PERGAMON

Phytochemistry 55 (2000) 191–195

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Pectin esterase, polygalacturonase and gel formation in peach pectin fractions

Hong-Wei Zhou, Ruth Ben-Arie, Susan Lurie *

Department of Postharvest Science, Agricultural Research Organization, The Volcani Center, Bet-Dagan, POB 50250, Israel

Received 19 January 2000; received in revised form 10 May 2000

Abstract

Peaches (*Prunus persica* cv. Hermoza) were stored at 0C in regular air (RA) or in controlled atmosphere (CA 10% CO_2, 3% O_2) for 4 weeks and then ripened for 4 days at 20°C. Woolliness developed in the regular air stored fruit while the controlled atmosphere stored fruit ripened normally. In the woolly fruit symptoms of the disorder were greater in the inner mesocarp than in the outer. Polygalacturonase (PG) and pectin esterase (PE) activities differed in the outer and inner mesocarp of the affected fruit. PG activity was low and PE activity was high in the inner mesocarp of the woolly fruit during ripening relative to the outer mesocarp, while in the healthy fruit, activities were similar in both areas. Cell wall fractions of water-soluble, CDTA-soluble and carbonate-soluble pectins were prepared from freshly harvested peaches and incubated with PE and PG from ripe peaches at different ratios. Only the CDTA-soluble fraction formed a gel with peach enzymes, and the rate of gelation increased with increasing amounts of PE relative to PG. Both water-soluble and CDTA-soluble pectin fractions formed gels with commercial PE (extracted from orange peel). The PE extracted from peaches was stable when stored at 0°C for 9 days, while PG activity was stable only for 1 day. We suggest that PE, acting on pectins in the cell wall in vivo may cause gel formation and that the CDTA-soluble polymers have the capacity to bind apoplastic water and create the dry appearance observed in woolly fruit.

Keywords: *Prunus persica*; Peach; Storage; Pectins; Chilling injury; Woolliness

1. Introduction

Woolliness is a physiological disorder of peaches and nectarines whereby they fail to ripen normally after prolonged periods of cold storage, resulting in a lack of juice and a dry, woolly texture. This undesirable texture of the flesh due to chilling injury has been associated with an imbalance in cell wall metabolism. Woolliness has been attributed to impaired solubilization of pectic substances with accumulation of insoluble low methoxyl pectin of high Mr (Ben-Arie and Lavee, 1971). Attempts to understand the development of woolliness has focused on studying the activities of polygalacturonase (PG) and pectin esterase (PE). During normal ripening, PG activity increases and PE activity decreases along with an increase in water soluble pectin (Ben-Arie and Lavee, 1971; Pressey and Avants, 1973; Buescher and Furmanski, 1978). Ben-Arie and Sonego (1980) found an increase in PE activity and inhibition of PG in cold stored peach fruit relative to their activities in normally ripening fruit. This has recently been confirmed in woolly nectarines (Zhou et al., 2000). This imbalance in activity causes the pectins to undergo less degradation (Dawson et al., 1992; Lurie et al., 1994; Zhou et al., 1999), and more de-esterification (Lurie et al., 1994) in woolly fruit than in normally ripening fruit.

The dry woolly texture of peaches can be measured as a lack of expressible juice (Lill and van Der Mespel, 1988). However, the water content of the woolly fruit is the same as that of healthy, juicy fruit (Zhou et al., 2000), and more expressible juice can be obtained by mild heating of the woolly fruit tissue (Ben-Arie and Lavee, 1971). Therefore, it has been hypothesized that the accumulation of high Mr pectin with a low degree of esterification may enable the binding of extracellular water into a gel-like form, causing the apparent lack of juice. However, gelation by specific pectins from peaches or nectarines has never been demonstrated.

In peaches, woolliness is more severe in the inner than in the outer mesocarp, similarly to the appearance of gel

* Corresponding author Tel.: +972-3-9683606; fax: +972-3-9683622.

