CHANGES IN THE PECTIC SUBSTANCES OF STORED ELBERTA PEACHES

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SUMMARY

Total and soluble pectic substances were determined in Elberta peaches ripened for 7 days at 70°F following storage at 30°F and 34°F for 7, 14, 19, 24 and 29 days. Some samples were gassed with ethylene (1000 p.p.m.) prior to storage. All fruit was mature-green when harvested.

A considerable loss of total pectic substances occurred in fruit which ripened normally following storage. Abnormal ripening of overstored fruit was associated with little or no loss in total pectic substances during ripening. A possible inactivation of the enzyme pectin methylesterhase is discussed in relation to this abnormal ripening.

A detailed account is given of an accurate and rapid method for the determination of total pectic substances in small quantities of marc prepared from fruit tissue. In this method, an infra-red gas analyser and a potentiometric recorder were used to measure the carbon dioxide evolved from fruit marc decarboxylated with hydrochloric acid.

I. INTRODUCTION

Fruit texture and juiciness appear to be related primarily to the composition and structure of the cell wall, of which the pectic substances are an important constituent. A characteristic change associated with normal ripening is a decrease of insoluble and a corresponding increase of soluble pectin. In overstored peaches, abnormal ripening occurs on removal from storage, resulting in fruit of a mealy texture, a condition known as woolliness. Practically no free juice can be expressed from such fruit. On sections of woolly peaches stained with ruthenium red, it can be observed that the intercellular spaces are filled with red masses of jelly-like pectins (de Haan 1957). It should be noted, however, that ruthenium red is not specific for pectic substances (Kertesz 1951). Working with several varieties of peaches, including Elberta, de Haan (1957) found that woolliness could be avoided if the fruit was ripened before storage to a stage where the ratio of soluble to insoluble pectin was approximately 2 : 1. Storage at as low a temperature as possible for the shortest period was recommended. In some samples of Boland and Beregrine peaches stored at 31° and 37°F, he found an increase in both insoluble and soluble pectin. Date and Hansen (1953) found an increase in total pectin in three varieties of pears stored at 30°–31°F.

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Elberta peaches grown in the Queensland Granite Belt are frequently cool-stored at temperatures approximating 34°F. A marked loss in quality due to woolliness often occurs in fruit stored longer than two weeks. In view of the evidence suggesting that the metabolism of the pectic substances is an important factor in determining the quality of fruit in relation to juiciness and texture, trials have been carried out over two seasons to determine the changes in the pectic substances of stored Elberta peaches.

II. EXPERIMENTAL DETAILS

Source and Treatment of Fruit.—Mature-green Elberta peaches were obtained from the one grower for each of the two seasons involved (1961 and 1962). Half-bushel case samples were subjected to the following prestorage and storage treatments:

(i) immediate storage at 30°F
(ii) immediate storage at 34°F
(iii) gassed with ethylene (1000 p.p.m.) for one day prior to storage at 30°F (designated as E1)
(iv) gassed with ethylene (1000 p.p.m.) for two days prior to storage at 30°F (designated as E2).

Removals from storage were carried out after 14, 19, 24 and 29 days' storage during the 1961 season and after 7, 14, 19 and 29 days' storage during the 1962 season. Each removal consisted of samples of 10 fruit per treatment and this fruit was held at 70°F for 7 days prior to analysis. Samples of gassed and non-gassed fruit were analysed for total pectic substances prior to storage during the 1962 season only. Both the total and the soluble contents of the fresh fruit were determined during the 1961 season. Total pectic substances only were determined during the 1962 season, using marc prepared by the method of Gee, McComb, and McCready (1958).

Analytical Methods, 1961 *Season.*—The 10 fruit comprising each sample were halved and peeled and macerated in a Waring blendor for 3 min. Duplicate samples (approximately 5 g) of macerated pulp were weighed for each of the soluble and total pectin determinations. To each sample was added 30–50 ml distilled water. The samples for soluble pectin determination were allowed to stand for 2 hr with occasional shaking and then filtered through a fast filter paper (Whatman No. 4). To the samples for total pectin determination were added four drops of concentrated hydrochloric acid (to bring the pH to approximately 1·5) and the samples boiled for 1½ hr; hot water was added periodically to maintain the initial volume. The samples were filtered through a fast filter paper.

