LXXIV. A COMPARISON OF VARIOUS METHODS OF OBTAINING ASH-FREE PECTIN.

BY ALICE MABEL EMMETT.

From the Food Investigation Board of the Department of Scientific and Industrial Research and the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.

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PECTIN contains mineral impurities, which vary in amount and character according to the source and the method of extraction. This is shown by the data given below in which the percentage of mineral impurity was obtained by heating the pectin to constant weight at dull redness in a platinum crucible:

						Percentage of ash
Pectin from lemon peel; extracted by (a) ammonium oxalate ...						3.08
(b) heating with N/20 HCl						3.57
Pectin from lemon pulp	...	...	...	...	...	4.65
Pectin from Lord Derby apples	...	...	...	...	...	2.41
Pectin from apple juice	...	...	...	...	...	5.64

A qualitative analysis of the ash of pectin obtained from lemon peel by extraction with ammonium oxalate showed the presence of aluminium, a trace of iron, calcium, sodium, potassium and a trace of copper. In the case of the apple-pectin, iron was always found in the ash.

In all the above cases the pectin extract was precipitated with alcohol (after acidification in the case of the ammonium oxalate extract), the pectin filtered off through muslin, redissolved in water and reprecipitated several times. The precipitate was then dried by standing with successive amounts of 95 % alcohol and finally with absolute alcohol and with ether. The final drying took place over sulphuric acid in a vacuum desiccator. In the course of experiments to test the completeness of precipitation of pectin by acidified alcohol [Emmett and Carré, 1926] it was necessary to use ash-free pectin, since the calcium pectate method of estimation [Carré and Haynes, 1922] used as a control to the alcohol method is not satisfactory in the presence of certain mineral impurities. The colloidal nature of pectin indicated that it should be possible to remove these impurities by dialysis and a comparison of the efficiency of the following three methods was undertaken.

- (1) Dialysis against distilled water.
- (2) „ „ N/50 hydrochloric acid.
- (3) Electro-dialysis.

The material for these experiments was obtained from a commercial pectin solution, "certo" (supplied by the Douglas Packing Company), precipitated and dried as previously described. A 1.0–1.5 % solution in distilled water was used in every case, and because of the extreme susceptibility of pectin to attack by fungi and bacteria, the solution was always covered by a layer of toluene and a little chloroform also added. The samples for ash estimation were precipitated with alcohol and dried at 100°.

Dialysis against distilled water and N/50 hydrochloric acid.

Dialysis against distilled water has been used for the purification of pectin by Fellenberg [1918] and Norris and Schryver [1925]. The last mentioned investigators do not advocate the method, since they have found it impossible to reprecipitate the purified material with neutral alcohol. In the investigations here described this difficulty was only experienced in the case of very dilute solutions, and even in these cases a gel was obtained which could be dehydrated by long standing with alcohol.

In the preliminary experiments on dialysis against distilled water and N/50 hydrochloric acid, the dialyser consisted of a parchment bag, fixed to a glass ring, the pectin being protected from dust by a cardboard cover. The toluene, however, evaporated rapidly, fungi developed and bacterial decomposition of the pectin was in some cases detected. Subsequent experiments were made with the pectin contained in a stoppered bell-jar, the open end being covered with parchment, and with this form of apparatus no contamination was experienced.

In the case of HCl dialysis, the pectin precipitate was washed free from acid, by standing with successive amounts of alcohol, before the ash was determined.

Electro-dialysis.

