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# Effect of candying on cell wall polysaccharides of plums (*Prunus domestica* L.) and influence of cell wall enzymes

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ABSTRACT

"Ameixa d'Elvas" is a candied plum (*Prunus domestica* L.) produced by a traditional process, using fruits of a specific 'greengage' variety, "Rainha Cláudia Verde". The candying process consists of boiling the intact plums in water for 15 min and then putting them in sugar syrup, which is successively concentrated until 75 °Brix. Although a loss of intercellular adhesion of parenchyma cells after boiling is observed, candied plums are able to recover their cell-to-cell adhesion, giving a final tissue with a consistency similar to that observed for the fresh fruit. In order to explain this observation, cell wall polysaccharides of plums harvested in two orchards, Vila Viçosa (VV) and Cano (CA), from the same geographic region and at the same stage of ripening, were analysed fresh, boiled and candied. Plum cell walls are composed mainly of pectic polysaccharides and cellulose that, during the boiling step, are degraded and solubilised. Highly esterified pectic polysaccharides undergo gelation inside the fruits in the presence of sucrose, leading to the recovery of the fruit's consistency. During the candying process diffusion of these methylesterified pectic polysaccharides to the sucrose syrup increase the syrup viscosity. The activity of pectin methylesterase, polygalacturonase, and cellulase of fresh fruits explains the observed higher extension of degradation of cell wall polysaccharides of the CA plum tissues after boiling. This higher degradation seems to prevent the complete recovery of the parenchyma cell structure, which was observed for the less degraded polysaccharides of VV plums.



## 1. Introduction

The relative amounts of cell wall polysaccharides, as well as the type, length, and branching pattern of their side-chains have a profound effect on the structure of the cell walls and, consequently, in the texture of fruits (Van Buren, 1979; Wakabayashi, 2000; Waldron, Smith, Parr, Ng, & Parker, 1997). Generally, fruit cell walls contain a large amount of pectic polysaccharides, being the major constituents of the middle lamella and thus contributing to the cell adhesion mechanism and cell packing (Harker, Stec, Hallett, & Bennett, 1997; Waldron et al., 1997). Hemicelluloses, like xyloglucans and cellulose, also play a significant structural function in the cell wall, thus influencing the textural properties of the fruits. Cellulose microfibrils and xyloglucans form a strong structure around each cell, which is embedded in a matrix of interconnected pectic polysaccharides (Vincken et al., 2003). These interactions between the semi-rigid cellulose microfibrils and the less rigid polysaccharide molecules also play a key role in the mechanical properties of the fruits. The cell wall polysaccharide composition is characteristic for each fruit and is important in understanding its texture. Concerning plums, as far as we know, no study is yet available on this subject.

Heat processing promotes softening of fruit tissue, resulting in the increase of middle lamella cell separation and/or wall weakening, due to the depolymerisation of the methylesterified pectic polysaccharides by a mechanism of β-eliminative degradation (Van Buren, 1979; Waldron et al., 1997), exhibiting a lower average molecular weight. The proportion of neutral sugar side-chains is also an important factor contributing to textural changes during the heating process, since the side-chains of the pectic polysaccharides have been shown to interact with hemicelluloses, e.g., xyloglucans and cellulose (Prasanna, Prabha, & Tharanathan, 2007). The amount and the molecular mass of xyloglucans decrease with heat processing and a partial breakdown of the cellulose–xyloglucan network could occur, decreasing the integrity of the cell wall architecture. In addition, the solubilisation of hemicelluloses and cellulose have been also related to texture loss after heating (Mafra, Barros, & Coimbra, 2006a; Mafra, Barros, & Coimbra, 2007). A relevant technological aspect of cell structure modification of fruits caused by heat treatment is the increase of the flow rate diffusion of sucrose during fruits osmotic dehydration, as was observed for apples (Nieto, Salvatori, Castro, & Alzamora, 1998).

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The decline in cell wall strength and cell-to-cell adhesion resulting in tissue softening in fresh fruits is also associated with fruit ripening, where structural changes in all cell wall polysaccharides occur (Brummell, Dal Cin, Lurie, Crisosto, & Labavitch, 2004; Femenia, Sanchez, Simal, & Rossello, 1998; Mafra et al., 2006b; Wakabayashi, 2000). Pectin methylesterase (PME) catalyses the hydrolysis of methylester groups from galacturonosyl residues of pectic polysaccharides. PME play an important role in determining the extent to which demethylesterified pectic polysaccharides are accessible to degradation by polygalacturonases (PG) (Fischer & Bennett, 1991). High pectin demethylesterification, as catalysed by PME, also has the purpose of modifying the pH and the cation-exchange properties of the walls, which modulates the activity of other wall-degrading enzymes (Ali, Chin, & Lazan, 2004; Barnavon et al., 2001; Micheli, 2001). Cell wall loosening has also been explained by the disruption of non-covalent bonds between xyloglucan and cellulose (Cutillas-Iturralde, Pena, Zarra, & Lorences, 1998). The remarkably high levels of cellulase have been implicated in the change of cellulose fibril organisation and decrease of xyloglucan molecular weight, as shown in a large number of fruits. The partial breakdown of the cellulose–xyloglucan network decreases the integrity of the cell wall, which may increase the wall pore size and could also enhance the mobility of other hydrolases in cell walls (Mafra et al., 2006b; Prasanna et al., 2007; Rosli, Civello, & Martinez, 2004; Wakabayashi, 2000; Yashoda, Prabha, & Tharanathan, 2005).

Plums (*Prunus domestica* L.) of a special type of 'greengage' variety, "Rainha Cláudia Verde", from Alto Alentejo (South-East of Portugal) can be utilised to obtain a traditional candied plum, "Ameixa d'Elvas", which has a protected designation of origin (PDO) recognised by the European Union. The candying process consists in boiling the intact plums in water for 15 min and then put them in sugar syrup, which is successively concentrated until 75 °Brix. The main objective of this work is to study the cell wall polysaccharides of plums and to evaluate the changes that occur during candying. Also, analysis of cell wall degrading enzymes was performed on the fresh fruits to better evaluate their stage of maturity. In addition, analysis of polysaccharides was performed in the final sucrose syrup, to evaluate polysaccharide diffusion from the fruits to the liquid media.

## 2. Materials and methods

### 2.1. Plant material

Plums (*P. domestica* L.) of 'greengage' variety 'Rainha Cláudia Verde' were collected at the established stage of ripening suitable for candying (16–17 °Brix, pH 3.3, and titratable acidity of 1 meq of malic acid per 100 g of fresh weight) in two orchards, Vila Viçosa (VV) and Cano (CA). The candying process was carried out by boiling the plums in water (water:fruit ratio of 4:1) for 15 min, followed by immersion of the intact boiled plums in 60 °Brix sucrose syrup (syrup:fruit ratio of 2:1) for one day. The sucrose solution was then concentrated until 65 °Brix and, after 7 days, until 75 °Brix. The plums were stored two months in the 75 °Brix sucrose syrup, its concentration being occasionally (2–3 times) corrected due to its hygroscopicity.

The analyses of cell wall polysaccharides were performed on fresh, boiled, and candied plums. The plums were collected in the factory (Confibor Lda; Estremoz, Portugal), brought immediately to the laboratory and were frozen with liquid nitrogen and maintained at −20 °C until analysis. The sucrose syrup was used for the study of polysaccharides and was also maintained at −20 °C until analysis.

### 2.2. Preparation of cell wall material

Plums (500 g) were destoned and the flesh was 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). The residue was dispersed again in ethanol, boiled for 10 min, and filtered. The residue was then washed with diethyl ether and allowed to dry at room temperature. The dried material was named the alcohol insoluble residue (AIR).