E-mail address: zeslov@netvision.net.il (S. Lurie).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00271-5

breakdown in plums (Taylor et al., 1994). In the latter case PG activity was found to be lower in the affected area. Therefore, it is possible to compare enzyme activities within the same fruit and relate the activities to the severity of the disorder.

In this study we have taken two approaches to investigate gel formation as a consequence of woolliness and its relationship to PE and PG activity. In the first, pectin fractions of harvested peaches were incubated with PE and PG extracted from ripe peaches, at different ratios, or with commercial enzymes. In the second approach the enzyme activity of inner and outer mesocarp of woolly and healthy fruit was compared.

2. Results

After storage for 4 weeks, fruit removed for ripening at 20°C from both storage regimes appeared healthy. However, after 4 days of ripening, all the fruits from RA were woolly, while only 5% of the CA stored fruits exhibited signs of woolliness. In the fruits from RA, woolliness often involved visible gel-like areas in the inner mesocarp, while the area near the peel appeared dry. Expressible juice reflected this dry appearance being 15% of tissue weight in RA fruits, and 40% from CA fruit.

At removal from storage, PG and PE in the inner and outer mesocarp were similar in RA fruit (Fig. 1). In CA stored fruit PE activity was similar in both areas of the flesh, but PG activity was higher in the inner mesocarp than in the outer. During ripening, PE activity increased in RA stored fruit along with woolliness development, and was higher in the inner than in the outer mesocarp, whereas it decreased equally in both areas of the CA fruit. In RA stored fruit during ripening, PG activity did not increase but the difference between the two areas increased, while CA stored fruit showed enhanced PG activity in both flesh areas and the difference between the two areas diminished. Due to increased PE activity and no change in PG in RA stored fruit during ripening, the ratio of PE to PG was higher in ripened fruit after RA where woolliness developed than in ripened fruit following CA, and particularly high in the inner mesocarp which was the most affected tissue.

When the water-, CDTA- and carbonate-soluble pectins were incubated with PG and PE at different ratios only the CDTA-soluble fraction formed a gel (Table 1). When incubated with PE alone a gel was formed within 2 days, and as PE was mixed with PG in increasing amounts the time for gel formation increased. With PG alone it required 6 days for the CDTA fraction to form a gel. However, when the enzyme fractions were compared for activity (Table 1) some low PE activity was found in PG enzyme preparation. To determine if this residual PE activity was responsible for gel formation,

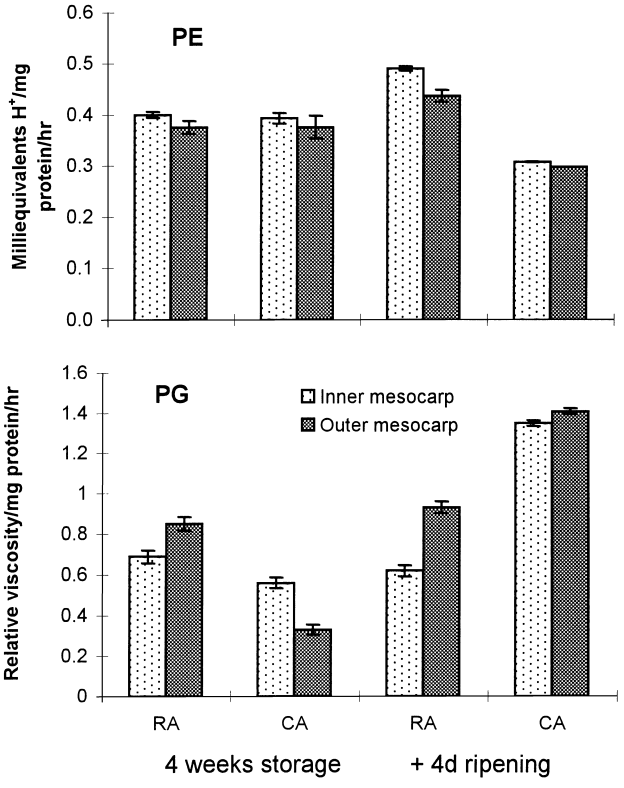

Fig. 1. Activity of PE and PG in the inner and outer mesocarp of Hermoza peaches after either RA or CA storage and 4 days of ripening at 20°C.

