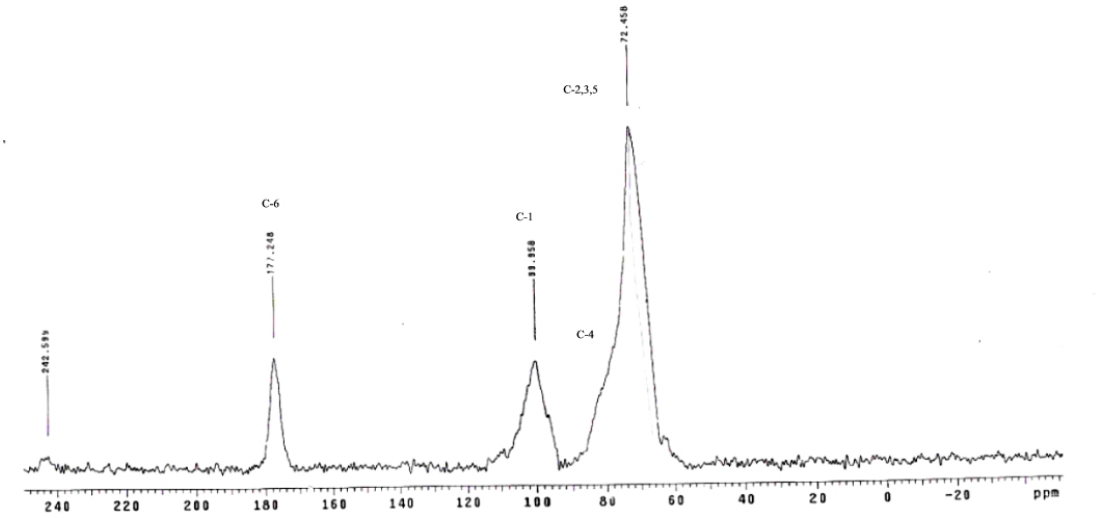

Fig. 7. ^{13} C CP/MAS solid-state NMR spectra of HCl containing SHMP-extracted Beauregard sweet potato pectin

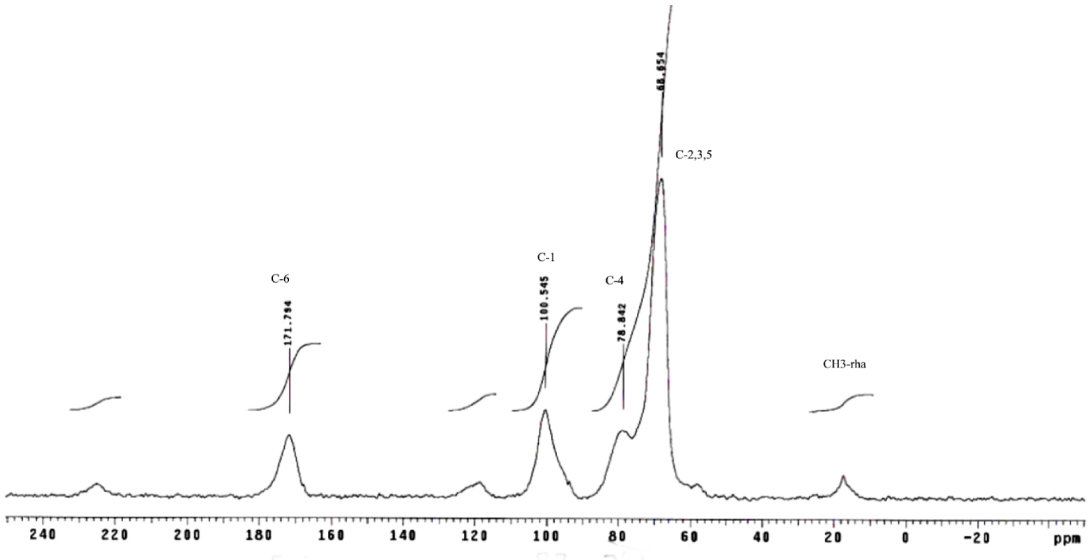

Fig. 8. ^{13} C CP/MAS solid-state NMR spectra of NaOH-extracted Beauregard sweet potato pectin

HCl-extracted pectins have peaks for the C-6 carbon of galacturonic units at ~ 170 – 171 ppm due to relatively high content of the methyl ester groups together with some free carboxyls [27,36,37] In contrast, peaks of the C-6 carbons of galacturonic units of pectin extracted using alkaline or SHMP had peaks between 174-176 ppm. This suggests that the C-6 carbons of these pectins were mostly in the form of carboxylate anion (COO^-) [27,36].

