Available online at www.sciencedirect.com
SCIENCE DIRECT®
Postharvest Biology and Technology 38 (2005) 213–222
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Effect of heat treatments on cell wall degradation and softening in strawberry fruit

Ariel R. Vicente [a], María L. Costa [a], Gustavo A. Martínez [b], Alicia R. Chaves [a], Pedro M. Civello [b,*]

[a] *Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (CONICET-UNLP), 47 y 116 (B1900AJJ) La Plata, Argentina*
[b] *(IIB-INTECH) Instituto de Investigaciones Bioquímicas-Instituto Tecnológico de Chascomús (CONICET-UNSAM), Camino Circunvalación Laguna Km 6, (B7130IWA) Chascomús, Argentina*

Received 28 December 2004; accepted 17 June 2005

Abstract

Strawberries (75% red color) were harvested and heat-treated at 45 °C, 3 h in an air oven. After treatment, fruit were stored at 20 °C for 2 days. Firmness, content of cell wall components and activity of enzymes related to cell wall degradation was determined in the external and internal fruit zones of control or heat-treated fruit. Therefore, the total content of pectins and hemicelluloses was measured, along with the water, EDTA and HCl soluble pectin fractions, and the enzyme activity of endo-1,4-β-D-glucanase (EGase), β-xylosidase (β-Xyl), polygalacturonase (PG), β-galactosidase (β-Gal), and pectin methylesterase (PME). Heat-treated fruit remained firmer than control fruit in both zones after 1 day of storage at 20 °C. After 2 days of storage, the difference was still observed in the external zone. Heat treatments reduced EGase and β-Xyl activity in both zones and delayed hemicellulose degradation. PG and β-Gal activity was also inhibited by the treatment. Heat-treated fruit maintained higher level of HCl soluble pectins while had lower amount of water-soluble pectins than control fruit. PME activity was increased by the treatments and heat-treated fruit showed higher amount of EDTA soluble pectins than the control. Results show that firmness, activity of assayed enzymes and cell wall composition are different in the external and internal zones of strawberry fruit. Furthermore, the application of heat treatment affected the solubilization of pectins and hemicelluloses.

Keywords: Strawberry; Softening; Heat treatment; Cell wall

1. Introduction

The primary cell walls of higher plants are composed predominantly of polysaccharides together with lower amounts of structural glycoproteins, phenolic esters, ionically bound minerals and proteins (Carpita

* Corresponding author. Tel.: +54 2241 424049; fax: +54 2241 424048.
E-mail address: civello@intech.gov.ar (P.M. Civello).

0925-5214/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2005.06.005

and Gibeaut, 1993). One of the most important changes in soft fruit like strawberries, raspberries and blackberries during postharvest storage is the reduction of firmness. Excessive softening influences shelf life, wastage and infections by postharvest pathogens (Brummell and Harpster, 2001). Fruit softening has been correlated with solubilization and depolimerization of cell wall constituents and the importance of different enzymes in fruit softening has been extensively reviewed (Fischer and Bennett, 1991; Brummell and Harpster, 2001). Many attempts have been made to delay softening in berry fruit. Refrigeration is the most extended technology used to delay ripening associated changes, including cell wall disassembly (Kader, 1992). Control and modified atmospheres (Smith, 1992; Gil et al., 1997) have been used to prevent softening in fresh strawberries. Recently, the reduction of fruit softening has been evaluated by suppressing the expression of genes encoding for cell wall degrading enzymes (Brummell and Harpster, 2001). In the case of strawberry, the suppression of PL resulted in a firmer fruit (Jiménez Bermúdez et al., 2002). On the contrary, down-regulation of an EGase gene in strawberry did not affect fruit firmness (Woolley et al., 2001). Heat treatments have been used to delay softening in many products (Lurie, 1998; Paull, 1990). In the case of strawberry, García et al. (1995) reported that submersion in water at 45 °C for 15 min retarded softening. Hot air treatments have been also used to maintain strawberry fruit firmness (Civello et al., 1997; Vicente et al., 2002). It is known that heat treatments could deeply modify the activity of cell wall degrading enzymes and consequently affect the modification of cell wall components (Lurie, 1998). The reduction of polygalacturonase (Chan et al., 1981; Yoshida et al., 1984) and α and β-galactosidase activity by heat treatments has been reported (Sozzi et al., 1996). However, the information is still scarce and in the case of strawberry, the effect of heat treatment on cell wall degrading enzymes has not been analyzed yet. Data obtained from strawberry varieties with contrasting fruit firmness suggest that strawberry fruit softening could be closely related to pectin solubilization and depolymerization (Rosli et al., 2004). Therefore, the effect of heat treatment on pectin degradation has been particularly focused in this study. The paper describes the effect of heat treatment on cell wall composition of strawberry fruit and on the activity of EGase and four enzymes related to pectin degradation (PME, PG, β-Gal and β-Xyl) in the internal and external zone of the receptacle.

