Cell Wall Metabolism in Cold-stored 'Somerset' Sweet Cherry Fruit

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Abstract

'Somerset' is a dark-red, sweet cherry (*Prunus avium* L.) cultivar displaying remarkable firmness levels, with concomitantly longer shelf-life potential in comparison to other varieties. It is generally accepted that fruit firmness depends mainly on the composition, structure and interconnections among cell wall polysaccharides. However, the biochemical mechanisms involved in cell wall disassembly vary widely among species, and the understanding of the processes underlying firmness loss in cherry fruit is particularly poor, although a critical role for β-galactosidase (β-Gal) activity has been suggested. In this study, 'Somerset' fruit were hand-collected at commercial maturity, and kept at 0 ºC for 14 or 28 days plus 3 additional days at 20 ºC to simulate commercial shelf life. Firmness, weight loss and juiciness were assessed in each case as indicators of fruit texture. Soluble and insoluble cell wall materials were extracted from lyophilized tissue, and a number of cell wall-modifying enzyme activities were also assessed therein. While β-xylosidase (β-Xyl), pectate lyase (PL), α-L-arabinofuranosidase (AFase) and pectin methylesterase (PME) activities were apparently connected to ripening-related firmness changes in this cherry cultivar, data obtained do not support a role for β-Gal in this process.

INTRODUCTION

Sweet cherry (*Prunus avium* L.) fruit must be harvested fully ripe in order to achieve good eating quality. While surface colour and soluble solids content (SSC) are the main criteria generally used to determine harvest maturity of these fruit (Romano et al., 2006), the eating quality of produce include additional indicators such as titratable acidity (TA), SSC/TA ratios, aroma and flavour. However, fruit handling options are determined largely by firmness, and sweet cherries are indeed highly perishable due to rapid softening rates associated to high susceptibility to infections and mechanical bruises. These characteristics restrict drastically their storage potential and marketing possibilities after harvest. In this context, 'Somerset' cherry fruit display remarkable firmness levels in comparison to other cultivars, with accordingly longer shelf-life potential.

Ripening-related firmness loss is commonly attributed to modifications in cell wall composition and structure, driven by the cooperative action of numerous related proteins. Yet profound differences apparently exist in the extent and enzyme regulation of ripening-related modifications of cell wall polysaccharides among fruit species, or even among cultivars of the same species (Goulao and Oliveira, 2008). For this reason, we undertook this preliminary study on cell wall-modifying activities during and after cold storage of

'Somerset' sweet cherry fruit, aimed at identifying enzymic factors possibly related to firmness loss and postharvest deterioration.

MATERIALS AND METHODS

Plant material and quality analyses

Cherry fruit (*Prunus avium* L. cv. 'Somerset') were sampled in 2011 at commercial harvest time (June 7th) from a commercial orchard located in Corbins, in the area of Lleida (NE Spain). Fruit were selected for uniform size and colour, checked for absence of infections, alterations and visual defects, and stored at 0 ºC and 92% relative humidity under regular air for 14 or 28 days. Samples were analysed immediately after harvest and upon removal from storage, with or without 3 additional days at 20 ºC to simulate commercial shelf life. Average fruit weight at harvest was 10.49 g. Weight of 30 cherries was determined jointly at harvest and at each analysis date in order to determine weight loss (%) regarding harvest date. Firmness was measured with a Durofel DFT 100 durometer (Agro-Technologie, Forges Les Eaux, France) fitted with a 5.64-mm tip, on two opposite faces on the cheek region of 30 fruit, and results were expressed as Durofel units (1-100) (Table 1). For the assessment of juiciness, three replicate samples (10 fruit each) per treatment were stoned and squeezed until no more juice was released. After filtration, the volume of juice recovered was measured, and expressed as mL 100 g^{-1} fresh weight. Fungal decay was expressed as percentage of fruit affected.