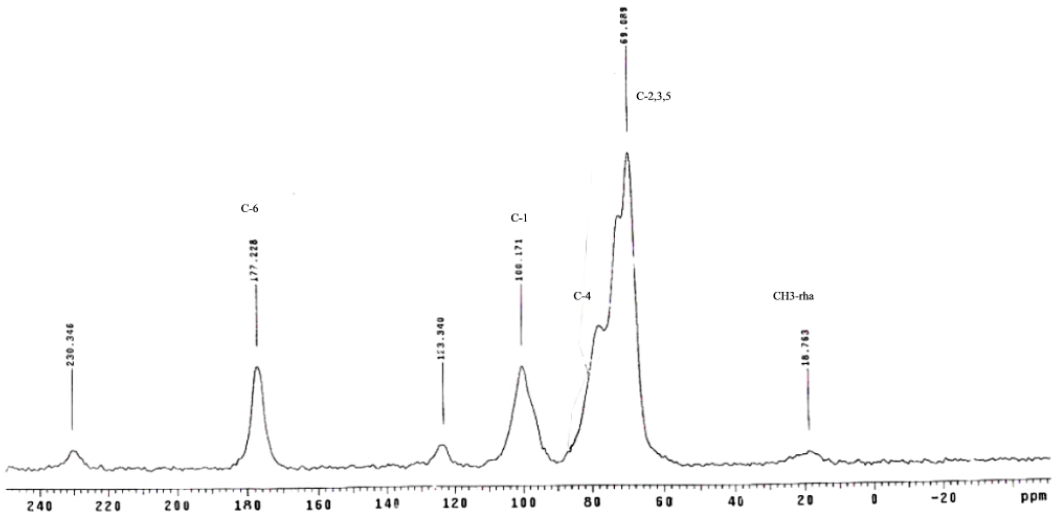

Fig. 9. ^{13}C CP/MAS solid-state NMR spectra of NaOH containing SHMP-extracted Beauregard sweet potato pectin

The spectra of pectin extracted using HCl containing SHMP, except those extracted from Beauregard variety, also had resonance signals at ~ 53 ppm representing methyl carbons of the methyl ester ($COO\underline{C}H_3$), whereas those extracted using alkaline or alkaline containing SHMP did not. Interestingly, pectins from Northern Star sweet potato extracted using HCl containing SHMP showed resonance at ~ 20 ppm (Fig. 10). According to Farago and Mahmoud [38]-these peaks are from the methyl carbon of acetyl groups ($O_2C\underline{C}H_3$).

