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Assam Lemon as A Source of Pectin

Part I: Effect of method of extraction, drying and storage of peel and pomace on the recovery and quality of pectin

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ABSTRACT

In the case of Assam lemons, two extractions with 0.2 per cent HCl in the case of fresh material, and three in the case of dried material, yield the maximum quantity of pectin of high quality. Drying of the peel and pomace at 55°C does not significantly affect the recovery of the pectin. Blanching of the fresh material results in the recovery of pectin of better quality, from the dried material. Rasping of the fruit for the cold extraction of peel oil prior to recovery of pectin does not materially affect the yield or the quality of the pectin. The pectin has a high jelly grade of 250-275. It contains d-galacturonic acid, d-galactose, l-arabinose and d-xylose just like any other citrus pectin.

The lemon is generally regarded as one of the best sources of pectin (Kertesz, 1951). The 'Assam lemon' commonly known as 'Kagzi lemon' (*Citrus limonum*) is one of the most widely grown among citrus fruits in Assam. No systematic investigation appears to have been made so far, regarding the utilization of this fruit. Citrus fruits are widely employed for the preparation of a variety of preserved products like canned and bottled juice, squash, concentrate, marmalade, candied peel, etc. The peel and pomace also find use as by-products for the recovery of oil and pectin. It was, therefore, of interest to study the possibility of utilizing the Assam lemons for similar purposes. Preliminary work on the utilization of the juice has already given encouraging results. These will be reported in a separate paper. In the present communication, only the results pertaining to the lemon as a possible source of pectin are reported.

Preliminary work has shown that after the extraction of the juice, the peel and pomace contain as much as 20-22 per cent of pectin as calcium pectate, which is quite good, when compared with other sources of pectin such as mandarin orange peel and pomace (Pruthi, Parekh and Girdhari Lal, 1961), guava (Damodaran and Rangachari, 1945; Pruthi, Mookerjee, and Girdhari Lal, 1960), papaya (Damodaran and Rangachari, 1945; Bhatia, Krishnamurthy and Girdhari Lal, 1959; Jain and Girdhari Lal, 1955), jack fruit (Damodaran and Rangachari, 1945; Jain and Girdhari Lal, 1955; Bhatia, Siddappa and Girdhari Lal, 1959), etc. Assam lemon is thus a promising source of pectin. In view of this and the strenuous attempts being made in the country to prepare pectin from indigenous sources so that it might not be necessary to import this important commodity, it was of interest to make a detailed study of the recovery and quality of pectin from these lemons. The data are presented under different sections.

Experimental

Fresh 'Assam lemons' were got from the Regional Research Laboratory, Assam, Jorhat. They were despatched by air, and it took 5-6 days for the consignment to

reach Mysore. They were handled promptly soon after receipt in the laboratory. The fruits were mature and light green in colour. They were washed, cut into small pieces, and the juice extracted in a basket press. 500 gm. lots of the peel and pomace were minced in a motorized mincer and then blanched for 5 minutes in boiling water to inactivate the pectic enzymes therein (Kertesz, 1951). The blanched material was then washed with cold water and pressed to remove as much as possible of the soluble solids. The pressed material was then added to four times its weight of water containing 0.2 per cent (w/w) of hydrochloric acid, and the mixture boiled gently for 45 minutes. The pH of extraction was 1.8-2.0. The slurry was then filtered through a piece of cotton cloth, applying gentle manual pressure to facilitate filtration. The residue was again heated for 45 minutes with 3 times its weight of water containing 0.2 per cent hydrochloric acid, and filtered through cloth to get the second extract. The two extracts were mixed, and pectin was precipitated by adding to the combined extract double its volume of acidified alcohol. The pectin, which was precipitated in the form of flocks, was collected on cloth by passing the liquid through it, then washed twice with 66 per cent alcohol and then thrice with a small quantity of absolute alcohol. The precipitate was then dried to a hard granular mass, which was subsequently powdered and sifted through a 100 mesh sieve.

In another set of experiments, several lots of the peel and pomace, after blanching, washing and pressing, were dried in a through-flow drier for a period of 5-6 hours at a temperature of about 55°C. The moisture content of the material was thereby reduced from 84-86 per cent to 4.3-5.8 per cent. The object of this was to study the possibility of storing the dried peel and pomace during the main season so that the dried material could be utilized for the preparation of pectin during the off season. Fifty gm. lots of the dried material were taken and added to 30 times their weight of water containing 0.2 per cent hydrochloric acid. In each case, the mixture was boiled gently for about 45 minutes, after which the slurry was filtered through cloth. Two more extractions were taken, boiling the residue each time for about 45 minutes, using the same quantity of water as before containing 0.2 per cent HCl. The first, second and third extracts were combined, and pectin precipitated as in the case of fresh material. Samples of pectin prepared from fresh as well as dried material were analysed for the various constituents.

