Phytochemistry, 1980, Vol. 19, pp. 2553–2555. Pergamon Press Ltd. Printed in England. 0031-9422/80/1201-2553 $02.00/0

PECTOLYTIC ENZYME ACTIVITY INVOLVED IN WOOLLY BREAKDOWN OF STORED PEACHES*

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(*Revised received* 2 *April* 1980)

Key Word Index—*Prunus persica*; Rosaceae; pectin-methylesterase; polygalacturonase; chilling injury.

Abstract—The development of woolly breakdown in peaches stored at 0° was accompanied by increased activity of pectinesterase (PE) and inhibition of polygalacturonase (PG) activity. With intermittent warming of the fruit, which delayed the development of woolly breakdown, PG activity increased to levels measured in normally ripened fruit. It is proposed that the development of woolly breakdown in cold-stored peaches derives from an imbalance of pectolytic activity, whereby low temperatures induce PE to cause the accumulation of de-esterified pectate (soluble in EDTA) and inhibit PG from degrading this substrate.

INTRODUCTION

It has been shown that woolly breakdown of peach flesh, occurring during cold storage, is related to the reduced ability of the fruit to convert insoluble pectic substances to soluble pectin and to the accumulation of EDTA-soluble pectin (de-esterified pectate) when the fruit is stored at temperatures below 8° [1]. It was also found that just prior to the appearance of disease symptoms, there was a rise in the activity of pectinesterase (EC 3.1.1.11) (PE), whereas in healthy fruit, stored at 8°, there was a steady decline in PE activity throughout the storage period. Although no PG activity was detected in the fruit, the gradual increase in free galacturonic acid in healthy fruit could be interpreted as the result of increasing pectolytic activity. On the other hand, the trace quantities of free galacturonic acid in the disordered fruit suggested that storage at 0° had inhibited pectolytic activity. Pressey *et al.* [2] have since detected increasing activity of PG in ripening peaches and have demonstrated [3] the presence of both an endo-PG (EC 3.2.1.15) and an exo-PG (EC 3.2.1.67).

The purpose of this work was to verify the hypothesis that storage of peaches, under conditions which induce the development of woolly breakdown, alter the natural metabolism of pectic substances by enhancing PE activity and inhibiting PG activity.

RESULTS

Intermittent warming of peaches at the critical stage, just prior to the onset of woolly breakdown symptoms, has been shown to prevent disease development [4, 5]. Pectolytic enzyme activity was therefore assayed in peaches stored under the following conditions: treatment A, continuous storage at 0°; treatment B, 10 days' storage at 0°, 24 hr at 25° followed by restorage at 0°; and treatment C, 10 days' storage at 0°, 24 hr at 25°, 10 days' storage at 0°, 24 hr at 25°, followed by restorage at 0°.

After 2 weeks' storage, there were no disease symptoms in fruit flesh in any treatment, but after an additional 3 days at 20°, woolly breakdown became apparent in fruit that had been stored continuously at 0° (treatment A—Fig. 1a). PE activity upon removal from storage was 50% higher in

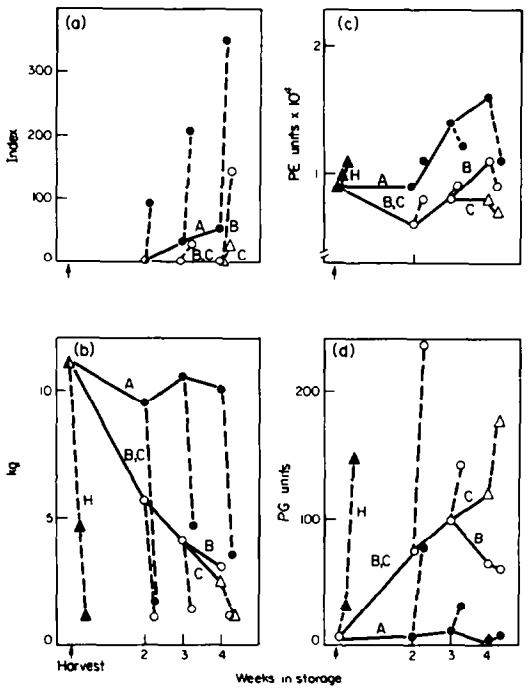

Fig. 1. Woolly breakdown development in stored Somerset peaches (a), as related to changes in firmness (b), PE activity (c), PG activity (d), during storage at 0° (——) and subsequent shelf-life at 20° (– – –). H = Fruit ripened at 20° after harvest (▲), A = continuous storage at 0° (●), B = storage at 0° with 24 hr intermittent warming at 25° after 10 days (○), C = Storage at 0° with two periods of 24 hr intermittent warming at 25° after 10 and 20 days (△).

* Contribution from the Agricultural Research Organization, The Volcani Centre, Bet Dagan, Israel, 1979. Series No. 298-E.

treatment A than in treatment B (Fig. 1c), and PG activity was more than 3 times greater in treatment B than in treatment A (Fig. 1d). As the extent of woolly breakdown increased in treatment A during further cold storage, PG activity remained constantly low and there was no additional fruit softening (Fig. 1b), but PE activity increased. In contrast, in healthy fruit from treatment B, there was an additional increase in PG activity accompanied by a decline in fruit firmness and, although PE activity also rose, it still remained much lower than in treatment A (Fig. 1c).