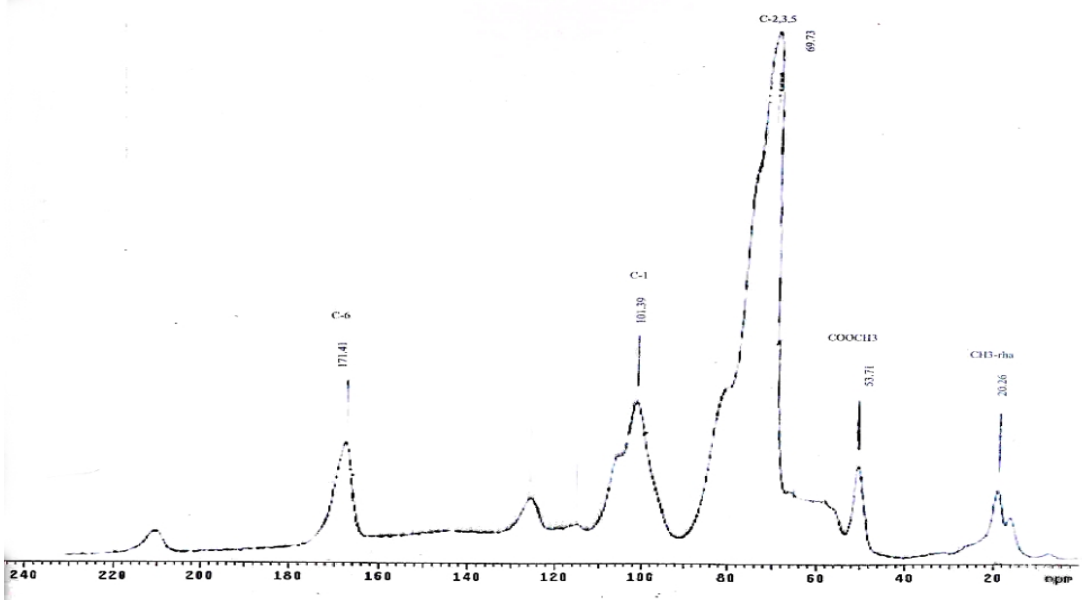

Fig. 10. ^{13}C CP/MAS solid-state NMR spectra of HCl containing SHMP-extracted Norhtern Star sweet potato pectin

Table 3. Chemical shift of sweet potato pectin

Sample	Chemical shift (ppm)						
	C-1	C-2,3,5	C-4	C-6	$COOCH_3$	O_2CCH_3	CH_3-Rha
Polygalacturonic acid	101.90	69.81	80.33	172.71	-	-	18.50
Esterified citrus pectin	101.68	69.51	79.00	171.15	53.80	-	-
Beauregard HCl	100.58	68.60	sh*	170.67	52.95	-	17.34
Beauregard HCl+SHMP	101.80	71.43	82.49	175.17	-	-	17.42
Beauregard NaOH	100.54	68.65	78.84	171.79	-	-	18.30
Beauregard NaOH+SHMP	99.96	72.46	sh	177.25	-	-	-
Northern Star HCl	102.15	70.06	sh	171.17	53.46	-	-
Northern Star HCl+SHMP	101.39	69.73	sh	171.41	53.71	20.26	-
Northern Star NaOH	102	70.08	80.32	172.77	-	-	18.48
Northern Star NaOH+SHMP	103	73	sh	177	-	-	-
Bis192 HCl	101.37	71.37	sh	171.17	53.59	-	18.48
Bis192 HCL+SHMP	100.26	69.08	sh	176.07	54.81	-	-
Bis192 NaOH	101.57	69.33	sh	172.14	-	-	-
Bis 192 NaOH+SHMP	100.41	69.62	sh	175.88	-	-	18.39
Bis183 HCl	101.43	69.72	sh	171.51	53.59	-	17.99
Bis183 HCl+SHMP	101.53	69.33	sh	171.61	53.93	-	18.28
Bis183 NaOH	100.70	63.81	80.26	175.44	-	-	-
Bis183 NaOH+SHMP	100.99	70.54	sh	175.98	54.17	-	18.19
Starch residue HCl	101.43	69.72	sh	171.51	53.69	-	17.99
Starch residue HCl+SHMP	101.62	69.28	sh	175.41	54.79	-	17.24
Starch residue NaOH	102.21	70.06	sh	173.11	-	-	-
Starch residue NaOH+SHMP	100.51	70.44	sh	176.95	-	-	18.87

sh=shoulder C-2,3,5

Other pectin spectra (Figs. 11-12) show that, in general, HCl-extracted pectin had peaks for the C-6 carbon of galacturonic units at ~ 170 – 171 ppm due to relatively high content of methyl ester groups together with some free carboxyls [27,36,37]. In contrast, the C-6 carbon signal of pectin extracted using alkaline or SHMP had a resonance signal between 174-176 ppm, which was attributed to sodium galacturonate. It was also observed that the spectra of pectins had intense signals ~ 17 ppm, which corresponds to methyl carbons of rhamnose [27].