Analytical Methods

Moisture, ash, and alkalinity of ash of pectin samples were determined by standard methods (AOAC, 1960).

Total pectin was estimated by the method of Carré and Haynes, 1922.

The fractionation of pectin was done according to the method of McColloch, 1952.

Equivalent weight, Methoxyl content and A.U.A. content were determined by the method of Owens, 1952 employing the following procedure.

0.5 g. of the pectic substance was weighed into a 250 ml. titration flask, moistened with 5 ml. of ethanol and 100 ml. of CO_2-free distilled water, and 6 drops of Hinton's indicator added, and the liquid titrated with 0.1 N NaOH to a definite change in the colour of the indicator. The equivalent weight was calculated as follows:

$$\text{Eq. wt.} = \frac{1000 \times \text{wt of sample (gm)}}{\text{N} \times \text{vol. of alkali (ml)}}$$

where N = the normality of the alkali used.

To the neutral solution from the equivalent weight determinations, 25 ml. of 0.25 N NaOH were added and the mixture shaken thoroughly and allowed to stand for 30

TABLE I. *Effect of concentration of acid in the extracting medium on the yield and quality of pectin*

	Concentration of HCl (w/w) %			
	0.1	0.2	0.4	1.0
Yield				
Pectin % F.W.B.	2.75	3.21	3.12	2.62
Pectin % D.W.B.	19.40	23.00	21.10	18.60
Analysis of pectin				
Ash	0.82	0.74	0.51	0.52
Eq. wt.	1,042.00	992.00	833.00	813.00
MeO %	9.20	9.40	8.70	8.60
A.U.A. %	70.11	72.23	73.64	72.23
Jelly grade	277	277	265	250

minutes at room temperature in a stoppered flask. 25 ml. of 0.25 N HCl were then added and the liquid titrated with 0.1 N NaOH to the same end point as before

$$\text{Per cent MeO} = \frac{\text{N} \times \text{vol. of alkali (ml.)} \times 3.1}{\text{wt. of sample (gm.)}}$$

Anhydrouronic acid was calculated as follows:

$$Z = \frac{\text{Wt. of sample (mg.)}}{\text{m.e. alkali for free acid} + \text{m.e. alkali for saponification} + \text{m.e. alkali for titratable ash.}}$$

$$\text{Per cent A.U.A.} = \frac{176 \times 100}{Z}$$

Jelly grade was determined by preparing test jellies with 50 gm. of sugar, different quantities of pectin and calculated quantity of citric acid solution (to adjust the pH to 3.0 ± 0.5). Total weight of each mix was kept constant (90 g.), distilled water being added to make up the difference. Cooking was done on an electric hot plate to a final weight of 77 g. in each case, and the jellies obtained were compared after standing overnight with those prepared under identical conditions from a standard pectin sample (200 grade Genu citrus pectin) using the finger test for comparison.

For the characterization of the pectin, 1 g. sample of the purified pectin was hydrolysed at 37°C for 48 hours with the enzyme preparation 'Pectinol' (Rohm & Hass). After 48 hours of hydrolysis, aliquots of the hydrolysate were spotted on Whatman Filter paper No. 1. The chromatogram was run using the ascending technique, employing acetone, n-butanol and water (7:2:1) as solvent mixture and benzidine trichloroacetic acid as developer (Bacon & Edelman, 1951). The spots were cut, eluted and then tested for individual sugars and uronic acid (Feigl, 1960).

Results and Discussion

1. *Effect of concentration of acid in the extracting medium and time of heating on the recovery of pectin*

Data regarding the effect of the concentration of acid in the extracting medium and the effect of time of heating on the yield and quality of pectin are presented in Tables I and II respectively. The following concentrations of hydrochloric acid (w/w) were used: 0.1 per cent; 0.2 per cent; 0.4 per cent and 1.0 per cent. The different times of heating tried were 30, 45 and 60 minutes.