Cell wall analyses

For biochemical analyses, 30 fruit were stoned at each sampling date, frozen in liquid nitrogen, freeze-dried, powdered, and kept at -80 ºC until processing. The phenol:acetic acid:water (2:1:1, w/v/v) (PAW) method (Redgwell et al., 1992) was used for the extraction of cell wall materials (CWM) from lyophilised tissue (3 g), with some modifications as explained elsewhere (Lara et al., 2004). The PAW-insoluble pellet was washed twice in acetone, recovered by vacuum filtration, lyophilised and weighed to determine yield of insoluble cell wall materials (CWM). The PAW-soluble fraction (PAW_{sf}) was recovered, lyophilised and weighed. Yields were expressed as % (w/w) FW. For the extraction of polygalacturonase (exo-PG; EC 3.2.1.67 and endo-PG; EC 3.2.1.15), pectinmethylesterase (PME; EC 3.1.1.11), pectate lyase (PL; EC 4.2.2.2), β-galactosidase (β-Gal; EC 3.2.1.23), α-L-arabinofuranosidase (AFase; EC 3.2.1.55), β-xylosidase (β-Xyl; EC 3.2.1.37) and endo-1,4-β-D-glucanase (EGase; EC 3.2.1.4) activities, a 10% (w/v) homogenate was prepared from lyophilised tissue (100 mg). All extraction and assay procedures were as described in Ortiz et al. (2011a). Results were given in terms of specific activity (activity units mg^{-1} protein).

Statistical analysis of data

All statistical analyses were performed using Minitab 15 (Minitab Inc., UK). Data were analyzed by analysis of variance (ANOVA), with storage and shelf life periods as factors, and the means were compared using the Fischer's LSD test at $P \leq 0.05$. Partial least squares regression (PLSR-2 technique) was used as a predictive method to relate a matrix of dependent variables (*Y*) to a set of explanatory variables (*X*). Unscrambler version 7.6 software (CAMO ASA, Trondheim, Norway) was used for developing these models.

RESULTS AND DISCUSSION

Fruit quality after cold storage

Juiciness of 'Somerset' cherries declined steadily during cold storage, in parallel to increased weight loss (Table 1), and this trend was accentuated upon transfer of fruit to 20 ºC for shelf life simulation. Samples retained high firmness levels throughout the experimental period (Table 1). Fruit firmness increased notably after 14 days at 0 ºC (86.5 units) in comparison with values at harvest (80.4 units), indicating a tightening effect of cold temperature on fruit tissues as also observed elsewhere for 'Pájaro' strawberries (Lara et al., 2004). In all cases, though, firmness of fruit declined after being kept 3 days at 20 ºC, this decline being statistically significant uniquely for fruit stored during 14 days.

Cell wall materials after cold storage

Insoluble and PAW-soluble cell wall materials were extracted from fruit samples in order to assess possible relationships to some attributes related to shelf life potential. Increased firmness levels in cold-stored samples were accompanied by similar increments in the yield of insoluble cell wall materials (CWM) recovered from fruit pericarp (Table 2), which suggests a possible relationship between both and, generally speaking, the changes in CWM yields were in accordance with differences in firmness. However, firmness of non-stored fruit decreased after remaining 3 days at 20 ºC after harvest (Table 1) in spite of a two-fold augment in CWM yields (Table 2). This suggests that the total amount of insoluble materials is not a reliable indicator of cell wall status, which would be more properly described by the specific composition and structure of the cell wall polymers. Actually, yields of the PAW_{sf}, indicative of the degree of *in vivo* solubilisation of cell wall polysaccharides, did not keep any apparent relationship to firmness values, an increase during shelf life at 20 ºC being found only after four weeks of cold storage (Table 2).

We found increases in the ratio between insoluble and PAW-soluble cell wall materials, both after cold storage and during shelf life at 20 ºC. This strongly disagrees with our previous observations on fruit displaying non-melting as well as melting softening patterns, such as apple (Ortiz et al., 2011a), peach (Ortiz et al., 2011b) and nectarine (Ortiz et al., 2010). In all these cases, we have consistently found declining $CWM:PAW_{sf}$ ratios during fruit softening, indicative of increased solubilisation of cell wall polymers during the process. The discrepancy with the observations for sweet cherry reported herein agrees with the view that noticeable differences exist among fruit species in the mechanisms involved in ripening-related firmness loss (Goulao and Oliveira, 2008), and suggests that additional processes may play a key role in this fruit.

