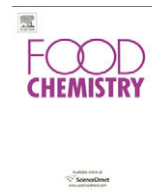
Food Chemistry 115 (2009) 1094–1101

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Analytical Methods

Effects of ripening on microstructure and texture of "Ameixa d'Elvas" candied plums

Cláudia Nunes [a], Conceição Santos [b], Glória Pinto [b], Sónia Silva [b], José A. Lopes-da-Silva [a], Jorge A. Saraiva [a], Manuel A. Coimbra [a,*]

[a] Departamento de Química, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[b] CESAM and Departamento de Biologia, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 12 February 2008
Accepted 24 December 2008

Keywords:
Prunus domestica
Green Gage
Microstructure
Histocytological studies
Pectin methyl esterase
Polygalacturonase
Cellulase

ABSTRACT

The plums used to produce a traditional candied product, "Ameixa d'Elvas", were obtained from two orchards, Vila Viçosa (VV) and Cano (CA). These orchards were selected because the fruits were behaving differently: (1) The day of harvesting for candying, established by the total soluble solids and titratable acidity, was one week earlier in VV; (2) VV yielded candied plums with good texture properties whereas CA gave poor processed fruits. In order to understand the origin of these differences, fruits from both orchards were harvested on the day established as the harvesting day for VV (day 1) and for CA (day 8). Comparable texture properties were obtained in firmness, rigidity and deformation work between the VV fresh plums harvested on day 1 and the CA plums harvested on day 8 but these were lower in CA when the flesh was analysed separately, in accordance with the activities of pectin methyl esterase (PME), polygalacturonase (PG) and cellulase (Cel). The increase of the intercellular area of parenchyma cells and the decrease in cell area caused by boiling, which resulted in a pronounced loss of textural properties, were partially recovered after the immersion of the fruits in sucrose syrup. The CA plums harvested on day 8 had a more pronounced degradation with boiling and lower recovery of cell shape, size and textural characteristics than had those of VV harvested on day 1. Upon candying, similar properties were observed for the fruits harvested on the same day: good candied products were observed for VV and CA fruits harvested on day 1 and poor candied products were observed for VV and CA fruits harvested on day 8. This work shows that the characteristics of the flesh of the fresh fruits are key parameters in defining the textural properties of the candied plum. The establishment of the harvesting moment for candying should take into account the changes that occur in the flesh of the plums during ripening.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

"Ameixa d'Elvas" is a protected designation of origin (PDO) recognised by the European Union for a candied plum (*Prunus domestica* L.) from Alto Alentejo (south–east of Portugal), obtained by a traditional candying process. Only the fruits of the 'Green Gage' variety, 'Rainha Cláudia Verde' can be utilised to produce candied "Ameixa d'Elvas". Briefly, the candying process consists of boiling the intact plums in water, followed by immersion in sugar syrup solutions of increasing concentrations up to 75 °Brix. The ripening stage of the plums, when harvested for candying, seems to be one of the most important factors influencing their final textural properties. For this reason, the plums used to produce "Ameixa d'Elvas" are harvested at a well defined maturation point evaluated by the total soluble solids (16 °Brix) and titratable acidity (1.0 meq malic acid/100 g fruit flesh weight).

The major textural changes, resulting in the softening of fruits, are due to enzyme-mediated alterations of the composition and structure of cell wall polysaccharides, such as pectic polysaccharides and cellulose, which leads to their partial solubilisation (Waldron, Smith, Parr, Ng, & Parker, 1997). The activity of certain enzymes is related to cell wall polysaccharide changes during the ripening process. Cell walls are complex in composition and structure and, thus, it is unlikely that any one particular enzyme would be able to significantly modify their properties. A combined action of a number of enzymes, acting synergistically, is a more plausible cause for the occurrence of the changes needed to impart any significant textural changes in fruits (Ali, Chin, & Lazan, 2004). Pectin methyl esterase (PME), polygalacturonase (PG), and cellulase (Cel) are among the enzymes generally recognised as having a crucial effect on fruit texture during ripening. The quantification of the activity of these enzymes is important to ascertain their contribution to the changes in texture due to the alteration of cell wall polysaccharides (Ali et al., 2004; Prasanna, Prabha, & Tharanathan, 2007; Wakabayashi, 2000).

* Corresponding author. Tel.: +351 234 370706; fax: +351 234 370084.
E-mail address: mac@ua.pt (M.A. Coimbra).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.12.089

The main contributors to textural losses during thermal processes are cell wall degradation, with the concomitant cell wall separation, and final collapse of the pectin network, extensively affecting cell adhesion. Cell disruption also influences a number of biochemical and chemical reactions, allowing substrate/enzyme and reagent contact, that also cause changes of texture (Aguilera, 2005). Cell structure modification of fruits caused by heat treatment has been shown to increase the rate of sucrose diffusion during fruit osmotic dehydration (Nieto, Salvatori, Castro, & Alzamora, 1998), which may also be relevant during the candying process.

In this work, plums from two orchards, Vila Viçosa (VV) and Cano (CA), within the PDO region, were studied. These two different orchards were selected because of the different behaviours of the plums during ripening and candying. CA plums usually had one week delay in ripening, evaluated by total soluble solids and titratable acidity, in relation to VV plums. Moreover, it is known that plums from the CA orchard, although presenting an adequate maturity stage, result in a candied product of low or even no commercial use, due to appreciable loss of tissue consistency and skin disruption (Nunes et al., 2008a). In contrast to CA plums, the fruits from VV give a candied product with good textural properties, when harvested at the appropriate maturity stage. In these fruits, differences in the structures of their cell wall polysaccharides and related enzyme activities were noticed (Nunes, Saraiva, & Coimbra, 2008b). In order to understand the origin of these differences, fruits from both orchards were harvested on the day established as the harvesting day for each orchard. The histocytological and textural properties of fresh plums were evaluated and the activities of the enzymes having a catalytic action on cell wall (PME, PG, and Cel) were quantified, to ascertain their influences on the texture of the plums. The plums from both orchards were candied and the textural properties of the tissues were measured in order to evaluate objectively the influence of the stage of ripening of the fruit at harvesting on the final textural quality of the candied products.