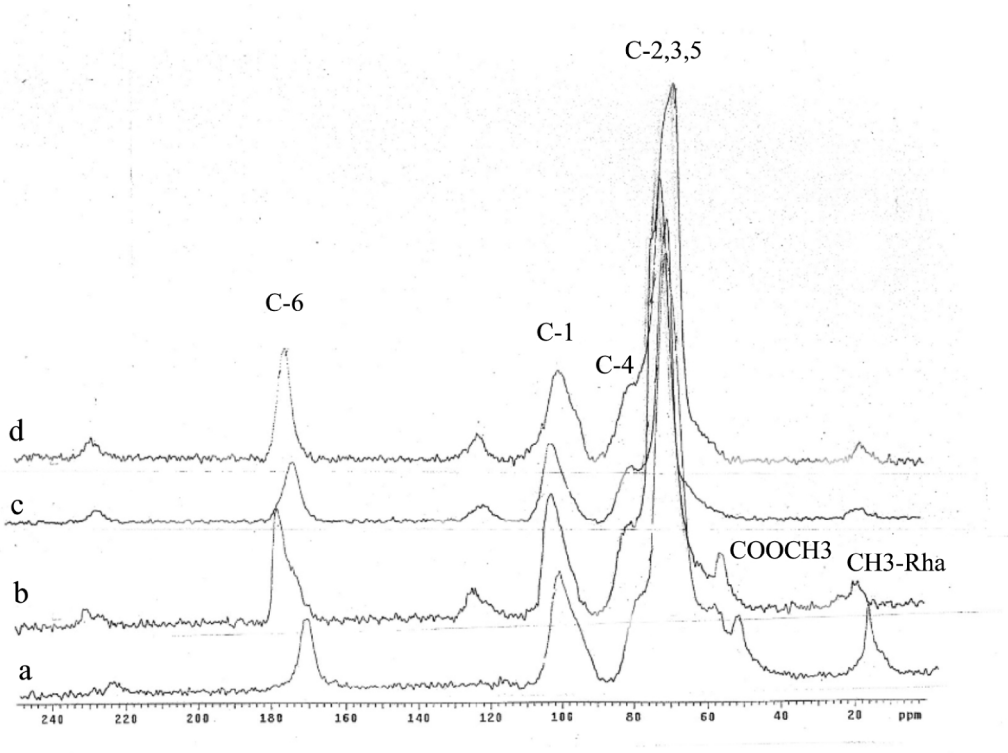

Fig. 11. ^{13}C CP/MAS solid-state NMR spectra of pectin extracted from Bis192 sweet potato

(a) extraction using HCl, (b) extraction using HCl containing SHMP, (c) extraction using NaOH, (d) extraction using NaOH containing SHMP

Fig. 11 shows that the spectra of pectin extracted using HCl as well as HCl containing SHMP had peaks at ~ 53-54 ppm, indicating these pectins contain significant amounts of methyl esters, whereas in pectins extracted using NaOH and NaOH containing SHMP, no $COO\underline{C}H_3$ peak was detected due to saponification. Unlike pectin from other sweet potato varieties, it was also observed that carboxyl C-6 carbons of galacturonic units of pectin extracted using NaOH solution were still in the acidic form of COOH, and did not shift downfield to 176 ppm (in the form of COO^-).

The spectra of pectin from Bis183 sweet potato (Fig. 12) show that extraction using NaOH containing SHMP had a resonance at ~53 ppm, representing the methyl carbon of methyl ester. This phenomenon is different from that extracted using the same method, where this peak was not detected. It was also observed that the peak of the C-4 carbon of pectin

extracted using NaOH was more resolved compared to that of the same pectin obtained using different extraction method.

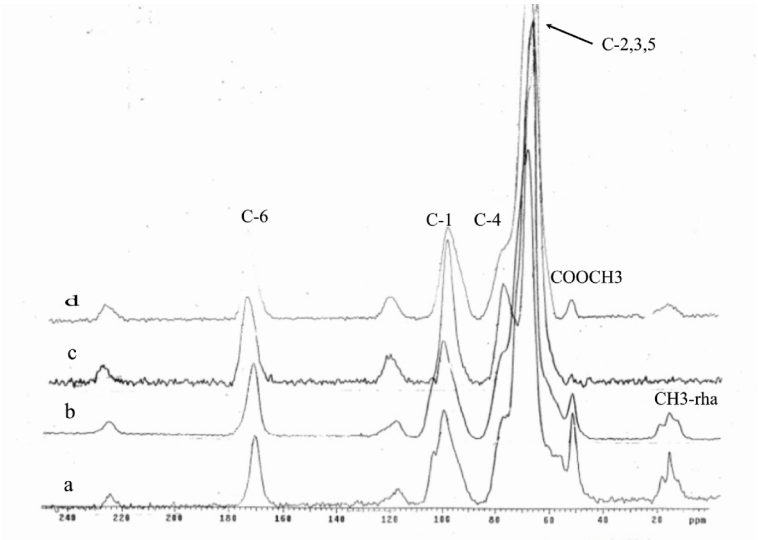

Fig. 12. ^{13} C CP/MAS solid-state NMR spectra of pectin extracted from Bis183 sweet potato.

(a) extraction using HCl, (b) extraction using HCl containing SHMP, (c) extraction using NaOH, (d) extraction using NaOH containing SHMP.