2. Materials and methods

2.1. Plant material

Strawberries (*Fragaria* x *ananassa* Duch., cv. Selva), having 75% surface red color were obtained from local producers (La Plata, Province of Buenos Aires, Argentina) and immediately transferred to the laboratory. Five hundred fruit were put in 50 plastic trays and covered with PVC (15 mm thick). Fruit were held in an air oven at 45 °C for 3 h. After treatment, the fruit were stored at 20 °C for 2 days. Corresponding control fruit were put in plastic trays and directly stored at 20 °C for 2 days.

2.2. Fruit dissection

Two discs (5 mm thick) from the equatorial zone of each fruit were obtained and then divided into two different zones (external and internal). The external zone was obtained by separating the external third of the receptacle while the internal zone was considered as the remaining central two thirds. Samples were taken immediately after the treatment or after 1 or 2 days at 20 °C, and immediately processed or frozen in liquid N_2 and stored at −20 °C until use.

2.3. Firmness

Firmness was measured after 0, 1 and 2 days at 20 °C using a texture analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY) fitted with a 3 mm diameter flat probe. Eighty discs obtained as described in Section 2.2 were compressed 2 mm at a rate of 0.5 mm s^{-1} and the maximum force developed during the test was recorded. Each disc was measured in the internal and external zone.

2.4. Enzyme extraction and activity assay

For each enzyme analyzed, two extracts were done per condition (control and 45 °C, 3 h), fruit zone (external and internal) and storage time (0, 1 or 2 days at 20 °C) analyzed. All the steps during extract

preparation were carried out at 4 °C. Determinations of enzyme activities were performed in duplicate, and the corresponding enzyme activity was expressed as $\Delta OD\,kg^{-1}\,s^{-1}$.

2.4.1. Endo-1,4-β-D-glucanase (Egase)

Approximately 5 g of frozen tissue was homogenized in an Omnimixer with 15 ml of buffer A (50 mM sodium acetate, 10 g l^{-1} PVPP, pH 6.0). The obtained suspension was centrifuged at 10,000 × *g* for 30 min and the supernatant was discarded. The pellet was washed twice with 15 ml of the same buffer and then it was suspended in 15 ml of buffer B (50 mM sodium acetate, 1 M NaCl, 10 g l^{-1} PVPP, pH 6.0) and stirred for 2 h at 4 °C. The homogenate was centrifuged at 10,000 × *g* for 30 min and the supernatant was used for assaying the enzyme activity. The assay mixture contained 50 mM sodium acetate buffer pH 6.0, 1 M NaCl, 0.5% carboxyl-methyl-cellulose and 1.5 ml of enzyme extract, in a total volume of 2 ml. The mixture was incubated at 37 °C and aliquots of 150 μl were taken after 0, 2, 5, 8 and 24 h and frozen until sugar analysis. Sugars were determined by the di-nitrosalicylic acid (DNS) method according to Miller (1959) measuring OD at 550 nm.

2.4.2. β-Xylosidase (β-Xyl), β-galactosidase (β-Gal) and Polygalacturonase (PG)

Approximately 5 g of fruit was homogenized with 15 ml of buffer B; the mixture was left under stirring for 2 h at 4 °C and then centrifuged at 10,000 × *g* for 10 min. The supernatant was used to determine the three enzyme activities. β-Xyl activity was measured using *p*-nitrophenyl β-D-xylopyranoside as substrate according to Cleemput et al. (1997) with slight modifications (Martínez et al., 2004). The β-Gal activity was assayed in a mixture containing 50 mM sodium acetate buffer pH 4.5, 3 mM *p*-nitrophenyl β-D-galactopyranoside and 500 μl of enzymatic extract in a total volume of 2 ml. The reaction mixture was incubated at 40 °C and aliquots of 150 μl were taken at different times and discharged into 500 μl of 0.4 M Na_2CO_3. The change of OD at 410 nm was followed. To measure PG activity, the supernatant was previously dialyzed overnight in 50 mM sodium acetate buffer pH 4.5. The dialyzed extract was used for assaying PG activity, in a mixture containing 50 mM sodium acetate buffer pH 6.0, 0.15% (w/v) polygalacturonic acid and 1 ml of the enzyme extract, in a total volume of 3 ml. The mixture was incubated at 40 °C and aliquots of 300 μl were taken at different times and immediately frozen in liquid N_2. Released galacturonic acid was measured with 2-cyano-acetamide according to Gross (1982), measuring OD at 276 nm.

2.4.3. Pectin methylesterase (PME)

Five grams of fruit was ground with 15 ml of 1 M NaCl and 10 g l^{-1} PVPP. The suspension obtained was stirred for 4 h and then centrifuged at 10,000 × *g* for 30 min. The supernatant was collected, adjusted to pH 7.5 with 0.01 M NaOH and used for assaying the enzyme activity. The activity was assayed in a mixture containing 600 μl of 0.5% (w/v) pectin, 150 μl of 0.01% bromothymol blue pH 7.5, 100 μl of water pH 7.5 and 100 μl of enzymatic extract. The mixture was incubated at 37 °C and the reduction of OD at 620 nm was followed.

