LXIV. A SIMPLE METHOD FOR THE PURIFICATION OF *CITRUS* PECTIN.

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THE preparation of highly purified pectin has hitherto depended upon the use of electrodialysis, prolonged settling of impurities [cf. Emmett, 1926] and similar methods. In any event, it is doubtful whether pure samples of esterified pectin have been obtained; only calcium pectate and pectic acid were regarded as obtainable in a pure state. The development of a method for preparing the esterified (soluble) pectin in a state of purity was, therefore, desirable. It has been found possible to do this readily by removing the impurities usually associated with pectins (prepared from *Citrus* fruits) by a method involving the use of iodine and bromine as oxidising agents in aqueous solution.

Norman and Norris [1930] had observed that pectin is very stable to oxidation with bromine in presence of calcium carbonate. Block and Cowgill [1932] had already used a method of oxidation to remove gums *etc.* from antineuritic vitamin extracts.

Iodine and bromine are usually employed as oxidising agents in alkaline solution. The presence of any alkali is evidently ruled out in this case on account of the extreme susceptibility of the ester groups in pectin to alkalis. However, it was found that oxidation of the impurities readily took place in a neutral or faintly acid medium without any appreciable effect on the pectin. Dilute solution of hydrogen peroxide was also tried without success.

EXPERIMENTAL.

Purification by partial oxidation with halogens.

General method. Pectin solution, about 0.5 %, was prepared by dissolving the pectin in boiling water. To 50 ml. of the cooled pectin solution about 3 ml. of *N*/10 iodine solution were added. The oxidation was allowed to proceed for varying periods and at different temperatures. The excess of iodine was then removed by shaking two or three times with ether until the aqueous layer was colourless. The excess could also be removed by warming *in vacuo* at 40°, or by bubbling air through the solution at the same temperature. Pectin was then precipitated by 95 % alcohol and after two hours filtered on muslin, finally passed through various grades of alcohol and dried in the oven at 100° for 20 hours.

Two commercial varieties of pectin were purified and a sample of grape fruit pectin, obtained by extraction of the rind with *N*/75 HCl, was similarly treated. In each series a control experiment was carried out, in which the pectin was precipitated without treating with iodine. The methoxyl value¹ and calcium pectate value² of the purified samples of pectin were determined (Table I).

¹ The MeO values were determined by the micro-Zeisel method [Pregl, 1930].

² The calcium pectate values were determined by taking an aliquot portion of the purified pectin dried at 100° for 20 hours, using the method of Carré and Haynes [1922].

Table I. *Purification with iodine.*

Sample of pectin	Time of oxidation	Temperature of oxidation ° C.	Yield of purified pectin g.	MeO %	Calcium pectate %
Series I:					
1. Commercial pectin I	Control	20	0.23	9.6, 9.6	94.9, 94.78
2. "	12 hours	20	0.21	10.6, 10.5	100.0, 100.15
3. "	20 "	20	0.20	11.0, 11.15	100.4, 100.4
4. "	48 "	20	0.19	11.4, 11.4	100.7, 100.8
5. "	72 "	20	0.19	11.6, 11.56	100.9, 100.8
6. "	$\frac{1}{2}$ hour	90-95	0.18	11.1, 11.0	100.5, 100.7
7. "	1 "	90-95	0.16	10.9, 10.8	100.2, 100.2
8. "	1 "	40	0.19	11.5, 11.4	100.8, 100.7
9. "	2 hours	40	0.18	11.4, 11.28	100.6, 100.38
10. "	6 "	40	—	10.9, 10.7	100.8, 100.7
Series II:					
1. Commercial pectin II	Control	20	0.22	7.9, 7.9	93.9, 94.0
2. "	24 hours	20	—	8.1, 8.2	96.0, 96.5
3. "	48 "	20	—	9.1, 9.2	100.0, 100.1
4. "	5 days	20	0.19	9.3, 9.2	100.4, 100.3
5. "	10 "	20	0.17	9.5, 9.4	100.5, 100.4
6. "	1 hour	40	0.20	9.4, 9.38	100.7, 100.6
7. "	2 hours	40	0.19	9.3, 9.3	100.3, 100.1
8. "	6 "	40	0.18	8.8, 8.5	100.3, 100.4
9. "	$\frac{1}{2}$ hour	90	0.18	9.1, 9.26	100.4, 100.3
10. "	4 hours	90	0.16	8.6, 8.5	100.2, 100.3
Series III*:					
1. Grape fruit† pectin	Control	20	0.22	9.4, 9.4	93.1, 93.2
2. "	1 day	20	0.20	11.2, 11.2	100.1, 100.2
3. "	2 days	20	0.20	11.3, 11.2	100.7, 100.5
4. "	6 "	20	0.18	11.3, 11.3	100.7, 100.8
5. "	1 hour	40	0.19	11.3, 11.38	100.4, 100.3
6. "	2 hours	40	0.17	11.6, 11.5	100.6, 100.6

