

Comparative chemical characteristics of hydrochloric acid- and ammonium oxalate-extracted pectin from roselle (*Hibiscus sabdariffa* L.) calyces

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Abstract

Recent research suggesting the existence of potential source of pectin from roselle calyces. Pectin was successfully extracted from seven different varieties of roselle calyces. Pectin extraction was conducted using hydrochloric acid (HCl, 0.03 N, pH 1.5) or ammonium oxalate (0.25% w/v, pH 4.6) at 85°C for 1 h. Chemical characteristics of the HCl- and ammonium oxalate extracted pectin were compared. Results indicated that ammonium oxalate exhibited greater efficiency in pectin extraction than HCl. Highest pectin yield at 18.7% was obtained by ammonium oxalate extraction of roselle calyx variety Acc.6 compared to only 9.77% by HCl extraction. The lowest pectin yield at 11.3% and 5.78% were observed respectively in ammonium oxalate and HCl extractions of roselle calyx variety UKMR-3. Some important characteristics of ammonium oxalate extracted pectin of roselle Acc.6 were 5.98% moisture, 3.81% ash, 4.64% methoxyl content, 42.24% anhydrouronic acid (AUA) and degree of esterification (DE) 60%. This study suggested that the high DE% roselle pectin is an alternative source of pectin for food industry.

Keywords

*Roselle calyces
pectin
hydrochloric acid
ammonium oxalate
chemical characteristics*

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Introduction

Roselle or *Hibiscus sabdariffa* L. is a native plant that can easily obtained in regions from India to Malaysia. The plant is commonly cultivated for the production of bast fiber from the stem of the plant. In fact, the plant itself has plenty of usage from the stem, leaves, calyces to the seeds. The leaves could be eaten raw or cooked in combination with other vegetables, while roselle fresh calyces for culinary purposes or in dried form being served as “tea”. Besides, the by-product of oil extraction of the seeds is valued as cattle feed. Today, roselle is attracting the attention of food and beverage manufacturers and even pharmaceutical industries who found the great potential of roselle as a natural biofunctional product and as a colorant to replace some synthetic dyes (Qi *et al.*, 2005; Plotto, 2007). Apart from that, pectin which always being neglected, in fact is another useful product that could be exploit from plant.

Pectin, a polysaccharide composing of D-galacturonic acids linked with b-1,4 glycosidic bonds, is found in middle lamella of plant cell membrane. Some of its galacturonide units are esterified as methyl galacturonate, whose extent is

reported as degree of esterification (DE) or methyl esterification degree (MED) (Seymour and Knox, 2002). One of the most important properties of pectin is gelling. The high-methoxyl pectin (HMP) (DE>50%) can form gel under acidic (pH 2.0–3.5) condition with the presence of sucrose at a concentration higher than 55%. While the low-methoxyl pectin (LMP) (DE<50%) can form gel with the presence of divalent ion, for example calcium ions. The presence of sucrose is not necessary in this case (Mishra *et al.*, 2001).

Pectin is used as a gelling agent in jams and jellies and as stabilizer in confections, dairy products, beverages, bakery fillings, icings and frostings. Pectin is also a fiber, which could lower the cholesterol, increase immunity and being applied as dentistry adhesive. The world consumption of pectin grows continuously and has exceeded 20,000 tons per year. Dried lemon, orange peel and apple pomace are important raw materials for pectin production. Citrus peel and apple pomace contain about 25% and 12% pectin respectively, and commercial extraction yield from citrus peel is about 25% (May, 1990; Walter, 1991).

Studies showed that the biochemical

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characteristics of extracted pectin depend on the plant source and the extraction method used. Pectin extraction is a multiple stage physical-chemical processes in which the hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, pH and time (Kertesz, 1951). Pectin can be extracted from fruit using acid such as HCl or HNO₃, or chelating agents for example EDTA, ammonium oxalate or sodium hexametaphosphate. Acid extraction seems to be the most widely used method (Mesbahi *et al.*, 2005; Koubala *et al.*, 2008a). It will be an another lucrative income earning if pectin can be produced alternatively from roselle calyces and most importantly could help in reducing waste production from agricultural origin. No or limited research was done on pectin extraction from roselle calyx. Hence, the aim of this study was to extract pectin from roselle calyces using HCl or ammonium oxalate. The chemical characteristics of HCl- or ammonium oxalate-extracted pectin were then compared.

Materials and Methods

Materials

Seven varieties of roselle: MARDI/Acc.3, Terengganu/Acc.6, PHR/Acc.12, Arab/Acc.21, UKMR-1, UKMR-2 and UKMR-3) were selected for pectin extraction study. All chemicals used were analytical graded.

Raw materials preparation

Roselle calyces were subjected to oven-dried at 55°C after separating from capsule and proper washing. Subsequently, the dried roselle calyces were ground to 100 µm particles prior to pectin extraction. The ground roselle calyx powder was then suspended in 85% (v/v) ethanol at 70°C for 20 min in a shaking water-bath. The resulting alcohol-insoluble-residue (AIR) was collected and oven-dried at 50°C. The dried AIR was ready for further pectin extraction.