### 2.3. Sequential extraction of AIR

The AIR was extracted according to the method described by Mafra et al. (2001). The AIR (8 g) was sequentially extracted with: (1) water, 600 ml for 16 h at 4 °C; (2) water, 500 ml for 6 h at room temperature; (3) 0.5 M imidazole/HCl pH 7.0, 600 ml of solution for 16 h at room temperature; (4) 0.5 M imidazole/HCl pH 7.0, 500 ml of solution for 2 h at room temperature; (5) 50 mM $Na_2CO_3$ + 20 mM $NaBH_4$, 600 ml, for 16 h at 4 °C; (6) 50 mM $Na_2CO_3$ + 20 mM $NaBH_4$, 500 ml, for 2 h at room temperature; (7) 0.5 M KOH + 20 mM $NaBH_4$, 500 ml, for 2 h at 4 °C; (8) 1 M KOH + 20 mM $NaBH_4$, 500 ml, for 2 h at 4 °C; (9) 1 M KOH + 20 mM $NaBH_4$, 500 ml, for 2 h at room temperature; (10) 4 M KOH + 20 mM $NaBH_4$, 500 ml, for 2 h at room temperature; (11) 4 M KOH + 3.5% $H_3BO_3$ + 20 mM $NaBH_4$, 500 ml, for 2 h at room temperature; and (12) 8 M KOH + 20 mM $NaBH_4$, 500 ml, for 2 h at room temperature. The KOH extractions were carried out with $O_2$-free solutions under $N_2$. After each extraction, the solubilised polymers were separated from the insoluble residue by centrifugation (24,400*g* for 10 min at 4 °C) followed by filtration of the supernatant through a glass fibre filter (Whatman GF/C). The $Na_2CO_3$ and KOH extracts were neutralised to pH 5–6, in the cold, with glacial acetic acid, prior to dialysis. The residue (cellulosic residue – CR) obtained after the alkali extractions was suspended in water, neutralised (pH 5–6) and dialysed. After dialysis, all extracts were concentrated under reduced pressure and precipitates were collected separately by centrifugation (24,400*g* for 10 min at 4 °C). The supernatant from the dialysis of the CR was collected separately from the residue by centrifugation and filtration. All extracts were frozen and freeze-dried.

### 2.4. Carbohydrate analysis

Monosaccharides were released from cell wall polysaccharides by a pre-hydrolysis in 0.2 ml of 72% $H_2SO_4$ for 3 h at room temperature, followed by 2.5 h hydrolysis in 1 M $H_2SO_4$ at 100 °C (Selvendran, March, & Ring, 1979). Neutral sugars were analysed after conversion to their alditol acetates by GC, using 2-deoxyglucose as internal standard (Coimbra, Deldadillo, Waldron, & Selvendran, 1996) and GC analysis as described by Nunes, Rocha, Saraiva, and Coimbra (2006). Cellulosic glucose was calculated as the difference between the content found with and without 72% $H_2SO_4$ pre-hydrolysis.

Uronic acids (UA) were quantified by a modification (Coimbra et al., 1996) of the 3-phenylphenol colorimetric method (Blumenkrantz & Asboe-Hansen, 1973). 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. A calibration curve was made with D-galacturonic acid.

The hydrolysis of all samples was done in duplicate and each one was injected twice. A third analysis was done for the few samples with higher variability.

### 2.5. Determination of the degree of methylesterification and acetylation

The determination of the degree of methylesterification and acetylation of the pectic polysaccharides was based on the estimate of methanol and acetic acid contents released after saponification (Waldron & Selvendran, 1990), as described previously by Nunes, Rocha et al. (2006). The methanol and acetic acid were extracted from the headspace of the HCl acidified (pH 2) aqueous solution by solid phase microextraction (HS-SPME), using a DVB/Carboxen/PDMS fibre. The analytes were separated by gas chromatography and detected using a flame ionisation detector (GC-FID). External calibration curves were used. All measurements were made with at least three replicates. Blanks were run in between each set of experiments. The degree of methylesterification was calculated in relation to mol% of uronic acids and the acetylation in relation to mol% of the total sugars determined for the extracts analysed.

### 2.6. Quantification of enzymatic activity

The extraction of plum enzymes was performed according to a slightly modified method described by Denès, Baron, and Drilleau (2000), to prevent loss of activity by phenolic inhibition. The flesh of plums (100 g) was homogenised with the addition of 100 ml 0.2 M Tris(hydroxymethyl)-aminomethane (Tris) buffer, at pH 7, containing 500 mg/l sodium metabisulfite ($Na_2S_2O_5$) and 1% PVPP (polyvinylpolypyrrolidone). 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 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 enzymatic extract. The pellet was used to determine the activity of the enzymes strongly linked to the cell wall, which were called cell wall covalently-linked enzymatic fraction.

Enzymatic activity quantification was determined in triplicate and expressed on plum fresh weight basis, for all enzymes. Total activity was calculated as the sum of the average activity of the three fractions.

#### 2.6.1. Pectin methylesterase (PME)

PME activity was measured by continuous recording of titration of carboxyl groups released from 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 apple pectin solution (degree of esterification 75%, 30 ml) containing 0.117 M NaCl at pH 7.0 and 25 °C. The quantification of activity was performed adding, to 15 ml of pectin solution, 0.5 ml of enzyme extract, for soluble and ionic extracts, or approximately 1 g solid residue, for the covalent fraction. The blank assays were performed by adding each enzymatic extract to 15 ml of water. The value obtained for the possible formation of carboxyl groups was subtracted from the previously measured enzyme activity. 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.

#### 2.6.2. Polygalacturonase (PG)

The quantification of PG activity was based on the method described by Gross (1982). The method consists of the measurement of the released reducing groups from polygalacturonic acid. The substrate solution contained 0.4% (w/v) polygalacturonic acid in 0.05 M sodium acetate buffer (pH 4.5). The reaction was carried out by adding 0.2 ml of enzyme extract (soluble and ionic fractions) or 0.1 g for covalent fraction, and incubating 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 blank assay was done adding the enzymatic extracts only, after the addition of borate buffer and 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 released reducing groups was determined by comparing with a calibration curve made with D-galacturonic acid, and the enzyme activity was expressed as nmol of galacturonic acid released per min.

#### 2.6.3. Cellulase (Cel)

The method of quantification of Cel activity consists on the measurement of the solution viscosity, using a Cannon–Fenske capillary viscometer (75 mm), of the substrate solution before and after the action of the enzyme during 60 min at 30 °C. The substrate solution consisted of 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 adequately diluted enzymatic extract (soluble and ionic fractions), a proper amount of solid residue (covalent fraction) and 0.50 ml more buffer were added to 4 ml of substrate solution. One Cel unit (U) was defined as 1% viscosity reduction per min.

#### 2.6.4. Viscosimetric analysis

The viscosity of the sugar syrups was determined using shear stress measurements, at 25 °C, with a TA AR-1000 rheometer (TA Instruments Ltd., Crawley, UK) fitted with a cone-plate device (4 cm and 6 cm, 2°).

### 2.7. Statistical analysis

Results are presented as mean value and the reproducibility of the results was expressed as standard deviation in tables and as error bars in figures. Statistical analysis between experimental results was based on Student's *t*-test. Significant difference was statistically considered at the level of $p < 0.05$.

## 3. Results and discussion

Cell wall polysaccharides composition of plums and the changes that occur during candying were studied. The fruits collected in two orchards, Vila Viçosa (VV) and Cano (CA), from the same geographic region within the PDO zone, and at the same stage of ripening, were analysed in fresh plums, after boiling, and after candying.

### 3.1. Cell wall material composition

Table 1 shows the physical characteristics of the fruits and the yield and sugars composition of the alcohol insoluble residues (AIR) prepared using fruits from two orchards, VV and CA. The fresh plums from VV had an average weight of 42 g and a volume of 34 ml/fruit, whereas those of CA were, on average, 33% lighter and 26% smaller. After the boiling process, the fruits increased their weight and volume by about 10–15%, due to the increase in their water content. However, the candied samples from the two orchards showed different weight and volume change tendencies with processing. In relation to the fresh fruits, those from VV decreased in weight (6%) and volume (12%), whereas those from CA increased in weight (14%) and recovered the volume measured for the fresh fruits. These results may be explained by the balance between the loss of water (75%) and the diffusion of sucrose into the flesh, resulting in a final product with only 22–23% of water. This balance was negative for bigger fruits and positive for the smaller ones.