2.5. Cell wall extraction and analysis

Cell wall polysaccharides were obtained as alcohol insoluble residue (AIR) according to d'Amour et al. (1993) with slight modifications. Approximately 10 g of frozen fruit was homogenized with 40 ml of ethanol and boiled for 30 min. The homogenate was filtered and the residue was washed three times with 15 ml of ethanol and then the solvent was evaporated at 20 °C. The dried residue obtained (AIR) was used to extract the different cell wall fractions.

2.5.1. Pectins

Polyuronides were isolated according to d'Amour et al. (1993) and Nara et al. (2001) with modifications. A 100 mg aliquot of AIR was homogenized in 100 ml of water and stirred overnight at 20 °C. The homogenate was filtered and the solid was washed three times with 10 ml of water. The filtrates were labeled water-soluble pectins (Wsp).

The residue was then resuspended in 100 ml of 0.05 M sodium acetate containing 0.04 M EDTA, and stirred during 4 h at 20 °C. The homogenate was filtered and the residue was washed three times with 10 ml of the same buffer. The filtrates were labeled EDTA soluble pectins (Esp).

Finally, the last residue was resuspended in 100 ml of 0.05 M HCl and heated with agitation at 100 °C for

1 h. The homogenate was cooled, then filtered and the residue was washed three times with 10 ml of 0.05 M HCl. The filtrates were pooled and labeled HCl soluble pectins (Hsp). The uronic acid concentration of all fractions was measured by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using galacturonic acid (GA) as standard.

2.5.2. Hemicelluloses

The washed residue from pectin extraction was stirred for 8 h with 100 ml of 4 M NaOH at 20 °C. The homogenate was filtered and the filter residue was washed three times with 5 ml of 4 M NaOH. The filtrates were pooled and labeled hemicellulose fraction (Hem). Quantification of Hem was done after complete hydrolysis with 66% (v/v) H_2SO_4 at 100 °C during 60 min and estimated as glucose by using the anthrone method (d'Amour et al., 1993).

2.6. Statistical analysis

Experiments were performed according to a factorial design, the main factors being the treatment (control and heat treatment), the receptacle zone (internal and external) and storage time (0, 1 and 2 days). Data were analyzed by ANOVA. The means were compared by a LSD test at a significance level of 0.05.

3. Results and discussion

3.1. Firmness

Fruit firmness was higher in the internal zone of the fruit. A reduction in firmness was found in the internal (Fig. 1A) and external zones (Fig. 1B) during storage. Fruit firmness changed from initial values of 1.57 and 0.65 to 0.92 and 0.39 after 2 days at 20 °C in the internal and external zone of control fruit, respectively. When the heat treatment was finished, there were no firmness differences between control and heat-treated fruit in either external or internal zone. However, after 1 day at 20 °C both zones were firmer in heat-treated than in control fruit. After 2 days at 20 ° C, no differences were observed in the internal tissues, but the external zone of heat-treated fruit still remained firmer than the corresponding control. It has been reported that heat treatments could delay softening in different fruits (Paull, 1990; Lurie, 1998). Civello et al. (1997) reported that hot air treatments (42 or 48 °C, 3 h) delayed softening in strawberry, and similar results were described in boysenberry (Vicente et al., 2004). Several authors have reported that heat treatments could reduce the activity of cell wall degrading enzymes and delay cell wall disassembly (Paull, 1990; Lurie, 1998; Sozzi et al., 1996). However, the effect of heat treatments on the activity of enzymes related to cell wall degradation in soft fruit had not been analyzed yet.

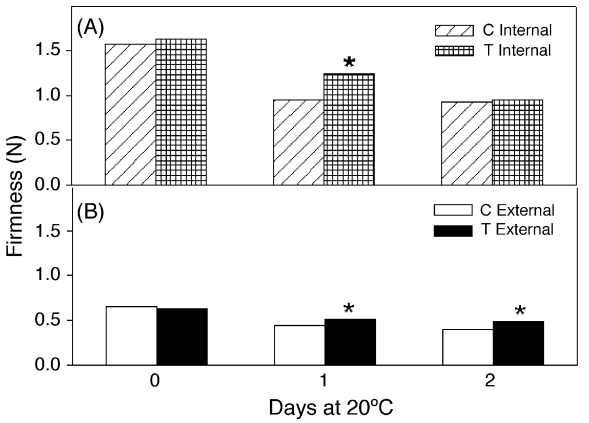

Fig. 1. Firmness in the internal (A) and external (B) zone of control (C) and heat-treated (T) strawberry fruit during incubation at 20 °C. The asterisk shows significant differences at $P = 0.05$.