* It will be recalled that Norman [1928], while attempting to prepare pure pectins from lemons and oranges, always obtained pectin having an apparent methoxyl value 8.5 %, although the real methoxyl content calculated from calcium pectate yield was 11.4. This low methoxyl value, he suggested, was due to admixture with partially de-esterified pectin and other degradation products produced by the action of the acid of the juice. This difficulty is readily overcome by using the rind instead of the entire fruit.

† Prepared by extraction of the grape fruit rind with *N*/75 HCl; MeO and Ca pectate values corrected for ash.

It will be seen that purification was slow at ordinary temperature, but at 40° it was complete in about 1-2 hours. Longer heating or heating at higher temperatures only tended to cause a slight hydrolysis of the methoxyl groups. The yield of oven-dried purified pectin in all cases was over 70 % of the starting material. Whilst the calcium pectate values showed a removal of 5-6 % of the "impurity," the methoxyl values were raised by 10-20 %. This would tend to show that halogens destroyed some pectin with lower methoxyl content. Also, in Series II, the highest methoxyl value obtained was 9.5 %; this was probably because the sample was a mixture of tri- and tetra-methoxypectic acid.

Purification with bromine water.

The purification was carried out exactly as described with iodine (p. 456) using approximately 20 ml. *N*/10 bromine water for each gram of pectin (Table II).

Table II. *Purification with bromine water.*

Sample of pectin	Time of oxidation	Temperature of oxidation ° C.	Yield of purified pectin g.	MeO %	Calcium pectate %
Series I:					
1. Commercial pectin I	Control	20	0.23	9.6, 9.6	94.9, 94.78
2. " "	24 hours	20	0.21	11.2, 11.2	100.3, 100.4
3. " "	48 "	20	0.20	11.5, 11.45	100.8, 100.9
4. " "	72 "	20	0.20	11.5, 11.6	100.9, 100.9
5. " "	1 hour	40	—*	11.4, 11.4	100.7, 100.6
6. " "	2 hours	40	0.18	11.2, 11.2	100.6, 100.4
7. " "	$\frac{1}{2}$ hour	90	—*	10.9, 10.8	100.3, 100.4
8. " "	1 "	90	0.16	10.5, 10.4	100.2, 100.3
Series II:					
1. Commercial pectin II	Control	20	0.22	7.9, 7.9	93.9, 94.0
2. " "	2 days	20	0.20	9.6, 9.58	100.1, 100.2
3. " "	4 "	20	—*	9.6, 9.5	100.6, 100.8
4. " "	1 hour	40	—*	9.6, 9.5	100.5, 100.5
5. " "	2 hours	40	0.18	9.2, 9.1	100.3, 100.4
Series III:					
1. Grape fruit pectin	Control	20	0.22	9.4, 9.4	93.1, 93.2
2. " "	24 hours	20	—*	11.3, 11.4	100.3, 100.4
3. " "	48 "	20	0.20	11.3, 11.5	100.6, 100.8
4. " "	1 hour	40	0.18	11.5, 11.4	100.3, 100.4
5. " "	2 hours	40	0.18	11.5, 11.4	100.5, 100.5

* Not determined.

On comparing the above results with those obtained with iodine, it will be seen that bromine water was even more satisfactory than iodine, for its action was quicker; also, the excess of bromine was more readily removed, and the purified pectin was perfectly white.

Purification with chlorine water.

Attempts were made to carry out the purification by means of chlorine water under conditions similar to those described above, but without success. Partial de-esterification of the methoxyl groups and degradation of the pectin molecule itself were found to occur. Complete degradation occurred in 3 days at room temperature and in 1 hour at 40° with the production of brown gummy substances whose nature has not been ascertained.

Purification with hydrogen peroxide.