**Table 1**
Physical characteristics and sugar composition of AIR extracts of fresh, boiled, and candied plums from Vila Viçosa (VV) and Cano (CA) orchards

| Orchard | Fresh | | Boiled | | Candied | |
|---|---|---|---|---|---|---|
| | VV | CA | VV | CA | VV | CA |
| Fruit weight (g) | 41.9 ± 0.2[a] | 27.7 ± 1.3[b] | 47.2 ± 1.7[c] | 30.2 ± 2.9[b,d] | 39.2 ± 0.2[e] | 32.3 ± 0.7[d] |
| Volume (ml) | 34 ± 1 | 25 ± 1 | 38 ± 2 | 29 ± 1 | 30 ± 2 | 25 ± 1 |
| Moisture (%) | 84 ± 1 | 85 ± 0 | 88 ± 0 | 92 ± 1 | 22 ± 1 | 23 ± 0 |
| AIR yield[1] (g/kg) | 33 | 33 | 37 | 41 | 15 | 19 |
| AIR yield[2] (mg/fruit) | 749 | 658 | 617 | 387 | 247 | 399 |
| AIR polysaccharides (mg/fruit) | 338 | 417 | 241 | 223 | 144 | 192 |
| AIR sugars (mg/fruit) | | | | | | |
| Rha | 7 ± 1 | 4 ± 0 | 5 ± 1 | 4 ± 0 | 3 ± 0 | 4 ± 0 |
| Fuc | 5 ± 0 | 3 ± 1 | – | 3 ± 0 | 2 ± 0 | 3 ± 0 |
| Ara | 56 ± 2 | 60 ± 1 | 33 ± 1 | 33 ± 2 | 25 ± 3 | 32 ± 1 |
| Xyl | 14 ± 1 | 13 ± 0 | 11 ± 1 | 11 ± 1 | 9 ± 0 | 11 ± 0 |
| Man | 9 ± 1 | 6 ± 1 | 5 ± 1 | 5 ± 1 | 3 ± 0 | 5 ± 0 |
| Gal | 58 ± 5 | 58 ± 1 | 43 ± 2 | 39 ± 1 | 29 ± 2 | 33 ± 1 |
| Glc | 43 ± 21 | 87 ± 1 | 41 ± 5 | 29 ± 1 | 14 ± 0 | 21 ± 1 |
| UA | 147 ± 11 | 185 ± 20 | 103 ± 10 | 100 ± 7 | 58 ± 5 | 82 ± 3 |

Mean ± standard deviation ($n = 4$).
Means with the same superscript letter are not significantly ($p > 0.05$) different.
[1] Yield is expressed in g of dry weight material per kg of fresh weight plum.
[2] Yield is expressed in mg of dry weight material per plum.

The AIR was used to obtain the polymeric material. On a fresh weight basis of the flesh, the AIR represented 3.3%, 3.7–4.1%, and 1.5–1.9% of fresh, boiled, and candied fruits. These values show that the amount of polymeric material present in the flesh of the plums in the different stages of processing is, apparently, independent of the size of the fruit. However, on a fruit basis, the AIR yield was only slightly higher in VV (0.75 g) than in CA (0.66 g), which suggests a relatively higher cell wall content in the smaller fruits. After boiling, the content of AIR in VV decreased 18%, whereas a higher decrease in the content of AIR (41%) was observed in CA. This result suggests a higher degradation and/or solubilisation of this polymeric material in aqueous media. The lowest AIR yields were obtained for the candied fruits. In these samples, the amount of AIR in VV was 0.25 g, decreasing its amount 67% in relation to the amount present in the fresh fruits, whereas the amount of AIR in CA was 0.40 g, decreasing its amount 40% in relation to the amount present in the fresh fruits and maintaining its amount in comparison to the boiled ones. These results show a larger loss of polymeric material from fresh to candied plums in VV (0.50 g/fruit) than in CA (0.26 g). According to the sugars analysis of the AIR (Table 1), the polysaccharides represent, in all samples, nearly 60% of the AIR. As a consequence, the relative loss of polysaccharides during processing was similar to that observed for the AIR: 0.50 and 0.26 g/fruit, in VV and CA, representing a 60% and 54% decrease, respectively, from fresh to candied fruits. The diffusing of the polymeric material to the aqueous media seems to explain its lower amount in the final product. This is in accordance with previous observations showing that, during osmotic dehydration of fruits in sucrose solutions, a two-way mass transfer is established. Water and water-soluble substances (sugars, vitamins, pigments, organic acids, mineral salts, etc.) flow out of the fruit into the solution, while in the opposite direction sucrose is transferred from the solution to the fruits (Giraldo, Talens, Fito, & Chiralt, 2003; Peiró, Dias, Camacho, & Martinez-Navarrete, 2006).

Uronic acids are the most abundant sugars of the cell wall polysaccharides of the plums. Also abundant are arabinose, galactose, and glucose. This sugar composition implies that pectic polysaccharides, composed of uronic acid, Ara and Gal, together with cellulose, are abundant in plums. All of these sugars decrease their amount per fruit during the processing, showing the same tendency observed for the amount of AIR and polysaccharides, that is, a higher decrease with boiling in CA, whereas in VV the higher decrease is observed from boiled to candied fruits. In order to observe the composition of the cell wall polysaccharides with processing, and explain the differences in the sugars composition of the AIR, this material was submitted to a sequential extraction with aqueous solvents and the sugars present in all extracts were analysed. The results are shown in Tables 2–4 for the fresh, boiled, and candied plums, respectively.

### 3.2. Pectic polysaccharides

The extracts rich in pectic polysaccharides are recovered with water, with aqueous solutions of chelating agents, and with solutions of dilute carbonate (Coimbra et al., 1996). Also, it has been shown that in the supernatant of the dialysis of the cellulosic residue obtained after neutralisation (snCR) is a fraction mainly composed of pectic polysaccharides (Ferreira, Mafra, Soares, Evtuguin, & Coimbra, 2006).

The extraction of the AIR with water and imidazole allowed the extraction of 7.4–9.3% of polymeric material from fresh, 9.5–9.8% from the boiled, and 22.3–27.1% from the candied plums. These extracts were very rich in polysaccharides in all samples (63–90%), and the main sugars were those characteristic of pectic polysaccharides. The ratio Ara/Gal was nearly 1 in all these pectic polysaccharide samples. However, the ratio of the amount of uronic acid to the amount of Ara + Gal changed from 4 in fresh to 2 in boiled and candied plums, showing that the pectic polysaccharides recovered with water and imidazole from boiled and candied fruits were more branched than those recovered in these extracts from fresh plums. The UA/(Ara + Gal) ratio was 8 in fresh plums in the extracts obtained from the dilute sodium carbonate solutions at 4 °C, and 2 in the extracts obtained at 22 °C. As observed for the water and imidazole extracts, in these carbonate-extracted pectic polysaccharides, the UA/(Ara + Gal) ratio also decreased. In boiled samples, the ratio was 5 and 1, for the carbonate extracts obtained at 4 °C and 22 °C, respectively, and 2 and 0.5 in candied samples. In the snCR fraction, the recovered pectic polysaccharides had an UA/(Ara + Gal) ratio of 0.5 in all samples. The relatively high proportion of Ara and Gal in the final residue, as well as the presence of UA, suggests the occurrence of a fraction of highly-branched pectic polysaccharides that were not recovered from the polymeric matrix with the solvents used. This was more relevant in the fresh fruits than in the processed ones. The increase of branched pectic polysaccharides with heat processing was also observed for other fruits and vegetables, which is related with the loss of firmness

**Table 2**
Sugar composition of fractions of fresh plum AIR obtained by sequential extraction with aqueous solvents for Vila Viçosa (VV) and Cano (CA) orchards