2. Materials and methods

2.1. Plant material and sample preparation

Plums (*P. domestica* L.) of 'Green Gage' variety, 'Rainha Cláudia Verde', were supplied by Fruteco-Fruticultura Integrada, Lda. (Borba, Portugal). They were collected in 2005 in two orchards, VV and CA, within the PDO region.

(a) VV and CA plums were collected in the day established as the harvesting day for the candying process, when the fruits reach 16 °Brix and a titratable acidity of 1.0 meq malic acid/100 g of fresh weight. The fruits harvested on day 1 (19th July), the harvesting day for VV plums, were designated VVf1 and CAf1, and the fruits harvested on day 8 (26th July), the harvesting day for CA plums, were designated VVf8 and CAf8, where f stands for fresh, and 1 or 8 stand for the collection day. Plums were brought immediately to the laboratory, the histocytological and texture analyses being carried out on the same day.
(b) VV plums were also collected 6 days before and 4 days after the first harvesting, on 13th and 22nd July, respectively.
(c) Fresh plums, harvested on day 1 and 8 from both orchards, were candied according to the traditional process, at the day of harvesting, on a laboratory scale. First, the plums were boiled in water for about 15 min (water:fruit ratio of 4:1), until their floatation occurred (some boiled plums were withdrawn to study the effect of the boiling step on texture and cell properties). Later, they were immersed in a 60 °Brix sucrose syrup (syrup:fruit ratio of 2:1). On the following day, the sugar solution was concentrated by addition of sucrose powder and heating to reach 65 °Brix and to reach 75 °Brix after 7 days. The plums were kept for two months in the 75 °Brix sugar syrup, at room temperature, the concentration of the sugar syrup being adjusted by heating when necessary (2 or 3 times), due to its hygroscopicity.

2.2. Total soluble solids, pH and titratable acidity

Total soluble solids, pH, and titratable acidity were determined in fresh plums. Plum juice was obtained by squeezing plum flesh and filtration through a glass fibre filter (Whatman GF/C). Total soluble solids and titratable acidity were determined on the filtrate. Total soluble solids (°Brix) were determined by measuring the refractive index of the juice with a hand refractometer (ATC-1E, Atago Co. Lda., Japan). Titratable acidity was measured using 6 g of juice diluted with 50 ml of distilled water, by titration with 0.1 M NaOH to an endpoint of pH 8.1, using an automatic pH–stat (Crison micro TT2022, Alella, Spain) and was expressed as meq of malic acid/100 ml juice.

2.3. Histocytological analyses

The histocytological analyses were performed on plums from VV and CA orchards without any processing (VVf1, VVf8, CAf1 and CAf8), boiled (VVb1, VVb8, Cab1 and Cab8) and candied (VVc1, VVc8, CAc1, and CAc8), where b and c stand for boiled and candied, respectively.

Material preparation and fixation, for light and scanning electron microscopy (SEM) analysis, was performed as previously described by Pinto (2007). Briefly, samples were fixed in 2.0 ml/100 ml glutaraldehyde in 0.04 M of PIPES buffer, pH 7.6 (Duchefa, Haarlem, The Netherlands) overnight at 4 °C and then washed in PIPES. Tissues were transferred to 1.0% (w/v) osmium tetroxide in PIPES buffer and dehydrated using increasingly concentrated ethanol solutions (30–100%, v/v).

For light microscopy, samples were embedded in an epoxi resin (Embed-812). Semi-thin sections (1 μm) were stained with aqueous 1% (w/v) methylene blue. Samples were analysed with a Nikon Eclipse 80i light microscope (Nikon Co., Kanagawa, Japan) and photographs were taken using a Leica DC 200 digital camera (Leica Microsystems AG, Germany).

For SEM analysis, ethanol dehydrated samples were further dehydrated by successive immersions in acetone solutions of increasing concentration (30–100%, v/v) and finally in a critical point device (Baltec CPD 030, USA), using CO_2 as transition agent. Samples were fixed on steel supports and coated with gold using a JEOL metalizer (FFC-1100, Japan) at 1100–1200 V, 5 mA for 10 min. Samples were observed in a scanning electronic microscope (Hitachi, S4100, Japan) at 20 kV.

Microstructure images were analysed using the image analysing program, UTHSCSA Image Tool, version 3.00 (University of Texas Health Science Center, USA). Cell axis, area, and intercellular area were estimated, based on optical microscopic and SEM images, at least, on three images from different pieces of each sample and five measurements were made in each image, resulting in a total of 15 measurements, for each of the three quantified parameters.

2.4. Fruit texture analyses

Fresh (VVf1, VVf8, CAf1 and CAf8), boiled (VVb1, VVb8, CAb1 and CAb8) and candied (VVc1, VVc8, CAc1 and CAc8) plums were

used for texture characterisation. Puncture tests were performed using a TA-Hdi texture analyser (Stable Micro Systems, Godalming, UK), equipped with a 5 kg load cell and a 2 mm diameter cylindrical stainless steel probe. Penetration was done at 1 mm/s to a depth of 15 mm. Each plum was cut by the longitudinal line and one half was analysed with skin and another half without skin, with puncturing carried out from the external plum surface, in both cases. At least six different fruits of each sample were used, and each half was punctured at five different locations, one in the middle and four around the middle, at the corners of a square, 7–10 mm apart from the middle punctured point. Firmness (the peak maximum force), rigidity (the maximum initial slope of the force–displacement curve obtained during the puncture tests), and the deformation work applied to the sample (calculated as the area below the force–displacement curve until the maximum force), during the downstroke puncture, were used to characterise the textural properties of the samples.

2.5. Preparation of cell wall material

Fresh plums (500 g) from both orchards (VVf1, VVf8, CAf1 and CAf8) were dispersed in ethanol (2 l) at a final concentration of 85% (v/v) and boiled for 10 min. The mixture was cooled and filtered through a glass fibre filter (Whatman GF/C), and the residue was then washed with diethyl ether and allowed to dry at room temperature. The dried material constituted the alcohol-insoluble residue (AIR).

