II. A MODIFICATION OF THE CALCIUM PECTATE METHOD FOR THE ESTIMATION OF PECTIN.

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In the accurate estimation of the pectin content of a solution, such as apple juice, by the calcium pectate method described by Carré and Haynes [1922] the presence of a substance giving with calcium a compound insoluble in dilute acetic acid introduces a serious difficulty. No pectin solution containing oxalates, for instance, could be estimated by direct precipitation with calcium chloride. On the other hand, the simple older method of precipitation by alcohol is exceedingly untrustworthy. If the imperfection of this method were solely due to the dragging down of other substances by the alcohol precipitate, the latter could be redissolved in water and the pectin estimated as calcium pectate. Carré and Haynes have shown, however, that the amount of pectin obtained by precipitation with alcohol is dependent upon dilution, and that in very dilute solutions precipitation does not take place.

In view of the fact that the alcohol method of estimating pectin is still used by many investigators¹, a further series of experiments has been carried out in order to confirm the results obtained with this method by Carré and Haynes. These are here described, together with a modification of the Carré-Haynes calcium pectate method involving a preliminary precipitation of the pectin by acidified alcohol. This method may be used when estimation by direct precipitation with calcium chloride is impossible, as in the case of extracts made with ammonium oxalate.

¹ Wichmann [1922, 1923] recommends a method of pectin estimation by precipitation with alcohol, the precipitate being finally washed with 80 % alcohol. It has been proved repeatedly, however, that alcohol of this dilution effects ready solution of the pectin precipitate [Carré and Haynes, 1922; and p. 10 of the present paper]. Wichmann, on the other hand, considers pectin in the most dilute solutions to be insoluble in alcohol, and supports his conclusions by demonstrating that a pectin solution which cannot apparently be precipitated by alcohol alone will immediately produce a precipitate on addition of sodium chloride. It is obvious, however, that this procedure does not support the contention that pectin is insoluble in alcohol, but merely shows that a colloidal solution of pectin in alcohol is precipitated by an electrolyte. A detailed criticism of Wichmann's conclusions will be published shortly.

Precipitation by 95 % alcohol.

Precipitations were carried out with pectin solutions of varying dilution, and the first estimations were made in the following way: 25 cc. of a pectin solution, made from crude dried pectin, were diluted with a measured volume of water in each case and precipitated with 100 cc. of 95 % alcohol. The solution was allowed to stand overnight and then passed through hardened filter papers, from which the precipitate could be scraped with ease. This method of removing the precipitate was employed since it was impossible to dry the filter paper, previously treated with alcohol, to constant weight at 100°, and the method of filtering directly into a Gooch crucible was extremely slow. After washing with alcohol, the precipitate was transferred to a weighed glass dish and dried at 100°.

Table I shows the results of two experiments carried out in this way, and it will be seen that the alcohol precipitates, in those dilutions from which an alcohol precipitate was obtained, are heavier than those obtained by the calcium pectate method. This was attributed to the presence of impurities, and the method of weighing the alcohol precipitate directly was therefore abandoned. In the third experiment of Table I, and in all subsequent experiments, the actual weight of pectin contained in the alcohol precipitates was estimated by dissolving the precipitate on the filter paper in hot water, and reprecipitating as calcium pectate.

In the first experiment "Certo," a commercial pectin solution (ash 2.6 %), supplied by the Douglas Packing Co., was used, and for the second and third the material was pectin from lemon pulp (ash 4.65 %). In each experiment a solution of pectin was made up of the required strength and 25 cc. used for each precipitation.

Table I. *Amount (g.) from 25 cc. solution.*

Exp.	Undiluted		Diluted with 25 cc. water		Diluted with 50 cc. water		Diluted with 75 cc. water	
	Ca salt	Alcohol	Ca salt	Alcohol	Ca salt	Alcohol	Ca salt	Alcohol
1	0.090 0.096	0.146 0.149	0.093 0.092	0.133 0.134	— —	— —	0.094 0.096	0.112 —
2	0.0443 —	0.0527 0.0515	0.0445 —	0.0519 0.0517	— —	— —	0.0443 —	No ppt. after standing a week
3	0.0456 0.0457 0.0458	0.0441 0.0440 —	— — —	0.0400 0.0448 —	— — —	0.0309 0.0290 —	— — —	No ppt. after standing 4-5 days

These experiments all serve to show the extreme untrustworthiness of alcohol precipitation, the two factors responsible for this being (1) dragging down of impurities by the alcohol precipitate, and (2) less complete precipitation with increasing dilution. At a concentration of about 0.04 % pectin is not precipitated at all by alcohol.