the pectin fractions were incubated with commercial enzymes which did not have any contaminating activity (Table 2). Among these three fractions, water-soluble and CDTA-soluble fractions formed a gel with PE in 4 d (Table 2) while PG alone did not induce gelation and no PE activity was detectable in PG. The carbonate fraction did not form a gel under any of the observed conditions. After gelation, the pH of gel was determinated and found to be 6.5 with peach enzymes and 6.3 with commercial enzymes. These values are those of the physiological environment in tissue (Ugalde et al., 1988).

The stability of PG and PE extracts held at 0°C was measured daily (Fig. 2). Results showed that PE activity remained high in vitro at the normal fruit storage temperature, while PG activity began to decrease after 1 day in storage.

Calculations indicate that if the CDTA-soluble polymers in the cell wall are involved in juice binding, then they are in a sufficient concentration to explain the lack of juice in woolly fruit. The pectins of the CDTA-soluble fraction could bind 10 times their dry weight as seen in Table 1. From 5.5 g flesh tissue used for extracting cell wall, 0.22 g dry CDTA material was obtained. A woolly fruit weighing 100 g would have enough CDTA-soluble polymers to hold 40 ml of juice. This is enough to bind the extracellular juice and create a dry, woolly fruit.

Table 1
Gelation of CDTA-soluble pectin extracted from Hermoza peaches, resuspended in water, and incubated with PG and PE extracts from ripe peach fruit[a]

Gelation mixture:	Final enzyme activities		Setting time (days)	pH
	Endo-PG[b] (units × 1000)	PE		
CDTA	0	0	00	
CDTA + PG	15.104	0.509	6	6.5
+ PE	0	2.804	2	6.5
+ PE/PG (4:1, v/v)	3.021	2.345	2	6.5
+ PE/PG (3:2, v/v)	6.042	1.886	3	6.5
+ PE/PG (2:3, v/v)	9.062	1.427	4	6.5
+ PE/PG (1:4, v/v)	12.083	0.968	5	6.5

[a] Two hundred microliters of 10% CDTA-soluble pectins were mixed with 50 μl of enzyme extracts and incubated at 20°C. The PG crude extract had specific activity of 3.49 relative viscosity/mg protein/h for PG and 0.12 meq H^+/mg protein/h for PE. The PE crude extract had no PG activity and 0.58 meq H^+/mg protein/h of PE specific activity.

[b] PG activity was relative viscosity changes per 50 μl crude enzyme extract in 1 h, while PE activity was 1 mM NaOH consumed per 50 μl crude enzyme extract in 1 h.

Table 2
Gelation of pectin fractions extracted from Hermoza peaches and incubated with commercial PG and PE (Sigma)

Gelation mixture[a]		Setting time (days)	pH
H_2O-soluble fraction	+ PG	∞	
	+ PE	4d	6.3
CDTA fraction	+ PG	∞	
	+ PE	4d	6.3
Na_2CO_3 fraction	+ PG	∞	
	+ PE	∞	

[a] 5% H_2O-soluble, 10% CDTA-soluble and 5% Na_2CO_3-soluble fractions 200 μl + 5 μl PG or PE containing 0.5 unit activity (as described by Sigma) in the gelation mixture.

3. Discussion

This is the first time it has been demonstrated that a pectin fraction from peach can be acted upon by its cell wall enzymes to form a gel in vitro. There are two kinds of gels formed from pectin substrates: one is a high ester pectin gel and another is a low ester pectin gel. The former requires extremely high sugar concentrations to interact with the pectin and it is unlikely to be formed in situ (Pilnik and Voragen, 1992). The latter could be considered as a possible gelation model for woolly fruits as long as a low degree of esterification (DE) exists.

