XXXIX. THE EXTRACTION OF PECTIN FROM THE FRUIT RIND OF THE LIME (*CITRUS MEDICA ACIDA*).

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INTRODUCTION.

THE extraction of pectin from plant tissues has recently been somewhat extensively investigated. In their work on the pectic substances contained in turnips, strawberries, rhubarb stem and apples, Schryver and Haynes [1916] employed a hot dilute solution of ammonium oxalate to extract pectinogen from the washed and dried material. Subsequently, Farnell [1923], working in conjunction with Schryver, showed that oxalic acid could be substituted for the salt, and indeed, is preferable in so far that it remains in solution when the extracted pectinogen is precipitated by alcohol, whereas ammonium oxalate is strongly adsorbed by the alcohol-gel. Farnell also demonstrated that solutions of ammonium sulphate and of carbonic acid are capable of extracting appreciable amounts of pectinogen from dried turnip, but that water alone is without effect. He suggested that pectinogen is loosely combined with calcium in plant tissues, and is liberated therefrom by any reagent which precipitates the metal. Carré [1922] has, however, shown that dilute hydrochloric acid is an effective extractor of pectinogen, a result which is not concordant with this generalisation.

In examining the factors which influence the extraction of pectinogen from the fibre of sugar cane, Farnell (private communication) has employed hydrochloric acid in preference to other reagents, and has demonstrated that the quantity of pectinogen extracted depends (*a*) on the degree of subdivision of the material, (*b*) on the length of time of extraction, and (*c*) on temperature. Maximum extraction was obtained in one to two hours; more prolonged treatment led to diminishing yield. Similarly, greater extraction was obtained at 98° than at 30°, but, when the material was extracted with acid in the autoclave at 120°, hydrolysis caused a marked reduction in the amount of pectinogen brought into solution. This last result was not observed, however, when acetic acid and when water were employed. Finally, lime water, of strong alkaline reaction, extracted no pectinogen from the fibre. Evidently the hydrogen ion concentration of the extracting agent is a factor of major importance in deciding the ultimate products of the reaction.

In order further to test this conclusion, and the more clearly to explain the results of other investigations, the writer has carried out a preliminary study of the behaviour of the pectic substance contained in the white inner rind of the fruit of the lime towards certain extracting agents.

EXPERIMENTAL.

The outer yellow rind of ripe lime fruits was removed with a sharp knife, and the inner white rind stripped off without rupturing the juice-containing pulp cells. The white rind was then minced in a machine, treated three or four times with alcohol to remove water, and finally twice extracted under a reflux condenser with boiling 95 % alcohol. The resulting material was air dried, passed through a sieve having circular holes of 2 mm. diameter, and bottled for future examination.

The following figures indicate the yield of rind residues obtained: 180 ripe lime fruits, of mass 9000 g. gave 288 g. of fresh white rind, which gave 100 g. of air-dry material, containing 9.3 g. of moisture, and 3.8 g. of ash (mainly calcium oxide).

The extracting agents employed in the investigation were water, dilute hydrochloric acid ($N/20$), citric acid solutions, and solutions of sodium hydroxide. The proportion of material to extracting solution was in every case 1 g. of material to 50 cc. of solution.

The amounts of pectinogen extracted are expressed in the accompanying tables as percentages (on the oven-dry basis) of calcium pectate, precipitated from measured volumes of the extracts by the procedure due to Haynes and Carré [1922]. For comparison, the percentages of alcohol-precipitable matter, and of total solids in the extracts were also determined. Alcohol precipitates were obtained by slowly delivering from a pipette small volumes of the extracts into ten times their volume of 95 % alcohol. Total solids were determined by evaporating known volumes of the extracts to constant mass, and correcting, where necessary, the mass of the residue for the amount of solid extracting agent present.

The well-known objections to the alcohol precipitation method of estimating pectic substances, and the errors involved in the method used for determining total solids, render the values obtained for these quantities unreliable. Hence trustworthy conclusions as to the relative effects of the various treatments described should mainly be drawn from the figures for calcium pectate precipitates, which are believed to be quantitative.

Viscosities were determined by observing the time of delivery of the extracts from a standard 50 cc. tubular pipette, and comparing the results with the time of delivery of water under constant temperature conditions. Reactions (p_H values) were estimated by the colorimetric method. In every case, the figures refer to the reaction of the extract and not to that of the extracting agent.

