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Carbohydrate Polymers **23** (1994) 231–234

0144-8617/94/$07.00

A preliminary characterization of some pectins from quince fruit (*Cydonia oblonga* Mill.) and prickly pear (*Opuntia ficus indica*) peel

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(Received 14 March 1993; revised version received 4 October 1993; accepted 12 November 1993)

The preliminary characterization of a hot acid extracted pectin from quince (*Cydonia oblonga* Mill.) and from prickly pear (*Opuntia ficus indica*) peel was carried out. The yield of the extraction, the galacturonic acid content and the neutral sugar composition were determined and compared with published data on apple and lemon pectins

The pectin yield from quince was on average 0·53% on fresh weight, which is of a similar order to apple. The quince pectin had a high galacturonic content (about 78%), and a degree of methoxylation of about 59% corresponding to a medium–high methoxyl pectin.

The prickly pear pectin yield was 0·12% on fresh weight. This pectin had a galacturonic acid content of 64%, a low degree of methoxylation (10%), a high acetyl (10%) and neutral sugar content (51% galacturonic). It might be related to similar polygalacturonides present in the mucilages of other Cactaceae.

INTRODUCTION

The optimization of pectin extraction from traditional raw materials and the search for new or not yet sufficiently used sources, with particular emphasis on the fibrous residues of fruit processing, is of substantial interest.

Quince (*Cydonia oblonga* Miller) is too acid, astringent and tough to be consumed as a fresh fruit. However, it can be consumed when cooked or when processed as jams, jellies and liquors (Roversi, 1983). Therefore quince fruit are widely used in the food industry, but rarely in the home. The main property of interest of quince fruit is its peculiar intense flavour when ripe (Schreyen *et al.*, 1979; Tsuneya *et al.*, 1983), therefore it is coprocessed with other fruit, such as apple and pear. Its juice can be blended with other fruit juices to diversify flavour characteristics (Schobinger *et al.*, 1982). A knowledge of the characteristics of the pectin extracted from the whole quince fruit and the evaluation of its suitability as an additive in the food industry, might result in another use for quince fruit.

According to the available literature, quince fruit pectin has not been extensively studied, probably because quince is not a widely cultivated crop. However, in Italy there are some areas where there is an interest in quince fruit production, and Schobinger *et al.* (1982) pointed out that, due to the development of new fruit cultivation methods as alternatives to traditional ones, the interest in quinces has increased substantially in recent years.

Prickly pear (*Opuntia ficus indica*) is a fruit grown in subtropical and tropical areas and is cultivated in Sicily. Because of the development of its cultivation, the extraction and utilization of natural red and yellow pigments contained in blood-red prickly pear pulp has been studied (Forni *et al.*, 1992). This processing yields a lot of waste in the form of peel. The utilization of this material before discarding it may be of interest from both economical and ecological points of view. As the fibrous plant material usually contains pectic substances, a survey of the characteristics and the possibility of extraction of pectin from prickly pear wastes should be taken into consideration. Also, no information on the pectin composition of this fruit has been found in the literature. Research on the mucilage composition of some Cactaceae, including some Oputiaceae, was reported by Saag *et al.* (1975), who pointed out the strong similarity of the mucilage polysaccharides to pectins, although they contained lower levels of galacturonic acid.

The aim of this research is to present a partial characterization, including the most important aspects (such as galacturonic acid, methoxyl and acetyl content) of the pectins extracted from quince fruit and from the peel of prickly pear, to evaluate the possible utilization of these fruits as a source of commercial pectin.

MATERIALS AND METHODS

Quince cv. Gigante di Vranja were obtained from two orchards in the River Po valley; one (M) at Isola Serafini (Piacenza, Italy) and the other (T) at Tencara di Pizzighettone (Cremona, Italy). Fruit were harvested at two different ripeness stages: 15 days before the market stage (stage 1) and at the market stage (stage 2), i.e. the suitable stage for both direct consumption and processing. The samples were stored at 2°C before extraction of the pectin. Whole fruit was used for the preparation of the pectin.