Fig. 13 shows the spectra of commercial starch residue pectin. It was observed that only pectin extracted using HCl had a prominent peak for the methyl carbon of methyl ester and rhamnose whereas others did not, indicating that extraction using SHMP or NaOH has eliminating the methyl ester content of the pectin.

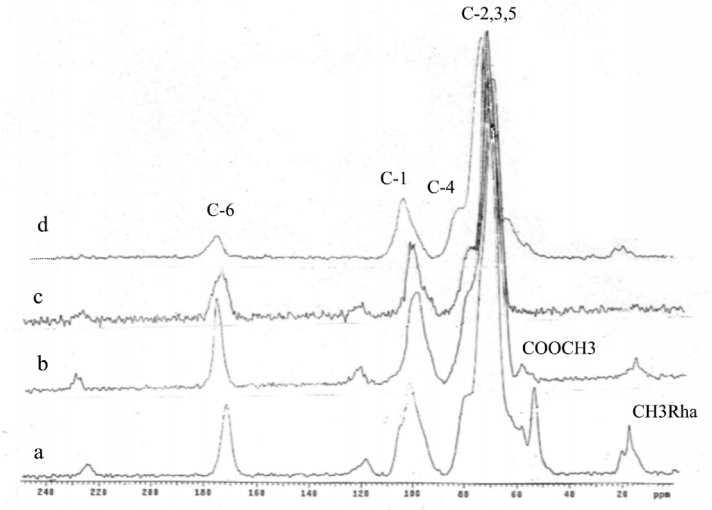

Fig. 13. ^{13}C CP/MAS solid-state NMR spectra of pectin extracted from commercial sweet potato starch residue.

(a) extraction using HCl, (b) extraction using HCl containing SHMP, (c) extraction using NaOH, (d) extraction using NaOH containing SHMP

The NMR spectra of the extracted pectins were then used for determining the galacturonic acid content, degree of esterification (DE) and acetylation (DA) [26,27]. Galacturonic acid content was calculated as the ratio of area of the C-6 to the average area of C-1,2,3,4, 5; DE was the ratio of area of $COO\underline{C}H_3$ to that average area of C-1,2,3,4,5, and DA was the ratio of integral intensity of $O_2C\underline{C}H_3$ carbon to that average area of C-1,2,3,4,5. The area of C-1 was converted to 100 and the other areas were based on that of C-1. The peak area of the pectin samples are presented in Table 4 and their corresponding galacturonic acid and degree of esterification are shown in Table 5.

Table 4. Peak area of the carbons obtained from the NMR spectra

Variety	Extraction method	Area of C-6	Area of C-1	Area of C2,3,4,5	Average area of C-1,2,3,4,5	Area $COOCH_3$
Beauregard	HCl	50.3	100	417.6	103.5	58.8
	HCl+SHMP	46.9	100	492.4	118.5	-
	NaOH	77.6	100	590	138	-
	NaOH+SHMP	64.4	100	437.9	107.5	-
Northern Star	HCl	49.5	100	527.7	125.5	27.5
	HCl+SHMP	56.68	100	587	137	42.5
	NaOH	80.75	100	595.2	139	-
	NaOH+SHMP	93.7	100	506.9	121	-
Bis 192	HCl	34.7	100	536.8	127.3	11.2
	HCl+SHMP	127	100	894	188	18
	NaOH	96.25	100	660.4	152	-
	NaOH+SHMP	75	100	364	92.8	-
Bis 183	HCl	53.5	100	639.7	148	35
	HCl+SHMP	54	100	575	135	41.7
	NaOH	52.9	100	645	149	11
	NaOH+SHMP	57.8	100	628	125.6	-
Starch residue	HCl	47.7	100	639.7	147.9	14
	HCl+SHMP	48.15	100	580.8	136.2	43
	NaOH	35.8	100	279.1	75.8	-
	NaOH+SHMP	86.3	100	639.6	147.9	-

The results (Table 5) show that there were large variations in galacturonic acid content. Galacturonic acid contents were between 27.3 and 80.8%. This is in contrast to Levigne *et al*. [14] where the galacturonic acid contents were reported constant for pectins obtained using a number of extraction conditions. The lowest content of galacturonic acid (27.3%) was found in Bis 192 pectin extracted using HCl and the highest 80.8 was also found in Bis 192 extracted using NaOH containing SHMP. Except for Bis 183, in all varieties, extraction using NaOH plus SHMP gave the highest galacturonic acid contents. It was also noted that the higher the yield of material extracted, the higher the percentage of galacturonic acid. This was attributed to the ability of SHMP to chelate Ca^{2+} and destruction of the alkali-labile linkages such as ester, some glycosidic linkages between methylated galacturonic residues and hydrogen bonds [34], leading to more galacturonic acid being liberated from the cell wall.

