LXII. PECTIN AND ITS HYPOTHETICAL PRECURSOR, "PROTOPECTIN."

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It has long been known that the expressed juice of unripe fruits contains very little pectin, and that when the crushed marc is subsequently extracted with cold water, only small, additional quantities of this substance are obtained. In contrast to this, when a soft, fully ripe fruit is pressed, the juice contains a considerable amount of pectin, and further and larger quantities may readily be obtained by extraction of the marc with cold water.

When, however, an unripe fruit is heated with dilute hydrochloric acid it becomes thoroughly disintegrated, and the liquid obtained by straining and pressing the resulting mixture is rich in pectin. This has led to the assumption that unripe fruits contain an insoluble compound which might be regarded as the parent substance of pectin, and which, by hydrolysis, during the process of ripening, or when heated with acid, becomes converted into ordinary, soluble pectin. To this insoluble compound the name "protopectin" was given by Fellenberg [1918], and it corresponds to the "pectose" of earlier investigators. It has, moreover, been assumed that the presence of this "protopectin" was a cause of the hardness of unripe fruits, and that its gradual change into soluble pectin was a feature of the process of ripening.

After having worked for some time with pectin, however, it occurred to the present author that the generally accepted explanation of the apparent deficiency of soluble pectin in unripe fruits might not be the correct one. To quote one observation: if it be attempted to filter a solution containing only a relatively small concentration (0.5 % or even less) of pectin through a reasonably efficient paper it will be found that, after a very few moments, filtration becomes extremely slow. The filtrate then collected contains only a very small proportion of pectin, the greater part of the latter substance being retained on the surface of the filter as a gel-like layer. Considerable quantities of distilled water may then, in course of time, be passed through this filter, when the pectin continues to be removed, but at an extremely slow and apparently constant rate. Now it seemed that conditions analogous to this might prevail in the case of hard, unripe fruit, where really efficient disintegration of the tissue is peculiarly difficult to attain, particularly when it is considered that

fruits contain products which, though soluble in alcohol, are insoluble in water, and would certainly hinder the access of the latter to the pectin occluded in the tissue.

In view of these considerations, and also taking into account the fact that no direct evidence of the existence of "protopectin" has ever been obtained, the following alternative explanation of the fact that fruits not fully ripe retain part of their pectin content in an apparently insoluble form, presented itself to the author: namely, that no such substance as "protopectin" exists, but, that the persistent retention of pectin in an apparently insoluble form by the tissue of incompletely ripe fruits is due partly to the presence of substances insoluble in water but soluble in alcohol, but more particularly to the great difficulty in attaining, by mechanical means, really efficient disintegration of the unripe tissue. In other words, it is suggested that the presence of "protopectin" is not a cause of the hardness of the tissue of unripe fruits, but that the behaviour of this tissue, described above, is the effect of its dense nature.

More than two years ago a few preliminary observations were made which tended to support this view. The material employed was a very finely ground sample of dried pomace (the press-cake from the cider press). Three quantities, each weighing 30 g., were exhaustively extracted in a Soxhlet apparatus; No. 1 with water only; No. 2, first with alcohol, and subsequently with water; whilst No. 3, after extraction with alcohol, was heated in an autoclave at 110° with dilute hydrochloric acid for one hour, and subsequently exhausted with boiling water. The amounts of pectin obtained from the three extracts were, respectively, 1.4 g., 1.6 g., 1.6 g. Had "protopectin" been present the last extract should have yielded the largest amount of pectin, whilst the inhibiting effect of the presence of alcohol-soluble substances is shown by the lesser amount of pectin obtained from the first extract.

It was therefore decided to conduct some experiments with unripe apple tissue, taking precautions to remove all the substances soluble in alcohol and also to ensure as complete a mechanical disintegration as was possible with the means available.

The variety of apple selected for this purpose was Bramley's Seedling. The fruit for the experiment described below was picked on August 29th, 1922, when it was far from being in a ripe condition. These apples, when pressed, yielded a juice which contained only a very small amount of pectin. A quantity of the fruit was peeled, deprived of all core tissue, thinly sliced, and then placed in alcohol, these operations being conducted as quickly as possible. The weight of fresh, sliced material taken was 286 g. The alcohol into which the sliced fruit had been placed was decanted, and replaced by fresh solvent, and this treatment was continued, at the ordinary temperature, so long as anything was dissolved from the apple tissue. The latter was then well crushed in a mortar, brought on to a filter, and well washed, first with absolute alcohol, and finally with ethyl acetate. After drying, first in the air, but subsequently in a vacuum over sulphuric acid, the material weighed only 8.9 g. This dry

