XXXIX. THE RELATION OF PECTOSE AND PECTIN IN APPLE TISSUE.

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It has long been maintained that pectose, or proto-pectin as it is called by some authors, is an insoluble constituent of plant tissues, associated with cellulose in the cell-walls but exhibiting none of the properties of that substance. It has also been claimed by the majority of investigators that during the ripening of fruit, pectose decomposes into a soluble form, generally known as pectin, which consequently appears in increasing amounts in the expressed cell-sap as the fruit matures. In the unripe fruit, however, pectin occurs in negligible quantities only.

Concurrently with this disturbance in the pectic constituents, the fruit exhibits signs of softening as ripening proceeds. Microscopic examination of the tissues shows that this softening is associated with structural relations and staining and chemical reactions of the cell-wall which can only be interpreted as due to the conversion of an insoluble substance like pectose into soluble pectin.

In advanced stages of over-ripeness and during senescence the pectic changes become very much pronounced. In this condition the tissues are readily disintegrated, the cell-walls become markedly thinner, and the cells ultimately separate from one another owing to solution of their middle lamella substance.

It has been found possible to locate the pectic substances in the cell-walls by staining with ruthenium red, and to follow their distribution throughout the development of the fruit¹. Pectic substances have been located in the cell-walls and middle lamella, and periodic examinations of the apple tissue as the season advances confirm the view that the physical modifications in mature fruit are due to pectic changes, and that the processes of ripening and physiological breakdown are intimately associated with the progressive change of an insoluble pectic substance into a soluble modification. As a result of chemical work already published in part [Carré, 1922] and the unpublished

¹ This work has been carried out in conjunction with Dr A. S. Horne and will shortly be ready for publication.

microscopical studies referred to above, it would seem difficult to hold any other view than that pectose (proto-pectin¹) is chemically different from the pectin to which it gives rise, both these substances occurring in the tissues of ripe fruit.

An exactly opposite view, however, has been put forward by F. Tutin [1923], who vigorously disputes the conception of pectose and pectin as chemically different substances in the tissues, and upholds an alternative explanation of the phenomena to be observed during the ripening processes of fruits.

Tutin regards the existence of pectose as "purely hypothetical," and considers that the pectic material of the tissues consists of pectin only, which is "held in its typical soluble form throughout the development of the fruit." He maintains that ripening is in no way associated with chemical changes in the pectic components of the tissues, but that the apparently insoluble condition of the pectin (especially in hard unripe fruit), which is usually explained by the existence of pectose, is really due "to the great difficulty in attaining by mechanical means, really efficient disintegration of the hard unripe tissue."

Tutin therefore suggests that extraction with water alone should be sufficient to dissolve out the whole of the pectin thus held in the tissue, provided that complete disintegration of the material can be obtained. He consequently regards the ease with which pectin is extracted from ripe fruit as compared with unripe to be solely due to alterations in the physical condition of the fruit, and in no way connected with any chemical change in the pectic constituents themselves. Tutin also suggests that apart from the difficulties of mechanical disintegration, another factor is at work, namely, the action of certain constituents of the tissues, which tends to retain the pectin and prevent its ready removal with water. In support of this, he states that solutions of pectin approximating to a concentration of 0.5 % cannot be filtered without undergoing change in concentration, owing to retention of some of the dissolved pectin by the filter paper. He suggests that when attempts are made to extract the pectin from the tissues by treatment with water, the cell walls retain pectin in a similar manner.

It would seem impossible, however, to accept such a view. The question of the retention of pectin during filtration through various media has been investigated by the author, and it has been found that the amount absorbed varies directly with the concentration of the solution employed, but that solutions containing less than 0.9 to 1.0 % of pure pectin exhibit no alterations when filtered through ordinary filter paper. If on the other hand the solution to be filtered has a concentration greater than 1.0 % of pectin, an appreciable amount may be taken up. Such concentrations of pectin are however greater than that obtained in normal juice or under the experimental conditions of the usual extraction methods.

¹ As the word "pectose" receives the support of the majority of workers in this field, it is here adopted in preference to "proto-pectin."

These results are illustrated by the following examples:

Effect of Filtration on Pectin Solutions. Concentration of Pectin Solution
(100 cc.).