3.2. Enzymatic activities

3.2.1. Endo-1,4-β-D-glucanase (EGase)

Endo-1,4-β-D-glucanase (EC 3.2.1.4) hydrolyzes internal linkages of 1,4-β-D-glucans. Its possible "in vivo" substrates include non-crystalline regions of cellulose, xyloglucans and glucomannans. EGase activity has been reported in fully ripe strawberry fruit (Abeles and Takeda, 1990). Several endoglucanase genes whose expression is high in red ripe fruit have been isolated (Harpster et al., 1998; Llop-Tous et al., 1999; Trainotti et al., 1999). EGase activity was found to be higher in the internal tissues of the fruit (Fig. 2A) and a reduction was found in both zones during storage at 20 °C. Immediately after treatment, heat-treated fruit showed lower EGase activity than control fruit in both external and internal zones. The same effect was found after 1 day at 20 °C, but after 2 days the lower EGase activity was found only in the heat-treated inter-

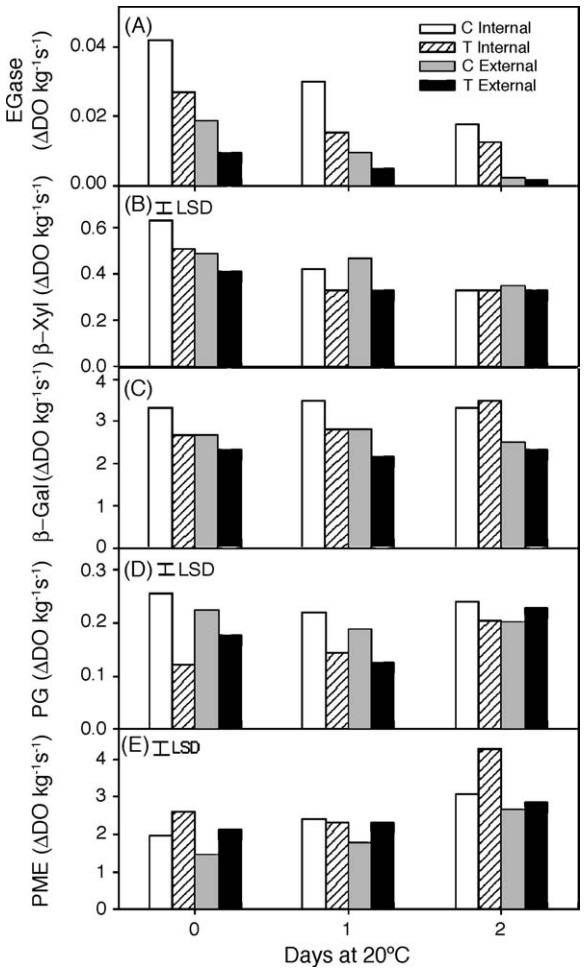

Fig. 2. Effect of heat treatment (45 °C, 3 h) on activity of enzymes related to cell wall degradation. (A) EGase; (B) β-Xyl; (C) β-Gal; (D) PG; (E) PME. The enzyme activity was measured in the internal and external zone of control (C) and heat-treated (T) strawberry fruit during incubation at 20 °C. The least significant difference at $P = 0.05$ is shown.

nal fruit zone. The amount of cellulose in strawberry fruit decreases from "green" to "ripe" stages (Rosli et al., 2004). However, contradictory results have been reported, and several authors have proposed that the cellulose amount remains relatively constant throughout ripening (Neal, 1965; Koh and Melton, 2002). As EGase is not active on crystalline cellulose "in vitro" (Barnes and Patchett, 1976), it has been proposed that the enzyme could be associated with degradation of xyloglucans and/or non-crystalline cellulose (Llop-Tous et al., 1999; Woolley et al., 2001). Therefore, the lower levels of EGase found in heat-treated fruit in this study could be related to a less extensive modification of xyloglucan structure.

3.2.2. β-Xylosidase (β-Xyl)

Xylose represents near 30% of hemicellulose composition and is present in small amount in pectins (Koh and Melton, 2002). It should be expected that approximately only 25% were β-xylose (xylan or xylogalacturonan). Although the percentage of β-xylose is low, it could be highly relevant in the structure of the cell wall since down-regulation of a β-xylosidase gene altered cell wall composition and plant development in *Arabidopsis thaliana* (Goujon et al., 2003). The activity of β-xylosidase (EC 3.2.1.37) has been reported in different fruits during ripening (Itai et al., 2003; Botondi et al., 2003; Martínez et al., 2004). Xylans and some pectic compounds found in the cell wall have been suggested as the main targets of β-xylosidases. Xylans are composed of a backbone of β-1,4-linked D-xylosil residues with or without arabinosyl residue side groups. Among pectins, xylose is present in xylogalacturonan, a polymer with an α-1,4-linked D-galactosyluronic acid backbone with β-D-xylosyl residues attached to C3 of the backbone residues (Nakamura et al., 2002). In strawberry fruit, β-xylosidase activity increases from large green to 50% red stage, and decreases thereafter (Martínez et al., 2004). In the present work, β-xylosidase activity decreased during storage at 20 °C in both control and heat-treated fruit (Fig. 2B). Since the initial ripening stage was 75% red, these results agree with data reported previously (Martínez et al., 2004). Just after treatment, the β-Xyl activity was lower in heat-treated fruit than in controls in both zones analyzed. The same trend was found after 1 day at 20 °C but no differences were observed after 2 days. Hemicelluloses show a substantial reduction of molecular mass during strawberry fruit ripening (Huber, 1984). The reduction of β-Xyl activity found in heat-treated fruit could contribute to delayed softening by decreasing xyloglucan or xylans degradation, or by diminishing the cleavage of β-xylose from side chains of pectin compounds.