To each of the filtrates from both the soluble and total pectin determinations were added two volumes of 95 per cent. alcohol; the alcohol was added slowly with constant stirring and the mixture allowed to stand overnight. The mixture was then filtered through a fast filter paper having appreciable wet strength

(Whatman No. 541). The pectin precipitate was washed several times with 70 per cent. alcohol and finally once with 95 per cent. alcohol. The washed precipitate was dissolved off the paper with hot water and the pectin solution de-esterified by adding 10 ml N sodium hydroxide. This solution was made up with distilled water to a final volume of 200 ml in a volumetric flask. The solution was allowed to stand at least 30 min at room temperature before being analysed by the colorimetric method of McCready and McComb, (1952).

Analytical Methods, 1962 *Season.*—According to McComb and McCready (1954), most of the conclusions based upon the role of total pectic substances in fruit texture have been drawn from the characterization of less than 50 per cent. to about 70 per cent. of the total pectic substances present. This is because it has not been possible to extract the pectic substances from the three-dimensional lattice of the cellular framework without changing their molecular and chemical composition. Gee, McComb, and McCready (1958) have outlined a procedure for the quantitative determination and partial chemical characterization of the total amount of the pectic substances without extraction from the plant tissue. This procedure was used for the analysis of the 1962 samples. The method is a titrimetric one and is valid only if the titratable acidity of the fruit marc is due to pectin only. This can be ascertained by analysing a sample of marc by a method specific for total pectic substances.

The determination of galacturonic acid by measuring the carbon dioxide evolved by decarboxylation with hydrochloric acid is one of the most dependable procedures used in analysing pectic substances (Kertesz 1951). This is the basis of a method that was used to check the titrimetric method. The evolved carbon dioxide was determined with an infra-red gas analyser and potentiometric recorder by the following procedure.

Procedure.—Nitrogen was passed at a known flow rate, approximately 300 ml per min, through a round-bottomed long-necked boiling flask A (Figure 1), above which was a refluxing condenser B. The effluent gas stream from the condenser was passed through a zinc trap C and thence through a U-tube D containing silica gel and connected to the inlet side of a flow-meter E; the outlet was connected to an infra-red gas analyser (1·17 per cent. CO_2 full-scale deflection). The analyser output was fed to a potentiometric recorder (O–4mV) with a chart speed of 24 in. per hr.

The boiling flask containing some boiling chips and 100 ml of 19 per cent. (w/w) hydrochloric acid was heated by an electrothermal heating mantle adjusted to keep the acid boiling moderately. The whole apparatus and infra-red analyser were purged with nitrogen until the system was free of carbon dioxide (indicated by a constant baseline on the recorder chart). A known amount (about 20–30 mg) of air-dried marc was weighed into a small receptacle and dropped into the boiling acid in the flask either through a side arm or down the neck of the flask (the refluxing condenser was momentarily raised). The receptacle used was a short section, approximately $1\frac{1}{2}$ in. of a 10-mm test-tube flared at the open end.