Pectin extraction

Roselle pectin was extracted using 0.03 N hydrochloric acid (HCl) or 0.25% (v/v) ammonium oxalate at 85°C for 1 h. HCl extraction was carried out at pH 1.5 while ammonium oxalate extraction at pH 4.6. Pectin extraction was initiated with the addition of 250 mL extractant to 10 g of AIR. The mixture was stirred continuously in shaking water-bath for 1 h. The resulting extract was then filtered through nylon cloth and precipitated with 96% (v/v) ethanol. Pectin was collected via centrifugation at 2600 × g for 10 min. The extracted pectin was washed

three times with 70% (v/v) ethanol, followed by 96% (v/v) ethanol and finally with acetone before drying at 55°C (Koubala *et al.*, 2008a). The pectin yield was calculated using Equation (1):

$$\text{pectin yield (\%)} = \frac{P}{B_i} \times 100 \quad (1)$$

where, p = extracted pectin in gram, B_i = weight of AIR in gram.

Determination of moisture and ash content

Moisture and ash content of extracted pectin were determined according to AOAC (1997) method.

Determination of methoxyl content

The determination of methoxyl content (MeO%) of the extracted pectin was conducted according the method described by Owens *et al.* (1952). Initially, 0.5 g pectin was moistening with 5 mL of ethanol (96% v/v) in a 250 mL conical flask. Subsequently, 1 g sodium chloride was added. One-hundred milliliters of deionized water and six drops of phenol red indicator were added thereafter. The mixture was then stirred to ensure all the pectic substance is fully dissolved. After that, the mixture was carefully titrated with 0.1 N NaOH until the color changed and this color-changed situation should continue for at least 30 s more (Titration A). Then, 25 mL of 0.25 N NaOH was added to the solution and shaken vigorously. The solution was allowed to stand for 30 minutes at room temperature in stopped conical flask. Next, 25 mL of 0.25 N HCl was added, and the mixture was titrated again with 0.1 N NaOH until the color changed (Titration B). The following Equation (2) was used to calculate the methoxyl content:

$$\text{MeO \%} = \frac{\text{meq Titration B} \times 31 \times 100}{\text{Weight of sample (mg)}} \quad (2)$$

where meq Titration B = miliequivalents of NaOH used in Titration B, 31 is the molecular weight of the methoxyl group.

Determination of anhydrouronic acid content

The anhydrouronic acid content (AUA%) could be calculated after the methoxyl content was determined according to the Equation (3):

$$\text{AUA\%} = \frac{176 \times 100}{z} \quad (3)$$

where 176 is the molecular weight of AUA and

$$z = \frac{\text{weight of sample (mg)}}{\text{meq Titration A} + \text{meq Titration B}}$$

Determination of degree of esterification

The degree of esterification (DE%) of extracted pectin was calculated using Equation (4):

$$\text{DE\%} = \frac{176 \times \text{MeO\%} \times 100}{31 \times \text{AUA\%}} \quad (4)$$

Statistical analysis

Statistical Analysis Systems software version 6.12 (SAS Institute, Cary, NC, USA) was used for analysis of variance. Values of $P < 0.05$ were considered to be significant.

Results and Discussion

Extraction of *roselle calyces pectin*

Pectin was successfully extracted from seven varieties of roselle calyces using HCl and ammonium oxalate. As shown in Table 1 and Table 2, significantly more pectin was obtained using the ammonium oxalate extraction method (17.70%) than HCl extraction method (9.77%). Almost similar results were reported by Koubala *et al.* (2008b) and Berardini *et al.* (2005). In fact, more pectin could be extracted under acidic condition and high extraction temperature as established by Kulkarni and Vijayanand (2010) and Koubala *et al.* (2008b). However, extraction at high temperature and low pH decreased the methoxyl content of the extracted pectin. This probably due to the partial degradation of pectin as suggested by Kulkarni and Vijayanand (2010). In addition, extended extraction time to more than 60 min did not help in increasing pectin yield, thus the pectin extraction time was maintained at 60 min as observed by Kulkarni and Vijayanand (2010).

Chemical characteristics of *roselle calyces pectin*

The chemical characteristics of the HCl- and ammonium oxalate-extracted pectin of seven roselle calyces varieties were compared and are shown in Table 1 and Table 2 respectively. Higher moisture ranging from 5.01–11.96% was observed in the HCl-extracted pectin compared to the ammonium oxalate-extracted roselle calyces pectin (5.10–6.42%). This situation may be associated with the hygroscopic characteristic of dried pectin powder. Both HCl- and ammonium oxalate-extracted pectin were low in ash content between 2.60–4.96% and 3.63–3.98% respectively.