3.2.3. β-Galactosidase (β-Gal)

β-Galactosidases (EC 3.2.1.23) are also involved in pectin degradation, removing non-reducing terminal

galactosyl residues from side chains of pectin polysaccharides. Three different galactosidases have been described in strawberry fruit (Trainotti et al., 2001). Galactosidase activity was higher in the internal than in the external fruit zone (3.33 and 2.66 ΔOD kg^{-1} s^{-1}, respectively) and no changes were found in the internal or external zone of control fruit during storage (Fig. 2C). Heat-treated fruit showed lower β-Gal activity than control fruit immediately after the treatment in the internal but not in the external zone. However, heat-treated fruit had lower β-Gal activity than control fruit in both zones analyzed after 1 day at 20 °C. When the fruit was held at 20 °C for 2 days no differences were found between control and heat-treated fruit in either zone analyzed. Sozzi et al. (1996) reported that high temperature stress reduced β-Gal activity in tomato. Galactosidase participation in pectin solubilization has been suggested (Ranwala et al., 1992). However, other enzymes like pectato lyases seem to play an important role in pectin degradation and softening in strawberry fruit (Jiménez Bermúdez et al., 2002). It has been proposed that β-1,4-galactosidases could remove backbones in the side chains of rhamnogalacturonans (Smith et al., 1998), then reducing noncovalent interactions between adjacent polysaccharide chains. Galactose residues can also be found in hemicelluloses located in side chains attached to the main polymeric molecules (Trainotti et al., 2001). Loss of either galactans or galactose during strawberry fruit ripening has been reported (Knee et al., 1977; Redgwell et al., 1997). Galactosidase activity may play a role in the softening of these fruit and the reduction of β-Gal produced by heat treatment could contribute to delay degradation of cell wall polymers.

3.2.4. Polygalacturonase (PG)

Polygalacturonases (EC 3.2.1.15) and pectate lyase (EC 4.2.2.2) catalize the cleavage of pectin backbones (Jiménez Bermúdez et al., 2002). In strawberry fruit, three different polygalacturonases have been partially characterized (Nogata et al., 1993). Two of them have exo-polygalacturonase activity whereas the other has both endo and exo-polygalacturonase activity. PG activity decreases during strawberry fruit growth and ripening, and low activity is found in red ripe fruit (Nogata et al., 1993). A fruit specific and developmentally regulated PG gene, *spG*, has been isolated from strawberry; the gene is expressed only at White stage during ripening, and the authors have proposed that its function would be related to the oligosaccharin production more than to extensive cell wall degradation during ripening (Redondo-Nevado et al., 2001).

In this work, a slightly higher PG activity was found in the internal fruit zone of control fruit (Fig. 2D). The PG activity remained essentially constant in control fruit during stay at 20 °C, but the application of heat treatment reduced PG activity in both zones. After 1 day at 20 °C the heat-treated fruit still displayed lower PG activity than the controls, but after 2 days of storage PG activity recovered and no differences were found between control and heat-treated fruit in both zones analyzed.

3.2.5. Pectinmethylesterase (PME)

PME (EC 3.1.1.11) is a ubiquitous plant enzyme that de-esterifies methoxylated pectin present in the cell wall, releasing sites accessible to further degradation by PG. Otherwise, de-esterification of homogalacturonans may induce the formation of Ca^{2+} bonds among pectins, then reinforcing the cell wall structure. Just after treatment, heat-treated fruit had higher PME activity than control fruit in both zones analyzed (Fig. 2E). After 2 days at 20 ° C, heat-treated fruit showed higher PME activity than control fruit in the internal zone but no differences were observed in the external zone. This differs from data obtained in apples, where no difference of PME activity between control and heat-treated fruit was found (Klein et al., 1995). However, the treatment applied in that case (38 °C, 4 days) was very different from the heat treatment used in this work. Furthermore, the response to heat treatment depends on the product and even on the cultivar used (Lurie, 1998).

3.3. Cell wall components

Cell wall was sequentially extracted, and main fractions (hemicelluloses, pectin soluble in water, EDTA and HCl) were prepared from control and heat-treated fruit. Results are shown in Table 1 and discussed below.

