LXXII. AN INVESTIGATION OF THE CHANGES WHICH OCCUR IN THE PECTIC CONSTITUENTS OF STORED FRUIT.

BY MARJORIE HARRIOTTE CARRÉ.

Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.

(Received July 27th, 1922.)

AN accurate method of estimating pectin in dilute solution by precipitating as calcium pectate, has recently been established. The method has been successfully applied to the estimation of pectin in some fruit juices and by the application of this method it was proved that a complete extraction of the soluble pectin of apples can be effected by a process of continuous washing out with water.

As a result of this preliminary work it was found possible to follow the changes which take place in the pectic constituents of apples kept in cold and ordinary storage and to determine if the improved keeping properties of the apples in cold storage is accompanied by any marked difference in pectin content.

The soluble pectin probably develops from an insoluble pectic substance contained in the cell wall, which is left behind in the pulp residues after the aqueous extraction of the soluble form. This insoluble pectin corresponds to the protopectin of Fellenberg [1918], and to the pectose of earlier investigators.

Estimations of the soluble pectin were systematically carried out at regular intervals throughout the period of storage. The apples chosen for the experiments were very different, Lane's Prince Albert, a hard and acid cooking apple, and the soft, sweet Cox's Orange Pippin. Equal quantities were stored in cupboards at the ordinary temperature, and in a refrigerator at 60° C.

In view of the possibility that the time of harvesting might influence the keeping properties of the fruit, the estimations were carried out on three pickings, which were subsequently kept in cold and ordinary store. Batches were gathered three weeks earlier than the usual harvesting, at the normal time in early September, and the third picking was left on the trees till October.

Estimation of the Soluble Pectin.

The following is a summary of the process of extraction and estimation of the soluble pectin; a more detailed account of the method has recently been published [Carré and Haynes, 1921].

Samples of ten apples were cut up, freed from skin and core, and thoroughly mixed. Fifty grams were used for each estimation throughout the season.

After killing the cells, by freezing the weighed portion of the material in an efficient freezing mixture, the extraction of the soluble pectin was carried out by washing with water and pressing out in a small hand press. These processes were repeated until all the pectin was extracted. The completeness of the extraction was verified in all cases by testing the last washings of the pulp for pectin by precipitation as calcium pectate.

The dilute juice was filtered through fluted papers, to remove insoluble matter and disintegrated cell substance. It was then boiled to destroy enzyme action, and after cooling, the solution was made up to a known volume.

Aliquot portions were taken for each estimation, the quantity used depending on the concentration of the pectin solution. It was found advisable to make a rough preliminary determination of the amount of pectin contained in the juice solution, since, as the fruit approached maturity, the development of the soluble pectin from an initial negligible quantity to as much as 0.8 % necessitated a careful adjustment of the conditions of estimation.

The best results were obtained by using a volume of solution yielding 0.02–0.03 g. of calcium pectate.

For this weight of pectin, 100 cc. *N*/10 soda were used for the hydrolysis, and after 24 hours' standing the pectin was precipitated as calcium pectate by acidification with 50 cc. of *N*/1 acetic acid, and subsequent addition of 50 cc. *M*/1 calcium chloride. During the later period of storage in the case of both Cox and Lane apples, it was observed that, although the pectin content did not appreciably differ and the conditions of experiment were exactly as before, the coagulation of the calcium pectate became ill defined, and the precipitate was slimy and difficult to wash and filter. It was found however that a satisfactory coagulation could again be obtained by using double the amount of *N*/10 soda previously found adequate for hydrolysis. The larger amount of soda was found to be necessary as long as the supply of fruit was available, and it seems reasonable to conclude from these observations that some change occurs in the state of aggregation of the pectin sol as the fruit develops, or possibly an alteration may take place in the constitution of the pectin.

THE DEVELOPMENT OF SOLUBLE PECTIN.

The accompanying graphs (Figs. 1 and 2) are representative of the results obtained from the estimations. Fig. 1 illustrates the development of soluble pectin in cold and ordinary storage, and Fig. 2 shows the very close similarity in the development of the soluble pectin in all three pickings of apples.