Precipitation with acidified alcohol.

In the course of experiments with apples on the conversion of the insoluble pectic constituent (which he calls protopectin) into soluble pectin, Fellenberg [1918] refers to the use of precipitation with acidified alcohol for the estimation of pectin in the presence of calcium salts. No details are given of the method of estimation, of the acid or concentration employed, or of the limits of accuracy of the method.

The following experiments were undertaken with a view of determining the effect of acidification upon the precipitation of pectin by alcohol. A preliminary experiment, conducted as Exp. 3 above, in which the pectin solutions were made up to $N/20$ with HCl before the addition of alcohol, indicated that acidification might lead to more complete precipitation. Acidification also greatly increased the rate of filtration of the alcohol precipitate, which in previous experiments was very slow. Experiments were next made to find out if at any concentration of acid there was a maximum precipitation, and whether this method of precipitation could be used as a preliminary to the calcium pectate method.

In all the experiments the alcohol itself was acidified, and, in order to avoid any effect due to the presence of a different amount of water in the alcohol at different concentrations of acid, the following procedure was adopted. For each estimation, 25 cc. of pectin solution were precipitated, the required concentration of acid being obtained by adding the necessary volume of 1.25 N HCl in 95 % alcohol, and making up the volume to 100 cc. with alcohol containing the same amount of water as the acid alcohol.

Table II. *Amount (g.) from 25 cc. solution.*

Conditions of precipitation		Exp. 1	Exp. 2	Exp. 3	Exp. 4
Directly as Ca salt		0-0322	0-0330	0-0330	0-0330
		0-0324	0-0330	0-0331	0-0330
N	HCl in alcohol	0-0329	0-0322	0-0335	—
		0-0326	0-0322	0-0335	—
$N/10$	„	0-0323	0-0333	0-0335	0-0343
		0-0321	0-0330	0-0338	0-0337
$N/25$	„	—	—	—	0-0335
		—	—	—	0-0318
$N/50$	„	—	—	—	0-0332
		—	—	—	0-0334
$N/75$	„	—	—	—	0-0328
		—	—	—	0-0325
$N/100$	„	0-0325	0-0323	0-0326	—
		0-0321	0-0323	0-0334	—
$N/1000$	„	0-0326	0-0328	0-0338	—
		0-0333	0-0321	0-0338	—
$N/10,000$	„	0-0330	0-0317	0-0336	—
		0-0340	0-0316	0-0321	—
$N/100,000$	„	Gelled	0-0329	0-0334	—
			0-0320	0-0339	—
Neutral alcohol		Gelled	0-0315	—	—
			0-0328	—	—

In Table II are given the results of representative series of experiments, made on electro-dialysed¹ "Certo" (ash 0.7 %). The acidity is that of the mixture, after the addition of alcohol. These series show surprisingly little difference in the amounts of pectin obtained by preliminary precipitation with alcohol in the presence of different concentrations of HCl, and there is reasonable agreement between the results so obtained and those obtained by direct precipitation as calcium pectate. The gel obtained in the first series in the case of precipitation without acid, and with $N/100,000$ HCl, was not repeated with fresh batches of material, although further experiments on the same material gave the same result. These results seem to point to the formation of a gel as an intermediate stage between solution and precipitation in the case of pectin, and acidification appears to aid flocculation [Haynes, 1914].

The results obtained by alcohol precipitation in the presence of the higher concentrations of HCl were shown by the above experiments to be in good agreement with those obtained by direct calcium pectate precipitation, and $N/10$ acid was the concentration selected for further experiments.