Before filtration			After filtration		
		%			%
1	...	0.025	1	...	0.025
2	...	0.115	2	...	0.116
3	...	0.227	3	...	0.226
4	...	0.760	4	...	0.759
5	...	0.870	5	...	0.873
6	...	0.170	6	...	0.148

Experimental evidence has also been obtained showing that pectin solutions of concentrations similar to the above are unchanged by passage through a dense mass of tissue from which pectin had been previously removed by water. Since the methods of extraction of pectic substances involve the use of a large bulk of water, the concentrations of pectin rarely exceeding 0.05 %, such solutions will be unchanged by filtration through paper or in their passage through the tissues themselves. It is therefore evident that the holding back of pectin cannot be regarded as responsible for the supposed retention of the pectic constituents of the unripe tissues.

Tutin describes two further experiments which he considers afford conclusive evidence for his views.

In an earlier communication, Carré [1922] showed that pectose and pectin could be removed separately from apple tissues by first extracting the soluble pectin with *cold* water, and subsequently treating the residue with dilute hydrochloric acid, thus converting pectose into pectin which can then be removed with cold water as before. Tutin has carried out a similar experiment but interprets it very differently. He treated three equal quantities of apple residue obtained during the production of cider. One sample was exhaustively extracted with boiling water in a Soxhlet apparatus, and 1.4 g. pectin was thus obtained. A second sample was autoclaved at 110° with dilute acid for 1 hour and subsequently extracted with boiling water as before. The extract yielded 1.6 g. pectin.

Tutin assumes that if both pectin and pectose had originally been present in the tissues, the water extraction would have yielded soluble pectin only, whereas the subsequent acid treatment should have produced a much larger amount, due to the decomposition of pectose. Since the weight of pectin in both cases was about the same, Tutin concludes that no pectose can have been present in the samples.

In criticism of these deductions the following observations may be made: (1) The material used for the experiment was the "pomace" or dry press-cake from the cider press. It is therefore obvious that the juice expressed from the apple during the production of the cider would have carried away with it the soluble pectin originally present in the fruit. Hence Tutin's experiment represents nothing more than two different methods of extraction of the

insoluble pectose remaining in the residue after the removal of the soluble pectin. (2) It has been proved by the present writer that *cold water only* can be used to extract the soluble pectin from tissues, since extraction with hot water induces slow conversion of the pectose into pectin, presumably by a process of hydrolysis analogous to that effected by dilute acid. It follows therefore that the 1.4 g. of pectin obtained by Tutin with boiling water actually represents a portion at least of the pectose present in the original sample. Traces of acid remaining in the "pomace" would also tend to accelerate the decomposition of the pectose. (3) The method of extracting pectin by autoclaving with hydrochloric acid at 110° has been found invariably to give unsatisfactory results, probably owing to the fact that such treatment tends to bring about the decomposition of the pectin thus produced. The method finally adopted for the quantitative extraction of pectose depends upon boiling the material with $M/100$ hydrochloric acid and this process is found to give reliable results.

The following experiment demonstrates the accuracy of this method as compared with the autoclave method employed by Tutin. Ten uniform samples of apple pulp treated with $M/100$ HCl at 100° gave a mean pectose yield of 0.76 %, while ten similar samples of material extracted by the autoclave method gave a mean of 0.67 % pectose, showing that the results obtained by the latter method tend to be too low.

This indicates that the 1.6 g. of pectin obtained by Tutin from his second sample using the autoclave method is also too low a result and does not represent the actual pectose content of the sample. It would also seem that the evidence derived from this experiment is insufficient to justify his assertion of the non-existence of pectose.

In his second experiment Tutin describes successive extractions carried out in four stages of pectin from unripe apple tissue. (I) A weighed quantity of dried material previously soaked in alcohol was pounded in a mortar and soaked for twenty-five successive periods of 24 hours each, the volume of water used for each extraction being 1300 cc. The resulting solutions of pectin were evaporated under diminished pressure and estimated. The pectin content of each of the last ten extracts was small and apparently constant. Tutin considers this slowing down in the removal of the pectin to be due to the resistance offered by the undisintegrated portions of the material. (II) The material was next dried and thoroughly ground with sand in order to increase disintegration and the residue was extracted as before till only negligible quantities were obtained. (III) The process of grinding with sand and extracting with water was repeated. The residue examined microscopically showed that disintegration had been very thorough, although groups of cells were observed to have escaped the grinding process. Tutin therefore concludes that a small amount of pectin necessarily remains in the residue. He suggests that complete disintegration "would be most readily accomplished by resorting to the treatment with dilute hydrochloric acid employed by Carré

for the hydrolysis of the supposed protopectin¹ (pectose). (IV) Tutin therefore treated the residue with $M/20$ HCl in an autoclave at 110° and the resulting solution contained very little pectin. It is probable that the yield is too low in consequence of the method of procedure. Subsequent treatment with hydrochloric acid by this method produced no further yield of pectin.