3.3.1. Hemicelluloses (Hem)

Hemicelluloses showed a reduction in the external zone of control fruit during stay at 20 °C. No differences in Hem were found between control and heat-treated fruit after the treatment. However, after 2 days

Table 1
Changes in hemicellulose (Hem), total pectins, HCl soluble (Hsp), water-soluble (Wsp) and EDTA soluble (Esp) pectins in the internal and external zone of control and heat-treated (45 °C, 3 h in air oven) strawberry fruit during incubation at 20 °C

Days at 20 °C				
		0	1	2
Hem (g kg^{-1})	C_{INT}	3.70	3.30	3.13
	T_{INT}	4.36	4.75	4.97
	LSD = 0.77			
	C_{EXT}	6.09	5.40	4.07
	T_{EXT}	5.86	5.49	5.77
	LSD = 0.91			
Total pectins (g kg^{-1})	C_{INT}	2.130	2.241	2.231
	T_{INT}	2.060	2.164	2.168
	LSD = 0.116			
	C_{EXT}	2.473	2.644	2.571
	T_{EXT}	2.509	2.567	2.584
	LSD = 0.108			
Hsp (g kg^{-1})	C_{INT}	0.979	0.727	0.628
	T_{INT}	0.807	0.765	0.695
	LSD = 0.055			
	C_{EXT}	1.186	1.127	0.860
	T_{EXT}	1.166	1.100	1.026
	LSD = 0.062			
Wsp (g kg^{-1})	C_{INT}	0.747	1.007	1.124
	T_{INT}	0.690	0.890	0.924
	LSD = 0.067			
	C_{EXT}	1.033	0.784	0.994
	T_{EXT}	1.002	0.820	0.904
	LSD = 0.069			
Esp (g kg^{-1})	C_{INT}	0.404	0.457	0.487
	T_{INT}	0.563	0.529	0.549
	LSD = 0.049			
	C_{EXT}	0.540	0.530	0.587
	T_{EXT}	0.653	0.607	0.634
	LSD = 0.042			

C_{INT}, control internal zone; T_{INT}, heat-treated internal zone; C_{EXT}, control external zone; T_{EXT}, heat-treated external zone. The least significant difference at P = 0.05 is shown.

at 20 °C Hem levels remained higher in heat-treated fruit in both zones. For long time cellulose was thought to be one of EGase "in vivo" substrates. However, it has not been proved that EGases could degrade crystalline cellulose and instead xyloglucans were suggested as one of the main targets for these enzymes (Woolley et al., 2001). EGases and β-Xyl acting on hemicelluloses, possibly in concert with cell wall-modifying proteins such as expansins, could contribute to the progressive loosening of the cell wall (Woolley et al., 2001). Therefore, the inhibition of EGase by heat treatments, discussed in Section 3.2.1, could reduce Hem catabolism. Furthermore, the reduction of β-Xyl activity found in heat-treated fruit could also contribute to delay xylan degradation.

3.3.2. Total pectins

No changes in total pectins were found in the internal or external zones during stay at 20 °C (Table 1). Nogata et al. (1996) described that total pectins change during fruit development and ripening but no significant changes occur between red ripe and over ripe stages. The heat treatment did not modify total pectin content, and similar levels were found in treated and non-treated fruit (Table 1). Similar results were reported by Ben Shalom et al. (1993), who did not

find differences in total pectin content between control and heat-treated apples. However, a classical pectin fractioning was done to find out if the treatment modified the relative amount of each pectin fraction.

3.3.3. HCl soluble (Hsp), water-soluble (Wsp) and EDTA soluble pectins (Esp)

Immediately after treatment, there was a reduction in Hsp in heat-treated fruit in the internal zone. During stay at 20 °C Hsp decreased in both internal and external fruit zone. However, the diminution of Hsp in heat-treated fruit was delayed and after 2 days at 20 °C the fruit retained higher levels of this pectin fraction. In the case of Wsp, no differences were found between control and heat-treated fruit when the treatment was finished. After 2 days at 20 °C heat-treated fruit showed lower Wsp than control fruit in the internal and external zones. The content of Hsp decreases while that of Wsp increases during ripening, indicating that covalently bound pectins are released and become soluble (Woodward, 1972; Knee et al., 1977; Huber, 1984; Regdwell et al., 1997). In this work, it was found that heat treatment reduced both the decrease in Hsp and the increase in Wsp. Previous works in persimmon reported a reduction in polyuronide solubilization by heat treatment application (Woolf et al., 1997). Moreover, cell wall studies in apple fruit showed that exposure to hot air delayed pectin solubilization (Klein et al., 1990). Both the reduction of PG and β-Gal activities, the latter being able to cleave side pectin chains, found in heat-treated fruit could contribute to decrease pectin solubilization. Higher levels of EDTA soluble pectins (Esp) were found after heat treatment in both zones analyzed, and the same was observed after 1 and 2 days at 20 °C. The higher level of ionically bound pectins in heat-treated fruit correlates with the increase in PME activity found in this work. PME catalyzes the elimination of methyl residues from esterified pectins, then generating available sites to form calcium bridges (Barnavon et al., 2001). The enzyme from tomato has an optimum temperature of 55 °C (Van den Broeck et al., 2000). Hence, it is possible that the increase of PME activity in response to the temperature rise had originated more Ca^{2+} binding sites, then increasing the amount of EDTA soluble pectins. In that sense, higher levels of calcium bound to the cell wall have been found in heat-treated apples (Lurie and Klein, 1992).