2.6. Carbohydrate analysis

Neutral sugars were obtained by sulfuric acid hydrolysis (Selvendran, March, & Ring, 1979) and analysed after conversion to their alditol acetates by GC, using 2-deoxyglucose as internal standard (Coimbra, Delgadillo, Waldron, & Selvendran, 1996). A Carlo Erba 6000 GC apparatus, with split injector and a FID detector, was used, equipped with a 30 m column DB-225 (J&W) with i.d. and film thickness of 0.25 mm and 0.15 μm, respectively. The oven temperature programme used was: initial temperature 200 °C, a rise in temperature at a rate of 40 °C/min to 220 °C and then 220 °C for 14 min, followed by an increase to 230 °C at rate of 20 °C/min, this temperature being maintained for 1 min. The injector and detector temperatures were, respectively, 220 and 230 °C. The flow rate of the carrier gas (H_2) was set at 1 ml/min.

Uronic acids (UA) were quantified by a modification (Coimbra et al., 1996) of the 3-phenylphenol colorimetric method (Blumenkrantz & Asboe-Hansen, 1973), using a calibration curve made with D-galacturonic acid. Samples were prepared by hydrolysis in 0.2 ml of 72% H_2SO_4 for 3 h at room temperature, followed by 1 h in 1 M H_2SO_4 at 100 °C. The hydrolyses of all samples were done in duplicate and each sample was injected twice.

2.7. Quantification of enzymatic activity

The enzymatic activities of PME, PG and Cel were quantified in all fresh plums harvested from both orchards. An enzymatic extract was obtained, based on a procedure described by Denès, Baron, and Drilleau (2000). One hundred grammes of plum pulp were homogenised in 100 ml of 0.2 M Tris(hydroxymethyl)-aminomethane buffer (Tris buffer), at pH 7.0, containing 500 mg/l of sodium metabisulfite ($Na_2S_2O_5$) and 1% polyvinylpolypyrrolidone (PVPP). After extraction (2 h at 4 °C), the suspension was centrifuged at 20,000 *g* for 15 min at 4 °C and the supernatant obtained was used as the source of soluble fraction (SF) enzymatic extract. The pellet was dispersed in the same buffer, but containing 1 M NaCl, and stirred for 2 h at 4 °C, followed by centrifugation (20,000 *g* for 15 min). The supernatant obtained was used as the source of cell wall ionically-linked fraction (IF) enzymatic extract. The remaining pellet was used to determine the activity of the cell wall strongly-linked enzymatic fraction, usually called covalently-linked enzymatic fraction (CF). Enzymatic activities were quantified for SF, IF and CF, because the relative activity of the three forms and the different interactions of the three forms with the cell wall components can imply a different mobility capacity and a different capacity to act on cell wall polysaccharides. Enzymatic activity was determined in triplicate and expressed on a plum pulp weight basis, for all enzymes. Total activity was calculated by summing the average activities of the three fractions.

Pectin methyl esterase (PME) activity was measured by continuous recording of the titration of carboxyl groups formed in a pectin solution, using an automatic pH–stat (Crison micro TT2022, Alella, Spain) and a 0.01 M NaOH solution (Nunes et al., 2006). Assays were performed with a 3.5 mg/ml of apple pectin solution (DE 75%, 30 ml) containing 0.117 M NaCl at pH 7.0 and 25 °C. Activity was quantified by adding, to 15 ml of pectin solution, 0.5 ml of enzyme extract, for SF and IF extracts, or approximately 1 g of the remaining pellet for the CF. One unit (U) of PME activity was defined as the amount of enzyme necessary to generate 1 μmol of carboxyl groups per min, under the previously mentioned assay conditions.

Polygalacturonase (PG) activity was quantified according to the method described by Gross (1982), which is based on the measurement of reducing groups formed using polygalacturonic acid as substrate. The substrate solution contained 0.4% (w/v) polygalacturonic acid in 0.05 M sodium acetate buffer (pH 4.5) and the reaction was carried out by adding 0.2 ml of enzyme extract (SF and IF) or 0.1 g (CF), followed by incubation at 35 °C for 10 min. The reaction was stopped with 2 ml of 10 mM borate buffer at pH 9 and 0.4 ml of 1% (w/v) 2-cyanoacetamide. The mixture was put in a boiled water bath for 10 min and, after cooling, the absorbance at 276 nm was measured. The amount of reducing groups formed was determined using a calibration curve made with D-galacturonic acid and the enzyme activity was expressed as nmol of galacturonic acid released per min. One unit (U) of PG activity was defined as the amount of enzyme that yielded 1 nmol of reducing groups per min.

The activity of cellulase (Cel, EC 3.2.1.4) was measured by determining the viscosity of a carboxymethylcellulose substrate solution, before and after the action of the enzyme. Viscosity was measured using a Cannon–Fenske capillary viscometer (75 mm), and the substrate solution consisted of a 0.1% (w/v) carboxymethylcellulose in 0.1 M acetate buffer at pH 4 (Lohani, Trivedi, & Nath, 2004). In a standard assay 0.50 ml of acetate buffer, 0.50 ml of enzymatic extract, adequately diluted (SF and IF), or a proper amount (typically about 1 g) of solid residue and 0.50 ml more of buffer (CF), were added to 4 ml of substrate solution. The enzymatic reaction took place at 30 °C during 60 min. Activity of Cel was expressed as the relative decrease of viscosity, in relation to an assay without enzyme. One unit (U) of Cel activity was defined as the amount of enzyme that caused a 1% reduction of viscosity per min.

2.8. Statistical analysis

Quantitative analyses are presented as mean values and the reproducibility of the results is expressed as standard deviation in Tables and as standard error bars in Figures. Statistical analysis of the experimental results was carried out based on the *F*-test and Student's *t* test (Microsoft Excel, Microsoft Corporation, Redmond, USA). Significant differences were considered at the level of $p < 0.05$.

Table 1
Weight, total soluble solids (°Brix), titratable acidity (meq of malic acid/100 g), and pH of fresh plums from Vila Viçosa (VV) and Cano (CA) orchards.

Sample	Day	Weight (g)	Total soluble solids (°Brix)	Titratable acidity (meq of malic acid/100 g)	pH
Vila Viçosa					
VVf-5	13 Jul 05	35 ± 1[a]	14.5 ± 0.1[a]	1.14 ± 0.03[a]	3.19 ± 0.01[a]
VVf1	19 Jul 05	35 ± 1[a]	16.1 ± 0.1[b]	1.03 ± 0.01[b]	3.22 ± 0.01[a]
VVf4	22 Jul 05	36 ± 1[a]	17.1 ± 0.2[c]	1.00 ± 0.04[b]	3.22 ± 0.01[a]
VVf8	26 Jul 05	36 ± 1[a]	19.6 ± 0.1[d]	0.90 ± 0.01[c]	3.32 ± 0.02[b]
Cano					
CAf1	19 Jul 05	33 ± 1[b]	13.7 ± 0.1[e]	1.18 ± 0.01[a]	3.16 ± 0.01[a]
CAf8	26 Jul 05	33 ± 1[b]	15.7 ± 0.2[b]	1.04 ± 0.01[b]	3.27 ± 0.02[b]

Means ± standard deviation ($n = 3$). Within columns, means with different superscripts are significantly ($p < 0.05$) different.