Pectins with a DE below 50% tend to bind Ca cations. If Ca^{2+} is added slowly to low ester pectins, a calcium-pectate gel is formed. Instead of adding calcium it is possible to slowly deesterfy high ester pectin with PE. Calcium pectate gels obtained by PE action are a phenomenon known in the citrus industry when concentrated juices gel by the action of residual PE (Pilnik and Voragen, 1992). The fact that only the CDTA-soluble fraction produced a gel after incubation with PE in vitro strongly supports the hypothesis that the increased Ca pectate of the middle lamella in woolly fruit is the site of gel formation in vivo (Ben-Arie and Lavee, 1971), taking into consideration that in vitro the Ca has been chelated, whereas in vivo it is still available to facilitate gelation.

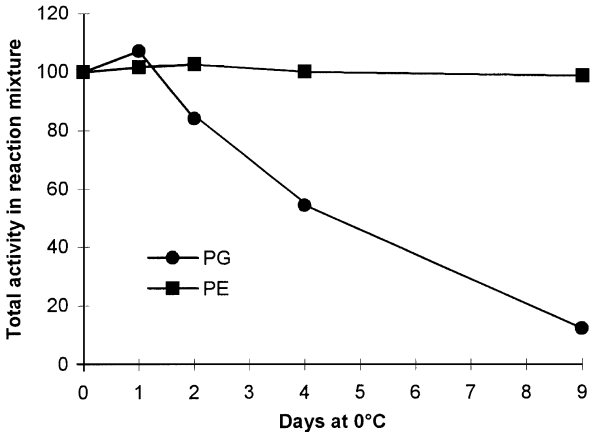

Fig. 2. In vitro total PG and PE activities during storage of the extracts at 0°C.

PE associates with the cell wall by ionic interactions, and can be extracted by NaCl treatment, as was done in this study. The affinity of PE for pectin is highly dependent on the DE of the pectin (Christensen et al., 1998). Citrus PE has a K_m of 0.7 mg ml^{-1} for citrus pectin with a DE of 70% and 17 mg ml^{-1} when the DE is only 25%. So as the enzyme progressively deesterifies the pectin its association with the substrate becomes weaker.

The deesterified pectins are able to interact with calcium ions to form gels, and are also susceptible to the subsequent action of polygalacturonase (Pressey and Avants, 1973). This sequence of events is what occurs during normal ripening, first PE and then PG, the sequence being a decrease in DE and then in size of the pectin molecules. In ripening of peaches after storage these two enzymes do not work in synchrony, for a number of reasons.

One reason is that PE activity continues during cold storage, whereas PG activity decreases. This study demonstrated that PE activity is very stable at low temperatures (Fig. 2), and in stored fruit a basal level of activity is also maintained (Ben-Arie and Sonego, 1980).

Therefore, the DE of pectins continually decreases during storage (Lurie et al., 1994). Nectarines have been shown to pass through a period of woolliness during ripening after storage which later disappears and the fruit becomes soft and juicy (von Mollendorff et al., 1992). The explanation for this transient woolliness is an imbalance between the PE and PG activity leading to an accumulation of large demethylated pectin molecules which are the substrate for gel formation and therefore woolliness. However, at longer times of ripening PG is able to hydrolyse these polymers to smaller units (von Mollendorff et al., 1993).

The greatest ratio between PE and PG activities was found in the inner mesocarp of RA stored fruits, where woolliness appeared most intensely (Fig. 1). This is similar to the localization of gel breakdown in plums, which also occurs in the inner tissue, and is associated with increased pectin viscosity (Taylor et al., 1994). However, in plums the CDTA fraction did not appear to influence the development of gel breakdown (Taylor et al., 1995), while in peaches it is the fraction most amenable to gel formation (Table 1). It is also a fraction which increases in proportion to other pectin fractions after storage (Lurie et al., 1994). Therefore, there may be significant differences in the etiology of gel breakdown in plums and woolliness in peaches and nectarines even though the disorders appear similar and both are stone fruits.

