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PECTIC CHANGES OCCURRING IN ELBERTA PEACHES SUFFERING FROM WOOLLY BREAKDOWN*

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Abstract—The processes which take place in peaches stored under conditions that favour development of woolly breakdown, were found to be connected with the metabolism of the pectic substances. In fruit held under conditions that ensure its normal ripening (above 8°), a gradual degradation of protopectin to soluble pectin occurs and the amount of pectates remains constant. However, in fruit that is held under conditions which favor woolly breakdown (below 8°), the insoluble pectic fractions begin to increase after approximately 2 weeks' storage. As a result, the total amount of pectic substances appears to increase. This change evidently occurs partially due to the action of pectin-methyl-esterase. During normal ripening the activity of this enzyme increases until the fruit attains full ripeness and then begins to decrease. In fruit with incipient woolliness, enzyme activity is initially low, and only begins to rise during the second stage of storage. Thus, an insoluble low-methoxyl pectin of high molecular weight is formed in the cell walls, which is capable of 'holding' water in a gel-like structure. It is suggested that this could be the reason for loss of juiciness in woolly peaches.

INTRODUCTION

WOOLLY breakdown of peaches is a physiological storage disorder occurring at temperatures below 7·2°.[1] The occurrence of the breakdown at low temperatures can be postponed by holding the fruit above 20° for at least 24 hr after harvest and prior to cold storage.[2] De Haan[3] associated this breakdown with the metabolism of the pectic substances and suggested that when the ratio of soluble to non-soluble pectin reached 2:1, woolly breakdown did not occur, this being the effect obtained by delayed storage. Support for this view was obtained by Reyneke,[4] who found that ripe juicy fruit was less susceptible to woolliness. However, another study regarding the effect of fruit maturity at harvest on the subsequent occurrence of woolly breakdown showed that the riper the fruit the more susceptible it was to breakdown.[5] It was therefore concluded that the changes occurring in fruit during delayed storage differ from those occurring in fruit ripening on the tree.

In their study of the pectic constituents of peaches, Appleman and Conrad[6] found that the protopectin in fruit left to ripen on the tree or in fruit held in cold storage did not solubilize as it did in fruit that ripened normally at 25° after harvest. However, in contrast to their findings that the total amount of pectic substances in the fruit was fairly constant irrespective of the ripening treatment, Watkins[7] reported a considerable loss of total pectic substances in normally ripened fruit, and associated abnormal ripening of overstored fruit

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[1] J. F. VAN DER PLANK and R. DAVIES, *J. Pomol.* **15**, 226 (1937).
[2] W. W. BOYES, *IX Int. Cong. Froid, Paris.* **4**, 533 (1955).
[3] I. DE HAAN, *S. African Ind. Chem.* **2**, 26 (1957).
[4] J. REYNEKE, *Am. J. Botany* **46**, 645 (1959).
[5] S. GUELFAT-REICH and R. BEN-ARIE, *Israel J. Agric. Res.* **16**, 163 (1966).
[6] C. O. APPLEMAN and C. M. CONRAD, *Univ. Md. Agri. Exp. Sta. Bull. No. 283* (1926).
[7] J. B. WATKINS, *Qd. J. Agric. Sci.* **21**, 47 (1964).

with an increase in total pectic substances. He postulated that this abnormal ripening was related to a possible inactivation of the enzyme pectin-methyl esterase (PME), which prevented subsequent pectin degradation by polygalacturonase (PG) to free galacturonic acid, found in increasing quantitites during post-harvest ripening of peaches at 20°.[8] However, the activity of these two enzymes has not been studied in relation to the quality of peach ripening. The present experiment was planned to investigate the metabolism of the pectic substances in harvested peaches stored under conditions which induce normal ripening or woolly breakdown.

RESULTS

The loss of juiciness accompanying woolly breakdown of stored peaches was found to be unconnected with the loss of water vapor from fruit occurring during storage but was correlated with the amount of expressible juice (Fig. 1). The latter in normally ripened fruit (stored above 8°) continually increased during storage for 3 weeks, whereas in

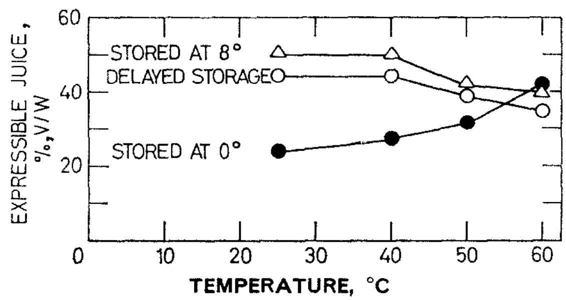

FIG. 1. THE EFFECT OF STORAGE CONDITIONS ON THE AMOUNT OF EXPRESSIBLE JUICE IN STORED ELBERTA PEACHES.

fruit stored below 8° it began to decrease with the onset of woolly breakdown, about two weeks after harvest. The amount of expressible juice from fruit from delayed storage treatment which postponed the onset of woolliness to the third week of storage at 0°, also began to fall just before signs of breakdown became apparent.