3. Results and discussion

3.1. General

According to Table 1, VV plums reached 16 °Brix and a titratable acidity of 1.0 meq of malic acid /100 g of fresh weight, the maturity parameters considered optimum to harvest these fruits for candying purposes, on the 19th of July (day 1) whereas CA achieved these values only on the 26th of July (day 8). On the 19th of July, the plums from the CA orchard showed only 13.7 °Brix and a titratable acidity of 1.18, which shows an earlier maturity stage than that presented by VV fruits on the 13th of July, almost one week before its optimum day for harvesting. This difference, which had already been noticed in previous years (Nunes et al., 2008a; Nunes et al., 2008b), shows that VV plums might be at a more advanced stage of ripening than CA.

3.2. Fresh plums analysis

3.2.1. Histocytological and texture analysis

In both orchards, VV and CA, parenchymatous tissue of fresh plums harvested at day 1 (VVf1 and CAf1) showed isodiametric parenchyma cells with large dimensions (diameter of 2.7–2.9 μm and area of 5.9–6.1 μm^2) and a well defined middle lamella with an intercellular area of 0.02 μm^2 (Table 2). Parenchyma cells with a more irregular shape and with a loss of cell-to-cell contact were observed on day 8, representing a decrease in cell area of 33–35% and an increase in intercellular area of 66% for VV and 34% for CA (Table 2) with evident cell separation (Fig. 1). With the evolution of the ripening stage, fruits show progressively more cells with irregular shapes and larger intercellular areas (Redgwell et al., 1997). Vascular strands, composed of xylem and phloem tissues, appeared to be diffusely distributed in the parenchyma and no changes were observed with ripening or between orchards. Comparing the VVf1 fruits with CAf8, significant histocytological differences were observed only in cell area (25% higher for VVf1). During fruit ripening, there is a change in the mode of tissue failure, from only cell rupture to some cell separation, a process already observed for other fruits (Heyes & Sealey, 1996; Mafra et al., 2001).

The values of firmness, rigidity, and deformation work of fruits are associated, respectively, with the cell wall strength, cell wall to cell wall adhesion and cell turgor (Heyes & Sealey, 1996). CAf1 tissues had firmness and deformation work values higher than VVf1 tissues only when the fruits were analysed with skin, but both fruits showed similar flesh texture (Table 3). Also, no significant differences were observed between CAf8 and VVf8. Ripening caused a 36–44% decrease in firmness and deformation work for the plums without skin whereas, with skin, the decrease was 32% for CA and no significant difference was observed for VV. A significant decrease in rigidity with ripening was observed only in the fruits without skin. Although no significant differences were observed for firmness, rigidity and deformation work between VVf1 and CAf8 plums analysed with skin, the texture parameters measured only in the flesh revealed that the tissues of VVf1 plums had significantly higher values than CAf8.

Table 2
Cell diameter, area, and intercellular area of parenchymatous cells of fresh (VVf and CAf), boiled (VVb and CAb) and candied (VVc and CAc) plums from Vila Viçosa and Cano harvested at day 1 and day 8.

Sample	Cell diameter (μm)	Cell area (μm^2)	Intercellular area (μm^2)
Fresh			
VVf1	2.93 ± 0.47[a]	6.08 ± 0.68[a]	0.02 ± 0.00[a]
VVf8	2.11 ± 0.45[a]	3.94 ± 0.62[b]	0.05 ± 0.01[b]
CAf1	2.67 ± 0.63[a]	5.88 ± 0.73[a]	0.02 ± 0.00[a]
CAf8	2.49 ± 0.27[a]	4.59 ± 0.23[b]	0.03 ± 0.01[a]
Boiled			
VVb1	2.46 ± 0.24[a]	4.79 ± 0.18[b]	0.11 ± 0.03[c]
VVb8	2.13 ± 0.28[a]	3.68 ± 0.25[b]	0.09 ± 0.02[c]
CAb1	1.97 ± 0.34[a]	3.51 ± 0.37[b]	0.21 ± 0.05[d]
CAb8	1.48 ± 0.17[b]	2.93 ± 0.09[c]	0.35 ± 0.05[e]
Candied			
VVc1	3.80 ± 0.66[c]	8.66 ± 0.37[d]	0.04 ± 0.01[b]
VVc8	3.22 ± 0.34[c]	7.23 ± 0.37[e]	0.06 ± 0.01[b]
CAc1	2.85 ± 0.49[a]	6.56 ± 0.74[e]	0.06 ± 0.02[b]
CAc8	2.27 ± 0.40[a]	5.15 ± 0.50[a]	0.05 ± 0.01[b]

Means ± standard deviation ($n = 15$). Within columns, means with different superscripts are significantly ($p < 0.05$) different.

3.2.2. Cell wall enzymatic activity

The activities of PME, PG and Cel were quantified in VVf1, VVf8, CAf1 and CAf8 plums. In addition, to have a more precise idea of the tendency of the activity of these enzymes, assays were also done for the VV plums harvested at an early stage of maturation (VVf-5) and between day 1 and day 8 (VVf4). All enzyme activities were quantified on the soluble (SF), ionically bound (IF), and strongly bound (CF) to cell wall fractions.