The whole pectic constituents of the tissues having been removed with water alone, Tutin therefore concludes that water-soluble pectin is the sole substance of pectic nature in the tissues, and that the problem of estimating the pectin content of fruits depends mainly on complete disintegration of the tissues in order to facilitate its extraction by water.

The explanation put forward by Tutin is the one which suggested itself to the writer in the earlier stages of pectin work, but it has been found totally inadequate to interpret the various facts observed during the course of a more exhaustive research on the pectic constituents of apples. Experiments similar in procedure to those described by Tutin but of a more searching nature had already been devised before the publication of his results. These experiments afford conclusive evidence for the existence of pectose and pectin as definite chemical entities, and suggest that although mechanical disintegration is an important factor in the extraction of pectin, the whole problem cannot be reduced to such simple terms.

In criticism of Tutin's deductions it may be suggested that pectin is not originally present as such in the tissues as he supposes, but arises from pectose by a process of hydrolysis, the amount of pectin produced by any method of extraction depending on the efficacy of the hydrolytic agent employed. Hence treatment of the tissues with cold water, as Tutin describes in the foregoing experiment, will convert pectose into pectin by an extremely slow process of hydrolysis. This would account for the long period of time, approximately 40 days, which he found necessary to extract by means of cold water the whole of the pectin from his sample of apple.

Tutin accounts for the increased production of pectin as a result of grinding the material by assuming that increased disintegration favours the dissolving out of the soluble pectin, but it must be pointed out that grinding will also promote increase of hydrolysis and therefore increased yields of pectin by bringing fresh supplies of pectose in closer contact with the water. When the tissues are subjected to a more vigorous process of hydrolysis—for instance by treatment with water at 45° , or by the use of dilute acids or alkalis—the production of pectin is found to increase according to the efficacy of the agent employed. The accompanying table illustrates this point. Approximately similar yields of pectin were obtained from each, but the time required for the operation varied markedly with the hydrolytic power of the reagent.

¹ It is difficult to understand why weak hydrochloric acid should be assumed to possess a disintegrating action on the tissues apart from chemical action. As a matter of fact such acid does not produce complete disintegration, but mere separation of the cells (*vide*, p. 264).

Method	Yield of pectin g.	Time required for operation hours
Cold water	0.80	600
Water at 100°	0.795	96
<i>M</i> /100 HCl at 45°	0.81	24
<i>M</i> /100 HCl at 100°	0.80	15

The following experiments (A and B) may also be quoted in support of the hydrolysis theory outlined above.

DETAILS OF EXPERIMENT A (Fig. 1).

100 g. of apple tissue was pressed in a hand press and washed with cold water to remove any soluble pectin initially present in the tissues. The total weight of pectin thus obtained in the three fractions amounted to 0.0400 g., the final fraction containing only a minute trace of pectin (0.0005). The volume of water used for the extractions on this and subsequent days was maintained at 500 cc. in order that the pectic content of successive fractions might be properly compared. The residue was then soaked in water for 24 hours and the pressing and washing repeated. It will be seen that this treatment produced an amount of pectin (0.0415 g.) as large as the yield on the previous day, although no mechanical disintegration had been resorted to. This marked development of pectin may be attributed to hydrolysis of pectose in consequence of: (*a*) the prolonged contact of the tissues with water, (*b*) the action of enzymes liberated from the cells crushed during the pressing operations, (*c*) the accelerating effect of any acid left in the tissues, and probably to a combination of all these factors.

Further extractions of the material (3rd–6th day) produced decreasing yields of pectin, the amount obtained on the 6th day being negligible. Grinding the residue with sand (7th and 8th day) resulted in a further small amount, probably in consequence of the exposure of fresh quantities of pectose to the hydrolytic action of the water.

If the extraction of the pectin was simply a matter of solution of the occluded material by the water, the grinding operations should have produced a far greater effect than was actually observed to be the case, since subsequent operations showed that approximately two-thirds of the total pectic material still remained in the tissue. This method of procedure was discontinued on the 8th day. A point was not reached at which no further amounts of pectin were produced, since hydrolysis with cold water, although extremely slow, is a continuous process. Microscopic examination of the residue at this stage showed that the tissues were almost entirely disintegrated, but staining with ruthenium red revealed substances of pectic nature abundantly present in the fragments of cell-walls and in the middle lamella regions. In order to hasten hydrolysis the residue was re-ground with sand (8th day) and boiled with water for 5 minutes. The amount of pectin then produced (0.0900 g.) was twice as great as that obtained on the first or second day of the experiment,

and nine times as much as the amount produced by cold water alone in the preceding fraction.