| Extract | Orchard | Yield[a] (%) | Cell wall sugars (mol%) | | | | | | | | Total (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | UA | |
| Water | VV | 4.3 | 2 ± 0 | 1 ± 0 | 18 ± 1 | 5 ± 0 | 3 ± 0 | 17 ± 1 | 6 ± 1 | 48 ± 2 | 783 |
| | CA | 5.7 | 1 ± 0 | 1 ± 0 | 16 ± 1 | 4 ± 0 | 2 ± 0 | 14 ± 0 | 10 ± 0 | 51 ± 1 | 767 |
| Imidazole | VV | 2.1 | 2 ± 0 | 1 ± 0 | 11 ± 1 | 3 ± 0 | 3 ± 0 | 12 ± 0 | 5 ± 0 | 64 ± 1 | 735 |
| | CA | 3.6 | 1 ± 0 | tr | 10 ± 0 | 2 ± 0 | 1 ± 0 | 7 ± 0 | 2 ± 0 | 76 ± 1 | 806 |
| $Na_2CO_3$ 4 °C | VV | 8.0 | 2 ± 0 | – | 7 ± 1 | 1 ± 0 | – | 4 ± 1 | tr | 85 ± 1 | 483 |
| | CA | 8.5 | 1 ± 0 | – | 8 ± 0 | tr | – | 3 ± 0 | – | 87 ± 1 | 794 |
| $Na_2CO_3$ 20 °C | VV | 1.7 | 2 ± 0 | – | 22 ± 2 | 1 ± 0 | – | 16 ± 1 | 1 ± 0 | 59 ± 4 | 466 |
| | CA | 2.3 | 2 ± 0 | – | 24 ± 1 | 1 ± 0 | – | 13 ± 1 | – | 61 ± 0 | 619 |
| 0.5 M KOH sn | VV | 0.7 | 2 ± 0 | 2 ± 0 | 13 ± 1 | 33 ± 1 | 5 ± 0 | 13 ± 1 | 13 ± 1 | 18 ± 1 | 451 |
| | CA | 1.3 | 4 ± 0 | – | 5 ± 0 | 34 ± 1 | 5 ± 0 | 10 ± 1 | 24 ± 0 | 18 ± 0 | 786 |
| 0.5 M KOH pp | VV | 2.0 | 2 ± 0 | 3 ± 0 | 29 ± 1 | 9 ± 0 | 1 ± 0 | 27 ± 0 | 12 ± 0 | 16 ± 1 | 326 |
| | CA | 2.0 | 1 ± 0 | 6 ± 0 | 14 ± 0 | 22 ± 0 | 1 ± 0 | 17 ± 0 | 26 ± 0 | 13 ± 0 | 387 |
| 1 M KOH sn | VV | 4.0 | 2 ± 0 | 3 ± 0 | 23 ± 1 | 15 ± 1 | 3 ± 0 | 22 ± 2 | 12 ± 0 | 20 ± 5 | 561 |
| | CA | 5.0 | 2 ± 0 | 2 ± 0 | 35 ± 0 | 11 ± 0 | 1 ± 0 | 28 ± 0 | 5 ± 0 | 16 ± 0 | 549 |
| 1 M KOH pp | VV | 0.3 | 3 ± 0 | 2 ± 0 | 33 ± 0 | 10 ± 1 | 3 ± 0 | 23 ± 1 | 11 ± 0 | 15 ± 1 | 265 |
| | CA | 0.3 | 2 ± 0 | 1 ± 0 | 39 ± 2 | 9 ± 0 | 1 ± 0 | 26 ± 0 | 10 ± 2 | 11 ± 0 | 235 |
| 4 M KOH sn | VV | 3.4 | 2 ± 0 | 2 ± 0 | 26 ± 0 | 11 ± 0 | 10 ± 0 | 20 ± 0 | 16 ± 0 | 12 ± 1 | 555 |
| | CA | 4.8 | 2 ± 0 | 2 ± 0 | 28 ± 0 | 11 ± 0 | 7 ± 0 | 26 ± 0 | 13 ± 0 | 11 ± 0 | 612 |
| 4 M KOH pp | VV | 0.5 | 3 ± 0 | 2 ± 0 | 36 ± 3 | 15 ± 1 | 5 ± 0 | 14 ± 0 | 14 ± 2 | 12 ± 1 | 234 |
| | CA | 0.3 | 1 ± 0 | 1 ± 0 | 42 ± 0 | 16 ± 0 | 2 ± 0 | 12 ± 1 | 6 ± 0 | 21 ± 1 | 437 |
| 8 M KOH sn | VV | 0.5 | 2 ± 0 | 2 ± 0 | 27 ± 0 | 9 ± 1 | 8 ± 1 | 22 ± 1 | 16 ± 1 | 13 ± 3 | 700 |
| | CA | 2.6 | 3 ± 0 | tr | 37 ± 0 | 2 ± 0 | – | 29 ± 0 | 1 ± 0 | 28 ± 0 | 770 |
| 8 M KOH pp | VV | 0.4 | 3 ± 0 | – | 52 ± 1 | 10 ± 0 | 2 ± 0 | 14 ± 1 | 7 ± 0 | 13 ± 1 | 265 |
| | CA | 0.8 | 2 ± 0 | – | 40 ± 1 | 2 ± 0 | – | 30 ± 0 | tr | 25 ± 1 | 637 |
| snCR | VV | 6.5 | 2 ± 0 | – | 32 ± 1 | 1 ± 0 | – | 31 ± 1 | – | 35 ± 1 | 958 |
| | CA | 1.2 | 2 ± 0 | – | 32 ± 1 | 1 ± 0 | – | 23 ± 1 | – | 42 ± 0 | 767 |
| CR | VV | 38.8 | 3 ± 1 | – | 28 ± 4 | 4 ± 1 | 1 ± 0 | 18 ± 1 | 24 ± 9 | 22 ± 5 | 303 |
| | CA | 14.0 | 2 ± 0 | – | 19 ± 0 | 2 ± 0 | 1 ± 0 | 13 ± 0 | 39 ± 0 | 25 ± 0 | 848 |

Mean ± standard deviation ($n$ = 4).
tr, trace amount.
sn, material soluble in water recovered after neutralisation and dialysis of the extract.
pp, material insoluble in water recovered after neutralisation and dialysis of the extract.
CR, cellulosic residue.
[a] Yield is expressed in mg of dry weight material per 100 g of AIR.

of the tissues (Hurtado, Greve, & Labavitch, 2002; Ratnayake, Melton, & Hurst, 2003; Stolle-Smits, Beekhuizen, Recourt, Voragen, & Van Dijk, 1997).

On a fruit basis, the amount of uronic acids, Ara, and Gal decreased with boiling and candying, although different behaviours were found for the fruits from the two orchards (Fig. 1). The fresh fruits of VV contained less uronic acids than CA in all combined extracts, except in snCR (Fig. 1a). In these snCR extracts, the amount of uronic acids of fresh plums from VV reached 20 mg/fruit whereas in CA it was only 4 mg/fruit. Lower values of uronic acids were also found in all boiled and candied fruits (1–4 mg/fruit). These results allow us to infer that a large amount of pectic polysaccharides of fresh VV plums were entrapped in the hemicellulosic and cellulosic matrix. In strong alkali, the ionisation of the $-CH_2OH$ groups on cellulose could prevent the diffusion of negatively-charged pectic polysaccharides enmeshed within the swollen cellulose matrix. Upon neutralisation of the cellulose-rich suspension, the loss of negative charges on cellulose facilitates diffusion of the entangled pectic polysaccharides (Coimbra, Waldron, & Selvendran, 1994). In all other plum samples, the previous degradation of pectic polysaccharides and/or the swelling of the cellulosic matrix could have allowed their solubilisation by the water, imidazole, and carbonate extracts. Fig. 1b shows that the amount of Ara + Gal of fresh plums is more evenly distributed than uronic acids through the combined pectic extracts (19 and 26 mg/fruit in VV and CA, respectively), KOH extracts (22 and 45 mg/fruit), snCR (31 and 4 mg/fruit), and cellulosic residue (42 and 37 mg/fruit). The exception was the snCR of CA, as already stated for the uronic acids. The fresh, boiled, and candied fruits were shown to have the same amount of Ara + Gal recovered by the pectic extracts. However, these two sugars, which mostly arise from the pectic polysaccharide side-chains, decreased their content on a fruit basis (70–80%) mainly in the cellulosic residue. The solubilisation observed for the uronic acids in the pectic extracts in boiled and candied plums was also observed in the Ara + Gal, which was more pronounced in the candied than in the boiled samples. These results show that an important amount of pectic polysaccharides, although degraded, was still present in the cell wall matrix and diffused during the osmotic process. The higher diffusion of UA-rich polymers during candying observed for VV than for CA is in accordance with the already reported loss of these polymers during the boiling process.

### 3.3. Degree of methylesterification and activity of cell wall pectic enzymes

The esterification with methanol or acetic acid is a very important structural characteristic of pectic polysaccharides, since it could be related with the change in texture of the fruits during processing. In order to evaluate if the different characteristics observed on cell wall pectic polysaccharides between the two orchards could be related to the methylesterification or acetylation

**Table 3**
Sugar composition of fractions of boiled plums AIR obtained by sequential extraction with aqueous solvents for Vila Viçosa (VV) and Cano (CA) orchards