It can be inferred from the effect of temperature on the release of expressible juice, that the fruit juice was retained in the woolly fruit in a gel-like structure, probably by the pectic substances. The amount of expressible juice from woolly fruit increased when the fruit was heated to above 40° (Fig. 2). The same treatment applied to non-woolly fruit had the opposite effect, probably due to the mushy structure that the fruit pulp acquired at higher temperatures.

Examination of the composition of the pectic substances and their characterization substantiated the above theory. The total amount of pectic substances in normally ripened fruit gradually declined during storage (Figs. 3a and 4a). However, in woolly fruit there was generally a rise in the pectic content, beginning with the second week of storage (Fig. 3a). Even when there was no actual rise throughout the storage period (Fig. 4a), the pectic content of woolly fruit was noticeably higher than that of non-woolly fruit. Fractionation

[8] A. S. F. ASH and T. M. REYNOLDS, *Australian J. Biol. Sci.* 7, 435 (1954).

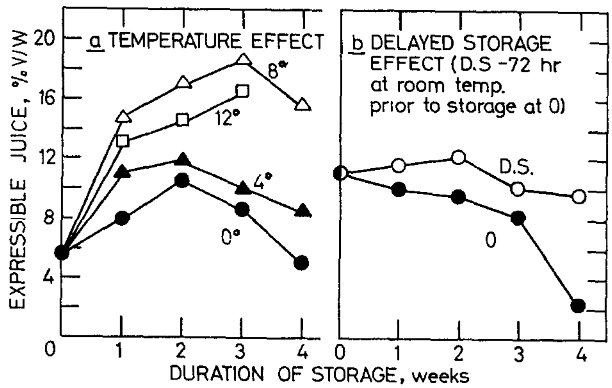

FIG. 2. THE EFFECT OF STORAGE TEMPERATURE ON THE AMOUNT OF EXPRESSIBLE JUICE IN ELBERTA PEACHES STORED FOR 3 WEEKS UNDER VARIOUS CONDITIONS.

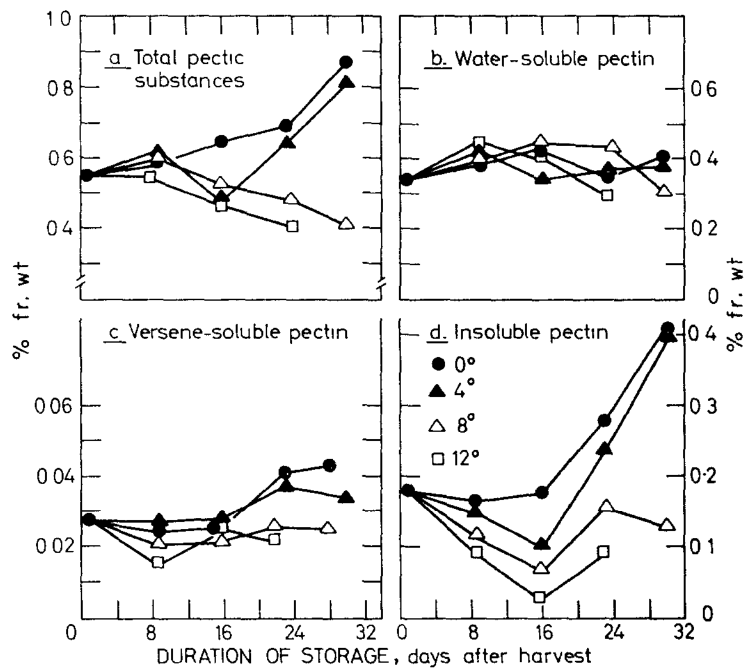

FIG. 3. THE EFFECT OF STORAGE TEMPERATURE ON THE CHANGES OCCURRING IN THE TOTAL PECTIC SUBSTANCES AND PECTIC FRACTIONS OF ELBERTA PEACHES.

of the pectic substances showed this difference to be due to quantitative changes in the water-insoluble fractions. The changes in the water-soluble fraction during storage were similar in both woolly and non-woolly fruits (Figs. 3b and 4b). As was to be expected, the rise in water-soluble pectin in fruit held at room temperature for 72 hr prior to cold storage was more rapid than in fruit stored immediately at 0°. However, during storage the difference became negligible.