The boiling was repeated daily till only small traces of pectin were extracted, the final fractions being approximately constant in amount. It will be seen from the table that the total weight of pectin obtained by boiling the residue with water is 0.4515 g., nearly three times the amount produced by cold water treatment.

It might be suggested on Tutin's theory that the large production of pectin with hot water is due to its greater solubility at the higher temperature, but experimental evidence indicates that the solubility of pectin is not appreciably greater in hot than in cold water. Neither can the production of

Fig. 1.

pectin be due to enzyme action in this case since the temperature of extraction is destructive to enzymes. It seems probable therefore that the enormous and rapid development of pectin can only be attributed to the increased hydrolytic action of the water on the pectose at the higher temperature.

This conclusion is borne out by the further marked increase which was produced when the residue was treated (18th–20th day) with a more powerful hydrolytic agent, *i.e.* boiling dilute hydrochloric acid ($M/100$) for 15 minutes. After two such treatments no further trace of pectin could be obtained, and microscopic examination of the residue treated with ruthenium red showed that all the pectin constituents of the tissues had been removed. This effect of the acid in removing the pectin cannot be attributed to solvent action since pectin is only very slightly soluble in dilute acids. The above experiment suggests that if dilute hydrochloric acid be used for the hydrolysis of the pectose from the outset, the extraction should be effected more rapidly than with water alone.

EXPERIMENT B.

A second experiment was therefore carried out on a 100 g. sample of apple, similar to that used in Exp. A. The pulp was extracted with $M/100$ HCl at 100° for successive 3-hour periods. After each treatment the residue was pressed and washed to remove the bulk of the pectin produced. It was found that after three such extractions no further pectin was obtained. The total extracted was 0.72 g., which agrees very closely with the 0.70 g. obtained from similar material in Exp. A, allowing for the experimental error necessarily involved in the latter (see Fig. 1).

Experiment B.

100 g. apple tissue. Material extracted with $M/100$ HCl at 100° .

Extraction	g. of pectin obtained
1	0.4810
2	0.1943
3	0.0448
4	0.0000
Total pectin ...	0.7201

Microscopic examination of the residue showed that all the pectic material had disappeared, although the tissues were not so markedly disintegrated by the acid as by the grinding with sand in the former experiment. It was found that the cells were separate from one another, in consequence of the solution of their middle lamellae, but the cell-walls themselves were almost invariably unbroken, and preserved their original outline.

Microscopic investigations of the pectic substances have been carried out by the author in conjunction with Dr A. S. Horne, and the results, which will be briefly alluded to, confirm the opinions arrived at from the chemical side. The decomposition of the pectic substances has been followed under the microscope by treating in various ways sections stained with ruthenium red, which colours the pectic constituents. Irrigation of these sections with water produced considerable swelling of the stained areas followed by their very slow disappearance. This process was observed to be much more rapid with hot water, and very much accelerated when either dilute acid or alkali was employed. Solution of the middle lamella and ultimate separation of the cells were attained, but complete removal of the pectic components of the cell-walls involved no fundamental alteration in the appearance of these walls.

It was also found that if sections were irrigated with dilute ammonium oxalate solution, which has long been recognised as a solvent for pectic substances, the solution of the stained areas was very rapidly effected. The cells were again observed to separate from one another, but the cell-walls themselves remained entirely unbroken.

It is therefore apparent from the foregoing discussion of results obtained by the author, that Tutin's theory of the non-existence of protopectin, or pectose, cannot be supported by experimental evidence. It may be concluded,

on the other hand, that pectose does actually exist as a practically insoluble constituent of the cell-walls of apple tissue, and that it can be converted into the soluble modification, known as pectin, by the action of water and by dilute acids or alkalis, the nature of the change being probably hydrolytic.

It is also evident that although mechanical disintegration is important in facilitating the process of hydrolysis, it is not, as Tutin claims, the essential factor in the removal of the pectic constituents of apple tissues, the necessity for disintegration being obviated if a sufficiently powerful reagent is used for extraction purposes.

SUMMARY.

It is shown by the author that Tutin's views on the non-existence of proto-pectin (pectose) cannot be maintained by the evidence afforded by his experiments. On the contrary, chemical investigations, borne out by microscopical work, indicate the presence of an insoluble pectic constituent in apple tissue, which is referred to as pectose, and which is converted by a process of hydrolysis into the soluble modification known as pectin.

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