| Extract | Orchard | Yield[a] (%) | Cell wall sugars (mol%) | | | | | | | | Total (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | UA | |
| Water | VV | 7.1 | 2 ± 0 | 1 ± 0 | 13 ± 1 | 3 ± 0 | 2 ± 0 | 11 ± 1 | 10 ± 1 | 57 ± 3 | 727 |
| | CA | 7.5 | 2 ± 0 | 1 ± 0 | 10 ± 0 | 3 ± 0 | 2 ± 0 | 11 ± 0 | 6 ± 1 | 66 ± 2 | 886 |
| Imidazole | VV | 2.4 | 2 ± 0 | 1 ± 0 | 18 ± 1 | 2 ± 0 | 2 ± 0 | 11 ± 1 | 3 ± 0 | 61 ± 2 | 753 |
| | CA | 2.3 | 1 ± 0 | 1 ± 0 | 25 ± 2 | 2 ± 0 | 2 ± 0 | 8 ± 0 | 2 ± 0 | 60 ± 2 | 902 |
| $Na_2CO_3$ 4 °C | VV | 6.5 | 1 ± 0 | tr | 6 ± 0 | 1 ± 0 | tr | 6 ± 0 | tr | 85 ± 1 | 926 |
| | CA | 17.5 | 2 ± 0 | 1 ± 0 | 19 ± 1 | 1 ± 0 | – | 16 ± 1 | – | 62 ± 1 | 716 |
| $Na_2CO_3$ 20 °C | VV | 2.2 | 3 ± 0 | 1 ± 0 | 26 ± 1 | 2 ± 0 | 1 ± 0 | 22 ± 1 | 1 ± 0 | 45 ± 2 | 573 |
| | CA | 4.6 | 1 ± 0 | tr | 27 ± 1 | 1 ± 0 | – | 26 ± 1 | tr | 44 ± 2 | 874 |
| 0.5 M KOH sn | VV | 0.7 | 2 ± 0 | 4 ± 0 | 10 ± 1 | 29 ± 0 | 3 ± 0 | 16 ± 1 | 23 ± 2 | 14 ± 2 | 822 |
| | CA | 0.9 | 2 ± 0 | 5 ± 0 | 7 ± 0 | 34 ± 2 | 3 ± 0 | 12 ± 0 | 12 ± 0 | 27 ± 3 | 953 |
| 0.5 M KOH pp | VV | 2.8 | 3 ± 0 | 2 ± 0 | 36 ± 1 | 4 ± 1 | – | 46 ± 1 | 2 ± 0 | 7 ± 1 | 225 |
| | CA | 1.3 | 4 ± 0 | 2 ± 0 | 24 ± 1 | 4 ± 0 | 2 ± 0 | 20 ± 1 | 4 ± 0 | 41 ± 2 | 197 |
| 1 M KOH sn | VV | 2.6 | 2 ± 0 | 3 ± 0 | 19 ± 1 | 16 ± 1 | 3 ± 0 | 28 ± 1 | 17 ± 1 | 11 ± 1 | 826 |
| | CA | 1.3 | 2 ± 0 | 5 ± 0 | 5 ± 0 | 27 ± 1 | 6 ± 1 | 15 ± 1 | 20 ± 0 | 21 ± 2 | 845 |
| 1 M KOH pp | VV | 1.2 | 2 ± 0 | 3 ± 1 | 31 ± 1 | 12 ± 1 | 2 ± 0 | 31 ± 2 | 5 ± 1 | 15 ± 1 | 428 |
| | CA | 1.8 | 4 ± 0 | 2 ± 0 | 24 ± 1 | 18 ± 2 | 2 ± 0 | 12 ± 1 | 3 ± 0 | 35 ± 2 | 197 |
| 4 M KOH sn | VV | 1.9 | 3 ± 0 | 2 ± 0 | 23 ± 1 | 7 ± 1 | 7 ± 0 | 28 ± 1 | 13 ± 1 | 17 ± 1 | 971 |
| | CA | 3.6 | 2 ± 0 | 3 ± 0 | 18 ± 1 | 11 ± 1 | 7 ± 0 | 22 ± 1 | 13 ± 1 | 26 ± 3 | 655 |
| 4 M KOH pp | VV | 0.4 | 3 ± 1 | 3 ± 1 | 36 ± 1 | 17 ± 0 | 3 ± 0 | 17 ± 1 | 9 ± 0 | 12 ± 2 | 213 |
| | CA | 0.8 | 3 ± 0 | 2 ± 0 | 18 ± 2 | 16 ± 1 | 3 ± 0 | 14 ± 1 | 12 ± 1 | 32 ± 2 | 560 |
| 8 M KOH sn | VV | 0.2 | 3 ± 0 | 2 ± 0 | 28 ± 1 | 7 ± 0 | 9 ± 0 | 31 ± 0 | 17 ± 1 | 4 ± 0 | 588 |
| | CA | 1.2 | 2 ± 0 | 2 ± 0 | 34 ± 1 | 6 ± 0 | 2 ± 0 | 31 ± 0 | 6 ± 1 | 16 ± 1 | 871 |
| 8 M KOH pp | VV | 0.3 | 2 ± 0 | 1 ± 0 | 37 ± 1 | 6 ± 0 | 1 ± 0 | 25 ± 0 | 4 ± 1 | 25 ± 1 | 614 |
| | CA | 0.2 | 3 ± 0 | 2 ± 0 | 21 ± 1 | 33 ± 2 | 2 ± 0 | 12 ± 0 | 6 ± 0 | 22 ± 2 | 405 |
| snCR | VV | 1.7 | 1 ± 0 | tr | 31 ± 1 | 1 ± 0 | tr | 28 ± 1 | 4 ± 0 | 34 ± 1 | 940 |
| | CA | 7.5 | – | – | 34 ± 2 | – | – | 26 ± 3 | – | 40 ± 5 | 450 |
| CR | VV | 14.6 | 2 ± 0 | 1 ± 0 | 19 ± 0 | 3 ± 0 | 1 ± 0 | 16 ± 0 | 43 ± 1 | 15 ± 2 | 544 |
| | CA | 9.8 | 2 ± 0 | 1 ± 0 | 15 ± 1 | 5 ± 0 | 2 ± 0 | 12 ± 0 | 35 ± 2 | 27 ± 1 | 494 |

tr, trace amount.
Mean ± standard deviation ($n = 4$).
sn, material soluble in water recovered after neutralisation and dialysis of the extract.
pp, material insoluble in water recovered after neutralisation and dialysis of the extract.
CR, cellulosic residue.
[a] Yield is expressed in mg of dry weight material per 100 g of AIR.

of pectic polysaccharides, the degrees of methylesterification and acetylation of the AIR and pectic polysaccharide-rich extracts not submitted to the alkali reagents were evaluated. Also, the activities of the enzymes pectin methylesterase (PME) and polygalacturonase (PG) were quantified in fresh plums from VV and CA orchards, since the activity of these enzymes can decrease the degree of methylesterification (DM) and the degree of polymerisation of pectic polysaccharides.

The DM, as well as the degree of acetylation (DA), determined for AIR, water, and imidazole extracts of fresh, boiled, and candied VV and CA plums are presented in Table 5. The DM of the pectic polysaccharides of AIR of VV fresh plums (57%) was higher than that of CA (38%). In the boiled plums, no statistical differences were observed, when compared to the values obtained for the fresh plums. However, the candying process caused a decrease of the DM of the pectic polysaccharides in the AIR to 44% and 26% for VV and CA plums, respectively.

The DM of the pectic polysaccharides present in the water extract of fresh plums of both orchards is similar to the DM observed for the AIR, which are lower than those determined for the imidazole extracts (65% and 75%, respectively, for VV and CA). After boiling, the DMs of the water extracts were significantly higher than those observed for the fresh plums, whereas the DMs of the imidazole extracts were significantly lower. The DM of the pectic polysaccharides present in the imidazole extract of boiled plums of both orchards is similar to the DM observed for the respective AIR. For the candied fruits, the DM of the pectic polysaccharides present in both water and imidazole extracts, of both orchards, is higher than the DM observed for their respective AIR. For VV plums, where no loss of pectic polysaccharides was observed between fresh and boiled fruits (Fig. 1a), these results indicate that the highly esterified pectic polysaccharides become more soluble with the heating treatment. During osmotic treatment some loss of these polysaccharides occurred, which is in accordance with the degradation of highly methylated regions of pectic polysaccharides by β-elimination due to heating (Stolle-Smits et al., 1997). The same process might have occurred in CA, although the results for this sample were not conclusive, due to the solubilisation of the pectic polysaccharides in the boiling step (Fig. 1a).

AIR and water extracts for fresh plums showed a DA between 5% and 7%, while for boiled and candied plums the percentage of acetyl groups per sugar residues tended to increase, reaching up to 15% in the water extracts of boiled plums from the two orchards (Table 5). The increase of the content of acetyl groups in pectic polysaccharides has been shown to prevent their gelling abilities (Renard & Jarvis, 1999). However, as in candied plums, the cell-to-cell adhesion of cells structure is recovered. It is possible that the high methylesterification of the pectic polysaccharides is an important factor for the recovery of the texture of the fruits after osmotic treatment. Boiled VV plums revealed a higher quantity of highly esterified (DM = 83%) soluble pectic polysaccharides,