The experiments indicate that in the early stages of the maturation of the apple there is no soluble pectin, but it gradually develops as ripening proceeds till it attains a maximum amount when the fruit reaches its fully ripe condition. The Cox apples ripened earlier than the Lane, and in both cases the fully ripe state was found to be coincident with the greatest amount of pectin developed. The maximum pectin content was maintained in all cases for

about 4 weeks, during which the apples were in their prime condition. This period was followed by a condition of general softening and a sudden drop in the pectin content was found to accompany the change of condition in the fruit. This softening process is the concluding phase in the apple's life, and it leads eventually to a state of physiological breakdown or of general decay brought about by fungal or bacterial action. During the whole softening

Fig. 1. Development of soluble pectin in cold and ordinary storage (Lane—1st picking).

Fig. 2. Development of soluble pectin in cold stored Lane's picked at different times.

period the soluble pectin content showed considerable variations. In order to ascertain if these variations could be accounted for by differences in the condition of the fruit estimations of pectin were made on sets of apples in different states of softness, each set being carefully selected to be as uniform as possible. The results obtained show that there is a marked decrease in pectin as the fruit softens. The following experiments illustrate this point:

	Hard sample	Soft sample
Weight of soluble pectin from 100 g. ...	0.51 g.	0.42 g.

It may therefore be concluded that the fluctuating values obtained for the soluble pectin are to be attributed to the great variability which was observed in the state of maturity of the individual apples taken for each estimation, and it follows that in samples of ten taken for each separate determination a large sampling error might easily result. It is proposed during the next season to carry out estimations on each of the individual apples and to calculate the probable error due to sampling.

The routine estimations showed that apples contained varying but appreciable amounts of pectin as long as supplies of fruit were available, and in no case where sound apples were examined did the pectin wholly disappear. On the other hand when diseased apples were examined there was found in most cases to be a complete absence of soluble pectin.

EFFECT OF COLD STORAGE ON THE DEVELOPMENT OF SOLUBLE PECTIN.

The curves showing the development of pectin in cold and ordinary stored fruit (Fig. 1) are very similar, the only effect of the low temperature being to prolong the period of ripening. The maximum point of soluble pectin content occurs six weeks to two months later in the cold stored fruit.

After the maximum was passed and during the breakdown of the fruit the pectin content of the cold and ordinary stored fruit was found to be practically identical as long as samples of both were available for comparison.

It was possible to carry out these comparisons up to the end of April on the cold and ordinary stored Lane's, but the Cox's Orange were so badly affected by Bitter Pit that the supply of ordinary stored fruit was exhausted by the end of December, soon after the maximum point had been reached. The greatly prolonged life of the cold stored Cox's as compared with those kept in ordinary storage was due to the effect of cold in retarding the progress of disease in the fruit.

If the results of the estimations of soluble pectin developed in cold and ordinary store during the normal life of the apple are added together, the quantity is found to agree very closely for apples of the same kind whether they have been picked at different stages of maturity or kept under different conditions of temperature.

Total of (12) estimations during six months.					
			Cold stored	Ordinary stored	
Lane I ¹	...	...	4.05 g.	4.07 g.	
Lane II	...	...	3.99	4.03	
Total of (7) estimations during three months.					
Cox I ...	...	...	3.01 g.	3.07	
Cox II	...	...	2.77	2.82	
Picking					
			1	2	3
Lane	...	...	4.29	4.11	4.45
Cox	...	...	4.95	4.70	4.64

¹ = the numerals indicate the different pickings of the apples.

This agreement is too close to be merely fortuitous, but it is impossible to attempt any complete interpretation at present. It may however be inferred from the foregoing results that a definite amount of soluble pectin is produced during the process of ripening and that it is subject to secondary change during the later stages of maturation.

Estimations of protopectin which are described below, throw some further light on the subject.

ESTIMATION OF PROTOPECTIN.

With a view to examining the pectin changes in the apple more thoroughly it became necessary to devise a quantitative method of estimating the insoluble pectin constituent or protopectin¹, as it will be called in the following account, and so to obtain some idea of the relations between the soluble and insoluble forms.