Prickly pear cv. Sanguigna were harvested in the area near Palermo (Italy) and were ripened until optimum conditions for pigment extraction were reached (Forni *et al.*, 1992). The peel obtained by manual peeling of fruit (a peeling depth of about 4 mm) was used for pectin preparation.

Extraction of AIS (alcohol-insoluble substances)

Samples (1000 g) of fruit or peel were homogenized with 4 l of boiling 96% ethanol. The mixture was kept overnight at room temperature, then filtered through a sintered glass G3 funnel using vacuum. The residue was washed five times with 500 ml of 60% ethanol, then three times with 500 ml acetone. The obtained AIS was air dried, weighed, and ground with a hammer mill to pass a 0·2 mm diameter sieve (Forni *et al.*, 1986).

Extraction of pectin

Pectin was extracted from AIS in hot-acid conditions similar to those used industrially (May, 1990). AIS (40 g) was suspended in deionized water acidified to pH 2 with HCl (10–20 g/litre). The mixture was incubated at 80°C in a water bath for 2 h with frequent agitation, then filtered through a G3 sintered glass funnel. From the filtrate, the dissolved pectin was precipitated by adding enough 96% ethanol to reach a final concentration of 60%. The precipitated pectin was left to settle for 1 h at 4°C, then centrifuged and washed with 500 ml 60% ethanol four times. The pectin was redissolved in a little water and then freeze-dried.

Determination of galacturonic acid

Galacturonic acid content was determined by HPLC on enzymically depolymerized pectins (Forni *et al.*, 1987). Pectin (50 mg) was dissolved in 10 ml distilled water by adding 1 ml 1 M NaOH and holding the solution at room temperature for 1 h with magnetic stirring to de-esterify the pectin. De-esterified pectin solution was acidified with 1 N H_2SO_4 to pH 4, transferred to a 25-ml volumetric flask; 2 ml of an enzymic solution containing 50 mg cellulase (TC; Serva) and 50 mg pectinase (Rohament P, Rhom) were added. Samples, brought to volume, were incubated at 40°C for 24 h with magnetic stirring. The enzymic reaction was stopped by heating at 100°C for 10 min. Depolymerized pectins were filtered through a Millex HA filter (Millipore) and injected (20 μl) on to an ION 300 column (300 × 7·8 mm), Alltech. Conditions were: column temperature, 66°C; eluent, 0·0085 N H_2SO_4; flow rate, 0·4 ml/min; refractometric detection with a Jasco 830 RI (att.8); quantitative analysis with a Shimadzu C-R6A Chromatopac utilizing standard water solutions of monohydrate galacturonic acid (Merck) at different concentrations (0·05%, 0·1% and 0·15%; values corrected for the water of hydration).

Neutral sugar composition

Individual neutral sugar composition was determined by HPLC after acidic hydrolysis of pectin: 4 mg of pectin was added to 2 ml of 2 N trifluor acetic acid (TFA) and heated for 1 h at 121°C. Products of hydrolysis were dried under a stream of air at 40°C, redissolved in 1 ml of distilled water, filtered on a Millex HA (Millipore) filter and injected (20 μl) onto an Aminex HPX 87-P column (300 × 7·8 mm, Bio Rad Labs) using the following conditions: column temperature, 75°C; eluent, water; flow rate, 0·35 ml/min; refractometric detection with a Jasco 830 RI (att.4); quantitative analysis with a Shimadzu C-R6A Chromatopac utilizing 0·05% and 0·10% standard solutions of glucose, xylose, arabinose, galactose, rhamnose and mannose (Merck). The RI response was linear with concentration for each sugar.

Degrees of methoxylation (DM) and acetylation (DAc)

The determination of the degrees of methoxylation and of acetylation were performed as described by Kravtchenko *et al.* (1992) with the following modifications.