**Table 4**
Sugar composition of fractions of candied plums AIR obtained by sequential extraction with aqueous solvents for Vila Viçosa (VV) and Cano (CA) orchards

| Extract | Orchard | Yield[a] (%) | Cell wall sugars (mol%) | | | | | | | | Total (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | UA | |
| Water | VV | 22.7 | 2 ± 0 | 1 ± 0 | 12 ± 0 | 4 ± 0 | 2 ± 0 | 15 ± 0 | 13 ± 0 | 50 ± 1 | 882 |
| | CA | 19.1 | 2 ± 0 | 1 ± 0 | 13 ± 1 | 4 ± 0 | 2 ± 0 | 12 ± 1 | 12 ± 1 | 54 ± 2 | 781 |
| Imidazole | VV | 4.4 | 2 ± 0 | 1 ± 0 | 20 ± 1 | 2 ± 0 | 2 ± 0 | 13 ± 1 | 3 ± 0 | 57 ± 1 | 781 |
| | CA | 3.2 | 2 ± 0 | 1 ± 0 | 14 ± 1 | 3 ± 0 | 2 ± 0 | 16 ± 1 | 3 ± 0 | 58 ± 1 | 630 |
| $Na_2CO_3$ 4 °C | VV | 6.0 | 2 ± 0 | 1 ± 0 | 13 ± 0 | 1 ± 0 | tr | 14 ± 1 | tr | 69 ± 1 | 886 |
| | CA | 12.2 | 2 ± 0 | 1 ± 0 | 14 ± 1 | 1 ± 0 | 1 ± 0 | 15 ± 1 | 1 ± 0 | 66 ± 2 | 682 |
| $Na_2CO_3$ 20 °C | VV | 3.3 | 2 ± 0 | – | 29 ± 1 | 1 ± 0 | – | 34 ± 1 | 1 ± 0 | 33 ± 1 | 658 |
| | CA | 5.0 | 3 ± 0 | 1 ± 0 | 33 ± 1 | 2 ± 0 | – | 34 ± 2 | 1 ± 0 | 27 ± 1 | 484 |
| 0.5 M KOH sn | VV | 3.9 | 3 ± 0 | 4 ± 0 | 14 ± 0 | 20 ± 0 | 2 ± 0 | 18 ± 1 | 15 ± 1 | 23 ± 2 | 300 |
| | CA | 1.0 | 1 ± 0 | 4 ± 0 | 7 ± 0 | 36 ± 1 | 4 ± 0 | 14 ± 1 | 19 ± 1 | 15 ± 2 | 817 |
| 0.5 M KOH pp | VV | 1.2 | 4 ± 0 | 2 ± 0 | 23 ± 1 | 7 ± 0 | 3 ± 0 | 25 ± 1 | 16 ± 2 | 21 ± 1 | 214 |
| | CA | 1.2 | 2 ± 0 | 2 ± 0 | 32 ± 1 | 6 ± 0 | 1 ± 0 | 31 ± 1 | 5 ± 1 | 20 ± 2 | 278 |
| 1 M KOH sn | VV | 3.9 | 2 ± 0 | 4 ± 0 | 13 ± 1 | 21 ± 1 | 5 ± 0 | 20 ± 2 | 18 ± 1 | 18 ± 2 | 695 |
| | CA | 5.1 | 2 ± 0 | 3 ± 0 | 15 ± 1 | 21 ± 1 | 6 ± 0 | 21 ± 1 | 14 ± 1 | 18 ± 1 | 713 |
| 1 M KOH pp | VV | 1.9 | 5 ± 0 | 1 ± 0 | 43 ± 2 | 8 ± 0 | 1 ± 0 | 26 ± 1 | 6 ± 1 | 12 ± 0 | 190 |
| | CA | 3.3 | 3 ± 0 | 3 ± 0 | 44 ± 2 | 8 ± 0 | 1 ± 0 | 30 ± 2 | 5 ± 0 | 7 ± 0 | 267 |
| 4 M KOH sn | VV | 4.8 | 2 ± 0 | 3 ± 0 | 16 ± 1 | 14 ± 1 | 10 ± 1 | 22 ± 1 | 23 ± 1 | 10 ± 1 | 735 |
| | CA | 6.9 | 3 ± 0 | 3 ± 0 | 20 ± 1 | 11 ± 0 | 5 ± 0 | 24 ± 1 | 13 ± 1 | 22 ± 1 | 544 |
| 4 M KOH pp | VV | 2.6 | 3 ± 0 | 3 ± 0 | 43 ± 2 | 23 ± 2 | 3 ± 1 | 10 ± 1 | 6 ± 0 | 9 ± 1 | 178 |
| | CA | 3.4 | 3 ± 0 | 2 ± 0 | 32 ± 1 | 6 ± 1 | 2 ± 0 | 26 ± 2 | 6 ± 0 | 23 ± 2 | 376 |
| 8 M KOH sn | VV | 0.7 | 2 ± 0 | 1 ± 0 | 34 ± 3 | 5 ± 0 | 4 ± 1 | 31 ± 1 | 8 ± 1 | 15 ± 2 | 937 |
| | CA | 2.5 | 3 ± 0 | 1 ± 0 | 31 ± 1 | 5 ± 0 | 3 ± 0 | 28 ± 2 | 5 ± 0 | 25 ± 2 | 535 |
| 8 M KOH pp | VV | 0.4 | 5 ± 0 | – | 55 ± 1 | 20 ± 0 | – | 10 ± 0 | 7 ± 1 | 3 ± 0 | 122 |
| | CA | 0.6 | 3 ± 0 | 1 ± 0 | 31 ± 1 | 10 ± 0 | 2 ± 0 | 30 ± 1 | 8 ± 0 | 13 ± 1 | 513 |
| snCR | VV | 2.3 | 2 ± 0 | 1 ± 0 | 23 ± 2 | 1 ± 0 | tr | 23 ± 3 | 3 ± 0 | 47 ± 5 | 931 |
| | CA | 1.9 | 2 ± 0 | – | 33 ± 1 | 1 ± 0 | – | 37 ± 1 | tr | 26 ± 0 | 678 |
| CR | VV | 37.7 | 2 ± 0 | 1 ± 0 | 19 ± 1 | 4 ± 0 | 1 ± 0 | 19 ± 0 | 38 ± 2 | 16 ± 1 | 564 |
| | CA | 21.7 | 2 ± 0 | 1 ± 0 | 22 ± 1 | 4 ± 0 | 1 ± 0 | 20 ± 0 | 22 ± 0 | 27 ± 2 | 484 |

tr, trace amount.
Mean ± standard deviation ($n = 4$).
sn, material soluble in water recovered after neutralisation and dialysis of the extract.
pp, material insoluble in water recovered after neutralisation and dialysis of the extract.
CR, cellulosic residue.
[a] Yield is expressed in mg of dry weight material per 100 g of AIR.

when compared to CA plums, which can explain the differences observed on the texture and microstructure of plums from the two orchards, where VV plums showed a higher recovery of cell size and shape and of cell-to-cell adhesion (Nunes et al., 2008).

In order to evaluate the involvement of pectin methylesterase (PME) and polygalacturonase (PG) in these properties, the activity of these enzymes in the fresh fruits was evaluated. According to Table 6, PME activity was 28% higher in VV than in CA whereas PG activity was 6-fold higher in CA than in VV. Usually, the activity of PME in fruits increases with ripening, up to a point where it decreases in a concerted action with the increase of PG activity. The activity of PME renders pectins more susceptible to the action of PG (Ali, Chin, & Lazan, 2004; Jain, Dhawan, Malhotra, & Singh, 2003; Majumder & Mazumdar, 2002; Wakabayashi, Chun, & Huber, 2000). As a result, although it should be taken into account that a higher PME activity quantified in vitro may not necessarily indicate an effective higher activity in vivo, due, for example, to possible restrictions to the access of the enzyme to its substrate, in CA plums the lower PME activity may be related to the lower DM of its pectic polysaccharides, which should be already de-esterified, justifying the increase in PG activity.

The higher PG activity could be related to the higher content of pectic polysaccharides in the water and imidazole extracts of CA than VV fresh plums (Table 2), as well as a higher content of the pectic polysaccharide extracts of CA plums in all stages of processing (Fig. 1a). This is in accordance with the increase of solubilisation and depolymerisation of pectic polysaccharides, which occur during ripening of the fruits and their correlation with the decrease in fruit tissue firmness (Priya Sethu, Prabha, & Tharanathan, 1996; Taylor, Rabe, Jacobs, & Dodd, 1995; Wakabayashi et al., 2000). Depolymerisation of chelator-soluble pectic polysaccharides during ripening has been reported in many fruit types, such as tomato, avocado, kiwi fruit, persimmon, apple, water melon, plum and nashi pear (Redgwell, MacRae, Hallett, Fischer, Perry & Harker, 1997), as well as in olive (Mafra et al., 2001; Mafra et al., 2006b). For both PME and PG, the activity was found to be mainly due to ionically and covalently bound cell wall fractions, indicating that these enzymes might be acting primarily on the cell wall. Also, the much higher activity of PG found for CA orchard plums indicates that these plums might be at a more advanced stage of ripening than is indicated by the soluble solids content and titratable acidity measurements.

In order to evaluate the extent of pectic polysaccharides that diffused to the sucrose syrup with the candying process, the amount of polymeric material and its sugar composition was analysed for both VV and CA samples.