PME activity was found mostly in the IF (about two thirds of the total activity) and the evolution of the total activity followed the evolution pattern of the IF (Fig. 2). PME activity increased slightly until the 1st day of harvesting for VV plums and then decreased at the 4th and 8th harvesting days to about half of its maximum activity value. In the CA orchard, total PME activity also decreased from CAf1 to CAf8. PG activity was mainly observed on the CF and an increase of the activity was observed throughout ripening. Total PG activity increased by around 40% from the 1st day to the 8th day of harvesting in both orchards. The increase of PG activity with ripening occurred when the activity of PME began to decrease. This is in agreement with many other reports of a similar pattern for the evolution of the activity of the two enzymes with ripening in other fruits (Barrett & Gonzalez, 1994; Lohani et al., 2004; Majumder & Mazumdar, 2002; Sethu, Prabha, & Tharanathan, 1996; Wakabayashi, Hoson, & Huber, 2003; Zhou, Ben-Arie, & Lurie, 2000). PME and PG actions on the methyl de-esterification and glycoside cleav-

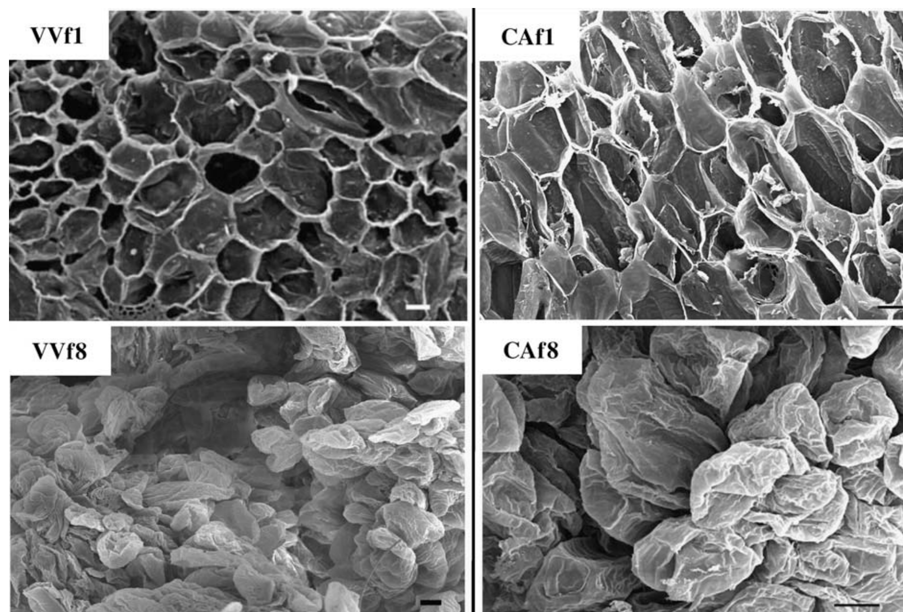


Fig. 1. Scanning micrographs of plums from Vila Viçosa and Cano orchard showing regions of parenchymatous cells (bar 100 μm). Plums harvested at day 1 (VVf1 and CAf1) and harvested at day 8 (VVf8 and CAf8).

Table 3
Firmness, rigidity, and deformation work values of fresh (VVf and CAf), boiled (VVb and CAb) and candied (VVc and CAc) plums from Vila Viçosa and Cano harvested at day 1 and day 8.

Sample	Firmness (N)		Rigidity (N/mm)		Work (J × 10^3)	
	Flesh + skin	Flesh	Flesh + skin	Flesh	Flesh + skin	Flesh
Fresh						
VVf1	5.01 ± 0.56^a	3.66 ± 0.48^a	2.28 ± 0.38^a	1.66 ± 0.29^a	5.07 ± 0.51^a	3.66 ± 0.42^a
VVf8	4.40 ± 0.47^a	2.44 ± 0.29^b	1.83 ± 0.37^a	1.03 ± 0.27^b	4.46 ± 0.46^a	2.36 ± 0.28^b
CAf1	6.32 ± 0.72^b	4.33 ± 0.52^a	3.00 ± 0.46^a	2.31 ± 0.43^a	6.31 ± 0.79^b	4.33 ± 0.58^a
CAf8	4.30 ± 0.58^a	2.36 ± 0.28^b	2.43 ± 0.29^a	1.36 ± 0.25^b	4.34 ± 0.58^a	2.44 ± 0.29^b
Boiled						
VVb1	0.93 ± 0.18^c	0.22 ± 0.05^c	0.17 ± 0.03^b	0.06 ± 0.01^c	1.39 ± 0.27^c	0.34 ± 0.08^c
VVb8	0.32 ± 0.07^d	0.19 ± 0.05^c	0.05 ± 0.01^c	0.04 ± 0.01^c	0.49 ± 0.11^d	0.29 ± 0.07^c
CAb1	0.91 ± 0.33^c	0.47 ± 0.15^d	0.14 ± 0.04^b	0.09 ± 0.04^c	1.36 ± 0.49^c	0.71 ± 0.22^d
CAb8	0.46 ± 0.09^d	0.31 ± 0.12^d	0.06 ± 0.01^c	0.04 ± 0.02^c	0.69 ± 0.14^d	0.47 ± 0.18^c
Candied						
VVc1	3.46 ± 0.56^a	1.60 ± 0.32^e	0.42 ± 0.09^d	0.26 ± 0.04^d	6.91 ± 0.81^b	3.20 ± 0.63^a
VVc8	2.46 ± 0.35^e	0.67 ± 0.16^d	0.20 ± 0.05^b	0.09 ± 0.03^c	4.93 ± 0.71^a	1.33 ± 0.33^b
CAc1	3.15 ± 0.46^a	1.05 ± 0.45^e	0.44 ± 0.09^d	0.34 ± 0.07^d	6.30 ± 0.91^b	2.10 ± 0.91^b
CAc8	2.35 ± 0.40^e	0.93 ± 0.31^d	0.19 ± 0.05^b	0.21 ± 0.04^d	4.69 ± 0.80^a	1.86 ± 0.61^b

Means ± standard deviation (n = 30). Within columns, means with different superscripts are significantly (p < 0.05) different.

age of the pectic polysaccharides of the middle lamella might contribute to the increase of the intercellular area observed in the histocytological studies and to the decrease of the measured texture parameters.

The total activity of Cel increased in both VV and CA plums during ripening, occurring mainly in SF and CF. The increase of Cel activity, in addition to the increase of PG activity, is a characteristic of ripened fruits (Fischer & Bennett, 1991; Iannetta, van den Berg, Wheatley, McNicol, & Davies, 1999; Jain, Dhawan, Malhotra, & Singh, 2003; Lohani et al., 2004; Prasanna et al., 2007; Sethu et al., 1996). Texture loss during ripening has also been correlated with the increase of Cel activity for other fruits (Barrett and Gonzalez, 1994; Lohani et al., 2004; Prasanna et al., 2007; Sethu et al., 1996).