Washing the precipitate.

The results obtained by the use of acidified alcohol preliminary to precipitation as calcium pectate showed a slight tendency to be higher than those obtained by direct calcium pectate precipitation. This could not be due to the greater precipitation of impurities by the alcohol precipitate than by the calcium pectate precipitate, since the alcohol precipitate was redissolved and reprecipitated as calcium pectate. It was more probably due to the occlusion of the NaCl formed when NaOH was added to the dissolved pectin, which, having been previously precipitated by acidified alcohol, would contain HCl. The calcium pectate precipitate, although washed until the filtrate gave no precipitate with silver nitrate, and once reboiled, filtered and again washed, apparently still retained a small amount of NaCl. The following experiments on electro-dialysed material showed that this was actually the case.

In each experiment 25 cc. of solution were precipitated:

- (1) directly as calcium pectate;
- (2) directly as calcium pectate, a trace of pure NaCl being added to the pectin solution;
- (3) by $N/10$ acid alcohol, hydrolysed with NaOH as in previous experiments—reboiled once;
- (4) as in (3), but hydrolysed, after redissolving the alcohol precipitate, by KOH;

¹ One of us (A.M.E.) has worked out in detail a method for the purification of pectin based on the electro-dialysis method employed by Knaggs, Manning and Schryver [1923] for the purification of gelatin. A full account of this method will be published shortly.

(5) and (6) like (3) and (4) respectively, but reboiled several times after first washing free from chlorides. The results obtained were as follows:

No. of boilings	Ca pectate g.	Ca pectate and NaCl g.	Acid alcohol: hydrolysed by NaOH g.	Acid alcohol: hydrolysed by KOH g.
1	0.0313 0.0315	0.0325 0.0322	0.0322 0.0320	0.0334 0.0320
3	— —	— —	0.0314 0.0313	0.0313 0.0313

Careful washing of the calcium pectate precipitate is therefore necessary, and as continuous reboiling of the precipitate introduced the possibility of loss, the following experiments were made to determine (a) whether loss does occur when a calcium pectate precipitate is continuously boiled and (b) whether washing the alcohol precipitate free from HCl with 95 % alcohol before redissolving makes unnecessary the reboiling of the calcium pectate precipitate.

The results given below indicate that reboiling has no effect on the weight of the calcium pectate precipitate¹ after the second boiling has removed all traces of CaCl_2 , and that washing the alcohol precipitate with alcohol tends to dissolve the pectin.

(1)

No. of boilings	1	2	3	4	5	6
Weight of Ca salt (g.) from 25 cc. solution	0.0349 0.0345	0.0335 0.0336	0.0336 0.0336	0.0334 0.0332	0.0334 0.0334	0.0337 —

(2) *Weight of calcium pectate (g.) obtained by precipitating 25 cc. of solution.*

Directly as calcium salt	By alcohol not washed at alcohol stage	By N/10 acid alcohol washed at alcohol stage
0.0313	0.0291	0.0302
0.0314	0.0286	0.0295

Time of standing of alcohol precipitate.

In all of the foregoing experiments, the alcohol precipitate was allowed to stand overnight before filtration. This made the method slow but the following experiment, in which the precipitate stood for only an hour, indicated that prolonged standing was necessary for complete precipitation.

25 cc. of solution estimated.

Directly as calcium pectate g.	By N/10 acid alcohol after standing 1 hour g.
0.0263	0.0246
0.0262	0.0250

¹ Wichmann [1923] criticises the Carré-Haynes method of estimation on the grounds that there is great difficulty in removing traces of CaCl_2 from the calcium pectate precipitate, and that the excessive washing thus entailed may bring about re-solution. The results quoted above afford a satisfactory answer to these criticisms.

Method for estimation of pectin.

The method of estimation adopted as a result of these investigations is therefore as follows.

A volume of solution containing about 0.02 g. pectin is precipitated with four times its volume of alcohol, containing the amount of HCl required to make the resulting mixture *N*/10. After standing overnight, the precipitate is filtered, washed once with acidified alcohol and dissolved off the filter paper with hot water. It is then hydrolysed with NaOH and estimated as calcium pectate, the precipitate being washed free from chlorides and reboiled with water three times before being filtered into a Gooch crucible and dried at 100°.