### 3.4. Polysaccharides of sucrose syrup

Table 7 shows that the material recovered after exhaustive dialysis with a large number of water exchanges of the sugar syrup of VV plums (1.3 mg/ml) was lower than the amount recovered in CA

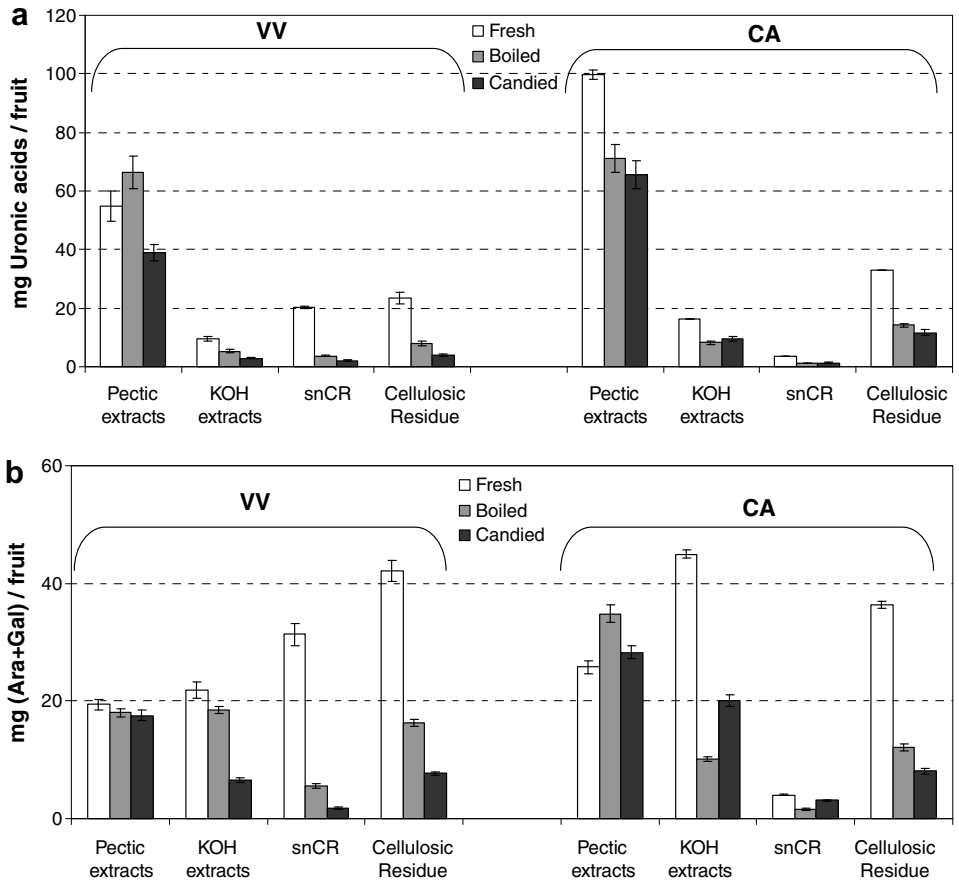


**Fig. 1.** Uronic acids (a) and Ara + Gal (b) content in combined cell wall extracts and cellulosic residue, expressed as mg per fruit, in fresh, boiled, and candied plums from Vila Viçosa (VV) and Cano (CA) orchards.

**Table 5**
Degree of methylesterification (DM) and acetylation (DA) of AIR and water and imidazole extracts of fresh, boiled, and candied plums of Vila Viçosa (VV) and Cano (CA) orchards

| Extract | Orchard | DM (%) | DA (%) |
|---|---|---|---|
| *Fresh* | | | |
| AIR | VV | 57 ± 1[a] | 6 ± 1[a] |
| | CA | 38 ± 2[b] | 5 ± 1[a] |
| Water | VV | 51 ± 5[a] | 5 ± 0[a] |
| | CA | 36 ± 2[b] | 7 ± 0[a] |
| Imidazole | VV | 65 ± 3[c] | – |
| | CA | 75 ± 2[d] | – |
| *Boiled* | | | |
| AIR | VV | 54 ± 4[a] | 10 ± 1[b] |
| | CA | 39 ± 2[b] | 11 ± 0[b] |
| Water | VV | 83 ± 3[e] | 15 ± 1[c] |
| | CA | 66 ± 2[c] | 15 ± 2[c] |
| Imidazole | VV | 50 ± 5[a] | – |
| | CA | 40 ± 2[b] | 5 ± 1[a] |
| *Candied* | | | |
| AIR | VV | 44 ± 1[b] | 3 ± 0[d] |
| | CA | 26 ± 2[f] | 13 ± 0[c] |
| Water | VV | 70 ± 1[g] | 6 ± 1[a] |
| | CA | 44 ± 2[b] | 12 ± 1[c] |
| Imidazole | VV | 62 ± 1[c] | 9 ± 1[b] |
| | CA | 39 ± 0[b] | 10 ± 2[b] |

Mean ± standard deviation ($n$ = 3).
Within columns, means with the same superscript letter are not significantly ($p > 0.05$) different.

**Table 6**
Pectin methylesterase (PME), polygalacturonase (PG), and cellulase (Cel) activities (U) in fresh plums of Vila Viçosa (VV) and Cano (CA) orchards

| Enzyme | Vila Viçosa | Cano |
|---|---|---|
| *PME* | | |
| Soluble | – | – |
| Ionic | 0.50 ± 0.04 | 0.63 ± 0.05 |
| Covalent | 0.74 ± 0.01 | 0.28 ± 0.04 |
| Total | 1.24 ± 0.05[a] | 0.90 ± 0.09[b] |
| *PG* | | |
| Soluble | 4 ± 1 | 23 ± 9 |
| Ionic | – | – |
| Covalent | 16 ± 4 | 101 ± 18 |
| Total | 19 ± 5[c] | 124 ± 28[d] |
| *Cel* | | |
| Soluble | – | tr |
| Ionic | tr | tr |
| Covalent | 0.26 ± 0.01 | 0.30 ± 0.01 |
| Total | 0.26 ± 0.01[e] | 0.31 ± 0.01[f] |

tr, trace amount.
Mean ± standard deviation ($n$ = 3).
Means with the same superscript letter are not significantly ($p > 0.05$) different.

(2.1 mg/ml). This material had a carbohydrate content of 48% for VV and 43% for CA, and was composed mainly of UA, Glc, Ara, and Gal. Man and Rha were also present. This sugars composition suggests that, in both samples, pectic polysaccharides account for 60–66% of the total polysaccharides recovered. The average ratio of UA/(Ara + Gal) was 2 for VV and 1 for CA, and the DM was 68% in VV samples and only 38% in CA. These results infer that the pectic polysaccharides present in the sugar syrup of VV plums contained less branched and more methylesterified pectic polysaccharides. The extent of branching and DM were comparable

**Table 7**
Yield, sugar composition, and degree of methylesterification of sucrose syrup for candied plums from Vila Viçosa (VV) and Cano (CA) orchards

| Orchard | Yield[a] (mg/ml) | Sugars (mol%) | | | | | | | | Total (mg/g) | DM (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | UA | | |
| VV | 1.3 | 2 ± 0 | – | 13 ± 0 | 2 ± 0 | 6 ± 0 | 7 ± 1 | 27 ± 2 | 44 ± 2 | 482 | 68 ± 5 |
| CA | 2.1 | 3 ± 0 | – | 20 ± 2 | 2 ± 0 | 5 ± 0 | 7 ± 0 | 34 ± 1 | 30 ± 3 | 432 | 36 ± 3 |

Mean ± standard deviation ($n = 4$).
[a] Yield is expressed in mg of dry weight material per ml of sucrose syrup.

to those observed in the pectic extracts of the fruit, which is in accordance with the hypothesis of diffusion of the cell wall polysaccharides from the fruits to the sucrose syrup during candying.

The presence of highly methylesterified pectic polysaccharides may be responsible for the high viscosity of the sugar syrup, when compared to the viscosity of a solution of sucrose with the same concentration. In fact, the viscosity of the plums' sugar syrup, measured at a shear rate of 280 $s^{-1}$, was 3.0 Pa, whereas the viscosity of the sucrose solution was 0.010 Pa. These results are in accordance with the results reported by Peiró et al. (2006), which showed a loss of UA from grapefruit tissues during osmotic dehydration, and a progressive increase of the pectin content and viscosity of the solution. Beyond pectic polysaccharides, other fruit compounds were also observed to diffuse to the syrup, namely, volatile compounds (Nunes et al., 2008).