The methoxyl content, yield and jelly grade were the highest when HCl of 0.2 per cent concentration was used (Table I). Both methoxyl content and jelly grade decreased when the extraction time was prolonged to 60 minutes. For the maximum recovery of pectin, without affecting

TABLE II. *Effect of time of heating on the yield and quality of pectin*

	30 minutes			45 minutes			60 minutes		
	I Extract	II Extract	Total	I Extract	II Extract	Total	I Extract	II Extract	Total
Yield									
Pectin % F.W.B. ...	1.72	1.10	2.82	2.16	0.87	3.03	2.35	0.70	3.05
Pectin % D.W.B. ...	11.92	7.65	19.52	15.00	6.07	21.07	16.30	4.89	21.19
Analysis of pectin									
Ash	0.68	0.52	...	0.62	0.74	...	0.58	0.51	...
Eq. wt.	946.00	868.00	...	1,003.00	741.00	...	1,042.00	641.00	...
MeO % ...	9.40	8.98	...	9.50	9.14	...	9.20	7.80	...
A.U.A. % ...	73.30	71.90	...	72.71	73.62	...	70.11	63.30	...
Jelly grade ...	294	265	...	294	265	...	277	227	...

its quality, the optimum period of heating will be approximately 45 minutes.

2. *Number of extractions*

Data regarding the effect of the number of extractions in the case of fresh and dried material are presented in Tables III and IV respectively.

The recovery from the first and second extractions was 95.6 per cent of the total pectin present in the material. In the third extraction, only 4.49 per cent of the total pectin was obtained and it was of lower jelly grade. It is, therefore, not necessary to employ a third extraction of the material. From Table IV it will be seen that in the case of the dried material, however, the recovery of pectin is considerable in the third extraction (16 per cent of total pectin). It will, therefore, be economical to carry out the third extraction as well, in this case, although the jelly grade of the resultant pectin might thereby be slightly lowered.

3. *Fractionation of pectin*

The results of fractionation of pectin from fresh and dried peel and pomace are presented in Table V. It will be seen from Table V that drying of the peel and pomace results in an increase of water soluble and oxalate soluble pectin and a decrease in the acid soluble pectin.

TABLE III. *Effect of number of extractions on the yield and quality of pectin from fresh peel and pomace*

	I Extract	II Extract	III Extract
Yield			
Pectin % F.W.B. ...	2.02	0.86	0.13
Pectin % D.W.B. ...	14.50	6.15	0.97
Recovery as per cent of total pectin ...	67.13	28.47	4.49
Analysis of pectin			
Ash	0.62	0.74	0.58
Eq. wt.	1,003.00	741.00	641.00
MeO %	9.50	9.14	9.20
A.U.A. % ...	72.70	73.60	75.91
Jelly grade ...	294	265	227

TABLE IV. *Effect of number of extractions on the yield and quality of pectin from dried peel and pomace*

	I Extract	II Extract	III Extract
Yield			
Pectin % F.W.B. ...	1.57	0.87	0.47
Pectin % D.W.B. ...	11.61	6.41	3.45
Recovery as per cent of total pectin ...	54.10	29.80	16.00
Analysis of pectin			
Ash ...	0.78	0.62	0.68
Eq. wt. ...	1,248.00	1,052.00	879.00
MeO % ...	9.10	8.60	7.80
A.U.A. % ...	66.80	66.80	65.90
Jelly grade ...	277	250	227

TABLE V. *Fractionation of pectin from lemon*

Raw material	Water soluble pectin expressed as per cent on			Oxalate soluble pectin expressed as per cent on			Acid soluble pectin expressed as per cent on		
	F.W.B.	D.W.B.	Total pectin	F.W.B.	D.W.B.	Total pectin	F.W.B.	D.W.B.	Total pectin
Fresh peel and pomace	0.16	1.18	5.7	0.72	5.33	25.7	1.92	14.22	68.6
Dried peel and pomace	0.26	1.89	9.98	0.79	5.74	30.21	1.59	11.40	60.0

TABLE VI. *Effect of different preliminary treatments on the recovery and quality of pectin from fresh peel and pomace*

Raw material and treatment	Moisture in raw material%	Method of precipitation	Yield of pectin		Ash	Eq. Wt.	MeO%	A.U.A. %	Jelly grade
			F.W.B.	D.W.B.					
Fresh peel and pomace unblanched, washed	85.5	Alcohol	3.33	23.04	1.03	1,002	9.40	72.70	277
Fresh peel and pomace blanched and washed	86.4	Alcohol	3.04	22.30	0.70	992	9.40	76.20	277
Fresh exhausted peel and pomace (from rumbling machine) blanched and washed	85.0	Alcohol	3.16	21.20	0.56	1,051	10.40	76.50	277

TABLE VII. *Effect of different treatments on recovery and quality of pectin from dried peel and pomace*