The following experiments show the application of the method to pectin solutions of varying concentration:

Amount (g.) from 25 cc. solution.

I		II		III	
Calcium pectate	Acid alcohol	Calcium pectate	Acid alcohol	Calcium pectate	Acid alcohol
0.0466	0.0462	0.0250	0.0249	0.0134	0.0134
0.0465	0.0466	—	0.0249	0.0128	0.0136
—	0.0465	—	0.0249	—	0.0131
—	0.0462	—	—	—	0.0134
—	0.0456	—	—	—	0.0128
Mean 0.0466	0.0463	0.0250	0.0249	0.0131	0.0133

Amount (g.) from 100 cc. solution.

IV	
Calcium pectate	Acid alcohol
0.0117	0.0108
0.0114	0.0112
—	0.0112
—	0.0110
—	0.0104
Mean 0.0115	0.0109

It will be seen that there is very good agreement between the two methods for pectin solutions as dilute as 0.05 %, but in the case of more dilute solutions the acid-alcohol method gives slightly lower results.

The application of the method to the estimation of pectin in the presence of oxalates, or other substances giving insoluble calcium salts, is quite satisfactory, as is shown by the following experiments. In each case 25 cc. of a 0.1 % pectin solution were used, 2 cc. of *N*/10 oxalic acid being added before precipitation of the pectin with acid alcohol.

Amount (g.) from 25 cc. solution.

Exp. 1		Exp. 2	
Calcium pectate (no oxalate)	Acid alcohol + oxalic acid	Calcium pectate (no oxalate)	Acid alcohol + oxalic acid
0.0230	0.0239	0.0246	0.0248
0.0235	0.0236	0.0246	0.0244
—	—	—	0.0240
Mean 0.0233	0.0238	0.0246	0.0244

Pectin precipitation using acids other than hydrochloric.

In view of the activity of the hydrogen ion in causing the precipitation of oppositely charged colloids, it appeared very probable that the large increase in hydrogen ion concentration in the case of alcohol acidified with HCl was responsible for the quantitative precipitation of pectin by acidified alcohol. A similar increase in the precipitating power of alcohol with increased hydrogen ion concentration was found by Pauli [1922] in the case of albumin and glutin although in this case a maximum of precipitation occurred at the isoelectric point, and a further increase in hydrogen ion concentration caused a decrease in the amount of precipitation obtained.

The following experiments, in which a weak acid (acetic) and another strong acid (sulphuric) were substituted for hydrochloric acid, supported the view that, in the presence of a sufficiently large concentration of hydrogen ions (as with $N/10$ HCl and $N/10$ H_2SO_4), pectin is quantitatively precipitated by alcohol, whereas if this concentration is not high enough (as with N and $N/10$ acetic acid) precipitation is not quantitative.

Amount (g.) from 25 cc. solution.

No.	Calcium pectate method	Acetic acid N in alcohol	Acetic acid $N/10$ in alcohol	HCl $N/10$ in alcohol	H_2SO_4 $N/10$ in alcohol
I	0.0259	0.0240	—	—	—
	0.0264	0.0244	—	—	—
II	0.0259	—	0.0247	—	—
	0.0256	—	0.0247	—	—
III	—	—	—	0.0238	0.0240
	—	—	—	0.0240	0.0241

SUMMARY.

Further evidence is presented, showing that precipitation of pectin by neutral 95 % alcohol is an exceedingly untrustworthy method of estimation owing to the failure of alcohol to precipitate a dilute solution.

Acidified alcohol precipitates pectin completely at practically all dilutions. This precipitate does not give directly a measure of the pectin content of the solution, owing to the difficulty of washing the precipitate free from HCl without bringing about solution of the pectin. In the case of pectin solutions containing substances precipitable by calcium, such as oxalates, it has been found very convenient to precipitate first with acid alcohol, and then after washing the precipitate with more acidified alcohol, to dissolve it and reprecipitate as calcium pectate.

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