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# Cell Wall Disassembly and Post-harvest Deterioration of 'Sweetheart' Sweet Cherry Fruit: Involvement of Enzymic and Non-enzymic Factors

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**Abstract**

Sweet cherries are very perishable, owing to rapid softening rates associated to significant deterioration of fruit. Ripening-related firmness loss is generally attributed to enzyme-catalysed modifications in cell wall composition and structure, but some experimental evidence suggests that non-enzymic mechanisms may be also involved in the process. In this study, 'Sweet Heart' cherries were picked at commercial maturity and kept at 0 ºC during 15 or 30 days, followed by up to 3 days at 20 ºC to simulate commercial shelf life. Cell wall materials were extracted, fractionated and analysed, and some cell wall-modifying enzyme activities were assessed for a possible role on firmness loss and overall deterioration of fruit. Radical scavenging activity (RSA) and both reduced and oxidised ascorbate contents were also analysed in each case. Regression analysis revealed that firmness was associated to reduced ascorbate and RSA, in turn inversely correlated to decay and weight loss. Higher firmness levels were found for fruit displaying higher RSA and yields of the cell wall fractions containing covalently-bound pectins and hemicelluloses, while decay correlated inversely with RSA and reduced ascorbate content. Polygalacturonase (PG) and pectate lyase (PL) activities were related to solubilisation of cell wall pectins and inversely to reduced ascorbate, but not to firmness-contributing cell wall fractions, which correlated rather to enzyme activities acting on pectin side-chains that

control the access to substrates of backbone-degrading enzymes. Data suggest that antioxidant status may play a major role in modulating cell wall disassembly of sweet cherry fruit.

**Keywords**: Ascorbic acid, Antioxidant capacity, Cell wall, Cherry, *Prunus avium*, Softening, Storage

## 1 Introduction

Sweet cherry (*Prunus avium* L.) fruit must be harvested fully ripe in order to achieve good eating quality. Surface colour and soluble solids content (SSC) are usually the main criteria used to determine harvest maturity of these fruit, and typical physical-chemical parameters such as SSC, titratable acidity (TA), SSC/TA ratios and firmness are generally used as indicators of the eating quality of produce. In turn, commercial quality also comprises aspects related to appearance, including colour of fruits and stems, and freedom from defects and infections (Romano et al., 2006). Standard and visual quality parameters, though, sometimes do not reflect completely the overall quality perceived by consumers. Aroma and flavour are also major attributes for the eating quality of the produce. Similarly, nutritional quality is an additional commercial value for consumers, and these fruit are particularly rich in bioactive compounds such as anthocyanins and polyphenolics (McCune et al., 2011) which have been shown to reduce the risk of a number of degenerative diseases (Tomás-Barberán and Andrés-Lacueva, 2012).

Sweet cherries are highly perishable due to rapid softening rates associated to high susceptibility to infections and mechanical bruises, which drastically restrict their storage potential and marketing possibilities after harvest. This attribute is thus a major factor determining fruit handling options. Ripening-related firmness loss is commonly considered to be largely due to modifications in cell wall composition and structure, driven by the cooperative action of numerous related proteins. Yet profound differences appear to 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). Additionally, there is some experimental evidence that non-enzymic mechanisms are also involved in the scission of cell wall polysaccharides, and particular attention has been focused on the role thereupon of ascorbic acid and its oxidation derivatives (Fry, 1998; Agius et al., 2003). In this study, we aimed at identifying factors related to firmness loss and postharvest deterioration during and after cold storage of sweet cherry fruit.

## 2 Materials and Methods

### Plant material and standard quality assessments

'Sweet Heart' sweet cherries (*Prunus avium* L.) were picked from an orchard located in Corbins (Segrià, NE Spain) at commercial maturity on the basis of fruit colour, according to the usual standards in the producing area. Harvest date was 25$^{th}$ June 2009. Defect-free, uniform colour fruit were harvested manually early in the morning, transported immediately thereafter to the ETSEA-UdL campus, and stored at 0 ºC and 92% relative humidity under regular air. After cold storage for 15 or 30 days, samples were placed at 20 ºC, and analyses were carried out 0 (henceforth, 15+0 and 30+0 fruit) and 3 (henceforth, 15+3 and 30+3 fruit) days thereafter. Three replicate samples (2 kg) were used per each combination of factors (cold storage period × shelf life period). 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.

Firmness was measured with a Durofel DFT 100 durometer (Agro-Technologie, Forges Les Eaux, France) fitted with a 5.64-mm tip. Two different measurements were performed on opposite sides of the equatorial zone of 30 fruit, and results were expressed as Durofel units (1, no resistance - 100, maximum resistance). 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 kg$^{-1}$ fresh weight. Soluble solids content (SSC) and titratable acidity (TA) were assessed in juice obtained as described above. SSC was determined with a hand-held refractometer (Atago, Tokyo, Japan), and results expressed as ºBrix. For TA determination, 10 mL of juice were diluted in 10 mL distilled water, and titrated with 0.1 M NaOH to pH 8.1; results were given as g malic acid L$^{-1}$. Skin colour was determined at two opposite equatorial points of 30 fruit using a portable tristimulus colorimeter (Chroma Meter CR-200, Minolta Corp., Osaka, Japan), with CIE $D_{65}$ illuminant and 8 mm aperture diameter. Hue angle was calculated from $a^*$ and $b^*$ parameters. The external appearance was also assessed on 30 fruit per treatment in terms of stem browning and incidence of fungal decay; results were expressed as percentage of fruit affected.