4. Experimental

Peaches (*Prunus persica*, cv. Hermoza) were sampled from the commercial harvest and divided into two lots; one lot was stored at 0°C in RA, and the second in CA (10% CO_2, 3%O_2). After 4 weeks storage, all fruits were transferred to 20°C for 4 days to ripen. At the end of the ripening period the fruits were halved and evaluated for woolliness, both by visual observation, and by determination of expressible juice by the method of Lill and van Der Mespel (1988). Woolly fruit where the inner mesocarp was more affected than the outer were sampled separately for both areas. Healthy fruit were also divided into inner and outer mesocarp for comparison. Forty fruits from each treatment were examined for woolliness, and samples were taken from 3 replicates of 5 fruit from each treatment. At harvest and after ripening without storage fruit flesh was also sampled as above. All samples were frozen in liquid nitrogen and held at −20° C until assaying.

4.1. Cell wall pectin extraction

Cell wall pectins in cell wall were extracted from mature fruit at harvest and separated into water-soluble, CDTA-soluble and Na_2CO_3-soluble fractions in the following manner: 5.5 g frozen fruit was extracted in boiling EtOH for 20 min, then transferred to distilled water with 0.02% sodium azide in which it was homogenized (Turrax) and then centrifuged (26,000 *g*) for 10 min. The supernatant was collected as the water-soluble fraction. The pellet was then washed twice and the collected supernatants were combined with the previous water-soluble fraction. The pellet was washed twice in Me_2CO, once in $CHCL_3$: MeOH (1:1) and finally in Me_2CO. The pellet was resuspended in 50 mM CDTA, pH 6.8, shaken for 3 h, and centrifuged. The supernatant was collected and the pellet was extracted twice more with CDTA. The three supernatants were combined as the CDTA-soluble fraction. The pellet was resuspended in 50 mM Na_2CO_3, 20 mM $NaBH_4$ at 4°C for 18 h plus 2 h at room temperature, centrifuged and the supernatant neutralized to pH 7.0 with HOAc as the carbonate-soluble pectin. CDTA and Na_2CO_3 fractions were dialyzed against double-distilled water with 6 water changes at 4°C. The three fractions were frozen and lyophilized. For gelation trials these fractions were dissolved into double-distilled water to final concentration of 5% (w/v) for water-soluble and Na_2CO_3-soluble and 10% (w/v) for CDTA-soluble fractions.

4.2. PG and PE extraction and activity determination

For enzyme extraction, 45 g of frozen fruit mesocarp were ground in 85 ml cold 12% PEG 4000, 0.2% Na bisulfite for 2 min. After centrifugation at 10,500 *g* for 10 min the pellet was washed with 0.2% Na bisulfite twice, collected and separated into 2 parts for extraction of each enzyme activity. For PG the pellet was incubated on a shaker at 4° C for 1 h in cold 50 mM NaAc buffer pH 5, 0.5 M NaCl. Following centrifugation as above, the supernatant was diluted with 50 mM NaAc buffer, pH 5, and used as crude extract. PG activity was measured as the reduction in viscosity of 4.5 ml 2% polygalacturonic acid in 50 mM NaAc, pH 4.4 mixed with 3 ml enzyme extract. Initial viscosity was measured with a Cannon-Fenske viscosometer and after a further 4 h incubation at 30° C. One activity unit was defined as the relative change in viscosity (initial viscosity–final viscosity)/viscosity of H_2O per mg protein per hour.

For PE extraction the 5 g pellet was resuspended in 15 ml 7.5% NaCl, 0.75% EDTA (pH 6.5) and stirred at 4° C for 1 h. Following centrifugation as above, the supernatant was collected. Five millilitres crude extract was mixed with 20 ml 1% citrus pectin and titrated with 0.01 N NaOH to maintain pH 7.4 while incubating at 30C. One unit of activity was defined as millequivalents of H^+ released per mg protein per hour.

PE and PG activities were generally determined immediately following extraction, but the crude enzyme extract from fruit ripened without storage were also held at 0°C with 0.2% sodium azide and aliquots were

assayed daily for a number of days in order to assess the decline in activity. PG activity at the beginning of the experiment was 2.50 relative viscosity/mg protein/h and PE was 0.34 milliequivalents H^+/mg protein/h.