Methanol and acetic acid from esterified galacturonic acid were released by saponification with alkali and quantified by HPLC on a cation exchange resin. Samples (30 g) of pectin were suspended in 1 ml of 0·8 N NaOH/isopropanol (1:1) solution and held at room temperature with magnetic stirring for 30 min. After centrifugation (5 min at 6000 rpm), the supernatant was filtered on Millex HA filter and injected (20 μl) on an ION-300 column (300 × 7·8 mm), Alltech at 66°C. The elution solution was 0·0085 N H_2SO_4; flow rate was 0·4 ml/min; peaks were monitored with a Jasco

Table 1. Pectin yields

Quince[a] (% wet wt)				Prickly pear[a] (% wet wt)	Citrus[b] (% wet wt)	Apple[b] (% wet wt)
M1[c]	M2	T1	T2			
0·42 ± 0·011	0·45 ± 0·032	0·58 ± 0·013	0·66 ± 0·018	0·12 ± 0·010	0·80	0·41

[a] Average ±SD.
[b] Cesalpinia (1979).
[c] M and T are the growing areas; 1 and 2 are the ripeness stages.

830 RI (att. 8), retention time and peak integration were recorded by a Shimadzu C-R6A Chromatopac. Standard solutions were prepared by dissolving 500 mg methanol and 500 mg acetic acid (Merk) in 100 ml of the NaOH/isopropanol solution.

The residue of de-esterified pectins after saponification and centrifugation was redissolved by adding 2 ml of 0·05 N oxalate to complex calcium which might hinder solubilization. The solution was then assayed for its content of galacturonic acid (AGA) using the enzymatic method. Degrees of methoxylation and acetylation were calculated as follows:

$$DM = \frac{CH_3OH}{AGA} \times 100$$

$$DAc = \frac{CH_3COOH}{AGA} \times 100$$

RESULTS AND DISCUSSION

Quince fruit

Pectin yield

Table 1 reports the pectin yields of quince and prickly pear and includes data on apple and citrus pectin for comparison. The quince pectin yield was on average 0·53% (wet wt). The second ripeness stage resulted in a higher pectin content; this fact suggests it may be desirable to delay the harvesting of the fruit. The quince pectin yield is intermediate between those of citrus and apple so quince can be considered a good source of pectin.

Galacturonic acid content

The galacturonic acid content was high (from 71 to 83%), with the highest value being for sample M2 (Table 2). The galacturonic acid content of quince was of the same level as that reported for industrial apple and lemon pectin samples (76·8% for apple and 60·8% for lemon; Kravtchenko *et al.*, 1992).

Degree of methoxylation and acetylation

The average degree of methoxylation was 59·1% which is similar to that of medium–high grade methoxyl pectins. In both samples (M and T) the degree of methylation was lower in the ripe material. The degree of methylation was lower than those quoted for pectin samples from lemon (72%) and apple (74%) (Kravtchenko *et al.*, 1992).

The values for the degree of acetylation were within the range of the lemon and apple pectins (1·5% and 5·0%, respectively).

The DM and DAc were less influenced than AGA by the growing area and the degree of ripening.

Neutral sugars

Table 3 shows the monosaccharide composition obtained by TFA hydrolysis of the quince pectin. The average neutral sugar content (29·24%) was twice that of lemon pectin, but 1·5 times lower than that of apple pectin (Kravtchenko *et al.*, 1992). The average glucose content was lower than in the apple pectin, while xylose was of the same order of magnitude. The HPLC method used could not separate galactose from rhamnose; however, for the purpose of this study it was sufficient to have the sum of the two sugars. Galactose and rhamnose, or one of these, was the most prominent neutral sugar in quince pectin, followed by arabinose, glucose and xylose. During ripening, galactose and rhamnose increased, while the other neutral sugars, which had standard deviations from the averages of the same order of magnitude as the degree of acetylation, are less influenced by growing area or ripeness stage.

The total neutral sugar content amounted to about 30% of the pectin. The sum of galacturonic acid and neutral sugars suggested that the pectin extracted from quince using the above-reported method appeared to be of high purity.