1 g. of the pectin dissolved in 200 ml. water was treated with 10 ml. of hydrogen peroxide (20 vols.) for varying periods at room temperature and the pectin was recovered as usual. At ordinary temperature no change in the methoxy and calcium pectate values was observed (this fact is in conformity with the observations of Norman and Norris [1930]). After treatment at higher temperatures, however, the pectin was completely oxidised, and no precipitate was obtained on addition of alcohol. The nature of these oxidation products is under investigation.

Action of halogens on sodium pectate and pectic acid.

As the halogens seemed to have slight action on partly de-esterified pectin it was of interest to study their action on other pectic substances such as sodium pectate and pectic acid.

(A) *Sodium pectate*. Preliminary experiments showed that the action of iodine on sodium pectate was very slow. Sodium pectate (2 g.) was dissolved in water and the solution divided into eight equal parts. Each part was treated with an excess (20 ml.) of *N*/10 iodine solution for varying periods at different temperatures, the excess of halogen was removed, and the sodium pectate was precipitated by alcohol (2 vols.), washed, dried at 100° for 20 hours and weighed (Table III).

Table III. *Action of iodine on sodium pectate.*

Time of treatment	Temperature (° C.)	Yield of sodium pectate recovered (g.)
Control	Room	0.24
24 hours	"	0.24
72 "	"	0.21
120 "	"	0.20
240 "	"	0.19
24 "	40-50*	0.22
72 "	40-50*	0.20
48 "	90*	0.20

* A fresh quantity of *N*/10 iodine solution was added when the solution became almost colourless.

Approximately 20 % of sodium pectate was destroyed in 10 days at ordinary temperatures, in 3 days at 40-50° and in 2 days at 90°.

(B) *Pectic acid*. 0.2 g. samples of pectic acid were weighed in a flask, suspended in water and treated with 20 ml. of *N*/10 bromine or iodine solution as shown in Table IV. (More halogen was added from time to time to replace the loss at higher temperatures.) Alcohol (2 vols.) was then added to precipitate any pectic acid dispersed in solution; the precipitate was collected, washed with alcohol, dried at 100° for 20 hours and weighed (Table IV).

Table IV. *Action of halogen on pectic acid.*

	Time of treatment	Temperature ° C.	Yield of pectic acid recovered g.
1. Control	—	—	0.20
2. Treatment with iodine	2 days	Room	0.20
3. "	3 "	"	0.19
4. "	10 "	"	0.19
5. "	3 "	50	0.18
6. "	6 "	50	0.17
7. "	3 "	90	0.16
8. Treatment with bromine	3 "	50	0.17
9. "	5 "	50	0.16

The action of halogen on pectic acid is negligible at room temperature and slight even at higher temperatures; this is probably due to its insolubility.

Action of halogens on the impurity isolated from a commercial Citrus pectin.

The "impurity," probably hemicellulose, from commercial pectin was obtained by allowing the pectin solution to stand overnight with sufficient lime water to render the solution just alkaline. After removing the precipitated calcium pectate, excess calcium was removed by adding oxalic acid till just

neutral, filtering and precipitating the filtrate with alcohol. 20 g. *Citrus* pectin gave 0.75 g. of the "impurity," *i.e.* 3.75 %; the latter contained about 23 % inorganic matter.

This isolated impurity (0.9 g.) was dissolved in water, the solution divided into three equal parts and then treated with excess of iodine or bromine solution at 40° for 6 hours. At the end of that period, alcohol (2 vols.) was added, and the insoluble impurity recovered was dried at 100° for 20 hours and weighed. As treatment with iodine failed to destroy about 33 % of the impurity the experiment was repeated, increasing the time of reaction to 12 hours. It was extraordinary to find that about 30 % of the impurity was still unattacked. This was possibly due to some change, physical or chemical, in part of the impurity during isolation. That this was actually the case was proved by an indirect procedure. A sample of pectin was first treated with iodine as on p. 456, and an attempt to isolate the impurity, by the method described above, was made. The product isolated from 20 g. of purified pectin weighed about 0.1 g. and consisted entirely of inorganic matter.

The results of treatment of the impurity with halogens are recorded below.

	Yield of ash-free impurity recovered g.
1. Control	0.17
2. Treatment with iodine for 6 hours	0.08
3. Treatment with iodine for 12 hours	0.07
4. Treatment with bromine for 6 hours	0.01

Application of the above methods to the preparation of pure pectin from plant materials.