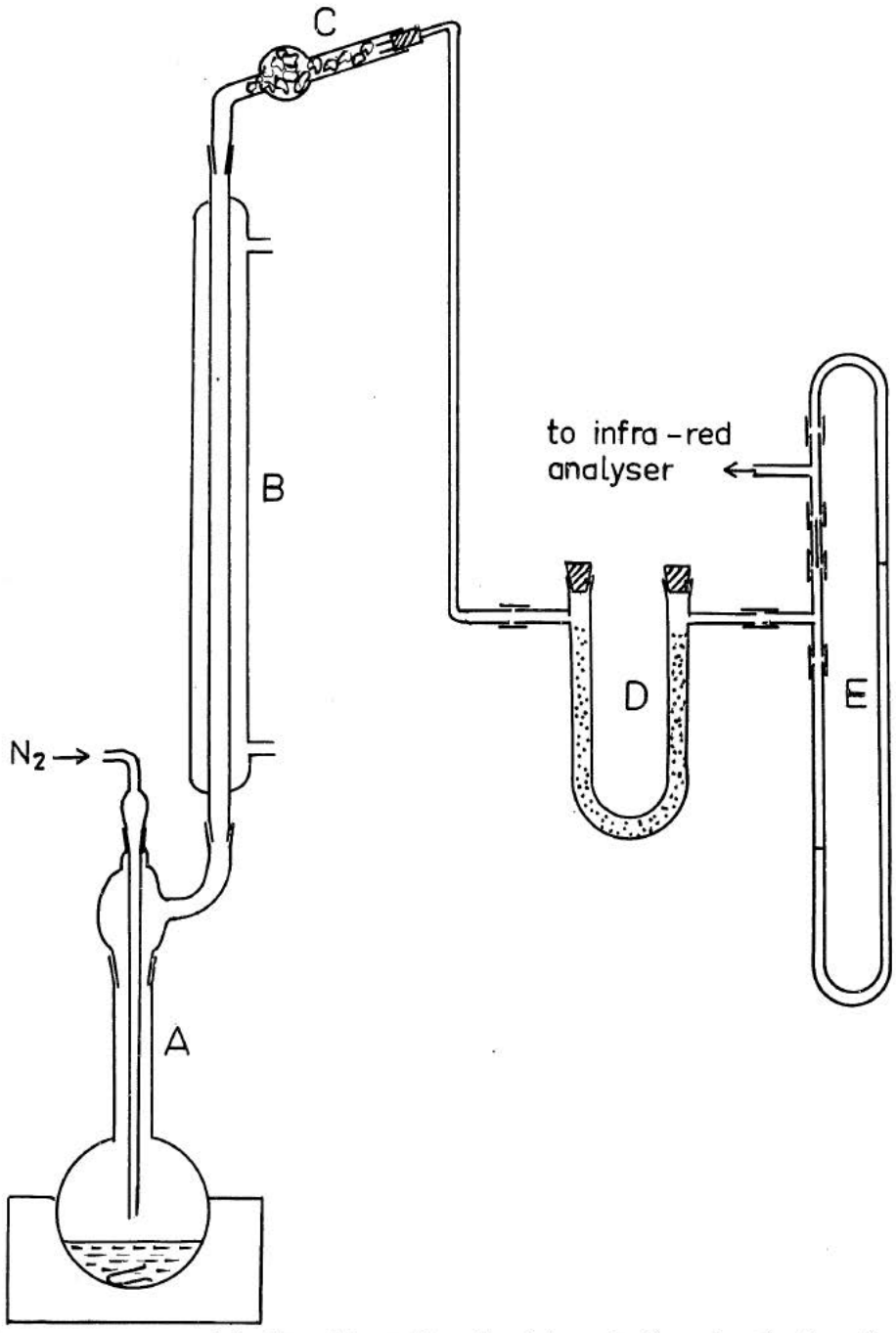

Fig. 1.—Apparatus used for determining total pectic substances by the carbon dioxide method.

The reaction took from 45–60 min. to reach completion as indicated by the recorder trace returning to the baseline. The area under the curve traced during the reaction represented the total amount of carbon dioxide evolved. This

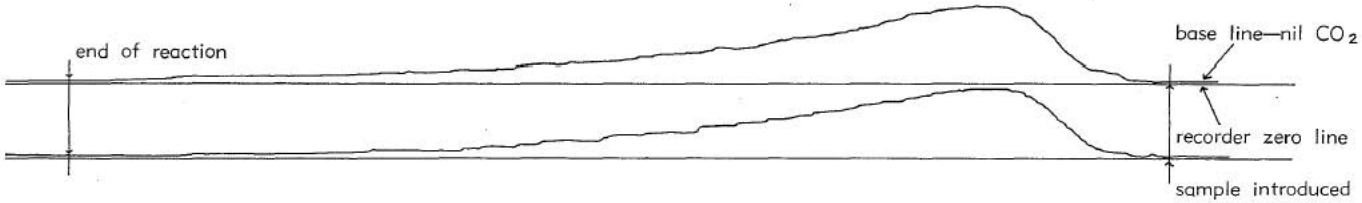

Fig. 2.—Recorder tracings of duplicate 20-mg samples.

was calculated by carefully cutting out the curve and weighing the recorder paper. A unit area of recorder paper was also cut out and weighed. The height of the unit area represents a known percentage of carbon dioxide and the length represents the time of evolution. The weight of carbon dioxide thus represented was calculated from the known flow rate. The amount evolved from the pectic substances was calculated from the weight of paper under the curve and the weight of the unit area. The weight of carbon dioxide multiplied by 4·0 gives the weight of anhydrouronic acid in the sample.