Prickly pear

Pectin yield

As reported in Table 1, the pectin yield was very low (0·12% wet wt), as compared with the yields of apple or

Table 2. Composition of quince pectin

	M1[a]	M2	T1	T2	Average ±SD
AGA[b]	81·2	83·3	76·3	70·7	77·9 ± 5·6
DM	60·9	58·1	59·4	57·9	59·1 ± 1·4
DAc	4·9	5·2	7·0	6·1	5·8 ± 0·95

[a] T: growing area; 1, 2: ripeness stages.
[b] Per cent dry weight.

Table 3. Neutral sugar composition of quince pectin (as % wt galacturonic acid)

	M1 ± SD[a]	M2 ± SD	T1 ± SD	T2 ± SD	Average ±SD
Glu	3·0 ± 0·64	3·4 ± 0·25	2·4 ± 0·10	5·2 ± 0·42	3·5 ± 1·20
Xyl	2·1 ± 0·71	2·5 ± 0·07	1·4 ± 0·30	3·9 ± 0·33	2·5 ± 1·05
Gal + Rha	12·5 ± 1·06	16·1 ± 0·61	16·8 ± 1·61	25·1 ± 0·88	17·6 ± 5·33
Ara	5·9 ± 0·62	5·2 ± 0·31	5·1 ± 1·83	6·2 ± 0·46	5·6 ± 0·53
Total	23·5 ± 2·54	27·2 ± 1·11	25·7 ± 2·56	40·4 ± 1·94	29·2 ± 5·73

Table 4. Composition of prickly pear peel pectin

AGA[a]	DM	DAc	Glu[b]	Xyl	Gal + Rha	Ara	Total sugars
64·30	10·0	10·4	3·9 ± 0·6	3·3 ± 0·6	34·5 ± 3·1	9·0 ± 1·7	50·7 ± 5·35

[a]Per cent dry weight,
[b]All neutral sugars are expressed as per cent weight galacturonic acid.

lemon pectin. The chemical features of this pectin were also very different from the other pectins examined.

Galacturonic acid, methoxyl degree and acetyl degree

The galacturonic acid content (Table 4) was lower than that of the quince pectin. However, the galacturonic acid content was within the limits (65%) recommended by May (1990) for the utilization of pectins as food or cosmetic additives. The very low degree of methoxylation (DM) (10%) could suggest a possible use as an additive for low calorie foods, though commercial low methoxyl pectin (LMP) pectins have DM values from 20% to 50%. In contrast, the degree of acetylation was high — about twice that of quince and apple, and seven times that of lemon. According to BeMiller (1986) acetyl values lower than 12·5% do not hinder gelation, although it would be expected to have some effect on gel rheology.

Neutral sugars

Total neutral sugars reported in Table 4 were the highest of the pectins examined although glucose, xylose and arabinose were of the same order of magnitude as in the other pectins. The rhamnose–galactose content gave a result equivalent to one-third of the galacturonic acid content; such a high figure suggests the presence in the pectin of some neutral polysaccharide chains, such as galactans, rhamnogalactans and arabinogalactans. These may be similar to the polysaccharides found in the mucilages of some species of cactaceae by Saag *et al.* (1975), even though they had a low galacturonic acid content.

CONCLUSION

Quince fruit can be considered to be an interesting source of commercial pectin. The yield of the hot acid extraction from whole fruit is quite good — slightly higher than that of apple. Based on its galacturonic acid content and its degree of methoxylation, this pectin can be graded as a medium–high methoxyl pectin with an acetyl content similar to that of apple pectin. The total neutral sugar content would suggest the presence of some co-extracted neutral polysaccharides in this pectin besides those linked to rhamnogalacturonic chains, but the purity of this pectin can be considered high enough for commercial use.

The prickly pear peel pectin as characterized by galacturonic acid content, was high enough to be utilized as a food additive, but had a low degree of methoxylation and a very high neutral sugar content, mainly consisting of rhamnose–galactose. This feature is similar to that found in the polygalacturonides present in some mucilages of cactaceae, therefore similar applications could be suggested, such as thickening agents in sauce production and pastry filling as well as in cosmetic and pharmaceutical preparations.

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