Cell wall-modifying enzyme activities after cold storage

Ripening- and postharvest-related disassembly of cell wall polymers is currently considered to arise from the finely coordinated action of a wide range of pectolytic and non-pectolytic enzyme activities. Historically, depolymerizing enzymes acting on the pectin backbone, such as PG and PME, had been considered to play a central role in fruit softening. Yet current experimental evidence shows that cell wall disassembly is far more complex than initially thought, and that other cell wall proteins may contribute significantly and decisively to this event. Actually, pectin depolymerisation during cherry fruit ripening has been reported to be very limited (Batisse et al., 1994), textural differences between crisp and soft cherry fruit being likely related to structure and

composition of pectin side-chains rather than to depolymerisation of the pectin backbone (Batisse et al., 1996). The removal of the pectin side-chains increases cell wall permeability and favours the access of PG and PME to their backbone substrates. A cell wall-associated sweet cherry β-galactosidase (β-Gal) has been characterised to be active against complex glycans (Gerardi et al., 2012). Because the enzyme was found to be the main glycosidase activity detected in sweet cherry extracts at different ripening stages, and to increase notably during ripening, it was suggested to play a significant role in fruit softening.

In this work, five pectolytic and two non-pectolytic cell wall-related enzyme activities were extracted and assayed. The changes in the activity of a pectin backbone-acting enzyme (PL), a pectin side-chain-acting enzyme (AFase) and a non-pectolytic enzyme (β-Xyl) are shown as an example (Fig. 1). For PL and β-Xyl activities, a decrease was observed after 14 days at 0 ºC, maybe in relation to increased fruit firmness levels (Table 1). A regression analysis was undertaken in order to aid the visualisation of relationships among the variables assessed, with fruit quality attributes as the *Y* variables and cell wall parameters as the potentially explanatory (*X* matrix) variables. The two first partial least squares (PLS) factors for this model explained up to 88% of total sample *Y*-variability. Yet results do not support a key role for β-Gal in postharvest deterioration of 'Somerset' cherries. Actually, the correlation loadings plot corresponding to this regression analysis (Fig. 2) shows that fruit firmness was associated to higher β-Gal activity levels. This result is not in accordance with the proposed role on sweet cherry softening (Gerardi et al., 2012), and it also disagrees with previous results on other fruit species (Ortiz et al., 2011a, 2011b). However, Gerardi et al. (2012) reported that β-Gal activity increased at early stages of sweet cherry ripening but decreased in over-ripe fruit. Similar results were obtained for nectarine (Ortiz et al., 2010), where β-Gal levels increased at the initial phase of softening, prior to the onset of the melting-like drop in firmness, to remain steady thereafter. Thus, the association between firmness and β-Gal activity might be indicating this early role in softening-leading events.

Another possibility arises from the observation that PME activity was also associated to firmness (Fig. 2). The β-Gal-catalysed removal of galactosyl-rich side-chains in pectins facilitates the access of PME to the pectin backbone. While the demethylating action of PME on uronic acid residues is required for subsequent PG- and PL-mediated depolymerisation, removal of methyl moieties also confers an anionic charge to polyuronides, which in the presence of calcium can aggregate non-covalently through calcium bridges, thus actually reinforcing the cell wall structure and favouring firmness retention.

EGase activity also seemed related to firmness levels. Contradictory reports on the relevance of this non-pectolytic activity for fruit softening have been published (Goulao and Oliveira, 2008). Activity levels were found to decrease progressively during fruit development of 'Mondial Gala' apples (Goulao et al., 2007) and 'Snow Queen' nectarines (Ortiz et al., 2010), and to be higher in more firm apple fruit (Ortiz et al., 2011c), thus de-emphasising its role in firmness loss, and suggesting an association to cell wall extensibility at early stages of fruit development.

The regression analysis revealed that firmness of 'Somerset' cherries was related mainly to the activity of the non-pectolytic enzyme β-Xyl, which acts on matrix glycans (Fig. 2). This observation suggests that the KOH-soluble fraction of insoluble CWM, which is enriched in hemicellulosic polymers, could be an important determinant of fruit firmness. However, CWM fractionations will be necessary to test this hypothesis. Finally,

and although to a lesser extent, fruit firmness was also related to PL activity, a lyase which removes demethylated uronic acid residues from the pectin backbone through a β-elimination mechanism, as well as to AFase, which cleaves arabinosyl residues from pectin side-chains. The latter observation highlights the relevance of pectin side-chains as key determinants of cell wall porosity, and thus as modulators of the mobility and substrate accessibility of several cell wall proteins. In contrast, PG activity, traditionally considered central to fruit softening, was apparently unrelated to post-harvest firmness changes in 'Somerset' cherries.