The method was checked with pure galacturonic acid and found to give theoretical yields of carbon dioxide within 1 per cent. This method gave consistently lower results than the titrimetric method. Esau, Joslyn, and Claypool (1962) found that the titrimetric method used for determining the pectic content of pears gave considerably higher results than those determined by the colorimetric versene pectinase extraction procedure.

A reproduction of the curves obtained from duplicate 20-mg samples of marc is shown in Figure 2. The percentage total pectic substances determined from these curves were 16·8 and 16·9 respectively.

The output signal from the infra-red analyser (Grubb-Parsons, Model SB2) was 1 mA at 0·5 V with an output resistance of 500 ohms, provided by a 500-ohm potentiometer from which a voltage can be fed to a potentiometric recorder. The potentiometer adjustment was too coarse to provide the 4 mV full-scale deflection required for the recorder. Consequently a 10 ohm wire-wound potentiometer was wired in series with the 500-ohm potentiometer, which was bridged with a 27,000-ohm resistor to reduce the resistance of the potentiometer and parallel resistor to 490 ohms. The recorder was fed from the 10-ohm potentiometer.

As the method was found to be reliable and suited to the serial determination of pectic substances, the 1962 samples were determined by this method as well as by the titrimetric method.

III. RESULTS

Results for both seasons appear in Tables 1 and 2 and are expressed graphically in Figures 3–6. The 1961 results, as determined by the colorimetric carbazole method, are expressed as optical density. As the results are for purely comparative purposes, it was not necessary to plot a standard curve for galacturonic acid to determine the results as percentage pectin. Results for both seasons can be compared from the slope, but not from the levels of the graphs in Figures 3–6. The 1962 results are expressed as percentage of total pectic substances in the fruit marc.

Although no account has been taken of any change in fresh fruit weight during storage and subsequent ripening, it is improbable that any such change could invalidate the results. Some loss in weight would undoubtedly occur during storage and the 1961 results probably show a higher level of total pectic substances than was actually present. The same trends, however, would still

exist and these same trends are evident in the 1962 season, in which the results would be independent of any change in fresh weight due to loss of moisture during storage and ripening.

All results in Tables 1 and 2, except those for the carbon dioxide method, are the means of duplicate samples. Both duplicates are shown for the carbon dioxide method to indicate the degree of accuracy obtained.

TABLE 1

TOTAL AND SOLUBLE PECTIN CONTENT OF FRUIT HELD FOR 7 DAYS AT 70°F FOLLOWING COOL STORAGE

Storage Temperature (°F)	Storage Time (days)	Total Pectic Substances			Soluble Pectin (1961 only) (Expressed as optical density)
		1961 Method	1962 Methods		
		Carbazole (optical density)	Titrimetric (%)	Carbon dioxide (%)	
30	7	—	22·8	17·9–18·2	—
	14	·315	19·7	12·0–12·1	·300
	19	·326	—	—	·249
	24	·362	27·6	19·4–19·7	·188
	29	—	29·1	19·7–20·2	—
34	7	—	—	20·1–20·4	—
	14	·354	23·4	18·8–18·9	·240
	19	·380	—	—	·178
	24	·494	30·5	22·9–23·3	·180
	29	—	32·8	25·0–25·4	—
E1 30	7	—	20·8	15·8–15·9	—
	14	·336	20·0	13·5–13·7	·274
	19	·253	—	—	·202
	24	·343	28·5	16·9–16·9	·221
	29	·298	—	16·6–16·7	·205
E2 30	7	—	20·8	15·1–15·1	—
	14	·381	23·1	14·1–14·4	·341
	19	·263	—	—	·222
	24	·235	21·0	12·7–12·9	·228
	29	·263	24·9	16·8–16·9	·232

TABLE 2

PERCENTAGE TOTAL PECTIC SUBSTANCES IN FRUIT PRIOR TO STORAGE (CARBON DIOXIDE METHOD)