Table 5. Galacturonic acid (GA) and degree of esterification (DE), and degree of acetylation (DA)

Varieties	Extraction methods	GA (%)*	DE (%)*	DA
Beauregard	0.1M HCl	48.6	57.0	
	0.1M HCl cont. 0.75%SHMP	39.6	No signal detected	-
	0.05M NaOH	56.0	No signal detected	-
	0.05M NaOH cont.0.75% SHMP	60	No signal detected	-
Northen Star	0.1M HCl	50.9	36	
	0.1M HCl cont. 0.75%SHMP	41.3	31.0	trace
	0.05M NaOH	58.1	No signal detected	-
	0.05M NaOH cont.0.75% SHMP	77.4	No signal detected	-
Bis192	0.1M HCl	27.3	9.0	-
	0.1M HCl cont. 0.75%SHMP	67.6	10.0	-
	0.05M NaOH	63.3	No signal detected	-
	0.05M NaOH cont.0.75% SHMP	80.8	No signal detected	-
Bis 183	0.1M HCl	36.0	23.8	-
	0.1M HCl cont. 0.75%SHMP	40.0	30.9	-
	0.05M NaOH	35.5	7.4	-
	0.05M NaOH cont.0.75% SHMP	46.0	No signal detected	-
Starch residue	0.1M HCl	32.3	9.5	
	0.1M HCl cont. 0.75%SHMP	35.4	32.2	-
	0.05M NaOH	47.2	No signal detected	-
	0.05M NaOH cont.0.75% SHMP	58.2	No signal detected	-

** Values are calculated from Table 4. GA = (Area of C-6/ Average area of C1-C5) x 100*
DE = (Area of $COOCH_3$/Average area of C1-C5) x 100

Although the galacturonic acid content of the extracted pectin generally was lower than that from commercial pectin as specified by EEC [39], FAO 40] and FCC [41] which is not less than 65%, these results were close to other reports on sweet potato pectin. Sasaki, Kishigami and Fuchigami [42], Noda *et al*. [20] Salvador *et al*. [21] reported that the content of galacturonic acid ranged from 0.03 to 0.26% for raw sweet potato, or from 47.1 to 31.3% of sweet potato cell wall materials.

The methyl ester groups were always detected in pectin extracted using HCl, and ranged from 9 to 57%. The highest was in Beauregard and the lowest in Bis 192 sweet potato (Table 5). In contrast, no esterification was detected in the samples extracted with NaOH containing SHMP. Esterification was found in pectins extracted using HCl containing SHMP, except for pectin from Beauregard. HCl containing SHMP-extracted pectins from Northern Star, Bis 192, and Bis 183 had degrees of esterification of 31, 10, 30.9 and 32.2%, respectively. The methyl ester in pectin extracted using NaOH was detected only in Bis 183 sweet potato. These results suggest that alkali extraction had saponified the pectin methyl esters [14, 43] leading to formation of the sodium salt of pectin.

Acetate groups were only detected in pectin from Northern Star and starch residue extracted using HCl containing SHMP, which was too low to quantify. This can be attributed to de-acetylation of pectin by mild acid or alkali hydrolysis during extraction [16].

4. CONCLUSION

The factory starch residue sample and the laboratory-prepared sweet potato starch residue samples both contained considerable amounts of cell wall material (35% to 52%, including pectin between 7 and 30%) and which makes this a feasible raw material for pectin production. The material extracted using HCl, NaOH, and HCl or NaOH containing SHMP, were primarily composed of pectins, since they contained large amounts of galacturonic acid (32% to 80%).

Pectin extracted using HCl as well as HCl containing SHMP contain significant amounts of methyl ester. On the other hand, in pectins extracted using NaOH and NaOH containing SHMP, no $COO\underline{C}H_3$ peak due to saponification. It was also observed that pectin extracted with HCl, produced galacturonic acid groups (-COOH), whereas extraction with HCl containing SHMP, NaOH, and NaOH containing SHMP, produced galacturonic acid groups in the form of carboxylate anion (COO^-), except in Bis 192, where the galacturonic units of pectin extracted using NaOH solution were still in the form of COOH.