Juice (5 mL) obtained from three replicate samples (10 fruit each) per treatment was introduced in 10-mL test tubes closed with elastic caps, and incubated at 65 ºC for 1 h for the analysis of ethanol and acetaldehyde content as previously described (Ke et al., 1994). A 1-mL headspace gas sample was taken with a syringe and injected into a gas chromatograph (Agilent Technologies 6890, Wilmington, Germany), equipped with a column containing Carbowax (5%) on Carbopack (60/80, 2m×2mm i.d.) as the stationary phase, and a flame ionisation detector. Nitrogen was used as the carrier gas (24 cm s$^{-1}$), and operating conditions were as follows: oven temperature 110 ºC, injector temperature 180 ºC, detector temperature 220 ºC. Acetaldehyde and ethanol were identified and

quantified by comparison with external standards, and results were expressed as $\mu L\ L^{-1}$.

**Extraction, fractionation and analysis of cell wall materials**

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 pellet obtained after PAW extraction was resuspended in water and centrifuged again. The PAW and water wash supernatants were combined, dialysed (mol. wt. cut-off 7000) for two days against Milli-Q water at 4 ºC, and centrifuged again to sediment out the precipitate formed during the dialysis. The combined supernatant (henceforth, PAW-soluble fraction; $PAW_{sf}$) was recovered, lyophilised and weighed. The PAW-insoluble pellet was washed twice in acetone, recovered by vacuum filtration, lyophilised and weighed to determine yield of CWM, expressed as % (w/w) FW. For further fractionation, CWM (100 mg) from each replicate were extracted sequentially with water, 0.05 M cyclohexane-trans-1,2-diamine tetra-acetate (CDTA), 0.05 M $Na_2CO_3$, and 4 M KOH (Selvendram and O'Neill, 1987), in order to fractionate water-soluble pectin, non-covalently-bound pectin, covalently-bound pectin and matrix glycans (hemicelluloses), respectively. Each fraction was filtered through Miracloth, extensively dialysed (mol. wt. cut-off 7000) for two days against Milli-Q water at 4 ºC, lyophilised and weighed. Yields were expressed as % (w/w) CWM.

Samples of CWM and of both CDTA- and $Na_2CO_3$-soluble fractions (30-35 mg each) were hydrolysed with 12 M sulphuric acid for further analysis as described previously (Ortiz et al., 2011). Uronic acid content in the hydrolysate was measured by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973), using galacturonic acid as a standard. Total neutral sugars were estimated at 490 nm by the phenol-sulphuric acid assay (Dubois et al., 1956), with galactose as the standard.

**Extraction and assay of cell wall-modifying enzyme activities**

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) and endo-1,4-β-D-glucanase (EGase; EC 3.2.1.4) activities, a 10% (w/v) pulp homogenate was prepared by homogenising 100 mg of freeze-dried pulp tissue in extraction buffer (Lohani et al., 2004). PG activity was determined on apple pectin (d.e. 70-75%) as described previously (Pathak and Sanwall, 1998), with galacturonic acid (GalUA) as a standard. One unit (U) of PG activity was defined as the liberation of 1 μmol of GalUA $min^{-1}$. PME activity was measured according to Hagerman and Austin (1986). For the assay, the reaction mixture contained enzyme extract, apple pectin and bromothymol blue (Alonso et al., 1997). One unit (U) of PME activity was defined as the decrease of one unit of $A_{620}\ min^{-1}$. PL activity was assayed with apple pectin as the substrate according to Moran et al. (1968) as modified in Lohani et al. (2004). One unit (U) of PL

activity was defined as the increase of one unit of $A_{235}$ $min^{-1}$. For the assessment of EGase activity, the DNS method (Miller, 1959), with carboxymethylcellulose as the assay substrate, was used to determine the amount of reducing sugars released, with glucose as a standard. One unit (U) of EGase activity was defined as the release of 1 μmol of glucose $min^{-1}$.

For the extraction of β-galactosidase (β-Gal; EC 3.2.1.23), β-xylosidase (β-Xyl; EC 3.2.1.37) and α-L-arabinofuranosidase (AFase; EC 3.2.1.55) activities, a 10% (w/v) pulp homogenate was prepared by homogenising 100 mg of freeze-dried pulp tissue in extraction buffer prepared according to Vicente et al. (2005). β-Gal, β-Xyl and AFase activity assays were undertaken in the crude extract as described elsewhere (Vicente et al., 2005; Wei et al., 2010). One unit (U) of β-Gal was defined as the liberation of 1 μmol of *p*-nitrophenol $min^{-1}$ from *p*-nitrophenyl-β-D-galactopyranoside. One unit (U) of AFase or β-Xyl was defined as the release of 1 nmol of *p*-nitrophenol $min^{-1}$ from *p*-nitrophenyl-α-L-arabinofuranoside or *p*-nitrophenyl-β-D-xylopyranoside, respectively.

Total protein content in the crude extracts was determined with the Bradford method (Bradford, 1976), using BSA as a standard. All analyses were done in triplicate, and results were expressed as specific activity (U $mg^{-1}$ protein).