### 3.5. Hemicellulosic polysaccharides and cellulase activity

In order to evaluate the changes occurring to the hemicellulosic polysaccharides during candying, the partially depectinated AIR was extracted with KOH solutions of increasing strength until a cellulosic residue was obtained. Upon neutralisation of the KOH extracts, the precipitated polymeric material was removed from the supernatant solutions. The sugars composition of the soluble and precipitated material was determined for all KOH extracts of fresh, boiled, and candied plums (Tables 2–4) and identical data

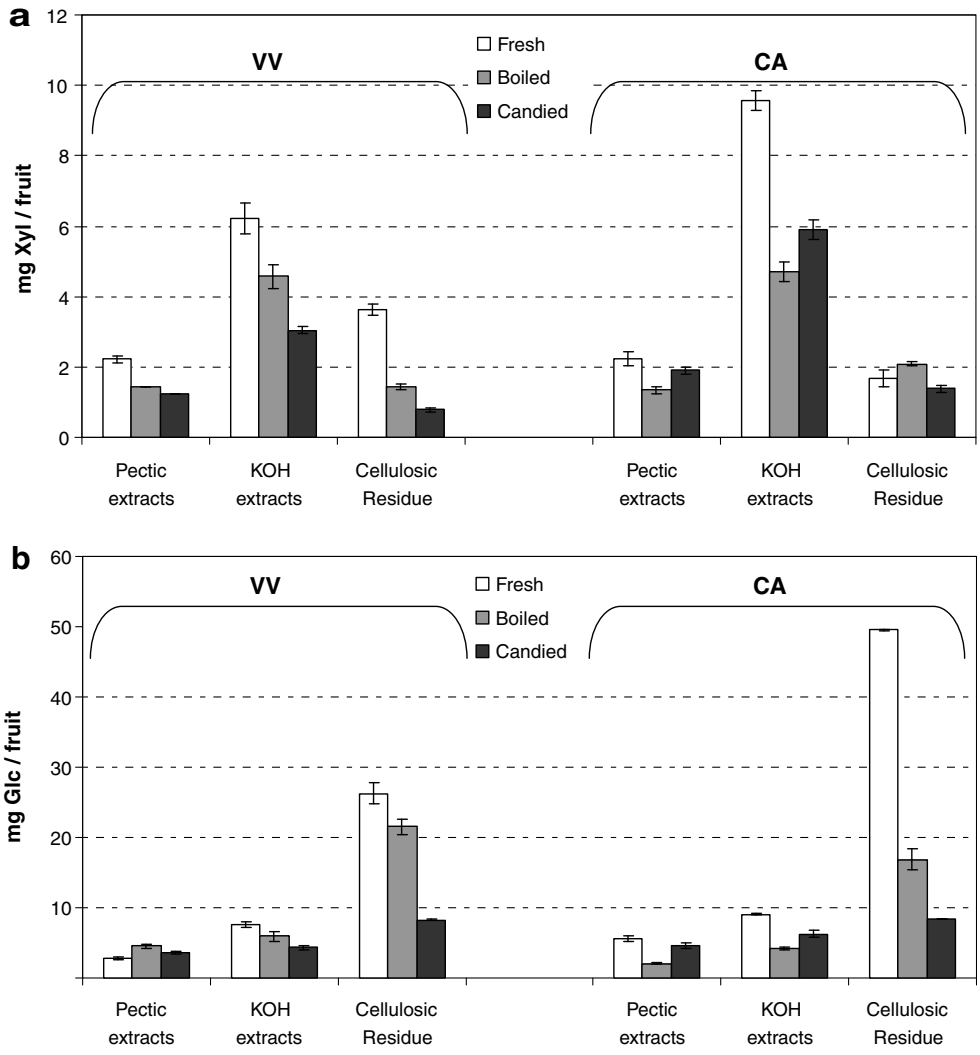


**Fig. 2.** Xylose (a) and glucose (b) content in combined cell wall extracts and cellulosic residue, expressed as mg per fruit, in fresh, boiled, and candied plums from Vila Viçosa (VV) and Cano (CA) orchards.

were obtained. The majority of the precipitated material was poor in sugars when compared to the supernatants, containing high amounts of UV-absorbing material, as observed for other fruits (Coimbra et al., 1994). Xyl was the main sugar present in soluble 0.5 M KOH extracts. The presence of a higher amount of Xyl than Glc is diagnostic of xylans (Mafra et al., 2001). However, the presence of Glc, Gal, and traces of Fuc, apart from Xyl, is diagnostic of xyloglucans. These polymers, although in small amount, occur in all KOH fractions. Soluble 1 M, 4 M, and 8 M KOH extracts were mainly composed of Ara and Gal, with lower contents of UA, Glc, and Xyl. The high amounts of Ara and Gal are indicative of the presence of arabinogalactans. The presence of lower quantities of mannans was indicated by the occurrence of Man in the KOH extracts, principally in soluble 4 M KOH. Based on these results, it can be inferred that the hemicelluloses constituent of the cell walls of plums are xylans, xyloglucans, arabinogalactans-rich polymers, and, in a minor quantity, mannans.

On a fruit basis, the amount of Xyl and Glc decreased with boiling (Fig. 2) and this decrease was higher in CA than in VV. After candying, a decrease in the amount of these sugars was also observed in VV plums, while in CA plums the decrease was only found in CR. These results suggest the solubilisation of the polysaccharides with processing. This different behaviour of solubilisation of these sugars is in accordance with the previous observation that the loss of cell wall polysaccharides in VV occurs mainly during the candying process whereas those of CA occur mainly during boiling.

In the cellulosic residue (CR), the main sugar present was Glc, which accounted for 24 and 39 mol% of CR sugars in fresh plums from VV and CA, 43 and 35 mol% in boiled plums, and 38 and 22 mol% in candied plums from VV and CA, respectively (Tables 2–4). The non-cellulosic Glc accounted for 10% of total Glc of the CR of fresh plums from VV, and 5% in CA. An increase of non-cellulosic Glc yield in CR was observed in boiled and candied fruits to 15% in VV and 22% in CA. Based on these results, the amount of cellulose can be estimated to be 24 and 47 mg/fruit for VV and CA fresh plums, respectively, 18 and 13 mg/fruit in boiled plums, and 7 mg/fruit in both processed plums. These results showed that upon boiling, cellulose degradation was observed in the fruits from both orchards, whereas a higher extent of degradation was observed in CA samples.

In order to study the importance of the activity of cellulase (Cel) of fresh plums to the observed degradation of cellulose, cellulase activity was quantified in both VV and CA fresh samples (Table 6). The Cel activity was significantly higher in CA than in VV plums. This higher activity could be related to the higher demethylesterification of pectic polysaccharides by the action of PME, since this enzyme could directly or indirectly assist other enzymes, besides PG, mainly by promoting accessibility of the enzymes, like Cel, to their substrates (Ali et al., 2004). The observed activity of Cel is in accordance with the results obtained for the analysis of cell wall polysaccharides. Cel could degrade both cellulose and the β-1,4-glucan backbone of xyloglucan, which leads to extensive polysaccharide depolymerisation (Iannetta, van den Berg, Wheatley, McNicol, & Davies, 1999; Priya Sethu et al., 1996; Wakabayashi et al., 2000). The higher recovery in CA plums of polysaccharides with KOH solutions seems to be associated to the higher Cel activity of these fruits which promoted solubilisation of the xyloglucans (Fig. 2). This is also in accordance with the higher amount of cellulosic Glc in VV when compared to CA plums.

## 4. Concluding remarks

Plum cell wall polysaccharides are composed mainly of pectic polysaccharides and cellulose. During the boiling step of the processing to Ameixa d'Elvas these polymers are degraded and solubilised. This may explain the observed decrease of cell wall adhesion and loss of firmness of the tissues. The plums' pectic polysaccharides are highly esterified. Their gelation in the presence of sucrose is responsible for the recovery of the fruit's consistency upon candying. During the candying process diffusion of these methyesterified pectic polysaccharides to the sucrose syrup increase the syrup viscosity, which may explain the retention of several fruit volatile compounds, which contribute to its aroma.

The higher solubilisation of CA plums cell wall polysaccharides after boiling, which was not observed in VV plum, should explain the lower firmness of these plums after candying. This higher susceptibility to the heat treatment could be related to the higher activity of CA plums cell wall enzymes. Although these plums have been produced in the same geographic region and had similar degree of ripening when evaluated by the soluble solids content and titratable acidity, the differences found seem to be related to different stages of ripening not highlighted by these parameters. The analysis of the cell wall polysaccharides and enzymes of CA plums show that these fruits might be in a more advanced stage of ripening than VV. A more adequate assessment of the ripening stage of the fresh fruits, perhaps based on cell wall polysaccharides and/or enzymes seems to be essential to avoid the lower texture quality of "Ameixa d'Elvas" produced using CA plums.

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