The method was used in the preparation of very pure samples of pectin from *Citrus* fruits (grape fruit and lemon). The white inner rind of these was used, and extraction was carried out by means of hot *N*/75 HCl. (Extraction with water gave very small yields, and 0.5 % ammonium oxalate removed an excessive amount of non-pectic substance.) The extraction was carried out by adding about 10 times the quantity of acid to the powdered rind, and after keeping at ordinary temperature overnight, boiling for 1–2 hours on a water-bath; the extract was then filtered off at the pump. The cooled pectin extract was then directly treated with a fair excess of *N*/10 bromine water or *N*/10 iodine solution (iodine is not as satisfactory as bromine, for reasons already stated), and kept for 2 days at room temperature. The excess of halogen was then removed by ether, the pectin precipitated with alcohol and treated in the usual manner.

In certain cases, the method detailed above gave a slightly coloured product, owing to the presence of certain impurities which gave red to violet colorations with iodine. Also in other cases impurities which readily absorb halogens were present, which necessitated the use of a large quantity of the oxidising agent. Both the above difficulties could, however, be avoided if the pectin was precipitated by alcohol immediately after extraction, redissolved in water and then treated with the oxidising agent.

The results of extraction and purification of grape fruit and lemon pectin are recorded in Table V.

Determinations of total furfuraldehyde and the uronic anhydride content of the several samples of pectin, after purification by the methods evolved, were

Table V.

Sample of pectin	% yield (calculated on wt. of air-dried rind)	MeO % (Mean of two deter- minations)	Ca pectate % (Mean of two deter- minations)	Remarks
Series I:				
1. Lemon pectin without purification	25	9.4	95	Deep yellow-coloured product
2. Lemon pectin directly treated with iodine	21.8	11.4	100.2	Slightly coloured product
3. Same as 2 with bromine	20.8	11.5	100.4	White product
4. Lemon pectin precipitated after extraction, redissolved and then treated with iodine	20.0	11.7	100.6	White product
5. Same as 4 with bromine	18.9	11.6	100.7	White product
Series II:				
1. Grape fruit pectin without purification	24.1	9.8	95.3	—
2. Grape fruit pectin directly treated with iodine after extraction	20.7	11.7	100.6	—
3. Same as 2 with bromine	20.0	11.6	100.7	White product
4. Grape fruit pectin precipitated after extrac- tion, redissolved and then treated with iodine	18.0	11.7	100.8	White product
5. Same as 4 using bromine	17.2	11.7	100.8	White product

carried out in order to test the agreement between the values obtained and those required by the formula given to the basal molecule of pectic acid by Nanji, D. R. *et al.* [1925] (Table VI).

Table VI.

	Furfuraldehyde %		Uronic acid anhydride %	
	Found	Calculated*	Found	Calculated*
Commercial pectin I	20.28	—	69.60	—
" II	20.40	—	69.80	—
Grape fruit pectin	20.38	20.45	69.80	69.60
Lemon pectin	20.31	—	69.70	—

* Based on Nanji, Paton and Ling's formulae.

DISCUSSION.

The purification of pectin with bromine and iodine in aqueous solution gives a rapid method of preparing pectin in a state of extreme purity, both from fairly pure commercial samples of pectin and also from *Citrus* fruits. The method would be particularly useful for investigations involving the structure of pectin, for unless a perfectly pure uniform sample of pectin were available conclusions regarding its ultimate constitution would have to be accepted with reserve. It is intended to extend the method to pectins from sources other than *Citrus* fruits.

The furfuraldehyde and carbon dioxide figures for purified pectin, as well as the action of halogens on sodium pectate and pectic acid, clearly show that no violent change has been produced in the pectins themselves by the action of halogens. The stability of the methoxyl groups in pectin towards oxidising agents is remarkable. Some evidence is already at hand to show that the fully methoxylated pectic acid is more stable to oxidation than the partly de-esterified pectin.

A full discussion of the function of methoxyl groups in pectin and the part they play in the genesis of pectin must be reserved till the work now in hand is completed; but it may be suggested that their function appears to be to prevent biological oxidation of pectin in plants.

SUMMARY.

1. Samples of pectin prepared from *Citrus* fruits and containing 90–95 % pectin can be further purified by treatment with bromine water or iodine at temperatures between 20° and 40°.
2. Chlorine water under similar conditions causes breakdown of the pectin: dilute hydrogen peroxide is without action.
3. The furfuraldehyde and carbon dioxide yields from pectins purified by this method agree with those usually accepted for highly purified pectin.
4. The methoxyl content of certain samples is found to approach very nearly that required by a fully methoxylated pectic acid.
5. The halogens have little action on sodium pectate and pectic acid.

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