Electro-dialysis has been found to be a very satisfactory method of purifying colloids and has been successfully used in the case of gelatin and glue [Schwerin, 1914, 1917; Knaggs, Manning and Schryver, 1923; Knaggs and Schryver, 1924]. The apparatus used varies slightly according to whether the colloid to be purified is in the form of a gel or in solution. Pectin does not form a gel with water alone, and in the experiments to be described the apparatus shown in the diagram (Fig. 1) was used. It consisted of a small parchment dialyser suspended within a bell-jar having a parchment-covered bottom. This dipped into a large glass trough filled with distilled water, at the bottom of which was a layer of mercury to serve as the cathode. The small inner dialyser contained distilled water, and dipped into the pectin solution contained in the bell-jar. Through the cork of the bell-jar passed tubes for filling and emptying the apparatus, and a glass tube filled with mercury in the end of which was fused a platinum electrode of about 2 cm.² area to serve as anode. The water in the dialyser was removed and replaced

through the tubes shown in the diagram. The pectin samples for ashing were similarly removed.

Fig. 1.

It was found important to make the platinum electrode the anode, a reverse current causing precipitation of the pectin.

The lighting circuit (220 volts D.C.) was used and the current was kept at 0.25 amp. by means of an adjustable external resistance. The heating effect with the given intensity of current was reduced as far as possible by placing the electrodes as close together as conditions would permit, and so decreasing the resistance. Cooling was also effected by exposing to the air a large surface of the water in the outer trough.

Comparison of efficiency of the three methods.

The following table of results gives a comparison of the efficiency of the different methods of ash removal:

Table I. *Ash of material before dialysis 3.1 %.*

Method of dialysis ...	Water	Hydrochloric acid			Electro-dialysis	
		I	II	III	I	II
Conc. of solution %	1.5	1.5	1.0	1.5	1.5	1.0
Water changed ...	Once a week	Twice a week	Twice a week	Twice a week	Twice a week	Twice a week
Duration of dialysis	Percentage of ash					
1 week	—	—	0.91	—	—	0.59
2 weeks	—	—	0.87	1.01	0.63	0.61
3 "	—	—	0.83	—	—	0.79
4 "	2.85	0.93	—	—	0.84	1.40
7 "	2.58	—	—	—	—	—
8 "	—	0.84	—	—	—	—
10 "	—	—	—	0.87	0.86	—
3 months	—	0.76	—	—	—	—
5 "	—	0.74	—	—	—	—

It will be seen from Table I that purification by dialysis against distilled water is very much the slowest of the three methods, and electro-dialysis the most rapid. Very satisfactory results are obtained by the electro-dialysis method, and the process is greatly hastened if the water in the dialyser is changed daily. In Table II are given the results of an experiment made in this way, and it will be seen that no appreciable change in ash occurred after the third day.

Table II.

Days of dialysis	Percentage of ash
Before dialysis	3.10
1	0.98
2	0.85
3	0.54
4	0.54
5	0.50
7	0.56

In using this method of purification care must be taken to change the water very frequently, so that the period of dialysis is not unduly prolonged. If this is not done, there is an initial fall of ash, followed by a rise, as will be seen from the results of the electro-dialysis experiments given in Table I. A similar increase in ash occurs if the dialyser is allowed to stand without current or change of water for several days, as shown in Table III. The reason for this increase in ash is rather obscure.

Table III.

	Exp. 1	Exp. 2
	%	%
Ash before dialysis	3.1	3.1
„ after 2 weeks' dialysis	0.74	—
„ „ 4 „ „	0.56	0.65
„ „ standing without current	0.89	1.14

In none of these experiments was it found possible to reduce the ash, which was found to be rich in iron, below 0.5 %. Other investigators [Fellenberg, 1918; Carré and Haynes, 1922; Nanji, Paton and Ling, 1925; Norris and Schryver, 1925] have called attention to the presence of iron in the ash obtained from pectin, but no evidence is available to decide whether this iron is present as a colloidal impurity not removable by dialysis, or whether it is in closer association with the pectin.

SUMMARY.

The following methods of removing mineral impurities from pectin are compared:

- (1) Dialysis against distilled water.
- (2) „ „ N/50 HCl.
- (3) Electro-dialysis.

Electro-dialysis is shown to be the most rapid and effective method, the ash of a sample of pectin being reduced from 3.1 % to 0.5 % in three days, when care is taken to change the water in the dialyser daily.

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