material was then submitted to a very thorough grinding in a mortar and the whole of it passed through a sieve the meshes of which were approximately $\frac{1}{200}$ th of an inch square. The ground product then weighed 8.8 g. This finely ground tissue was then well stirred, at frequent intervals, for about 2 hours with 1300 cc. of cold distilled water¹, and subsequently allowed to settle for about 22 hours. The supernatant liquid was then decanted, and the process repeated. In all, 25 extractions were made. The pectin content of each of the last ten extracts was very small, and apparently constant. The residual, wet marc was then mixed with about a litre of alcohol, collected on a Buchner filter, well washed, first with alcohol and subsequently with ethyl acetate, pressed, and allowed to dry. The dry material was re-ground with fine, sifted sand² in a very thorough manner, so that the sand became crushed to microscopic, sharp-edged, flint-like fragments. After again passing the product through the above-mentioned sieve the extractions with water were repeated as before. In this case it was found that only ten extractions were necessary to reach the point where only a very small amount of pectin was removed. The marc was then washed with alcohol and ethyl acetate, and dried, as previously described, after which it was again very thoroughly ground with the finely comminuted sand which had passed through the sieve after the first re-grinding. On repeating the treatment with water, as before, only seven extractions were found necessary. At this stage the material was carefully examined under a microscope, samples being taken two minutes after it had been vigorously stirred with distilled water, some from near the surface of the mixture, and others from the lowest portion. The former showed only fragments of cell walls together with minute particles of silica, whilst the latter contained, in addition to silica, some small fragments of vascular tissue which has escaped complete disintegration, together with one or two groups of about three partially broken cells still adhering together. It was evident that disintegration had been very thorough, although not absolutely complete, and it was concluded that a small amount of soluble pectin would still be retained in the tissue. Nevertheless, it was not deemed necessary to continue the laborious process of grinding and extracting in order to obtain proof of the absence of "protopectin." The residual marc was therefore heated in an autoclave at 110° with *N*/20 HCl under the conditions described by Carré [1922] and subsequently extracted with distilled water four times, in the manner described. The fourth extract contained an amount of pectin that was scarcely detectable. The pectin present in all the four series of extracts, obtained as above described, was, in each case, directly estimated as such. After concentration under diminished pressure, each extract was treated with a large volume of alcohol, the pectin collected, dried in a vacuum and weighed. The method proposed by Carré and Haynes [1921] was not adopted, as their procedure represents

¹ In order to ensure that the dry powder would readily mix with the water it was found convenient in the first instance to moisten it with a little alcohol.

² Purified by extraction with acids followed by strong ignition.

as pectin any acid which might happen to be present and which yields a calcium salt insoluble in dilute acetic acid. The following is a summary of the results obtained by the present author:

Weight of fresh apple tissue 286 g.	Weight of ground and dried tissue after treatment with alcohol 8-80 g.	Volume occupied by dry ground material About 28 cc.
	Weight of pectin from 8-80 g. of dry tissue g.	Volume occupied by wet tissue on subsidence from water (approximate) cc.
1. After fine grinding	1.9347*	330
2. After re-grinding with sand	1.5104*	220
3. Again re-ground with sand	0.3238	110
4. Autoclaved with HCl	0.1005	115

* These two weights, particularly the first one, are very slightly greater than the actual weight of pectin obtained. This is because, during the decantation of the pectin solutions from the marc, a very small amount of the latter was unavoidably removed with the pectin solution, and it was not subsequently separated owing to the above-mentioned difficulty of filtering, through a good paper, solutions of pectin which are not very dilute. The liquids decanted after the third and fourth treatments, however, were each exactly filtered, so that the weights of pectin yielded by them are strictly comparable. Nevertheless, the total percentage of pectin calculated on the weight of fresh tissue taken appears to be high in comparison with results previously obtained. This is attributed, in part, to the efficiency of the grinding and extraction, but also, probably, to the unripe condition of the fruit, since fully ripe fruit from the same trees was found to contain at least 50 % more water.

The volumes occupied by the marc are given as supplying additional evidence of the change produced by the various treatments.

It is seen from the above results that, after the removal of the "soluble pectin" from the original, finely ground tissue, a further, and almost as great an amount may readily be dissolved by treatment with cold, distilled water when the material has merely been submitted to a process of more efficient mechanical disintegration. The amount of pectin subsequently obtained after treatment with acid is insignificant, and its retention by the marc is obviously due to the fact that quite complete disintegration of the tissue had not been attained, even after the drastic grindings that had been resorted to.

The only conclusion that can be arrived at is, therefore, that "protopectin" does not exist, but that all the pectin present in the apple occurs in the ordinary, soluble form. The problem of estimating pectin in fruits therefore appears to resolve itself, in the first instance, into one of attaining complete disintegration of the tissue. It seems likely that this would most readily be accomplished by resorting to the treatment with dilute HCl employed by Carré [1922] for the hydrolysis of the supposed protopectin.

After the commencement of this work a paper, referred to above, was published by Carré, dealing with the changes which occur in the "pectic" constituents of stored fruit. The conclusions arrived at during the present investigation offer a ready explanation of some of the results there described. In the first place, it is probable that the "considerable variations" in the pectin content of stored fruit observed by Carré were due, not so much to a large sampling error, as suggested by her, but to differences in the degree of mechanical disintegration of the various samples of tissue before extraction

of the "soluble pectin." The great deficiency of pectin found by her in the early stages of ripening would appear to be chiefly due to lack of efficient disintegration, which is especially difficult to attain when the fruit is in an unripe condition. No special precautions seem to have been taken by Carré to ensure thorough grinding, and, in fact, she appears to recognise that this had not been attained. Thus, on p. 708, when describing the estimation, by treatment in an autoclave at 110° with dilute HCl, of protopectin in tissue which was supposed to have been already deprived of soluble pectin, she states: "The material became thoroughly disintegrated during the process...." Then again, she states that "A series of estimations of pectin and protopectins carried out at regular intervals on the same apples, showed that a very definite relationship exists between them and that the changes in the two constituents tend to be equal and opposite in amount." This, also, is obviously another result of varying degrees of inefficient mechanical disintegration. Naturally, the more pectin that can be removed after the first process of partial disintegration, the less there is left to be removed when the product has been submitted to a process which ensures complete disintegration.

It was observed by Carré that after crushing, and thoroughly washing apple tissue with water, a small amount of pectin continued to be removed during several weeks, on further treatment with water. This behaviour is attributed by the present author to the slow rate of diffusion of pectin from insufficiently disintegrated tissue.

REFERENCES.

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