**Determination of antioxidant capacity and ascorbic acid content**

Total antioxidant capacity was measured in terms of radical scavenging activity (RSA) by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described in Oms-Oliu et al. (2009). Dry material (200 mg) was added 10 mL 100% (v/v) methanol, homogenised, and centrifuged at 4 ºC and 4000 × *g* for 20 min. An aliquot (200 μL) of the supernatant was mixed in 7.8 mL DPPH solution (63 μM in 100% (v/v) methanol) in a light-tight tube, and allowed to react during 2 h at room temperature. The absorbance of the reaction mixture was measured at 515 nm against a methanol (100%, v/v) blank, and RSA was calculated according to:

$$RSA\ (\%) = [1 - (Abs_{sample}/Abs_{control})] \times 100$$

where $Abs_{sample}$ and $Abs_{control}$ are the absorbances of DPPH with and without sample, respectively.

The colorimetric ascorbate assay (Gillespie and Ainsworth, 2007) was used to analyse the contents of ascorbic and dehydroascorbic acid. Lyophilised tissue (40 mg) was used as the starting material, the absorbance at 525 nm was measured, and ascorbate content was estimated as nmol $gDW^{-1}$ from the corresponding calibration curve.

**Statistical analysis**

A multifactorial design with storage and shelf life periods as factors was used to statistically analyse the results. All data were tested by analysis of variance (GLM-ANOVA procedure) with the Statistical Analysis System (SAS version 9.1, SAS Institute, Inc., Cary, NC, USA), and means were separated by the Fisher's

LSD test at $P \leq 0.05$. Principal component analysis (PCA) was used to provide a general overview of the information contained in the dataset obtained. Partial least squares regression (PLSR) was used as a predictive method to relate a matrix of dependent variables (*Y*) to a set of explanatory variables (*X*). Unscrambler version 9.1.2 software (CAMO ASA, Trondheim, Norway) was used for developing these models.

## 3 Results and Discussion

**Commercial quality and antioxidant properties of 'Sweet Heart' cherries after storage**

Cherries were stored for up to 30 days in order to cause differences in firmness and other storability-related parameters, with the purpose of dissecting the biochemical mechanisms involved in the softening and deterioration process. Firmness at harvest was over 80 Durofel units (Table 1), and declined significantly, though not sharply, in stored fruit regardless of storage or shelf life period. This observation may have been originated by a hardening effect arising from weight loss, which was quite important, particularly after 3 days at 20 ºC (Table 1). The highest juiciness levels were found for 30+0 samples. Although fruit juiciness is generally regarded as desirable, high levels of this attribute might be also indicating over-ripening and tissue disintegration. Actually, these samples also had higher contents of acetaldehyde, which has been shown to promote fruit softening in some fruits (Janes and Frenkel, 1978; Pesis, 2005), and were associated to higher incidence of off-flavours (data not shown).

Total antioxidant activity, measured in RSA terms, decreased significantly after harvest, although no differences were found between fruit stored for 15 and 30 at 0 ºC. RSA declined further during the subsequent post-storage period at 20 ºC (Table 2). Contrarily, levels of ascorbic acid, both reduced and oxidized, were significantly affected by storage period. Reduced ascorbic acid contents were slightly higher in fruit stored for 15 days than in freshly harvested samples, but decreased after 3 days at 20 ºC. Levels of reduced ascorbate declined noticeably after 30 days at 0 ºC, concomitantly with strong increases in dehydroascorbic acid (Table 2), the oxidized form of ascorbate.

When quality and antioxidant properties were included in a PCA model, the corresponding biplot (Fig. 1) revealed that higher firmness was associated to higher levels of reduced ascorbate and RSA, which in turn correlated inversely to decay and weight loss, giving a hint that antioxidant properties may be important for delaying firmness loss and associated alterations. Therefore we extracted, fractionated and analysed cell wall materials in order to assess the possible relationship between cell wall metabolism and antioxidant properties of fruit.

**Cell wall composition in 'Sweet Heart' cherries after storage**

Soluble and insoluble cell wall materials were extracted from the pericarp

tissue and fractionated in order to determine yields in each case (Table 3). Yields of the PAW-soluble fraction, indicative of cell wall materials solubilised *in vivo*, were significantly higher in fruit stored for 30 days, whereas they were apparently unaffected by post-storage period at 20 ºC. Contrarily, no clear trend was found for insoluble cell wall materials (CWM) (Table 3A), yields in 15+3 samples being approximately three-fold in comparison with those at harvest. While this result might have arisen from an experimental error, it may also be reflecting that structure and composition of cell wall materials is more relevant than total yields for explaining firmness-related attributes of these fruit. When insoluble materials were further fractionated, yields of the fractions soluble in $Na_2CO_3$ and KOH, indicative respectively of covalently-bound pectins and matrix glycans, were observed to be generally in correspondence with higher firmness levels (Table 3B). Contrarily, yields of the CDTA-soluble fraction, which contains mainly pectins bound non-covalently to the cell wall, were higher in fruit stored at 0 ºC during 30 days than in those stored for 15 days, or even higher than at harvest, although they decreased again during shelf life at 20 ºC. This observation suggests that some remobilisation of insoluble cell wall materials from the $Na_2CO_3$- to the CDTA-soluble fraction may have taken place during softening, consistent with the view that a large part of polysaccharides solubilized during ripening remain linked transiently to the cell wall by ionic bonds, as reported for other stone fruit species such as peach (Brummell et al., 2004; Ortiz et al., 2011) and nectarine (Dawson et al., 1992; Ortiz et al., 2010). The finding that the contents of total neutral sugars and uronic acids in the $CDTA_{sf}$ increased significantly after harvest is also supportive of this idea (Table 3C).