Plums from the CA orchard presented comparable values for total PME and PG activities when compared with those from VV for the same day (Fig. 2). The total Cel activity at the 1st harvesting day was 60% higher for CA plums but similar on day 8. These results show that CA and VV plums were at the same stage of ripening on the same day regarding cell wall enzyme activity, despite differences in the total soluble solids content and titratable acidity previously discussed. Comparing the enzymatic activity of VVf1 with CAf8, the harvesting days to processing the plums according to these parameters, a 32% higher value was obtained for PME activity whereas 44% and 55% lower values were found for PG and Cel activities, respectively. A similar tendency was previously observed for these plums harvested in 2003 (Nunes et al., 2008b). These results are in accordance with the observed lower texture parameters found for the flesh of CAf8 when compared to VVf1. The increase of PG and Cel activities during ripening should contribute to the loss of texture through the action on cell wall pectic polysaccharides and cellulose, changing their structure and solubilising the cell wall components (Ali et al., 2004; Waldron et al., 1997).

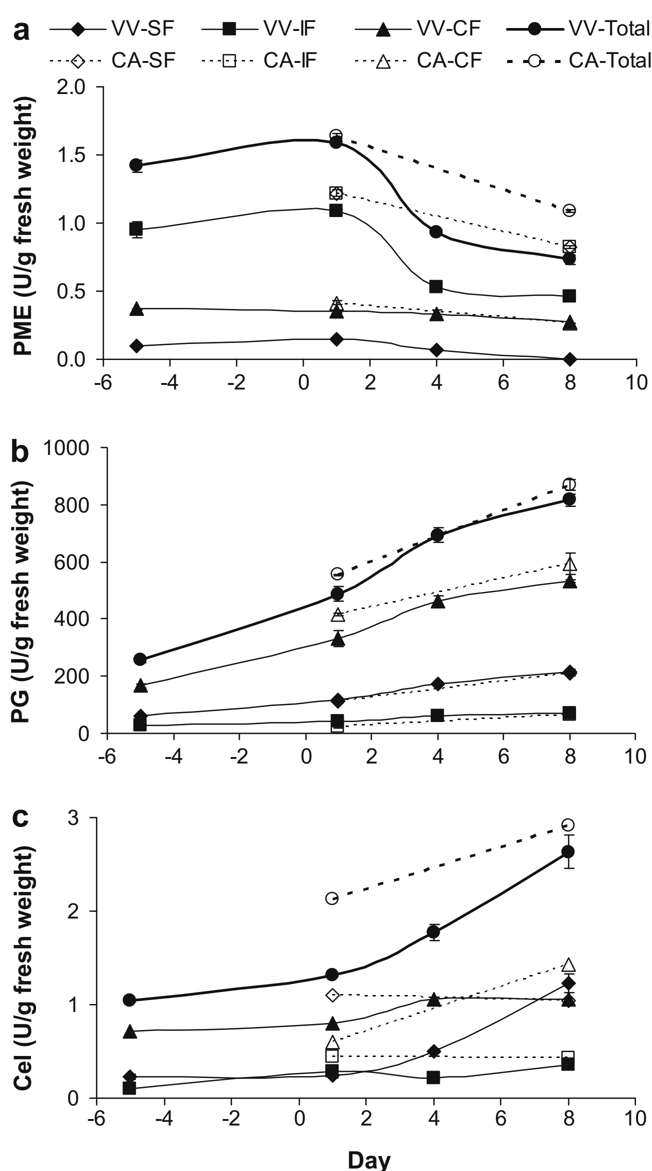


Fig. 2. Changes in activity of: (a) PME, (b) PG, and (c) Cel in fresh plums from Vila Viçosa and Cano orchards during ripening. SF, soluble fraction; IF, ionically-linked fraction; CF strongly-linked enzymatic fraction.

3.2.3. Cell wall composition

The AIR was used to obtain and characterise the polymeric material of fresh plums of both orchards at days 1 and 8 (Table 4). An average of 4–5% of polymeric material was extracted for the four samples of fresh plums analysed. The total sugars content ranged from 20 to 28 mg/g of fruit flesh and increased with ripening by about 30% for the VV and 20% for the CA orchards (Table 4). Uronic acids (UA) are the most abundant components of the plum cell wall polysaccharides, followed by glucose, arabinose and galactose. This sugars composition allows us to infer that pectic polysaccharides, composed of galacturonic acid, Ara, and Gal, together with cellulose, are abundant in plums. The main effect of ripening, in both orchards, is an increase in the AIR of the amounts of all sugars, but mainly UA (35–40%). The ratio UA/(Ara + Gal), which relates the proportion of galacturonic acid residues of pectic polysaccharides to the proportion of sugar components of their side chains, increased in both orchards, from 1.7 in VVf1 to 2.0 in VVf8, and from 1.5 in CAf1 to 1.8 in CAf8, indicating that the pectic polysaccharides became less branched with ripening. These results, associated with the results of the activity of PME and PG, imply solubilisation and depolymerisation of the cell wall pectic polysaccharides with ripening. These changes in cell wall composition are related to the decrease in fruit tissue firmness during the ripening process observed by the histocytological and texture analyses.

Between orchards, no significant differences were found for any sugars analysed when samples VVf1 and CAf1 and VVf8 and CAf8 were compared. Although no significant differences were observed between the AIR of VVf1 and CAf8 in the ratio UA/(Ara + Gal), total sugars were 26% higher in CAf8, as well as the proportion of UA that was 29% higher, suggesting that cell wall polysaccharides of CA plums were in a more advanced stage of ripening than that indicated by the total soluble solids and titratable acidity of the fruit. A similar tendency was previously observed for these plums harvested in 2003 (Nunes et al., 2008b).

3.3. Boiled plums analysis

In order to evaluate the effect of ripening of the fruits on the textural properties of the candied products, fruits of VV and CA orchards at the two harvesting days, 1 and 8, were processed, to give the samples VVb1 and CAb1, and VVb8 and CAb8, respectively. Boiled plums from both orchards showed degradation of parenchyma tissue and vascular strands, as shown by Nunes et al. (2008a). The shapes of the parenchyma cells (constituted now by cell walls and protoplast debris) became irregular and cell walls appeared detached from neighbouring cells when compared to fresh plums. A decrease in parenchyma cell area with the boiling treatment was observed for all plums analysed, this reduction being lower for VV (7–21%) than for CA (36–40%) plums at both stages of ripening (Table 2). Boiling also increased intercellular areas significantly for both orchards (45–85% in VV and 86–91% in CA), at the two stages of ripening.