The highest amount of pectin was extracted from the roselle Acc.6 calyx regardless of the type of extractant used. While roselle variety UKMR-3 contained the lowest amount of pectin compared to other varieties with 5.78% and 11.3% by HCl and ammonium oxalate extraction respectively. The chemical characteristics of HCl- and ammonium oxalate-extracted pectin from roselle Acc.6 calyx were compared and the results are shown in Table 3. Despite of higher efficiency of ammonium oxalate in pectin extraction compared to HCl, but extracted

Table 1. Chemical characteristics comparison of HCl-extracted pectin from seven varieties of roselle calyces

	Acc.3	Acc.6	Acc.12	Acc.21	UKMR-1	UKMR-2	UKMR-3
Yield (%)	8.01 ^d	9.77 ^a	7.50 ^e	9.02 ^c	6.56 ^f	9.54 ^b	5.78 ^g
Moisture (%)	5.66 ^e	8.23 ^c	5.96 ^d	5.01 ^f	5.33 ^f	11.96 ^a	11.87 ^b
Ash (%)	4.13 ^c	4.52 ^b	4.96 ^a	4.02 ^d	3.87 ^e	2.60 ^g	3.19 ^f
MeO (%)	3.41 ^f	7.11 ^a	2.33 ^g	6.52 ^b	3.98 ^e	6.26 ^c	4.92 ^d
AUA (%)	56.22 ^d	68.05 ^a	51.72 ^f	60.03 ^c	42.33 ^g	62.64 ^b	55.63 ^e
DE (%)	48.01 ^e	59.09 ^a	38.72 ^g	55.03 ^c	41.48 ^f	56.71 ^b	50.25 ^d

^{a-g} Values are means of triplicate measurements. Values with the different letter in the same row are significantly different ($P < 0.05$)

Meo, methoxyl; AUA, anhydrouronic acid; DE, degree of esterification

Table 2. Chemical characteristics comparison of ammonium oxalate-extracted pectin from seven varieties of roselle calyces

	Acc.3	Acc.6	Acc.12	Acc.21	UKMR-1	UKMR-2	UKMR-3
Yield (%)	14.90 ^c	18.70 ^a	17.60 ^d	17.70 ^c	18.30 ^b	14.40 ^f	11.30 ^g
Moisture (%)	5.41 ^e	5.98 ^d	6.03 ^e	5.17 ^f	5.10 ^g	6.16 ^b	6.42 ^a
Ash (%)	3.95 ^b	3.81 ^f	3.98 ^a	3.93 ^d	3.94 ^c	3.86 ^e	3.63 ^g
MeO (%)	3.41 ^d	4.64 ^a	1.36 ^g	3.91 ^b	3.72 ^c	3.22 ^e	2.67 ^f
AUA (%)	42.24 ^a	42.24 ^a	32.74 ^f	39.42 ^b	36.61 ^d	38.72 ^c	35.90 ^e
DE (%)	45.83 ^c	60.00 ^b	23.66 ^g	56.25 ^c	57.69 ^b	47.27 ^d	42.16 ^f

^{a-g} Values are means of triplicate measurements. Values with the different letter in the same row are significantly different ($P < 0.05$)

Meo, methoxyl; AUA, anhydrouronic acid; DE, degree of esterification

Table 3. Chemical characteristics comparison of HCl- and ammonium oxalate-extracted pectin from roselle calyx variety Acc.6

	HCl-extracted pectin	ammonium oxalate-extracted pectin
Yield (%)	9.77 ^b	18.70 ^a
Moisture (%)	8.23 ^a	5.98 ^b
Ash (%)	4.52 ^a	3.81 ^b
MeO (%)	7.11 ^a	4.64 ^b
AUA (%)	68.05 ^a	42.24 ^b
DE (%)	59.09 ^b	60.00 ^a

^{a-b} Values with the different letter in the same row are significantly different ($P < 0.05$)

pectin with higher MeO% (7.11) and AUA% (68.05) was observed in HCl extraction of roselle calyces. Koubala *et al.* (2008a) and Kratchanova *et al.* (1991) reported similar observations. The comparison of DE% of HCl- and ammonium oxalate-extracted pectin is shown in Table 3. Slightly higher DE% (60.00) was shown by ammonium oxalate-extracted pectin compared to HCl-extracted pectin with DE% of 59.09. This high DE% value ($> 50\%$) indicated both HCl- and ammonium oxalate-extracted pectin from roselle Acc.6 calyx may be an alternative source of pectin in food industry as most of the pectin with high DE% are having good gelling properties.

Conclusion

Remarkably, pectin was successfully extracted from roselle calyces using HCl and ammonium oxalate. Ammonium oxalate appeared to be the most effective extractant in solubilizing and releasing pectin from roselle calyx compared to HCl. The resulting high degree of esterification (> 50 DE%) of ammonium oxalate-extracted pectin indicated the roselle calyx pectin can be useful to the food industry. The utilization of roselle calyx for commercial pectin production will not only solve the problems of agricultural waste disposal but also considered profitable yet challenging to be discovered.

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