Cell wall fraction yields, together with RSA, ascorbate and dehydroascorbate contents, were used as the potentially explanatory variables (*X*-matrix) to develop a regression model of firmness and associated attributes. The corresponding correlations loadings plot (Fig. 2) shows that the model explained up to 80% of total variability in the *Y*-matrix, with principal component 1 (PC1) accounting alone for 69% thereof. Firmness was associated to RSA and $Na_2CO_3$ and KOH yields and, to a lower extent, to reduced ascorbate content and $CDTA_{sf}$ yields. In turn, weight loss was inversely correlated to the yields of these firmness-contributing cell fractions and to RSA, while decay showed an inverse correlation to RSA and reduced ascorbate content, suggesting a role for oxidative status in the progress of fungal infections. Interestingly, higher levels of cell wall solubilisation, as indicated by higher yields of PAW- and water-soluble fractions, were associated to higher contents of oxidised ascorbate (dehydroascorbic acid) and acetaldehyde, consistent with previous reports on the relationship between fruit softening and these metabolites (Fry, 1998; Janes and Frenkel, 1978; Pesis, 2005).

**Cell wall-modifying enzyme activities and their role in cell wall composition of 'Sweet Heart' cherries after storage**

Ripening-related modifications in cell wall composition and structure are

commonly attributed to the coordinated action of a number of specific enzymatic and non-enzymatic proteins on different cell wall polysaccharides. In this work, seven enzyme activities (five pectolytic; two non-pectolytic) were analysed at each sampling date. In order to assess a possible role in determining yield and composition of each cell wall fraction isolated from fruit (Table 3), a regression analysis was undertaken in which these activities were used as the potentially explanatory variables. Because data suggested that antioxidant status may have a role in determining some storage potential-related attributes, these results were also included in the *X*- matrix. This model explained up to 84% of total variability in yield and composition of cell wall fractions considered, and revealed some interesting correlations (Fig. 3).

PG and PL isoforms catalyse the cleavage of demethylated galacturonic acid residues from the backbone of pectic polymers through hydrolysis and β-elimination, respectively. Large research efforts have been traditionally focused on demonstrating a major role for PG action on fruit softening, whereas the possible involvement of pectate lyases in ripening-associated softening has received little attention until relatively recently. PL expression or activity has been reported during ripening of a few fruit species, including apple (Goulao et al., 2007; Ortiz et al., 2011a), strawberry (Medina-Escobar et al., 1997), banana (Domínguez-Puigjaner et al., 1997), peach (Brummell et al., 2004; Ortiz et al., 2011b) and nectarine (Dawson et al., 1992; Ortiz et al., 2010). In this work, a strong association was found between PG and PL activities and the water-soluble fraction of cell wall materials (Fig. 3), with correlation coefficients of 0.92 and 0.80, respectively. These two pectin backbone-acting enzyme activities were also highly correlated to total neutral sugars and uronic acid contents in the $CDTA_{sf}$, indicative of progressive solubilisation of cell wall pectins during ripening. Interestingly, these two enzyme activities, together with the hemicellulose-degrading EGase, showed an inverse correlation with the contents of ascorbic acid, again suggesting that antioxidant activity may have a role in preventing extensive cell wall disassembly. Ascorbate-generated hydroxyl radicals can cause non-enzymic scission of cell wall polysaccharydes and thus contribute to fruit softening. The ability to release ascorbate into the apoplast of tomato fruit has been shown to increase during ripening (Dumville and Fry, 2003), and de-esterified pectin was moreover more susceptible to ascorbate-induced scission *in vitro* than methylesterified pectin. This is also in agreement with results of this study, where we found a good correlation (r = 0.83) between PME activity and the contents of dehydroascorbic acid. PME catalyses demethylation of uronic acid residues, this demethylating action being required for subsequent PG- and PL-induced depolymerisation of polyuronides. PME activity and mode of action is highly dependent on apoplastic pH (Denès et al., 2000), which is known to decline during ripening (Almeida and Huber, 1999) thus approaching the optimal pH for this enzyme. PME action itself contributes to this pH decrease, and modifies substantially the activity and mobility of other cell wall proteins.

However, despite the finding that PG and PL activities were associated to

pectin solubilisation (Fig. 3), their correlation with $Na_2CO_{3sf}$ and $KOH_{sf}$ yields, observed previously to contribute largely to firmness (Fig. 2), was poor. It has been suggested that the highly branched structure of pectins may restrict the access of pectin backbone-acting enzymes to their substrates, thus limiting pectin degradation and solubilisation in spite of high levels of pectolytic activity (Goulao and Oliveira, 2008). Particular attention has been focused on galactosyl- and arabinosyl-containing side-chains, which are thought to control pore size in the cell wall and to protect cell wall polysaccharides from extensive depolymerisation by pectolytic enzymes. β-Gal and AFase act on these neutral sugar-rich pectin side-chains, and may therefore have a major role in pectin solubilisation. Actually, sweet cherry β-Gal has been partially purified and characterised, and suggested to contribute to cell wall hydrolysis during ripening-related fruit softening (Andrews and Li, 1994). Interestingly, pH optimum for sweet cherry β-Gal was found to be 4.0, which means that a steady decrease in apoplastic pH during ripening would favour progressive removal of galactosyl residues from pectic polymers, hence facilitating the solubilisation process. In this work, indeed, both β-Gal and AFase activities were found to correlate inversely with the yields of the pectin-containing fractions (Fig. 3), therefore supporting the idea of a major role in firmness loss. Particularly indicative in this regard was the observation of a good inverse correlation between uronic acid contents in the $Na_2CO_3$-soluble fraction and AFase activity levels (Fig. 3), consistent with the view that previous removal of arabinosyl-rich side-chains be required for subsequent PG- and PL- action on polyuronides.