The method adopted was to treat the residue after extraction of the soluble pectin with 100 cc. *N*/20 HCl in an autoclave for about an hour at a temperature of 110°. The material became thoroughly disintegrated during the process and the protopectin was converted into the soluble form, presumably by a process of hydrolysis. It was easily washed out with very little pressing, and the extract was estimated by the calcium pectate method. That the treatment removes all the insoluble pectic substances capable of being transformed by the acid was proved by repeating the autoclaving with a similar amount of *N*/20 HCl in which case no soluble pectin was found in the second extract. Subsequent treatments with greater concentrations of acid *N*/5 and *N*/1 also failed to produce any further trace of pectin.

To test the accuracy of the method equal amounts of apple pulp were taken, and the soluble pectin in them was extracted and estimated. The residues were treated as described above in order to convert the protopectin into soluble pectin, and the extracts thus obtained were also estimated. The following results illustrate the measure of agreement in the amounts obtained from two samples of uniform material. Both protopectin and soluble pectin are estimated in terms of the calcium pectate obtained from them.

Weight (g.) obtained from 100 g. of pulp.

	Soluble pectin	Protopectin	Total pectin (Protopectin and pectin)
(a)	0.43	0.36	0.79
(b)	0.385	0.45	0.835

It has been found possible to estimate the total pectin directly from the apple pulp without preliminary washing out of the soluble form.

¹ The adoption of this name for the hydrolysable pectin present in the cell wall implies that this is the precursor of soluble pectin. While there is much reason to believe that this is so, the fact cannot be regarded as finally established, and the present nomenclature must therefore be considered provisional.

Apple Sample I from Lane.

(Weight obtained from three similar samples of 100 g. of apple.)

		Picking		
		1	2	3
Soluble pectin	...	—	—	0.348
Protopectin	...	—	—	0.094
Total	...	0.404	0.418	0.442

Apple Sample II from Cox.

Soluble pectin	...	—	—	0.52
Protopectin	...	—	—	0.24
Total pectin		0.71	0.71	0.76

The amounts estimated in this way are in fairly close agreement with the total arrived at by adding the weights obtained by separate estimation of the soluble and insoluble pectin. The slightly lower values obtained in both these examples where the total pectin is extracted from the pulp may be entirely due to experimental error, but it is possible that some change may be brought about by the acids and salts contained in the juice.

ESTIMATION OF PROTOPECTIN OF APPLES KEPT IN COLD
AND ORDINARY STORE.

During the latter half of the period of storage this method was systematically used in a preliminary examination of the protopectin content of apples. Estimations were carried out on the residue of the weighed sample from which all the soluble pectin had been extracted. The residue was then autoclaved with acid as described above, and the resulting soluble pectin precipitated as calcium pectate and weighed. In this way the weight of protopectin and soluble pectin was determined in a given sample of apple.

The results obtained are of necessity incomplete, and the following account must be regarded as in the nature of a preliminary survey.

The protopectin determinations were made after the maximum soluble pectin content had been passed and the protopectin shows the tendency to fluctuate which was observed in the case of the soluble pectin.

The following estimations of the protopectin content of hard and soft apples show that there is less in the softer fruit. As the samples contained individuals differing greatly in their degree of maturity these fluctuations may be attributed, as in the case of the soluble pectin, to the variation of individual apples in each set taken for estimation.

Weight of protopectin in 100 g. of samples	Hard sample	Soft sample
A. Lane II ordinary stored	0.45	0.35
B. Lane II cold stored	0.28	0.18

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. The accompanying graph (Fig. 3) is typical of the results obtained:

The foregoing results show that after the maximum soluble pectin content is passed the quantity of soluble pectin tends to decrease and that the decrease is roughly proportional to a simultaneous increase in the insoluble pectin. On the other hand both the protopectin and soluble pectin have been shown to decrease markedly in soft over-ripe fruit as compared with harder less ripe fruit.

These observations seem to indicate that there is a balance between the protopectin and soluble pectin during the normal life of the apple, but that in the later stages of breakdown the total pectin tends to decrease. On the other hand there is some evidence to show that the total pectin content tends to *increase* as the apple ripens. The following estimations were carried out

Fig. 3. Relationship of pectin and protopectin in Lane II ordinary store.

on cold stored "Cox's" when the apples were in the early stages of their development and during the period of fluctuation when the fruit was beginning to get over-ripe.