Table I. *Extraction of pectinogen from white lime rind with water.*

Treatment	Calcium pectate	Alcohol ppt.	Viscosity	Total solids	Ash in solids	pH
1. Material allowed to remain in contact with water at 30° for 19 hours ...	18.1	22.7	1.093	27.3	1.2	5.2
2. Material put into water at 98°, and maintained at this temp. for 20 mins. ...	13.6	17.2	1.094	24.9	1.7	4.7
3. Material put into water at 98°, temp. then raised to 126° in autoclave and maintained for 20 mins. ...	33.5	45.9	1.031	59.4	1.4	4.8

Table II. *Extraction of pectinogen from white lime rind with dilute hydrochloric acid.*

Treatment	Calcium pectate	Alcohol ppt.	Viscosity	Total solids	Ash in solids	pH
1. Material allowed to remain in contact with N/20 HCl at 30° for 19 hours ...	30.1	33.2	1.242	36.1	4.2	1.3
2. Ditto; temp. then raised to B.P. and maintained for 1 min. ...	35.9	43.5	1.355	57.0	4.6	1.3
3. Ditto; temp. then raised to 98°, and maintained for 1 hour on boiling water-bath (Carré method) ...	47.7	50.1	1.242	75.1	3.9	1.3
4. Material put into boiling N/20 HCl; temp. then raised to 126° in autoclave, and maintained for 1 hour ...	11.5	26.2	1.000	86.5	4.0	1.3
5. Material successively extracted by Carré method (see treatment 3):						
1st extract ...	48.2	51.7	1.182	78.3	2.8	1.3
2nd „ ...	22.7	29.9	—	34.0	1.7	—
3rd „ ...	11.5	6.5	—	18.0	0.6	—
4th „ ...	5.0	3.2	—	7.2	0.4	—
10th „ ...	0.1	—	—	—	—	—

Table III. *Extraction of pectinogen from white lime rind with solutions of citric acid.*

Treatment: (a) Material put into the acid solution at 98°; maintained at this temp. for 20 mins.; then allowed to cool for 10 mins.

(b) Material put into the acid solution at 98°; temp. then raised to 126° in an autoclave (10 mins.); maintained at this temp. for 20 mins.; then allowed to cool for 10 mins.

	Calcium pectate	Alcohol ppt.	Viscosity	Total solids	Ash in solids	pH
(For comparison) N/20 HCl (Carré) ...	47.7	—	—	—	—	1.3
1.5 N citric acid 98° ...	42.8	52.8	1.182	—	—	2.0
126° ...	37.3	37.2	1.030	—	—	2.0
N/2 citric acid 98° ...	37.7	75.3	1.394	53.1	8.5	2.2
126° ...	41.8	55.1	1.091	61.8	6.2	2.2
N/20 citric acid 98° ...	18.6	25.7	1.090	34.4	8.6	3.2
126° ...	34.8	47.2	1.054	61.1	2.1	3.2
N/500 citric acid 98° ...	8.7	14.4	1.094	28.9	2.6	4.7
126° ...	30.6	35.4	1.063	57.3	2.0	4.8
(For comparison) water 98° ...	13.6	17.2	1.094	24.9	1.7	4.7
126° ...	33.5	45.9	1.031	59.4	1.4	4.8

Treatment: (c) Material allowed to remain in contact with N/2 citric acid at 30° for 19 hours; temp. then raised to boiling point, and maintained for 1 min.

(i) ...	48.3	56.2	—	—	—	2.2
(ii) ...	35.6	41.8	1.402	—	—	2.2

Table IV. *Extraction of pectinogen from white lime rind with solutions of sodium hydroxide.*

Treatment:—As with citric acid solutions (Table III)							
		Calcium pectate	Alcohol ppt.	Viscosity	Total solids	Ash in solids	pH
N/75 NaOH	98°	... 29.8	46.2	1.121	—	—	6.2
	126°	... 31.2	47.9	1.152	—	—	5.0
N/50 NaOH	98°	... 25.3	44.4	1.020	—	—	6.9
	126°	... —	—	—	—	—	—
N/10 NaOH	98°	... none	28.7	1.000	—	—	brown, > 7.0
	126°	... none	26.4	1.000	—	—	brown, > 7.0
N/2 NaOH	98°	... none	67.6	1.000	—	—	brown, > 7.0
	126°	... —	—	—	—	—	—

Table V. *Extraction of pectinogen from white lime rind under reduced pressure.*

Treatment: Material allowed to remain in contact with the extracting solution at 30° for 18 hours, then heated under reduced pressure (50 mm.) for 30 mins.