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## References

[1] F. Agius, R. González-Lamothe, J.L. Caballero, J. Muñoz-Blanco, M.A. Botella and V. Valpuesta, Engineering increased vitamic C levels in plants by overexpressing of a D-galacturonic acid reductase, Nature Biotechnology, 21 (2003), 177-181.

[2] D.P.F. Almeida and D.J. Huber, Apoplastic pH and inorganic ion levels in tomato fruit: a potential means for regulation of cell wall metabolism during ripening, Physiologia Plantarum, 105 (1999), 506-512.

[3] J. Alonso, N. Howell and W. Canet, Purification and characterisation of two pectinmethylesterase from persimmon (*Diospyros kaki*), Journal of the Science of Food and Agriculture, 75 (1997), 352-358.

[4] P.K. Andrews and S. Li, Partial purification and characterization of β-D-galactosidase from sweet cherry, a nonclimacteric fruit, Journal of Agricultural and Food Chemistry, 42 (1994), 2177-2182.
[5] N. Blumenkrantz and G. Asboe-Hansen, New method for quantitative determination of uronic acids, Analytical Biochemistry, 54(1973), 484-489.
[6] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, Analytical Biochemistry, 72 (1976), 248-254.
[7] D.A. Brummell, V. Dal Cin, C.H. Crisosto and J.M. Labavitch, Cell wall metabolism during maturation, ripening and senescence of peach fruit, Journal of Experimental Botany, 55 (2004), 2029-2039.
[8] D.M. Dawson, L.D. Melton and C.B. Watkins, Cell wall changes in nectarines (*Prunus persica*). Solubilization and depolymerization of pectic and neutral polymers during ripening and in mealy fruit, Plant Physiology, 100 (1992), 1203-1210.
[9] J.M. Denès, A. Baron, C.M.G.C. Renard and C. Péan, J.F. Drilleau, Different action patterns for apple pectin methylesterase at pH 7.0 and 4.5, Carbohydrate Research, 327 (2000), 385-393.
[10] E. Domínguez-Puigjaner, I. Llop, M. Vendrell and S. Prat, A cDNA clone highly expressed in ripe banana fruit shows homology to pectate lyases, Plant Physiology, 14 (1997), 1071-1076.
[11] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, Colorimetric method for determination of sugars and related substances, Analytical Chemistry, 28 (1956), 350-356.
[12] J.C. Dumville and S.C. Fry, Solubilisation of tomato fruit pectins by ascorbate: a possible non-enzymic mechanism of fruit softening, Planta, 217 (2003), 951-961.
[13] S.C. Fry, Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals, Biochemical Journal, 332 (1998), 507-515.
[14] K.M Gillespie and E.A. Ainsworth, Measurement of reduced, oxidized and total ascorbate content in plants, Nature Protocols, 2 (2007), 871-874.
[15] L.F. Goulao and C.M. Oliveira, Cell wall modifications during fruit ripening: when a fruit is not the fruit, Trends in Food Science and Technology, 19 (2008), 4-25.
[16] L.F. Goulao, J. Santos, I. de Sousa and C.M. Oliveira, Patterns of enzymatic activity of cell wall-modifying enzymes during growth and ripening of apples, Postharvest Biology and Technology, 43 (2007), 307-318.
[17] A.E. Hagerman and P.J. Austin, Continuous spectrophotometric assay for plant pectin methyl-esterase, Journal of Agricultural and Food Chemistry, 34 (1986), 440-444.
[18] H.W. Janes and C. Frenkel, Promotion of softening processes in pear by acetaldehyde, independent of ethylene action, Journal of the American Society for Horticultural Science, 103 (1978), 397-400.

[19] D. Ke, E.M. Yahia, M. Mateos and A.A. Kader, Ethanolic fermentation of 'Bartlett' pears as influenced by ripening stage and atmospheric composition, Journal of the American Society for Horticultural Science, 119 (1994), 976-982.
[20] I. Lara, P. García and M. Vendrell, Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria* × *ananassa* Duch.) fruit, Postharvest Biology and Technology, 34 (2004), 331-339.
[21] S. Lohani, P.K. Trivedi and P. Nath, Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA, Postharvest Biology and Technology, 31 (2004), 119-126.
[22] L.M. McCune, C. Kubota, N.R. Stendell-Hollis and L.M. Thomson, Cherries and health: a review, Critical Reviews in Food Science and Nutrition, 51 (2011), 1-12.
[23] N. Medina-Escobar, J. Cárdenas, E. Moyano, J.L. Caballero and J. Muñoz-Blanco, Cloning, molecular characterization and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants, Plant Molecular Biology, 34 (1997), 867-877.
[24] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31 (1959), 426-428.
[25] F. Moran, S. Nasuno and M.P. Starr, Extracellular and intracelular polygalacturonic acid trans-eliminase of *Erwinia carotovora*, Archives of Biochemistry and Biophysics, 123 (1968), 298-306.
[26] G. Oms-Oliu, I. Odriozola-Serrano, R. Soliva-Fortuny and O. Martín-Belloso, Effects of high-intensity pulsed electric field processing conditions on lycopene, vitamin C and antioxidant capacity of watermelon juice, Food Chemistry, 115 (2009), 1312-1319.
[27] A. Ortiz, G.B. Seymour, G.A. Tucker and I. Lara, Cell wall disassembly during the melting phase of softening in 'Snow Queen' nectarines, Postharvest Biology and Technology 58 (2010), 88-92.
[28] A. Ortiz, J. Graell and I. Lara, Preharvest calcium applications inhibit some cell wall-modifying enzyme activities and delay cell wall disassembly at commercial harvest of 'Fuji Kiku-8' apples, Postharvest Biology and Technology, 62 (2011a), 161-167.
[29] A. Ortiz, M. Vendrell and I. Lara, Softening and cell wall metabolism in late-season peach in response to controlled atmosphere and 1-MCP treatment, Journal of Horticultural Science and Biotechnology, 86 (2011b), 175-181.
[30] N. Pathak and G.G. Sanwall, Multiple forms of polygalacturonase from banana fruits, Phytochemistry, 48 (1998), 249-255.
[31] E. Pesis, The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration, Postharvest Biology and Technology, 37 (2005), 1-19.
[32] R.J. Redgwell, L.D. Melton and D.J. Brasch, Cell wall dissolution in ripening kiwifruit (*Actinidia deliciosa*), Plant Physiology, 98 (1992), 71-81.