Non-gassed	Gassed with Ethylene (1000 p.p.m.) for 1 Day	Gassed with Ethylene (1000 p.p.m.) for 2 Days
24·6	22·2	22·9

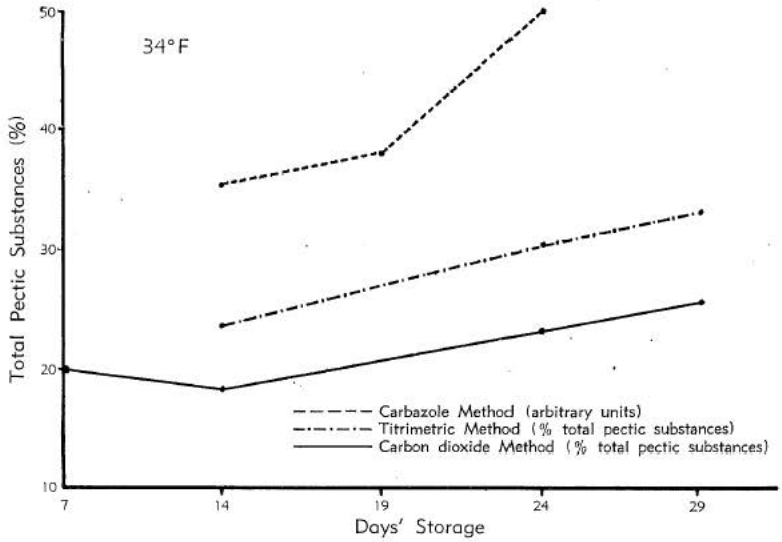

Fig. 3.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 34°F.

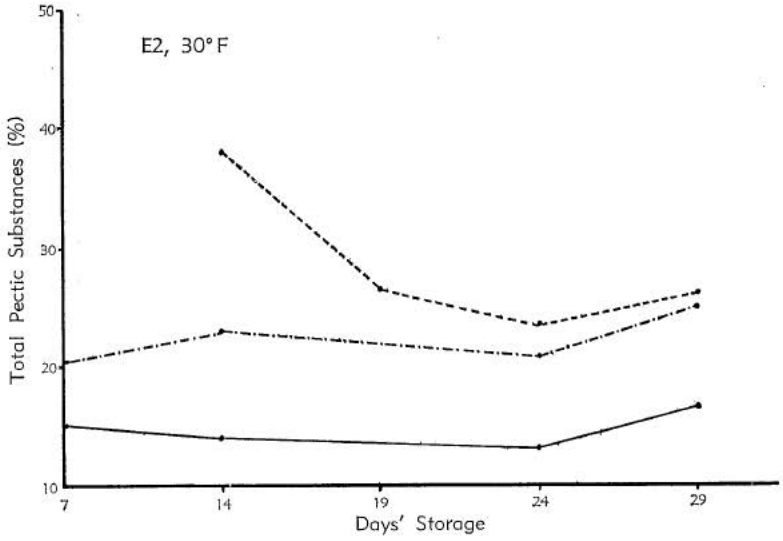

Fig. 4.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 30°F. Legend as for Fig. 3.

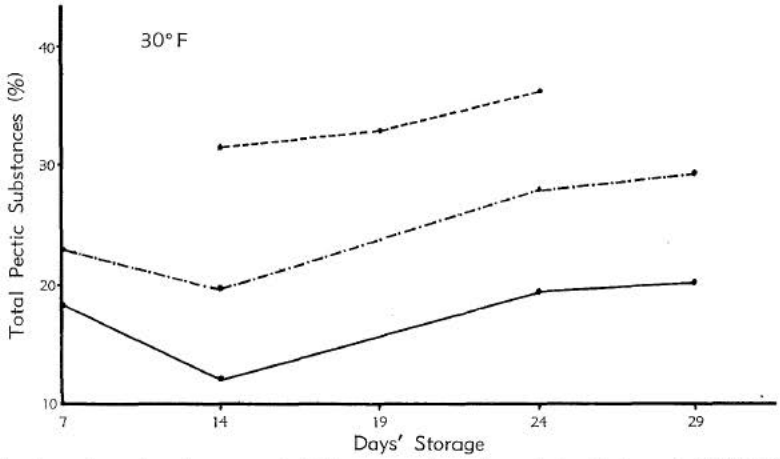

Fig. 5.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 30°F. Gassed with ethylene for 1 day prior to storage. Legend as for Fig. 3.