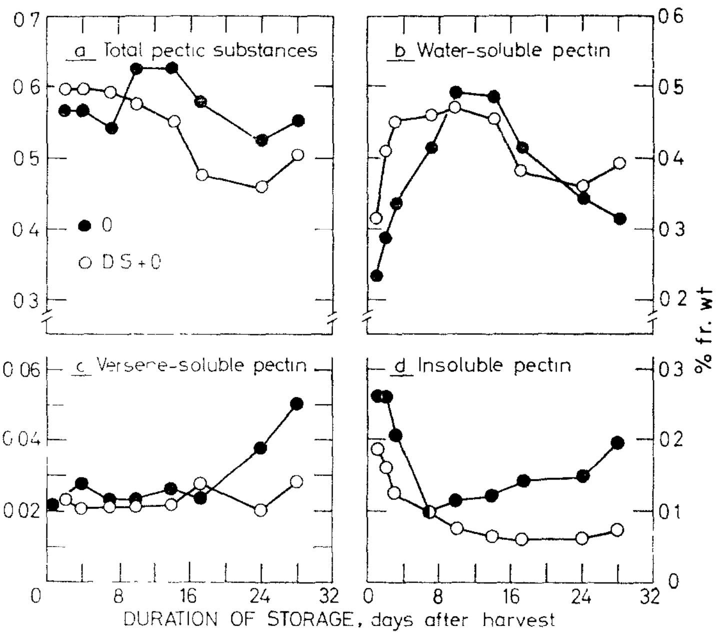

FIG. 4. THE EFFECT OF DELAYED STORAGE ON THE CHANGES OCCURRING IN THE TOTAL PECTIC SUBSTANCES AND PECTIC FRACTIONS OF ELBERTA PEACHES.

The percentage of versene-soluble pectin was fairly constant in the non-woolly fruit throughout the storage period (Figs. 3c and 4c), whereas a sharp rise in its content began to occur during the third week of storage in fruit suffering from woolly breakdown. At the same time an even more considerable rise was discerned in the versene-insoluble fraction extracted from the woolly fruit, as compared to a continuous decline in the same fraction from normally ripened fruit (Figs. 3d and 4d).

As the above changes occur almost simultaneously with the appearance of the initial symptoms of woolly breakdown, previous changes in certain characteristics of the pectic substances were thought to take place, bringing about differential solubility of the pectic components. It was found that whereas the amount of free carboxylic groups of the pectic substances from healthy fruit gradually increased during storage on account of the immediate decrease in the amount of saponifiable ester groups, characterization of the pectic substances from woolly fruit showed a more gradual decrease in the latter resulting in a delayed increase of the former. As there were no noticeable changes or differences in the acetyl content of the pectic substances from either type of fruit, the degree of esterification in the healthy fruit decreased throughout the storage period, whereas in the woolly fruit it began to decrease noticeably only after 2 weeks' storage (Fig. 5).

These changes indicate a difference in activity of the enzyme pectin-methyl-esterase (PME) during the first 2 weeks' storage between fruit that will ripen normally and fruit that will suffer from woolly breakdown. In normally ripened fruit the PME activity gradually rose to a peak during the 10–12 days' storage and later fell quite sharply, especially in fruit stored at 8° (Fig. 6). Temperatures below 8° were found to inhibit PME activity in fruit that was stored immediately after harvest during 12–15 days' storage. However, after this

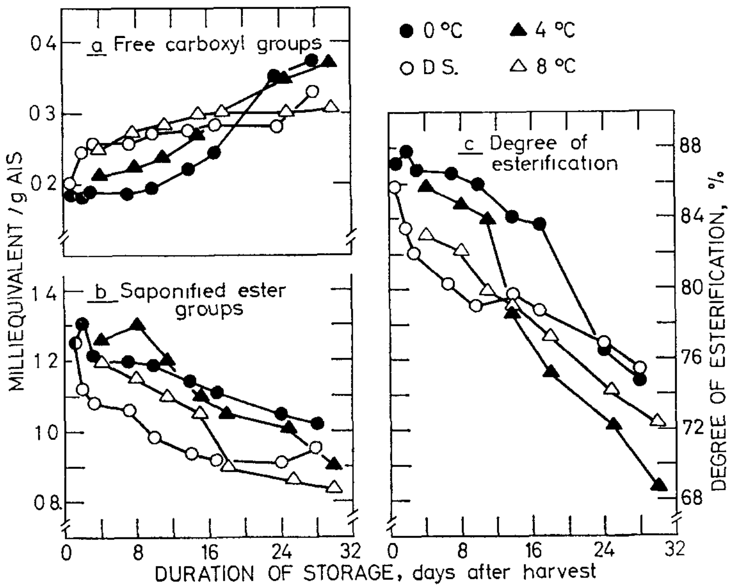

FIG. 5. CHARACTERIZATION OF THE PECTIC SUBSTANCES EXTRACTED FROM STORED ELBERTA PEACHES.

there occurred a very marked rise in the PME activity in these fruits, which increased with the increasing incidence of woolliness.