During 3 days' ripening at 20°, disease symptoms developed rapidly in treatment A. The differences in firmness, PG and PE activities between treatments A and B were maintained up to 3 weeks' storage. After 4 weeks' storage, symptoms of disorder began to appear in fruit from treatment B when held for 3 days at 20°. PG activity in this fruit also declined, but PE activity was relatively high. However, fruit that had received two periods of intermittent warming (treatment C) was still virtually free of woolly breakdown at this time: PE activity was lower than at harvest and PG activity was even higher than in fruit ripening naturally at 25° after harvest.

DISCUSSION

The necessity of adequate PG activity for the normal ripening and softening of fruits has been well demonstrated by its absence in the non-ripening *rin* tomato mutant [6]. On the other hand, the direct role of PE in fruit softening and pectin solubilization is not fully understood. In a recent review, Pressey [7] suggested that increased PE activity alone would in fact lead to decreased solubility of the pectin, due to the increase in free carboxyl groups and greater interaction with Ca^{2+}. However, it has generally been shown that PGs in higher plants require de-esterified pectate as their substrate [8–11], and therefore the action of PE is considered a prerequisite for PG activity [7]. For this reason, Watkins [12] postulates that the changes in pectin metabolism involved in the development of woolly breakdown in peaches derived from the inactivation of PE at low temperatures and the subsequent inability of PG to degrade the esterified pectin. It was, however, later shown [1], and is herein shown once again, that PE activity was not inhibited by low temperatures, and was in fact even somewhat higher under conditions that induced disease symptoms. A low temperature-induced increase in PE activity has also been reported in avocado fruits [13].

Pressey *et al.* [2] showed that PG activity, undetectable in immature peaches, became measurable as the fruit began to soften and thereafter increased sharply as the fruit ripened. Our data substantiate this finding, but show that the apparent correlation between fruit softening and PG activity, which was apparent during natural ripening at 25° after harvest, was masked by the onset of woolly breakdown due to cold storage at 0°. PG activity was in fact irreversibly inhibited by 3 weeks' storage at 0°, and could not be correlated with fruit softening during subsequent shelf-life. It is postulated that at temperatures which induce the development of woolly breakdown, increased PE activity is the initial cause of the accumulation of an EDTA-soluble de-esterified pectate [1], which cannot be further degraded due to the inhibiting effect of low temperatures on PG activity. Transfer of the fruit to higher temperatures within the critical period before PG has been irreversibly inactivated (10 days for the Somerset cv) enables a resumption of enzyme activity, resulting in the dissolution of the accumulated substrate, thereby preventing the undesirable change in fruit pulp texture.

EXPERIMENTAL

Plant material. Peach fruits (*Prunus persica* cv Somerset) of uniform size were harvested mature but not fully ripe. The fruit was stored at 0° either continuously (treatment A) or with one or two periods of intermittent warming every 10 days (treatments B and C, respectively). Upon removal from storage after 2, 3 and 4 weeks, and after an additional 3 days' ripening at 20°, the fruit was examined as follows. *Firmness* was determined with a Hunter penetrometer equipped with a 11-mm tip on two pared cheeks of each fruit. *Woolly breakdown* was determined visually in the halved fruit and graded as slight (< 25% of surface), moderate (25–50%) or severe (> 50%). The woolly breakdown index was calculated as follows: W.B. index = (% fruit with slight breakdown × 1) + (% of fruit with moderate breakdown × 2) + (% of fruit with severe breakdown × 4).

Enzyme extractions and assays. PE was extracted and assayed as described previously [1]. Units were expressed in milliequivalents of ester hydrolysed per hr per g of fr. wt. The extraction and assay of PG were based on the method described in ref. [2]. Fruit flesh (50 g) was homogenized with 50 ml cold 12% polyethylene-glycol and 0.2% sodium bisulfite. The homogenate was centrifuged for 10 min at 8000 *g* and the residue was washed twice with cold distilled water. The final residue was extracted with 0.1 M citrate–phosphate buffer, pH 4.0, and 0.5 M NaCl during 15 min at room temp. The supernatant obtained after 10 min at 8000 *g* was used for enzyme assay, by incubating it with 0.5% polygalacturonic acid at pH 4.0 for 20 hr in final vol. of 2 ml. Reducing sugars released were determined with the Sumner reagent [14]. A unit of activity was defined as 1 mmol of galacturonic acid released by 1 mg protein during 20 hr. Boiled extractions were assayed as controls for each determination of both enzymes. Protein was determined by the method of Bradford [15].

Replicates: Firmness determination and woolly breakdown evaluations were made on 3 replicates of 10 fruits. After the fruit had been halved for breakdown evaluation, median slices were cut from each fruit for enzyme assays. The experiment was conducted during two seasons with similar results. The data from one season only are presented.

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