Texture analysis of boiled plums tissues showed a sharp decrease in firmness (82–94%), rigidity (93–98%), and deformation work (72–91%), when compared to the fresh plums for the two orchards and the two days of harvesting (Table 3), in accordance

Table 4
Sugars composition of AIR extracts of fresh plums from Vila Viçosa (VVf) and Cano (CAf) orchards, at the 1st and 8th harvesting days.

Sample	Yield[a] (mg/g)	Cell wall sugars (mg/g fruit)								
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	Total
Vila Viçosa										
VVf1	43	0.4 ± 0.1	0.3 ± 0.0	2.4 ± 0.1	0.7 ± 0.0	0.5 ± 0.0	2.2 ± 0.1	5.3 ± 0.4	7.8 ± 0.8	19.6
VVf8	48	0.4 ± 0.0	0.2 ± 0.0	3.5 ± 0.2	0.8 ± 0.0	0.6 ± 0.1	3.4 ± 0.2	6.0 ± 0.4	13.5 ± 1.3	28.4
Cano										
CAf1	40	0.4 ± 0.0	0.3 ± 0.0	2.6 ± 0.4	0.9 ± 0.0	0.6 ± 0.1	2.4 ± 0.2	6.7 ± 0.3	7.3 ± 0.6	21.0
CAf8	42	0.4 ± 0.0	0.2 ± 0.0	2.9 ± 0.3	0.8 ± 0.1	0.7 ± 0.0	3.1 ± 0.2	7.2 ± 0.2	11.0 ± 1.3	26.4

Means ± standard deviation ($n = 4$).
[a] Yield is expressed in mg of dry weight material per g of fresh weight plum.

with the observations made for the plums harvested in 2003 (Nunes et al., 2008a). This result was expected, since thermal processing at high temperatures (100 °C and 15 min in the present study) causes considerable softening of vegetable tissues. This was due to the loss of turgor pressure and cell adhesion of parenchyma cells, as was observed by the decrease of cell area and the increase of the intercellular area by the microstructure analysis (Table 2).

For both orchards, the values obtained for the three texture parameters were significantly higher on the 1st harvesting day (with skin) when compared to the 8th harvesting day. For plums analysed without skin, no significant differences were observed with ripening. These results indicate that the extent of texture loss of the plum tissue, caused by boiling, is affected by the stage of ripening of the fruits, and that the skin has an important influence on the results obtained for the texture parameters. The lower firmness and rigidity obtained for the boiled plums at day 8 are in accordance with the smaller cell area and greater intercellular area of the plums from the 8th day of harvesting.

Boiled CA plums, compared to VV, showed a 48–74% higher intercellular area and 20–30% lower cell diameter and 20–27% lower area (Table 2). These differences are particularly pronounced for the 8th day of harvesting. Although, for the same day of harvesting, the values for the three texture parameters obtained with skin showed no significant differences between orchards, the firmness values measured without skin were different for VV and CA boiled samples harvested on the same day. Also, higher values for the deformation work were observed for CAb1 when compared to VVb1. Firmness, rigidity, and deformation work were significantly higher for VVb1 than for CAb8 plums analysed with skin. It seems that the extensive softness of the flesh, in all samples, prevented significant texture differences between them.

3.4. Candied plums analysis

The irregular shape of the boiled plum cells changed to a more spherical shape in candied plums (Nunes et al., 2008a), considerably increasing their diameter (65–75%) and area (53–65%), compared to the boiled plums (Table 2). Concomitantly with the recovery of cell wall diameter and area, candied plums showed an important reduction in intercellular area (34–64% for VV and 70–86% for CA). The recovery of the tissue structure, shown by the microstructure analysis, was also confirmed by the texture analysis of candied plums, which revealed much higher values for the three texture parameters (67–87% for firmness, 56–81% for rigidity, and 66–90% for deformation work), when compared with the boiled plums (Table 3). However, the values were lower than those obtained for the fresh plums, with the exception of the deformation work with skin, where values similar to those obtained for the fresh plums were observed. Similar results were obtained for these plums harvested in 2003 (Nunes et al., 2008a).

For both orchards, plums harvested at the 8th harvesting day presented a cell area of candied plums significantly smaller, 16% for VV and 21% for CA, than plums harvested at the 1st day. Firmness, rigidity, and deformation work values were significantly lower for plums from the 8th harvesting day compared with the 1st harvesting day. As was observed for the boiled plums, these results implicate the importance of the ripening stage of the plums for the recovery of cell shape and size after candying and consequently for the texture of candied plums. Plums in a more advanced stage of ripening give rise to a candied product with lower texture quality.

VV candied plums from both harvesting days showed cell diameters and areas even higher than those of fresh plums, while CA candied plums showed cell diameters and areas similar to those obtained for the CA fresh plums (Table 2). VV candied plums presented higher cell diameters and areas than CA candied plums. The values of all texture parameters studied were significantly higher for VVc1 than for CAc8, except the flesh rigidity. However, no significant differences in the texture parameters of the plums were found between orchards, with or without skin, on the same day of harvesting (Table 3). These results indicated that candied plums showed a recovery of the texture parameters similar to those on the same day of harvesting for both orchards, which is in accordance with enzymatic activity and cell wall polysaccharide analysis.

4. Conclusions

Cell wall enzymes activity and changes in cell wall polysaccharides are associated with changes in the tissue structure and texture of the fruits during ripening, which influence the characteristics of the plums during the thermal and candying process.

Histocytological data (cell diameter, cell area and intercellular area), texture data (firmness, rigidity and deformation work), activities of PME, PG and Cel, and cell wall polysaccharide composition, revealed that CA orchard plums are, probably, at a more advanced stage of ripening than that ascertained by the total soluble solids content and titratable acidity. Therefore, these conventional established parameters alone are not enough to accurately evaluate the stage of ripening of these plums for candying purposes.

Since the ripening stage is of prime importance for the textural characteristics of "Ameixa d'Elvas" candied plums, it is essential to precisely identify the most adequate maturation stage of plums to be processed.