Extractions with HCl resulted in a fairly high galacturonic acid content, greater degree of esterification, and, in addition, Beauregard appeared to be superior in comparison with other varieties because they had high galacturonic acid content as well as the degree of esterification. In contrast, extraction using NaOH containing SHMP resulted higher pectin yield and galacturonic acid content but very low in esterification. Northern Star had the highest pectin yield (30%), and Bis 192 had the highest galacturonic acid (80%). The overall results show that sweet potato variety had less effect on the yield, galacturonic acid and degree of esterification of pectin compared with the effect of extraction methods.

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COMPETING INTERESTS

Authors declare that no competing interests exist.

REFERENCES

1. Kertesz ZI. The pectic substances. Interscience Publ. Inc. New York; 1951.
2. Lapasin R, Pricl S. Rheology of Industrial Polysaccharides: Theory and Application. Blackie & Professional, Chapman & Hall, London. p.620. 1995.
3. Rolin C, De Vries J. Pectin. In H.W.S. Chan (Ed.), Food Gels, Chapt.10, pp.401-434. Elsevier Applied Sience, Barking, U.K; 1990.
4. Glicksman M. Gelling hydrocolloids in product applications. In J.V.M. Blanshard and J.R. Mitchell (Ed.). Polysaccharides in Foods. Butterworths, London. 1979;pp.185-204.
5. Pedersen JK. Textural ingredients for food. Business Briefing. Innovative Food Ingredients. 2002;1-4

6. Yamaguchi F, Shimizu N, Hatanaka C. Preparation and physiological effect of low-molecular weight pectin. Biosci. Biotech. Biochem. 1994;58:679-682.
7. Tian C, Wang G. Study on the anti-tumor effect of polysaccharides from sweet Potato. Journal of Biotechnology. 2008;136S,S351.
8. Inngjerdingen M, Inngjerdingen KT, Patel TR, Allen S, Chen XY, Rolstad B. Pectic polysaccharides from Biophytum petersianum Klotzsch, and their activation of macrophages dendritic cells. Glycobiology. 2008;18:1074–1084.
9. Simpson BK, Egyankor KB, Martin AM. Extraction, purification , and determination of pectin in tropical fruits. J. Food Preserv. 1984;8,63-72.
10. Matora VA, Korshunova VE, Shkodina OG, Zhemerichkin DA, Ptitchikina NM, Morris ER. The application of bacterial enzymes extraction of pectin from pumpkin and sugar beet. Food Hydrocoll. 1995;9:43-46.
11. Turquois T, Rinaudo M, Taravel FR, Heyraud A. Extraction of highly gelling pectic substances from sugar beet pulp and potato pulp: Influence of extrinsic parameters on their gelling properties. Food Hydrocoll. 1999;13:255-262.
12. Gnanasambandam R, Proctor A. Preparation of soy hull pectin. Food Chem. 1999; 65,461-467.
13. Golovchenko VV, Ovoda RG, Shashkov AS, Odovov YS. Studies of the pectic polysaccharide from duckweed Lemna minor L. Phytochem. 2002;60(1):89-97.
14. Levigne S, Ralet MC, Thibault JF. Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. Carbohydr. Polym. 2002;49:145-153.
15. Yapo BM, Robert C, Etienne I, Wathelet B, Paquot M. Effect of Extractions on the yield, purity and surface properties of sugar beet pulp pectin extracts. Food Chem. 2007;100:1356-1364.
16. Dea ICM, Madden JL. Acetylated pectic polysaccharides of sugar beet. Food Hydrocoll. 1986;1:71-98.
17. Endress, H.U., and Rentschler, C. Chances and limit for the use of pectin as emulsifier- Part 1. The European Food and Drink Review. Summer. 1999; 49-53.
18. Byg I, Diaz J, Øgendal LH, Harholt J, Jørgensen B, Rolin C, Svava R, Ulvskov P. Large-scale extraction of rhamnogalacturonan I from industrial potato waste. Food Chem. 2012;131:1207–1216
19. Yenovsky E. Pectin from sweet potato pulp. Food Industry. 1939;710.
20. Noda T, Takahata Y, Kumamoto, Nagata T, Shibuya N, Ibaraki. Chemical composition of cell wall material from sweet potato starch residue. Starch/starke. 1994;44:232-236.
21. Salvador DL, Saganuma T, Kitahara K, Tanoue H, Ichiki M. Monosaccharide composition of sweet potato fiber and cell wall polysaccharides from sweet potato, cassava, and potato analyzed by the high performance chromatography with pulse amperometric detection method. J. Agric. Food Chem. 2000;48:3448-3454.
22. Salvador LD, Suganuma T, Kitahara K, Fukushige Y, Tanoue H. Degradation of cell wall materials from sweet potato, cassava, and potato by a bacterial protopectinase and terminal sugar analysis of the resulting solubilized products. J. Biosci. Bioeng. 2002;93:64-72.