	Soluble pectin	Protopectin	Total
Early unripe state (Sept. 20) ...	0.05	0.48	0.53
Fully ripe state (Feb. 16-Mar. 16) ¹	0.65	0.36	1.01

¹ These figures represent the mean of the weights obtained during this period.

These results need confirmation but appear to show that in the few weeks following the picking of the apples the total pectin increases rapidly owing to the marked development of soluble pectin. The change in the protopectin content is much less pronounced and is insufficient to account for the increase of the soluble form. It may therefore be inferred that the total increase in pectin is due to a third source of pectin, which either gives rise to soluble pectin directly, or is transformed into it through the intermediate state of protopectin.

THE FACTORS CONTROLLING THE DEVELOPMENT OF SOLUBLE PECTIN.

The following investigations were made to obtain some information as to whether the production of soluble pectin is due to enzyme activity.

Samples of apple which had been previously killed by freezing were crushed with sand and thoroughly washed and pressed free from soluble pectin. The residues were left with water and a little thymol added to prevent bacterial action. At the end of a week the residues were again pressed out and tested for soluble pectin. In all cases examined pectin was found to have developed. The samples were put back in water and the process repeated every week till no more pectin was found to have developed. The following examples carried out on ordinary stored "Cox's" illustrate the results so obtained.

WEIGHT OF SOLUBLE PECTIN DEVELOPED FROM 100 G. OF APPLE PULP.

			A	B
			Cox III	Cox II
			Ordinary stored	Ordinary stored
1st week	...	...	0.22	0.30
2nd week	...	...	0.35	0.05
3rd week	...	...	0.04	0.05
4th week	...	...	0.00	0.06
5th week	...	...	0.00	0.02
6th week	...	...	0.00	0.00

A series of experiments on similar lines was carried out on swedes previously killed by ether and washed free from soluble pectin. As with the experiments on apple material a sample left standing with water showed a steady development of soluble pectin. Another portion was boiled with water for three hours and then pressed out and examined. Much soluble pectin was found in the extract. The residue was washed free and then again boiled for another three hours. No soluble pectin was obtained in this case. The residue was divided into two parts for different treatment. One portion was autoclaved with a solution of $N/20$ HCl at 110° and subsequent examination showed that a great deal of pectin had been produced in the process. The second portion was kept in water for one month but no soluble pectin was developed. It was then autoclaved with acid and a great deal of pectin was produced.

The foregoing observations show that a certain amount of pectin appears, if a residue previously washed free from pectin is left with water for a time, but that a steady state is finally reached when no more pectin is produced however long the sample is kept. Similar results are obtained by boiling; a certain amount of pectin is brought into solution but subsequent boiling for long periods has no further effect and no more pectin is developed after prolonged standing with water. On the other hand in both these cases large quantities of pectin are produced by autoclaving with acid. A great deal of further investigation is necessary before any definite conclusions can be made, but such facts as have already been ascertained support the theory that the

production of soluble pectin is due to the action of an enzyme which must be supposed to be present in the cell walls in a form which it was not possible to extract.

SUMMARY.

An account is given of the development of soluble pectin in different kinds of apples kept in cold storage and at ordinary temperature. It is shown that the pectin reaches a maximum during the process of ripening and then gradually falls as the apple becomes over-ripe. The date of picking of the fruit has no effect on the development of the pectin in either cold or ordinary store.

A method is described for the quantitative estimation of protopectin by hydrolysis with weak acid. Preliminary work carried out by the application of this method suggests that there is a definite relationship between the amounts of soluble and insoluble pectin constituents. The experiments indicate the possibility of a third source of pectin.

Preliminary experiments tend to show that the development of the soluble pectin may be attributed to enzyme activity.

This investigation has been carried out for the Food Investigation Board of the Scientific and Industrial Research Department. The author wishes to express her gratitude to Dr Haynes of this Department for her valuable criticism and advice.

REFERENCES.

- Carré and Haynes (1921). *Biochem. J.* 15, 60.
Fellenberg (1918). *Biochem. Zeitsch.* 85, 118.