[33] G.S. Romano, E.D. Cittadini, B. Pugh and R. Schouten, Sweet cherry quality in the horticultural production chain, Stewart Postharvest Review, 6 (2006), 2.
[34] R.R. Selvendran and M.A. O'Neill, Isolation and analysis of cell walls from plant material. In: D. Glick (Ed.), Methods of Biochemical Analysis, vol. 32, John Wiley Interscience, New York, USA, 1987, 25-153.
[35] F.A. Tomás-Barberán and C. Andrés-Lacueva, Polyphenols and health: current state and progress, Journal of Agricultural and Food Chemistry, 60 (2012), 8773-8775.
[36] A.R. Vicente, M.L. Costa, G.A. Martínez, A.R. Chaves and P.M. Civello, Effect of heat treatments on cell wall degradation and softening in strawberry fruit, Postharvest Biology and Technology, 38 (2005), 213-222.
[37] J. Wei, M. Fengwang, S. Shi, X. Qi, Z. Zhu and J. Yuan, Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit, Postharvest Biology and Technology, 56 (2010), 147-154.

**Table 1.** Quality attributes of 'Sweet Heart' sweet cherry fruit at harvest and after storage.

| | | Days (0 ºC + 20 ºC) | | | |
|---|---|---|---|---|---|
| | **Harvest** | **15 + 0** | **15 + 3** | **30 + 0** | **30 + 3** |
| **Firmness** (Durofel units) | 81.15 a | 74.60 c | 73.22 d | 76.90 b | 76.40 b |
| **TA** ($g\ L^{-1}$) | 11.02 a | 9.75 b | 8.60 c | 8.63 c | 8.21 d |
| **SSC** (º Brix) | 19.07 a | 17.13 c | 18.33 b | 17.33 c | 18.13 b |
| **Juiciness** (mL 100 $g^{-1}$ FW) | 61.26 c | 61.87 c | 63.22 b | 65.72 a | 56.28 d |
| **Hue** (º) | 14.64 b | 15.56 a | 11.20 c | 15.46 a | 15.42 a |
| **Weight loss** (%) | - | 1.05 d | 5.10 b | 1.80 c | 6.93 a |
| **Stem browning** (% fruit affected) | - | 60.0 c | 80.0 b | 76.6 b | 90.0 a |
| **Decay** (% fruit affected) | - | 0.0 c | 26.7 b | 23.3 b | 60.0 a |
| **Acetaldehyde** ($\mu L\ L^{-1}$) | 1.52 bc | 1.53 bc | 1.43 c | 2.10 a | 1.67 b |
| **Ethanol** ($\mu L\ L^{-1}$) | 1.75 d | 6.59 b | 8.01 a | 5.78 c | 8.04 a |

Values represent means of three (SSC, TA, juiciness, weight loss, alterations, acetaldehyde, ethanol) or 30 (firmness, colour) replicates. Means followed by different letters within a row are significantly different at $P \leq 0.05$ (LSD test).

**Table 2.** Radical scavenging activity (RSA) and ascorbate content in 'Sweet Heart' sweet cherry fruit at harvest and after storage.

| | | Days (0 ºC + 20 ºC) | | | |
|---|---|---|---|---|---|
| | **Harvest** | **15 + 0** | **15 + 3** | **30 + 0** | **30 + 3** |
| **RSA** (%) | 37.33 a | 27.81 b | 23.70 c | 27.07 b | 23.19 c |
| **Ascorbic acid** (nmol $gDW^{-1}$) | 2013.9 b | 2172.7 a | 1728.6 c | 930.6 e | 1001.9 d |
| **Dehydroascorbic acid** (nmol $gDW^{-1}$) | 0.0 d | 72.9 c | 0.0 d | 1260.7 a | 574.3 b |

Values represent means of three replicates. Means followed by different letters within a row are significantly different at $P \leq 0.05$ (LSD test).