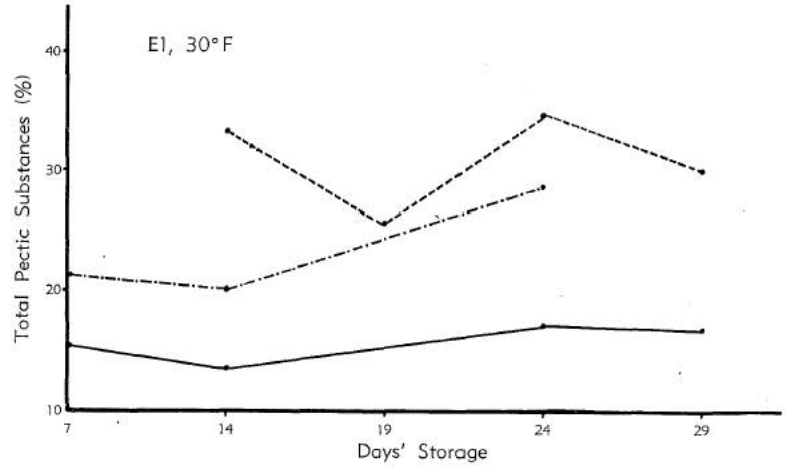

Fig. 6.—Total pectic substances in Elberta peaches ripened for 7 days at 70°F following storage at 30°F. Gassed with ethylene for 2 days prior to storage. Legend as for Fig. 3.

IV. DISCUSSION

Previous trials (unpublished data) carried out by the author have shown that the storage life of mature-green Elberta peaches grown in the Queensland Granite Belt is approximately 2 weeks at 32–34°F. Beyond 2 weeks' storage, the incidence of woolliness increased to 100 per cent. after 4 weeks' storage. Holding the harvested fruit at air temperatures for 2 days prior to storage decreased the incidence of woolliness to some extent and in fruit gassed with ethylene (1000 p.p.m.) for 2 days at 70°F prior to storage, the incidence of woolliness was negligible. Overstorage of this fruit resulted in a mushy breakdown of the flesh.

In the trials under discussion here, the condition of the fruit ripened after storage confirmed the observations made in previous trials. Unless otherwise stated, fruit referred to in the following discussion is fruit which has been ripened for 7 days at 70°F following storage. From the results, it is apparent that the storage life of mature-green Elberta peaches grown in the Queensland total pectic substances in the ripe fruit compared with the level in fruit which ripens normally following storage. In both seasons, fruit which was stored at 34°F contained the highest level of total pectic substances, irrespective of the storage time. Some degree of woolliness was evident in this fruit after 14 days' storage. With the exception of fruit gassed for 2 days prior to storage, the lowest level of total pectic substances for each treatment occurred after 14 days' storage, but rose as the storage time increased further. The highest level occurred in fruit stored for 29 days at 34°F; total pectic substances in this fruit was approximately the same level as in the green-mature fruit prior to storage and all fruits were in a woolly condition. The lowest level of total pectic substances after 29 days' storage occurred in both samples gassed with ethylene. The incidence of woolliness in these samples was negligible.

According to McCready and McComb (1954), the anhydrouronic acid contents of ripe peaches, pears and avocadoes were essentially the same as those of unripe fruits. Reference to Table 1, however, shows that the anhydrouronic acid (total pectic substances) content of peaches ripened at 70°F after storage for 2 weeks at 30°F, irrespective of the prestorage treatment, was approximately only half that of the green fruit. This finding is in agreement with the work of Ash and Reynolds (1954), who reported the presence of free galacturonic acid in fruit ripened at 20°C when removed from the tree but not in tree-ripened fruit. Two significant points emerged from their work. Firstly, a number of samples of pears and peaches ripened in a constant-temperature room at 20°C contained much higher concentrations of free galacturonic acid than any previously recorded. Secondly, where there was an appreciable variation in picking maturity, the amount of free galacturonic acid present after ripening was greater in fruit which was originally less mature and consequently required a longer time to ripen at 20°C. Ash and Reynolds postulated that the most probable mode of formation of free galacturonic acid in fruit would be by the enzymic degradation of pectin, which would require the presence of both pectin methylesterase (PME) and polygalacturonase (PG).