Extracting agent	Temp.	Calcium pectate	Viscosity	Reaction pH
Water	50°	19.8	1.065	5.5
N/20 HCl	63°	27.6	1.350	1.3
N/2 citric acid ...	65°	28.6	1.402	2.2

CONCLUSIONS AND DISCUSSION.

The following conclusions are drawn from the data contained in the tables.

I. *Extraction with water.*

1. Water extracts considerable amounts of pectinogen from white lime rind. In this respect, the pectic substance of lime rind appears to differ somewhat from that contained in turnip, etc. which is insoluble in water. The reaction of the water extracts in the case of lime rind was distinctly acidic; this may account, at least in part, for the result observed.

2. Length of time of contact with water, and degree of temperature are factors that also affect the amount of pectinogen extracted.

3. The three factors, reaction, time, and temperature, apparently do not act altogether in the same direction. The data in Table I are not sufficient, however, to warrant definite conclusions as to the relative effects of these factors.

II. *Extraction with dilute hydrochloric acid.*

1. Dilute hydrochloric acid, at temperatures up to boiling point, extracts considerably more pectinogen from white lime rind than does water. [See also Report of Food Investigation Board, 1920, p. 24.]

2. Maximum extraction was obtained by the Carré method of treatment, but a single extraction is not sufficient to remove all the pectinogen from the material. Even after ten successive extractions with hot dilute hydrochloric acid, an appreciable amount of pectinogen, precipitable as calcium pectate, remained in the rind (Exp. 5, Table II). The substance remaining after extraction was partly soluble in cuprammonia and in a solution of zinc chloride;

it therefore presumably contained cellulose in addition to a resistant fibrous residue, probably of ligno-cellulose type.

3. Heating with dilute hydrochloric acid to high temperature in the autoclave brings about pronounced destruction of pectinogen in white lime rind. This result is similar to that observed by Farnell (private communication) for the pectic substance of sugar cane fibre.

III. *Extraction with solutions of citric acid.*

The series of results recorded in Table III for the amounts of pectinogen (calcium pectate) extracted by solutions of citric acid of different hydrogen ion concentration indicate the following relationships:

1. At 98° the amount of pectinogen extracted varies directly with the H ion concentration.

2. Destruction of pectinogen appears to be brought about at the higher H ion concentrations (p_H 2.0) by heating in the autoclave to 126°.

3. On the other hand, more pectinogen is extracted by the autoclave treatment when the H ion concentration is less than that represented by p_H 2.0 than by treatment at a lower temperature in open vessels. The difference is progressively less, however, the more acidic the solution.

IV. *Extraction with solutions of sodium hydroxide.*

1. Because of the acid reaction of solutions of pectinogen in water, treatment of lime rind with very dilute soda may yield extracts containing considerable quantities of pectinogen, provided the acidity is not neutralised by the alkali added.

2. When the final reaction is alkaline, a red-brown colour¹ develops, and the extract contains no pectinogen precipitable as calcium pectate. This result is in agreement with the observation of Clayson, Norris and Schryver [1921] that normal soda extracts no pectinogen from dried plant tissues, but a substance that is probably allied to the hemicelluloses, and with that of Farnell, that soda of medium dilution fails to extract pectinogen from sugar cane fibre (private communication).

V. *Relationship between alcohol precipitate, total solids and calcium pectate.*

The amount of alcohol-precipitable matter contained in the extracts is considerably greater than the amount of calcium pectate obtained, except in those extracts that possess low pectinogen contents, in which case, alcohol fails to precipitate the pectinogen completely. Haynes and Carré [1922] had previously recorded a similar observation.

¹ The alkaline extracts were similar in colour and odour to solutions obtained when glucose and certain gums, notably gum tragacanth, are heated with caustic soda. The colour may be due to the caramelisation of sugars produced by the hydrolysis of hemicelluloses, pentosans, etc. contained in the citrus rind, although a sample of rind after repeated extraction with $N/20$ HCl to remove pectinogen, gave a much less intense coloration than did unextracted rind. The colour is partially discharged by acidifying.

The total solid matter exceeds in amount both the calcium pectate and the alcohol precipitate, the difference being especially great in extracts obtained by autoclave treatment.

The above facts may be explained as due to the properties of the products of decomposition and of hydrolysis of the pectic substance occurring in white lime rind, and to the different amounts of ash components extracted by the various treatments.