CONCLUSIONS

PME activity was closely related to fruit firmness of 'Somerset' cherries, while β-Xyl, PL and AFase activities showed an inverse relationship to this attribute. Total amount of insoluble cell wall materials was not a good predictor of firmness in 'Somerset' cherries. Further fractionation of these materials will give additional insight on cell wall modifications after fruit harvest. Alternative mechanisms possibly underlying firmness changes are being explored as well, including cuticle composition and antioxidant status. These data will help understanding the evolution of fruit quality during the marketing period, and give hints for suitable strategies to preserve key attributes.

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Tables

Table 1. Some quality attributes of 'Somerset' cherries at harvest and after cold storage.

Cold storage (0 ºC)	0 days		14 days		28 days	
Simulated shelf life (20 ºC)	0 d.	3 d.	0 d.	3 d.	0 d.	3 d.
Firmness (Durofel units [a])	80.4bc	77.2c	86.5a	82.1b	82.4b	81.8b
Weight loss (%)	-	1.2	7.2	15.4	11.3	21.5
Decayed fruit (%)	-	-	8.5	21.4	10	12.8
Juiciness (mL $100g^{-1}$ FW)	65.5a	62.3abc	64.4ab	57.7c	59.6bc	55.7c

[a] (Durofel units: 1, no resistance - 100, maximum resistance).

Values represent means of 30 (firmness) or 3 (juiciness) replicates. For weight loss assessment, the same 30 fruit were weighed jointly at harvest, upon removal from cold storage and 3 days thereafter. Decay incidence was evaluated on the total number of fruit. Mean values followed by different letters within the same row are significantly different at $P \leq 0.05$ (LSD test).

Table 2. Cell wall solubilisation in 'Somerset' cherries at harvest and after cold storage.

Cold storage (0 ºC)	0 days		14 days		28 days	
Simulated shelf life (20 ºC)	0 d.	3 d.	0 d.	3 d.	0 d.	3 d.
CWM (% FW) [a]	0.890d	1.654a	1.615a	1.587ab	1.385bc	1.258c
PAW_{sf} (% FW) [b]	0.017ab	0.014b	0.021ab	0.012b	0.022ab	0.025a
Ratio CWM:PAW_{sf}	53.1	118.2	76.1	132.3	63.1	50.8

[a] CWM: insoluble cell wall materials.

[b] PAW_{sf}: PAW-soluble materials (PAW: phenol:acetic acid:water 2:1:1, w/v/v).

Values represent means of 3 replicates. Mean values followed by different letters within the same row are significantly different at $P \leq 0.05$ (LSD test).