Even though no polygalacturonase (PG) activity was found in any of our experiments, other studies[8] indicated that pectic substances in ripening peaches are degraded to soluble galacturonic acid, which increases with the progress of maturation. A semi-quantitative evaluation of the amount of free galacturonic acid occurring in healthy and woolly peaches throughout the storage period showed that though it gradually increased in fruit stored immediately at 0° during the first 2 weeks, in the latter 2 weeks its amount somewhat decreased as woolliness became more marked (Fig. 7). However, in fruit stored at 8° the galacturonic acid content increased throughout the storage period and in fruit stored at 0° after 72 hr exposure at room temperature, it remained constant after the initial marked rise during the delay period.

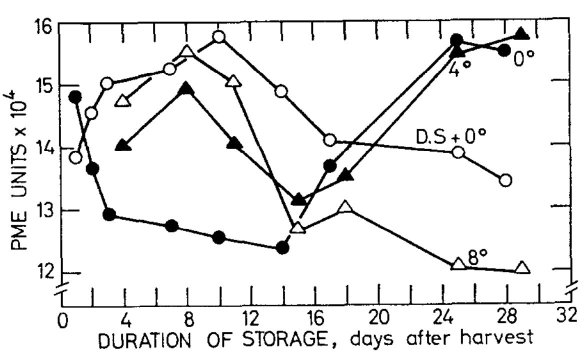

FIG. 6. PECTIN-METHYL-ESTERASE (PME) ACTIVITY IN ELBECHERTA PEAS STORED UNDER DIFFERENT CONDITIONS.

DISCUSSION

The normal post-harvest metabolism of pectic substances in ripening fruit is the degradation of insoluble protopectin to soluble pectin,[9] and there is considerable evidence that the rate of this process is slowed down by low temperatures.[6,10,11] This temperature effect was barely noticeable in this study, even during the first 10–15 days' storage, when the process followed its normal course, probably because the temperature range examined was rather narrow. The total amount of pectic substances during this period remained fairly constant, and no quantitative changes in the pectates of the middle lamella (the versene-soluble fraction) could be discerned.

The differences in the metabolism of the pectic substances between healthy and woolly fruit began to appear after 2 weeks' storage. Whereas the total amount of pectic substances decreased in healthy fruit, due to a degradation of the soluble pectin in addition to a continued decrease in protopectin, in woolly fruit they began to rise as a result of an increased quantity of the water-insoluble fractions (protopectin and pectates), and in spite of a continued decrease in water-soluble pectin. The loss of fruit juiciness was parallel to the above changes and it appears that it could be explained as a binding of the water (which is usually released during fruit ripening) by the pectic substances, in a gel-like structure.

Calculation of the ratio between the additional amount of insoluble pectin formed during the latter 2 weeks' storage and the loss of expressible juice during the same period shows it to be 2–3:100. This ratio is reasonable for the formation of a pectinic gel.[12] Though most of the pectin in question is water insoluble, Joseph[13] states that destabilized pectin forms a gel "as a result of an unsuccessful attempt to precipitate". In Hinton's[14] opinion, gel formation occurs as the result of precipitation of a part of the pectin from the solution. The large binding surface-forces then developed hold the solution of other incredients, and the remaining soluble pectin, with sufficient power to confer on the whole system the rigidity and normal physical characteristics associated with a jelly.

Nearly all studies of pectinic gels have been conducted *in vitro*, with all the components known and defined. However, some of the conclusions drawn can be used to substantiate the above theory. Apart from pectin concentration, the formation of a pectin gel depends on molecular weight (i.e. chain length), degree of esterification, pH and temperature.[15,16] Though the effect of molecular weight on gelation is clear,[15,17] the minimum degree of polymerization required for gel formation is difficult to determine, because of the heterogenicity of the pectic substances. In this case the molecular weight was not determined but viscosity measurements (data not given), the increased insolubility of the pectin, and the decrease in free galacturonic acid, suggest that the chain length of the pectic substances in woolly fruit was considerably greater than in healthy fruit. The strength of pectic gels has been shown to increase with the decrease in the degree of esterification of the pectin, especially in the range of 70–80 per cent esterification.[18] The final degree of esterification found for the pectic substances in woolly fruit was within this range. The effect of pH on