23. Dongowski G, Stoof G. Investigations on potato pulp as dietary fibre source-composition of potato pulp after influence of pectinases and cellulases and enzymatic degradation of starch. Starch/Starke. 1993;45:230-234.
24. Massiot P, Rouau X, Thibault JF. Characterisation of the extractable pectins and hemicelluloses of the cell-wall of carrot. Carbohydr. Res. 1988;172:229-242.
25. Stolle-Smits T, Beekhuizen JG, Recourt K, Voragen AGJ, Dijk CV. Changes in pectic and hemicellulosic polymers of green beans (Phaseolus vulgaris L.) during industrial processing. J. Agric. Food Chem. 1997;45:4790-4799.
26. Sullivan MJ. Industrial applications of high resolution solid-state 13C NMR techniques. Trends in Anal. Chem. 1987;2:31-37.
27. Sinitsya A, Copikova J, Pavlikova J. 13C CP/MAS NMR spectroscopy in the analysis of pectins. J. Carbohydrate Chem. 1998;17(2):279-292.
28. Steel RGD, Torrie JH. Principles and procedures of statistics. McGraw-Hill, Singapore. 563 p. 1980.
29. Ryden P, Selvendran RR. Structural features of cell wall polysaccharides of potato (Solanum tuberosum). Carbohydr. Res. 1990;195:257-272.
30. MSDS. Material safety data sheets (MSDS) for DMSO. 2001. Accessed 03 July 2001. Available: http://physchem.ox.ac.uk/MSDS/DM/DMSO.html.
31. Rolin C, De Vries J. Pectin. In H.W.S. Chan (Ed.), Food Gels, Chapt.10, pp.401-434. Elsevier Applied Sience, Barking, U.K; 1990.
32. Rombouts FM, Thibault JF. Feruloyated pectic substances from sugar beet pulp. Carbohydr. Res. 1986;154:177-188.
33. May CD. Industrial pectins: sources, production and applications. Carbohydr. Polym. 1990;12:79-99.
34. Renard CMG., Voragen AGJ, Thibault JF, Pilnik W. Studies on apple protopectin I: extraction of insoluble pectin by chemical means. Carbohydr. Polym. 1990;12:9-25.
35. Doesburg JJ. Pectic substances in Fresh and Preserved Fruit and vegetables. I.B.V.T., Wageningen, The Netherlands; 1965.
36. Catoire L, Goldberg R, Pierron M, Morvan C, du Penhoat CH. An efficient procedure for studying pectin structure which combines limited depolymerization and 13C NMR. Eur. Biophys. 1998; J. 27, 127-136.
37. Jarvis MC, Apperley DC. Chain conformation in concentrated pectic gels: evidence from 13C NMR. Carbohydr. Res. 1995;275:131-145.
38. Farago ME, Mahmoud I. E.A.D.A.W. Metal compounds of pectin. Inorg. Chim. Acta. 1983;80:273-278.
39. E.E.C. Specific criteria of purity for emulsifier, stabilizers, thickeners and gelling agents for use in foodstuffs. European Economic Council. 1978;78/663/EEC.
40. F.A.O. Specifications for identity and purity of thickening agents, anticaking agent, antimicrobial agent, antioxidants and emulsifiers. FAO Food and Nutrition Paper 4. Rome; 1978.
41. FCC. Food Chemical Codex. III Monographs, vol. 215. National Academic Press, Washington D.C; 1981.
42. Sasaki A, Kishigami Y, Fuchigami M. Firming of cooked sweet potatoes as affected by alum treatment. J. Food Sci. 1999;64(1):111-115

43. Kohn R. Binding of divalent cations to oligomeric fragments of pectin. Carbohydr. Res. 1987;160:343-353.

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