4.3. Gelation experiment

Crude PG and PE extracts from fruit ripened without storage were incubated at 20° C with the pectin fractions from harvest. The pectin solutions of 200 μl were mixed with 50 μl of the enzyme extracts and 0.2% sodium azide in different proportions and observed daily for gel formation. In addition, commercial enzymes, PG from *Rhyzopus* (Sigma, EC 3.2.1.15) and PE from citrus peel (Sigma, EC 3.1.1.11) were incubated with the pectin fractions. The pH was measured at the end of the incubation period using pH paper strips.

Acknowledgements

This research was funded by BARD: Binational Agricultural Research and Development Fund between United States and Israel.

References

Ben-Arie, R., Lavee, S., 1971. Pectic changes occurring in Elberta peaches suffering from woolly breakdown. Phytochemistry 10, 531–538.

Ben-Arie, R., Sonego, L., 1980. Pectolytic enzyme activity involved in woolly breakdown of stored peaches. Phytochemistry 19, 2553–2555.

Buescher, R.W., Furmanski, R.J., 1978. Role of pectinesterase and polygalacturonase in the formation of woolliness in peaches. Journal of Food Science 43, 264–266.

Christensen, T.M.I.E., Nielson, J.E., Kreiberg, J.D., Rasmussen, P., Mikkelsen, J.D., 1998. Pectin methyl esterase from orange fruit: characterization and localization by in-situ hybridization and immunohistochemistry. Planta 206, 493–503.

Dawson, D.M., Melton, L.D., Watkins, C.B., 1992. Cell wall changes in nectarines (*Prunus persica*): Solubilization and depolymerization of pectic and neutral polymers during ripening and in mealy fruit. Plant Physiology 100, 1203–1210.

Lill, R.E., van Der Mespel, G.J., 1988. A method for measuring the juice content of mealy nectarines. Scientia Horticultura 36, 267–271.

Lurie, S., Levin, A., Greve, L.C., Labavitch, J.M., 1994. Pectic polymer changes in nectarines during normal and abnormal ripening. Phytochemistry 36, 11–17.

Pilnik, W., Voragen, A.G.J., 1992. Gelling agents (pectins) from plants for the food industry. Advances in Plant Cell Biochemistry and Biotechnology 1, 219–270.

Pressey, R., Avants, J.K., 1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiology 52, 252–256.

Taylor, M.A., Rabe, E., Dodd, M.C., Jacobs, G., 1994. Effect of storage regimes on pectolytic enzymes, pectic substances, internal conductivity and gel breakdown in cold stored 'Songold' plums. Journal of Horticultural Science 69, 527–534.

Taylor, M.A., Rabe, E., Jacobs, G., Dodd, M.C., 1995. Effect of harvest maturity on pectic substances, internal conductivity, soluble solids and gel breakdown in cold stored 'Songold' plums. Postharvest Biology and Technology 5, 285–294.

Ugalde, T.D., Jerie, P.H., Chalmers, D.J., 1988. Intercellular pH of peach and apricot mesocarp. Australia Journal of Plant Physiology 15, 505–517.

von Mollendorff, L.J., Jacobs, G., De Villiers, O.T., 1992. Effect of temperature manipulation during storage and ripening on firmness, extractable juice and woolliness in nectarines. Journal of Horticultural Science 67, 655–662.

von Mollendorff, L.J., De Villiers, O.T., Jacobs, G., Westraad, I., 1993. Molecular characteristics of pectic constituents in relation to firmness, extractable juice, and woolliness in nectarines. Journal of the American Society of Hortitcultural Science 118, 77–80.

Zhou, H.W., Sonego, L., Ben-Arie, R., Lurie, S., 1999. Analysis of cell wall components in juice of 'Flavortop' nectarines during normal ripening and woolliness development. Journal of the American Society of Horticultural Science 124, 424–429.

Zhou, H.W., Lurie, S., Lers, A., Khatchitski, A., Sonego, L., Ben-Arie, R., 2000. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent woolliness. Postharvest Biology and Technology 18, 133–141.