Raw material and treatment	Moisture in the material %	Method of precipitation	Yield of pectin		Ash	Eq. Wt.	MeO%	A.U.A. %	Jelly grade
			F.W.B.	D.W.B.					
Unblanched, washed and dried peel and pomace ...	6.4	Alcohol	2.94	20.25	0.84	972	8.0	65.9	179
Blanched, washed and dried peel pomace ...	4.2	Alcohol	2.92	21.40	0.62	946	8.74	68.3	265
		Aluminium sulphate	1.88	13.40	1.46	918	8.46	66.4	250
Exhausted (rumbling machine), blanched, washed, and dried peel and pomace ...	4.6	Alcohol	2.82	18.70	0.50	972	8.97	70.0	265
		Aluminium sulphate	1.96	13.30	1.43	788	8.50	71.3	250

4. *Effect of different treatments on the recovery and quality of pectin*

Data regarding the effect of different treatments, such as (*i*) blanching of the material (*ii*) rasping of the fruit for cold extraction of oil, etc., on the recovery and quality of pectin from fresh as well as dried material are presented in Table VI and VII

TABLE VIII. *Comparative data for pectin from some important sources*

Source	Apple[15]	Grape fruit[15]	Raw papaya[5]	Commercial citrus pectin[16]	Lemon[16] (U.S.A.)	Mandarin orange[3] pomace and exhausted peel, Indian	Assam lemon
Yield %	...	...	0.72 (F.W.B.) 9.0 (D.W.B.)	...	...	15.8 (D.W.B.)	3.04 (F.W.B.) 22.3 (D.W.B.)
Moisture %	...	...	8.58	8.7	14.1	2.82	7.08
Ash %	...	...	4.79	8.3	0.4	0.50	0.70
Eq. wt.	577	518	...	...	...	...	992
Methoxyl %	9.1	9.8	7.5†	7.3	7.9	8.5	9.4
Uronic acid	...	...	66.8†	62.9	79.5	85.0	76.2
Jelly grade	264*	265*	227	185‡	280‡	175	277

* Moisture and ash free basis. † Commercial finger test. ‡ Delaware method.

respectively. It will be seen that drying of the blanched peel and pomace did not significantly affect the recovery or the quality of the pectin. In the case of the material dried without blanching, the jelly grade of the pectin decreased considerably. Rasping of the fruit for cold extraction of oil prior to pectin recovery had practically no effect on the recovery or the quality of pectin in the case of fresh as well as dried material. Although the quality of the pectin, obtained by precipitation with aluminium sulphate (Deluca and Joslyn, 1957), and purified by repeated washing with acidified alcohol (Bhatia, Krishnamurthy and Girdhari Lal, 1959) was not much affected, the yield was comparatively lower than in the alcohol precipitation method. The peel and pomace could thus be dried after blanching and washing and utilized later for the recovery of pectin.

5. *Characterization of pectin*

Paper chromatographic studies on the characterization of the pectin showed that it contains d-galacturonic acid, d-galactose, l-arabinose and d-xylose. In the case of citrus pectin also Savur (1956) has reported the same constituents. For comparison of Assam lemon pectin with other pectins data regarding the characteristics of pectin from other sources such as apple, grape fruit, mandarin orange, papaya, etc., as reported in literature are given in Table VIII. It will be seen that the pectin from Assam lemon compares well with other pectins of good quality. The ash content is, however, low, but the methoxyl content is practically similar to that of pectin from other sources. As regards uronic acid content, it is comparable with that of other citrus pectins. The jelly grade is quite high and compares very well with that of lemon and grapefruit and also of apple pectin. It is considerably higher than that of mandarin or papaya pectin. Assam lemon pectin is thus a pectin of high quality.

Conclusion

It will thus be seen that the peel and pomace, which are by-products in the utilization of the Assam lemons for extraction of juice for preservation, can be profitably employed for the preparation of pectin of high quality. This pectin resembles, in every way, high grade citrus pectin. Further work is in progress to study the stability of the pectin during prolonged storage under various conditions and also to investigate the technologial aspects concerned in developing the process on a large scale, so that the pectin could find a use in the fruit preservation industry.

Summary

1. In the case of Assam lemons, the recovery of pectin of good quality is the maximum when 0.2 per cent of hydrochloric acid (w/w) is used for extraction and the heating is for about 45 minutes. Two extractions in the case of fresh material are quite adequate.

2. Drying of the peel and pomace at a temperature of about 55°C does not significantly affect the recovery or the quality of the pectin.

3. Blanching the fresh material helped in the recovery of pectin of better quality from the dried material.

4. Rasping of the fruit for the cold extraction of oil prior to the recovery of pectin did not materially affect the yield or the quality of the pectin.

5. The pectin contained d-galacturonic acid, d-galactose, l-arabinose and d-xylose, just like any other citrus pectin. Its jelly grade was quite high, being in the range of 250-275, and compared well with that of other citrus pectins and also of apple pectin. It was even superior to that of pectin from papaya or mandarin orange.

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