Figures

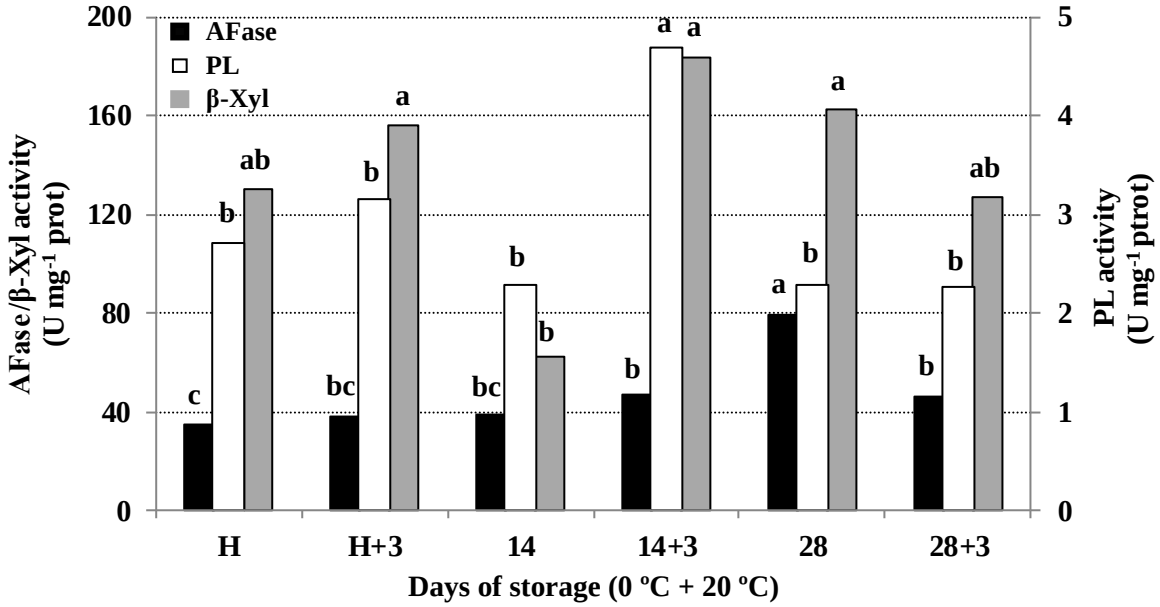

Fig. 1. Pectate lyase (PL), α-L-arabinofuranosidase (AFase) and β-xylosidase (β-Xyl) activities in 'Somerset' cherries at harvest (H) and after cold storage. Bars represent means of 3 replicates. Mean values bearing different letters for a given enzyme activity are significantly different at $P \leq 0.05$ (LSD test).

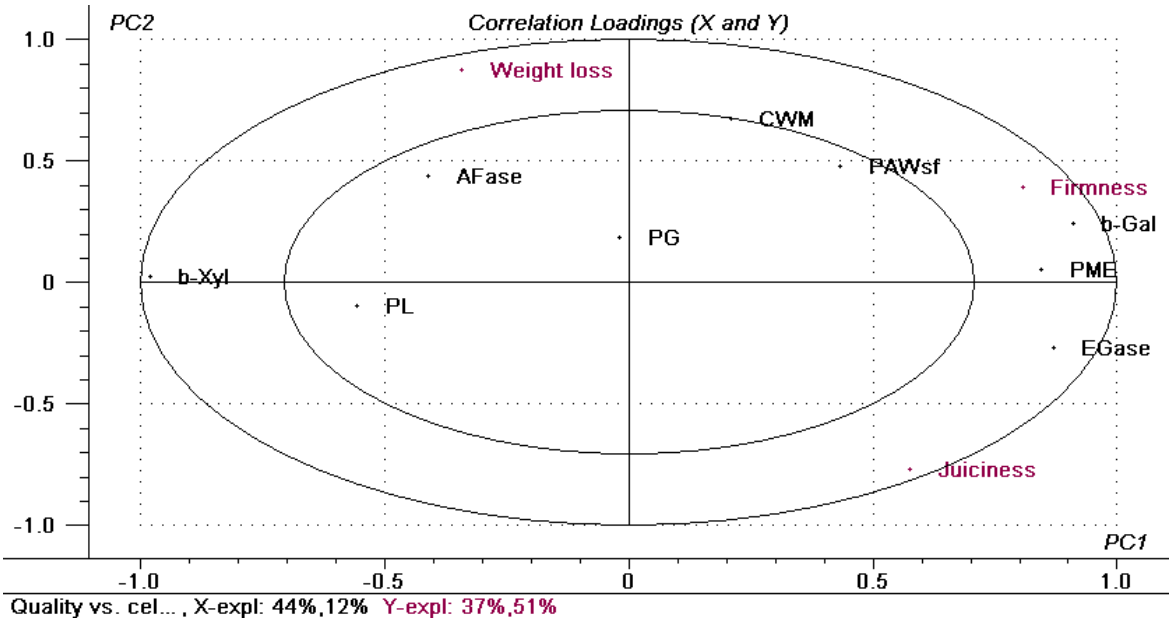

Fig. 2. Correlation loadings plot of factor 1 *vs*. factor 2 corresponding to a PLSR model for fruit quality attributes (*Y* variables) *vs*. cell wall fractions and cell wall-modifying enzyme activities (*X* variables) in 'Somerset' cherries at harvest and after cold storage (CWM, insoluble cell wall material; PAWsf, PAW-soluble cell wall material).