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[16] J. SOLMS, *Adv. Chem. Ser.* **25**, 37 (1960).
[17] H. DEUEL and J. SOLMS, *Adv. Chem. Ser.* **11**, 62 (1954).
[18] E. L. PIPPEN, T. H. SCHULTZ and H. S. OWENS, *J. Colloid Sci.* **8**, 97 (1953).

pectic gel formation depends on a number of other factors (pectin concentration, temperature, salt and acid concentration), but the optimum for gel formation is usually within the range of 2·8–3·4.[9] This is lower than that measured in the juice and blended pulp of peaches (approximately 4·0), but Pilnik[19] is of the opinion that this should not be a limiting factor to gel formation in the cell wall. Hinton[20] also found that as pH values rise, the temperature for successful gel formation *in vitro* decreases.

The stability of pectic gels does not alter within the temperature range of 0°–50°.[12] Therefore, the inference to be drawn from the increase in the amount of expressible juice from woolly peaches held at temperatures above 50°, is that the water was held in the fruit, in a gel-like structure, by the pectic substances.

The special structure of the pectic substances within the woolly fruit which enabled gel formation, appears to be the result of the effect of low temperatures on PME activity. The initial inhibition of the enzyme was followed by a markedly increased activity which upset the mechanism of pectin degradation to free galacturonic acid and gave rise to long, low-methoxyl chains of pectic acid. The PME activity in the peach is much lower than in many other fruits,[21–24] and the decrease in pectin esterification is therefore much less in the normally ripened peach than, for example, in ripened pears and avocados.[25] In the latter fruits noticeable PG activity has been found.[25] As this enzyme acts only on low-methoxyl pectin, the relatively high esterification of normally ripened peach pectin is probably also evidence of PG absence from peaches. Likewise, it does not seem likely that depolymerase or α-transeliminase is active in pectin degradation, as the final product appears to be free galacturonic acid. It may therefore be quite as Kertesz[9] states, that the final stages of pectin degradation in the peach are not enzymatic.

EXPERIMENTAL

Elberta peaches, harvested hard-ripe or ripe, as defined previously,[5] were either stored on the same day at 0°, 4°, 8° and 12° or held for 72 hr at ambient room temp. (20–26°), prior to storage at 0°. The following tests were conducted during 1 month's storage every 3–4 days upon removal from storage, or weekly after an additional 2 days' shelf-life.

The progress of woolliness was expressed as the amount of juice expressed from fruit placed under constant pressure, with the aid of a succulometer. In one trial, before expressing the juice, the fruit was brought to a constant temperature by enclosing it in a hermetically closed container and immersing the container in a thermostatted water bath for up to 1 hr. Fruit marc, for determination of pectic substances, was prepared as follows: 250 g fruit pulp from four randomly chosen fruit was blended with 500 ml boiling aceton for 5 min and boiled for an additional 20 min. After filtering on a Buchner funnel, the precipitate was re-extracted three times with 70% ethanol and twice with acetone. The eluates were discarded and the colorless powder obtained in the Buchner was weighed and ground in a Wiley mill to pass through a 40-mesh screen. Total pectic substances and the pectic fractions were extracted according to the methods described by McComb and McCready[26] and Postlmayr *et al.*,[27] respectively, and were determined by a modification of the colorimetric carbazole method.[21]

Characterization of the pectic substances was carried out according to the methods described by McCready and McComb with marc previously washed with 1N HCl, to free the non-esterified, bound carboxylic groups.

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[20] C. L. Hinton, *J. Sci. Food Agri.* **1**, 300 (1950).
[21] A. H. Rouse, C. D. Atkins and E. L. Moore, *J. Food Sci.* **29**, 34 (1964).
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Free galacturonic acid was extracted with 80% ethanol[8] and separated by paper chromatography.[28] It was identified and a semi-quantitative evaluation made by comparison with known quantities (5–10 μg) of anhydro-galacturonic acid.

PME activity was determined by titrating the carboxylic groups released from a pectin substrate by the enzyme extract.[22] The optimal conditions for extraction were: 1 M NaCl at pH 8·0 and 25° for 1 hr. The optimal conditions for activity determination were: 1% pectin substrate in 0·2 M NaCl at pH 7·5 and 30°. (Optimum enzyme activity was obtained at 45°, but 30° was chosen for convenience.)

[28] R. M. McCREADY and E. A. McCOMB, *Agri. Food. Chem.* **1**, 1165 (1953).