4. Conclusion

Heat treatments delay softening in the internal and external zones of strawberry fruit. A treatment at 45 °C for 3 h reduced EGase and β-Xyl activity and delayed Hem degradation. The activities of PG and β-Gal were also reduced by the treatment while the PME activity was enhanced. The combined effect of heat treatment on these enzymes could slow down pectin solubilization by reducing the pectin cleavage and by increasing the amount of putative sites for calcium bridge formation within the cell wall.

Acknowledgements

The authors thank the support from CONICET (PIP 02209), ANPCYT-SECYT (PICT 08-07088 and PICT 09-8760) and IFS (E/3068-1).

References

Abeles, F.B., Takeda, F., 1990. Cellulase activity and ethylene in ripening strawberry and apple fruits. Sci. Hortic. 42, 269–275.

Barnavon, L., Doco, T., Terrier, N., Ageorges, A., Romieu, C., Pellerin, P., 2001. Involvement of pectin methyl-esterase during the ripening of grape berries: partial cDNA isolation, transcript expression and changes in the degree of methyl-esterification of cell wall pectins. Phytochemistry 58, 693–701.

Barnes, M.F., Patchett, B.J., 1976. Cell wall degrading enzymes and the softening of senescent strawberry fruit. J. Food Sci. 41, 1392–1395.

Ben Shalom, N., Hanzon, J., Klein, J., Lurie, S., 1993. A postharvest heat treatment inhibits cell wall degradation in apples during storage. Phytochemistry 34, 955–958.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54, 484–489.

Botondi, R., DeSantis, D., Bellincontro, A., Vizovitis, K., Mencarelli, F., 2003. Influence of ethylene inhibition by 1-methylcyclopropene on apricot quality, volatile production, and glycosidase activity of low- and high-aroma varieties of apricots. J. Agric. Food Chem. 51, 1189–1200.

Brummell, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. 47, 311–340.

Carpita, N.C., Gibeaut, D.M., 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J. 3, 1–30.

Chan, H.T., Tam, S.Y.T., Seo, S.T., 1981. Papaya polygalacturonase and its role in thermally injured ripening fruit. J. Food Sci. 46, 190–197.

Civello, P.M., Martínez, G.A., Chaves, A.R., Añón, M.C., 1997. Heat treatments delay ripening and postharvest decay of strawberry fruit. J. Agric. Food Chem. 45, 4589–4594.

Cleemput, G., Hessing, M., van Oort, M., Deconynck, M., de Cour, J.A., 1997. Purification and characterization of a β-xylosidase and an endo-xylanase from wheat flour. Plant Physiol. 113, 377–386.

d'Amour, J., Gosselin, C., Arul, J., Castaigne, F., Willemot, C., 1993. Gamma-radiation affects cell wall composition of strawberries. J. Food Sci. 58, 182–185.

Fischer, R.L., Bennett, A.B., 1991. Role of wall hydrolases in fruit ripening. Annu. Rev. Plant Physiol. 42, 675–703.

García, J.M., Aguilera, C., Albi, M.A., 1995. Postharvest heat treatment on Spanish strawberry (*Fragaria* x *ananassa* cv. Tudla). J. Agric. Food Chem. 43, 1489–1492.

Gil, M.I., Holcroft, D.L., Kader, A.A., 1997. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. J. Agric. Food Chem. 45, 1662–1667.

Goujon, T., Minic, Z., ElAmrani, A., Lerouxel, O., Aletti, E., Lapierre, C., Joselau, J., Jouanin, L., 2003. AtBXL1, a novel higher plant (*Arabidopsis thaliana*) putative b-xylosidase gene, is involved in secondary cell wall metabolism and plant development. Plant J. 33, 677–690.

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

Harpster, M.H., Brummell, D.A., Dunsmuir, P., 1998. Expression analysis of a ripening specific, auxin repressed endo-1,4-β-glucanase gene in strawberry fruit. Plant Physiol. 118, 1316–1317.

Huber, D.J., 1984. Strawberry fruit softening: the potential role of polyuronides and hemicelluloses. J. Food Sci. 49, 1310–1315.

Itai, A., Ishihara, K., Bewley, J.D., 2003. Characterization of expression, and cloning, of beta-D-xylosidase and alpha-L-arabinofuranosidase in developing and ripening tomato (*Lycopersicon esculentum* Mill.) fruit. J. Exp. Bot. 54, 2615–2622.

Jiménez Bermúdez, S., Redondo-Nevado, J., Muñoz-Blanco, J., Caballero, J.L., López-Aranda, J.M., Valpuesta, V., Pliego-Alfaro, F., Quesada, M.A., Mercado, J.A., 2002. Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiol. 128, 751–759.

Kader, A.A., 1992. Quality and its maintenance in relation to the postharvest physiology of strawberry. In: Luby, J.J., Dale, A. (Eds.), The Strawberry into the 21st Century. Timber Press, Portland, OR, pp. 145–152.