The increasing activity of PG as the methyl ester is progressively removed from pectins has been demonstrated by several authors (Colowick and Kaplan 1955). Glasziou, Sacher, and McCalla (1960), in studying the effect of auxins on membrane permeability, considered it possible that middle-lamella pectins are attacked by a combined PME-PG action and that PME activity may be a prerequisite for middle-lamella dissolution; one of the several types of PG which have been described acts very slowly or not at all on high-ester pectins. It appears then that the action of polygalacturonase must be preceded by the de-esterification of pectin by pectin methylesterase.

It is interesting to note that although the incidence of woolliness was negligible in fruit gassed for two days prior to storage, there was little or no loss of total pectic substances in this fruit prior to storage (see Table 2), although a considerable loss occurred during subsequent ripening after removal from storage, irrespective of the storage time. This suggests that during at least the first two days of the ethylene-induced ripening of the detached green-mature fruit, the pectic substances are de-esterified by pectin methylesterase allowing a subsequent breakdown to galacturonic acid by polygalacturonase during the ripening which occurs following storage.

From the foregoing discussion it appears that the normal ripening process in detached Elberta peaches involves the breakdown of part of the pectic substances to free galacturonic acid. The 1962 results show a lower level of total pectic substances in fruit ripened after 14 days' storage than after 7 days' storage. A possible explanation is that as the ripening period at 70°F was a constant 7 days in each case, fruit ripened after 14 days' storage may have been in a more advanced stage of ripeness than fruit ripened after 7 days' storage. (Unpublished data of the author have shown that the number of days taken to reach the climacteric in pears ripened at 70°F following storage decreases with increasing storage time). With the exception of fruit gassed for two days prior to storage, the rise in total pectic substances with further storage suggests that one aspect of overstorage in peaches is a partial to complete failure of the mechanism, probably enzymatic, whereby the pectic substances are catabolized to galacturonic acid.

In the 1961 season, soluble pectin in fruit stored immediately at 30°F and 34°F decreased, with increasing storage time, as the total pectin increased. The lowest level of soluble pectin occurred in fruit stored at 34°F for 19 days, the level remaining constant with further storage. Approximately the same level occurred in fruit stored at 30°F after 24 days' storage. The soluble pectin levels also fell in fruit gassed with ethylene prior to storage but this fall paralleled the fall in total pectin which occurred after 14 days' storage. As in the 1962 season, fruit stored for 24 days may have been in a more advanced stage of ripeness than fruit stored for 19 days when both samples were held for 7 days at 70°F. A fall in total pectin resulting from a more complete breakdown to alcohol-soluble constituents could also result in a lower level of soluble pectin in the riper fruit.

Glasziou (1957*a*), in studying the effect of indole acetic acid (IAA) on the binding of pectin methylesterase (PME) to the cell walls, considered it possible that the binding of PME to the cell wall would lower the activity of the enzyme in the cell, decreasing the rate of hydrolysis of pectins. Glasziou (1957*b*), when carrying out similar studies on the tubers of the Jerusalem artichoke, found a very low non-specific adsorption of PME to cell-wall preparations from freshly dug tubers but similar preparations from the same batch of artichokes showed high non-specific adsorption after storing in damp sand in a cold room at 5°C for 1 week.

If an increased degree of adsorption of PME to cell walls is general for any cool-stored tissue, then one aspect of the abnormal ripening of overstored Elberta peaches may be an irreversible binding, beyond a certain critical storage time, of PME to the cell walls, the degree of binding increasing with storage time. The increasing level of total pectic substances with increasing storage time beyond 14 days in fruit stored immediately at 30°F and 34°F supports this theory. The theory is tenable only if PME is inactive in the bound state. Jansen, Jang, and Bonner (1960), in studying orange pectinesterase (PE) binding and activity, considered that their results clearly indicated that cell walls firmly bind PE and that, in the bound state, the enzyme is inactive at the pH of orange juice on the *in situ* pectic material.

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CORRECTIONS

Vol. 21, page 56: Line 5 should read "that the development of woolliness is associated with an increase in the level of"

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