Acknowledgements

We thank Fruteco-Fruticultura Integrada, Lda., Borba, Portugal, for providing the samples and Prof. João Mota Barroso and Ana Elisa Rato, from Universidade de Évora, Portugal, for helpful discussions about "Ameixa d'Elvas" plums. Cláudia Nunes and Sónia Silva thank FCT (Portugal) for the PhD Grants No. SFRH/BD/12928/2003 and SFRH/BD/32257/2006 and Glória Pinto for the Post-Doctoral grant SFRH/BPD/26434/2006. Thanks are also due to Project AGRO 220 and FCT, Portugal, for funding the Research Unit 62/94 "Química Orgânica, Produtos Naturais e Agro-Alimentares".

References

Aguilera, J. M. (2005). Why food microstructure? *Journal of Food Engineering, 67*(1–2), 3–11.

Ali, Z. M., Chin, L.-H., & Lazan, H. (2004). A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Science, 167*(2), 317–327.

Barrett, D. M., & Gonzalez, C. (1994). Activity of softening enzymes during cherry maturation. *Journal of Food Science, 59*(3), 574–577.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484–489.

Coimbra, M. A., Delgadillo, I., Waldron, K. W., & Selvendran, R. R. (1996). Isolation and analysis of cell wall polymers from olive pulp. *Modern Methods of Plant Analysis, 17*, 19–44.

Denès, J. M., Baron, A., & Drilleau, J. F. (2000). Purification, properties and heat inactivation of pectin methylesterase from apple (cv Golden Delicious). *Journal of the Science of Food and Agriculture, 80*(10), 1503–1509.

Fischer, R. L., & Bennett, A. B. (1991). Role of cell wall hydrolases in fruit ripening. *Annual Review of Plant Physiology and Plant Molecular Biology, 42*, 675–703.

Gross, K. C. (1982). A rapid and sensitive spectrophotometric method for assaying poligalacturonase using 2-cyanoacetamide. *HortScience, 17*, 933–934.

Heyes, J. A., & Sealey, D. F. (1996). Textural changes during nectarine (*Prunus persica*) development, and ripening. *Scientia Horticulturae, 65*(1), 49–58.

Iannetta, P. P. M., van den Berg, J., Wheatley, R. E., McNicol, R. J., & Davies, H. V. (1999). The role of ethylene and cell wall modifying enzymes in raspberry (*Rubus idaeus*) fruit ripening. *Physiologia Plantarum, 105*(2), 338–347.

Jain, N., Dhawan, K., Malhotra, S., & Singh, R. (2003). Biochemistry of fruit ripening of guava (*Psidium guajava* L.): Compositional and enzymatic changes. *Plant Foods for Human Nutrition, 58*(4), 309–315.

Lohani, S., Trivedi, P. K., & Nath, P. (2004). 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*(2), 119–126.

Mafra, I., Lanza, B., Reis, A., Marsilio, V., Campestre, C., De Angelis, M., et al. (2001). Effect of ripening on texture, microstructure and cell wall polysaccharide composition of olive fruit (*Olea europaea*). *Physiologia Plantarum, 111*(4), 439–447.

Majumder, K., & Mazumdar, B. C. (2002). Changes of pectic substances in developing fruits of cape-gooseberry (*Physalis peruviana* L.) in relation to the enzyme activity and evolution of ethylene. *Scientia Horticulturae, 96*(1-4), 91–101.

Nieto, A., Salvatori, D., Castro, M. A., & Alzamora, S. M. (1998). Air drying behaviour of apples as affected by blanching and glucose impregnation. *Journal of Food Engineering, 36*(1), 63–79.

Nunes, C. S., Castro, S. M., Saraiva, J. A., Coimbra, M. A., Hendrickx, M. E., & Van Loey, A. M. (2006). Thermal and high-pressure stability of purified pectin methylesterase from plums (*Prunus domestica*). *Journal of Food Biochemistry, 30*(2), 138–154.

Nunes, C., Santos, C., Pinto, G., Lopes-da-Silva, J. A., Saraiva, J. A., & Coimbra, M. A. (2008a). Effect of candying on microstructure and texture of plums (*Prunus domestica* L.). *LWT - Food Science and Technology, 41*, 1776–1783.

Nunes, C., Saraiva, J. A., & Coimbra, M. A. (2008b). Effect of candying on cell wall polysaccharides of plums (*Prunus domestica* L.) and influence of cell wall enzymes. *Food Chemistry, 111*, 538–548.

Pinto, G. (2007). Eucalyptus globulus plant regeneration via somatic embryogenesis. Ph.D. Thesis. Aveiro: University of Aveiro.

Prasanna, V., Prabha, T. N., & Tharanathan, R. N. (2007). Fruit ripening phenomena – An overview. *Critical Reviews in Food Science and Nutrition, 47*(1), 1–19.

Redgwell, R. J., MacRae, E., Hallett, I., Fischer, M., Perry, J., & Harker, R. (1997). In vivo and in vitro swelling of cell walls during fruit ripening. *Planta, 203*(2), 162–173.

Selvendran, R. R., March, J. F., & Ring, S. G. (1979). Determination of aldoses and uronic acid content of vegetable fiber. *Analytical Biochemistry, 96*(2), 282–292.

Sethu, K. M. P., Prabha, T. N., & Tharanathan, R. N. (1996). Post-harvest biochemical changes associated with the softening phenomenon in *Capsicum annuum* fruits. *Phytochemistry, 42*(4), 961–966.

Wakabayashi, K. (2000). Changes in cell wall polysaccharides during fruit ripening. *Journal of Plant Research, 113*(1111), 231–237.

Wakabayashi, K., Hoson, T., & Huber, D. J. (2003). Methyl de-esterification as a major factor regulating the extent of pectin depolymerization during fruit ripening: A comparison of the action of avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) polygalacturonases. *Journal of Plant Physiology, 160*(6), 667–673.

Waldron, K. W., Smith, A. C., Parr, A. J., Ng, A., & Parker, M. L. (1997). New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends in Food Science and Technology, 8*(7), 213–221.

Zhou, H.-W., Ben-Arie, R., & Lurie, S. (2000). Pectin esterase, polygalacturonase and gel formation in peach pectin fractions. *Phytochemistry, 55*(3), 191–195.