**Table 3.** Cell wall solubilisation (% FW, w/w) (**A**), yields of cell wall fractions (% CWM, w/w) (**B**), and neutral sugars and uronic acid contents in pectin-containing cell wall fractions (%, w/w) (**C**) isolated from 'Sweet Heart' sweet cherry fruit at harvest and after storage.

| | | Days (0 ºC + 20 ºC) | | | |
|---|---|---|---|---|---|
| **A** | **Harvest** | **15 + 0** | **15 + 3** | **30 + 0** | **30 + 3** |
| **CWM** [a] | 9.59 b | 6.16 c | 26.05 a | 9.66 b | 7.56 c |
| **$PAW_{sf}$** | 0.16 b | 0.18 b | 0.16 b | 0.26 a | 0.22 a |
| **B** | | | | | |
| **$Water_{sf}$** | 0.45 d | 2.55 b | 0.65 d | 1.65 c | 4.20 a |
| **$CDTA_{sf}$** | 19.40 b | 17.30 c | 13.45 d | 21.45 a | 15.90 c |
| **$Na_2CO_{3sf}$** | 12.90 a | 12.70 a | 4.90 c | 9.40 b | 10.45 b |
| **$KOH_{sf}$** | 3.00 a | 3.00 a | 0.50 c | 2.35 b | 1.90 b |
| **C** | | | | | |
| **Neutral sugars** | | | | | |
| **$CDTA_{sf}$** | 18.59 d | 23.77 b | 10.77 e | 20.42 c | 49.96 a |
| **$Na_2CO_{3sf}$** | 37.05 d | 40.42 c | 44.56 b | 39.97 c | 49.95 a |
| **Uronic acids** | | | | | |
| **$CDTA_{sf}$** | 10.72 e | 16.25 c | 13.39 d | 31.25 b | 39.92 a |
| **$Na_2CO_{3sf}$** | 45.83 b | 43.21 c | 33.05 d | 54.56 a | 45.94 b |

Values represent means of three replicates. Means followed by different letters within a row are significantly different at $P \leq 0.05$ (LSD test).

[a] CWM, insoluble cell wall material; $PAW_{sf}$, PAW-soluble cell wall material; $Water_{sf}$, water-soluble fraction; $CDTA_{sf}$, chelator-soluble fraction; $Na_2CO_{3sf}$, $Na_2CO_3$-soluble fraction; $KOH_{sf}$, KOH-soluble fraction.

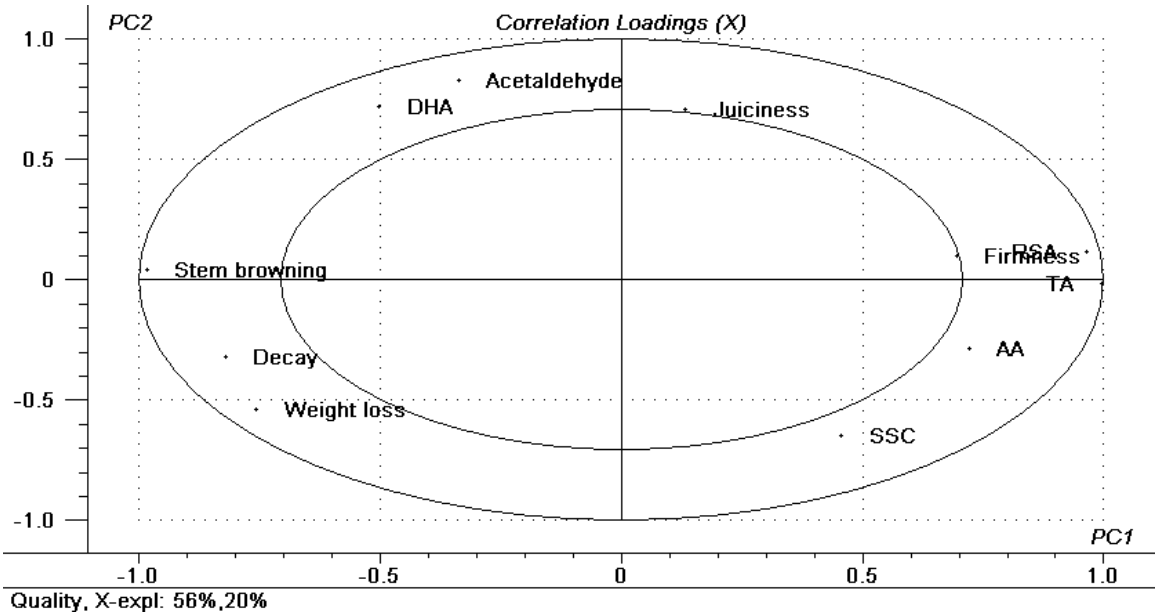


**Figure 1:** Correlation loadings plot corresponding to a PCA model for commercial quality and antioxidant capacity in 'Sweet Heart' sweet cherry fruit at harvest and after storage (AA, ascorbic acid; DHA, dehydroascorbic acid; RSA, radical scavenging activity; SSC, soluble solids content; TA, titratable acidity).