VI. *Relationship between viscosity and pectinogen content.*

Although the viscosity measurements recorded in the tables are merely relative, they indicate that the physical structure of the colloidal matter present in the extracts is decided largely by the conditions under which the extracts were prepared. Thus, in every case where an extract was obtained by treatment in the autoclave, its viscosity was measurably lower than that of extracts obtained by other means, even though its pectinogen content were greater. Furthermore, extracts prepared under identical conditions of time and temperature, but in the presence of different concentrations of hydrogen ion, exhibited viscosities that appear to be functions of the degree of acidity rather than of pectinogen content. The correlation between viscosity and hydrogen ion concentration, however, is apparently not simple; the figures suggest an optimum p_H value at which the extracts exhibit maximum viscosity for constant pectinogen content. Finally, the time factor, with which should be associated the extent of the surface of contact between the pectinogen-containing material and the extracting solution, appears also to be a factor controlling, not only the quantity of pectinogen extracted, but also the viscosity of the extract obtained.

The complex circumstances that affect the viscosities of colloidal solutions have recently received considerable attention from investigators who have attempted to explain the properties of gels and sols in terms of physical structure. The now widely accepted micellar theory evolved by these investigators postulates the presence in colloidal solutions of primary particles of solvated matter which may possess various sizes and shapes, and which may become progressively aggregated to form granules or chains, fibrils or filaments, depending on the prevailing conditions. According to this theory, viscosity is believed to be the expression of the extent to which solvation and aggregation have progressed, and gelation, the final stage of the process. The fact that the viscosity of a colloidal solution depends not only on its concentration, but also on its previous treatment and history, is readily explained on these assumptions.

VII. *Possible relationship between viscosity and jellying power.*

Although the preliminary investigation described has not included direct determinations of the jellying power of pectinogen extracts prepared under different conditions, it seems reasonable to suppose that this property is

positively correlated with viscosity, since, in terms of the micellar theory, viscosity is a measure of the degree of incipient gelation. Hence, the values given for viscosities might be taken to indicate the jellying powers of the various extracts. Considered in this light, the data contained in the tables fall into line with certain experimental results of other investigators. Thus, in their researches on the jellying power of citrus pectin extracts, Johnston and Denton [1923] noted that extracts possessing high jellying powers exhibited greater viscosities than extracts with less pronounced jelly-forming properties. They obtained no definite correlation, however, between the quantity of alcohol-precipitable matter and jellying power, and therefore concluded that the alcohol precipitation method is not a reliable means of estimating "effective pectin." Recently, H. A. Noyes [1923], in reviewing the efficacy of different processes for manufacturing jams and jellies, has stated that the best results are obtained by minimum heating, especially when carried out *in vacuo*, and that heating in open vessels destroys pectin, at least in so far as jelly producing power is concerned. The "vacuum cook process" is stated to be superior even to the "one minute cook process." In order to test this conclusion, the writer has submitted white lime rind to a vacuum extraction process with water, dilute hydrochloric acid and dilute citric acid solution. The results are set out in Table V. On comparing them with results obtained by other treatments, it is seen that the vacuum extracts possess the higher viscosities, considering their relatively lower pectinogen contents, and that, of the other treatments described, the one minute cook process is decidedly superior. These facts are in accordance with Noyes' experience.

It ought to be possible to replace by more exact means of procedure the empirical methods for preparing pectic products at present in vogue, by careful control of the complex factors involved in the processes of pectinogen extraction and jelly formation, and by the employment of precise quantitative methods for following the course of these processes. The application of the Haynes-Carré method of estimating pectinogen, and the use of the viscometer for comparing the colloidal conditions of pectic extracts should aid materially in bringing about this result.

SUMMARY.

1. The primary object of the investigation was to examine the relative efficacy of water, hydrochloric acid, citric acid and caustic soda solutions of varying hydrogen ion concentration, in extracting, under different heat treatments, pectinogen from dried white rind of lime fruits.

2. Under similar time conditions, *the total quantity of pectinogen* extracted appears to vary directly with H ion concentration when extraction is carried out at temperatures below boiling point, and directly with temperature when the final reaction of the extract is less acidic than p_H 2.0.

Alkaline extracts possess a brown colour, and contain no pectinogen.

Autoclave treatment (126°) at concentrations of H ion greater than that represented by p_H 2.0, brings about destruction of pectinogen by hydrolysis.

3. The *viscosity* of the extracts appears to depend largely on the conditions of extraction rather than directly on pectinogen content. If viscosity be considered as a measure of the degree of incipient gelation, this observation is concordant with the experience of jelly manufacturers who have found that the jellying power of pectinogen solutions depends on the treatment to which the raw material is subjected.

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