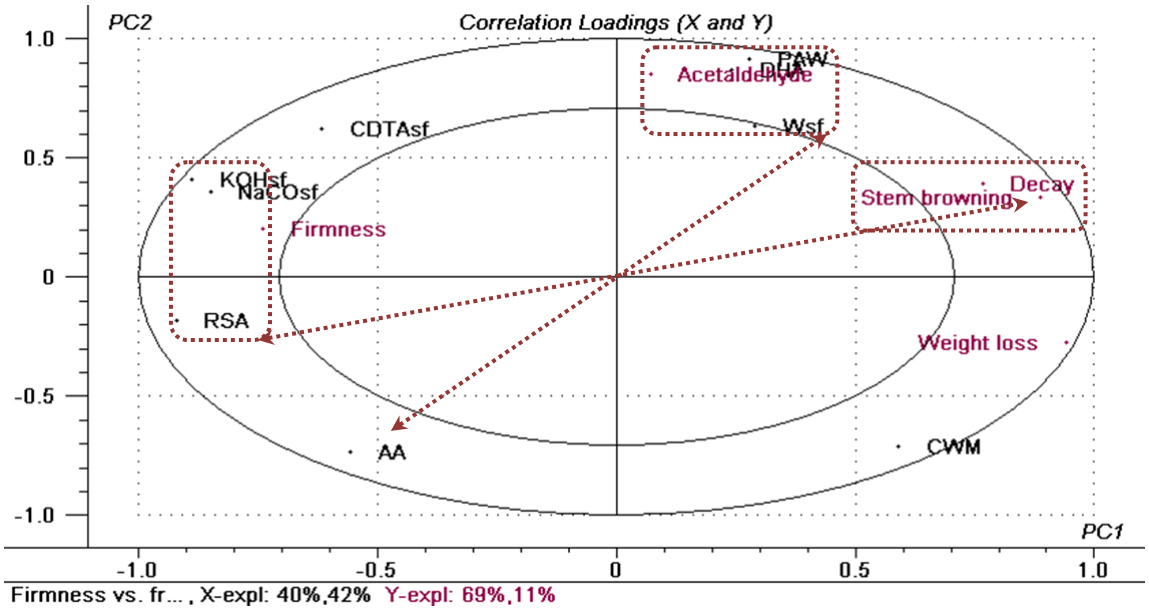


**Figure 2:** Correlation loadings plot of PC1 *vs.* PC2 corresponding to a PLSR model for storage potential-related attributes (*Y* variables) *vs.* cell wall fractions and antioxidant properties (*X* variables) in 'Sweet Heart' sweet cherry fruit at harvest and after storage (AA, ascorbic acid; DHA, dehydroascorbic acid; RSA, radical scavenging activity; CWM, insoluble cell wall material; PAW, PAW-soluble cell wall material; Wsf, water-soluble fraction; CDTAsf, chelator-soluble fraction; NaCOsf, $Na_2CO_3$-soluble fraction; KOHsf, KOH-soluble fraction).

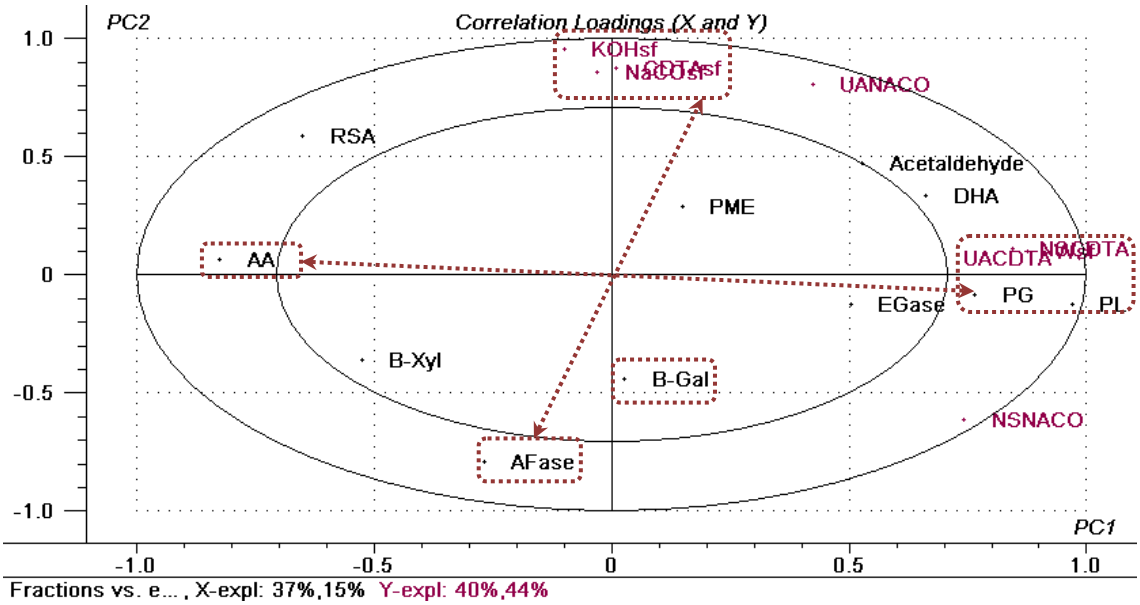


**Figure 3:** Correlation loadings plot of PC1 *vs.* PC2 corresponding to a PLSR model for yields and contents of uronic acids (UA) and neutral sugars (NS) of cell wall fractions (*Y* variables) *vs.* cell wall-modifying enzyme activities and antioxidant properties (*X* variables) in 'Sweet Heart' sweet cherry fruit at harvest and after storage (AA, ascorbic acid; DHA, dehydroascorbic acid; RSA, radical scavenging activity; CWM, insoluble cell wall material; PAW, PAW-soluble cell wall material; Wsf, water-soluble fraction; CDTAsf, chelator-soluble fraction; NaCOsf, $Na_2CO_3$-soluble fraction; KOHsf, KOH-soluble fraction; UACDTA and NSCDTA, uronic acid and neutral sugar contents in CDTAsf; UANACO and NSNACO, uronic acid and neutral sugar contents in NaCOsf).

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