Klein, J.D., Lurie, S., Ben-Arie, R., 1990. Quality and cell wall components of "Anna" and "Granny Smith" apples treated with heat, calcium and ethylene. J. Am. Soc. Hort. Sci. 115, 954–958.

Klein, J.D., Hanzon, J., Irwin, P.L., Ben Shalom, N., Lurie, S., 1995. Pectin esterase activity and pectin methyl esterification in heated golden delicious apples. Phytochemistry 39, 491–494.

Knee, M., Sargent, J.A., Osborne, D.J., 1977. Cell wall metabolism in developing strawberry fruits. J. Exp. Bot. 28, 377–396.

Koh, T., Melton, L., 2002. Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues. Postharvest Biol. Technol. 26, 23–33.

Llop-Tous, I., Domínguez-Puigjaner, E., Palomer, X., Vendrell, M., 1999. Characterization of two divergent endo-β-1,4-glucanase cDNA clones highly expressed in the nonclimacteric strawberry fruit. Plant Physiol. 119, 1415–1421.

Lurie, S., Klein, J.D., 1992. Calcium and heat treatments to improve storability of Anna apple. HortScience 27, 36–39.

Lurie, S., 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14, 257–269.

Martínez, G.A., Chaves, A.R., Civello, P.M., 2004. β-xylosidase activity and expression of a β-xylosidase gene during strawberry fruit ripening. Plant Physiol. Bioch. 42, 89–96.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Nakamura, A., Furuta, H., Maeda, H., Takao, T., Nagamatsu, Y., 2002. Analysis of the molecular construction of xylogalacturonan isolated from soluble soybean polysaccharides: biosci. Biotechnol. Biochem. 66, 1155–1158.

Nara, K., Kato, Y., Motomura, Y., 2001. Involvement of terminal arabinose and galactose pectic compounds in mealiness of apple fruit during storage. Postharvest Biol. Technol. 22, 141–150.

Neal, G.H., 1965. Changes occurring in the cell walls of strawberries during ripening. J. Sci. Food Agric. 16, 604–611.

Nogata, Y., Ohta, H., Voragen, A.G.J., 1993. Polygalacturonase in strawberry fruit. Phytochemistry 34, 617–620.

Nogata, Y., Yoza, K., Kusumoto, K., Ohta, H, 1996. Changes in molecular weight and carbohydrate composition of cell wall polyuronide and hemicellulose during ripening in strawberry fruit. In: Visser, J., Voragen, A.G.J. (Eds.), Pectins and Pectinases. Elsevier, Amsterdam, pp. 591–596.

Paull, R.E., 1990. Postharvest heat treatments and fruit ripening. Postharv. News Info. 1, 355–363.

Ranwala, A.P., Suematsu, C., Masuda, H., 1992. The role of β-galactosidases in the modification of cell wall components during muskmelon fruit ripening. Plant Physiol. 100, 1318–1325.

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

Redondo-Nevado, J., Moyano, E., Medina-Escobar, N., Caballero, J.L., Muñoz-Blanco, J., 2001. A fruit-specific and developmentally regulated endopolygalacturonase gene from strawberry (*Fragaria x ananassa* cv. Chandler). J. Exp. Bot. 52, 1941–1945.

Rosli, H.G., Civello, P.M., Martínez, G.A., 2004. Changes in cell wall composition of three *Fragaria* x *ananassa* cultivars with different softening rate during ripening. Plant Physiol. Bioch.

Smith, R.B., 1992. Controlled atmosphere of Redcoat strawberry fruit. J. Am. Soc. Hort. Sci. 117, 260–264.

Smith, D.L., Starret, D.A., Gross, K.C., 1998. A gene encoding for tomato fruit β-galactosidase II is expressed during fruit ripening. Plant Physiol. 117, 417–423.

Sozzi, G.O., Cascone, O., Fraschina, A.A., 1996. Effect of a high temperature stress on endo-β-mannanase and α-and β-galactosidase activities during tomato fruit ripening. Postharvest Biol. Technol. 9, 49–63.

Trainotti, L., Spolaore, S., Pavanello, A., Baldan, B., Casadoro, G., 1999. A novel E type endo-β-1,4-glucanase with a putative cellulose-binding domain is highly expressed in ripening strawberry fruits. Plant Mol. Biol. 40, 323–332.

Trainotti, L., Spinello, R., Piovan, A., Spolaore, S., Casadoro, G., 2001. β-galactosidases with a lectin like domain are expressed in strawberry. J. Exp. Bot. 52, 1635–1645.

Van den Broeck, I., Ludikhuyze, L.R., Van Loey, A.M., Hendrickx, M.E., 2000. Effect of temperature and/or pressure on tomato pectinesterase activity. J. Agric. Food Chem. 48, 551–558.

Vicente, A.R., Civello, P.M., Martinez, G.A., Chaves, A.R., 2002. Quality of heat-treated strawberry fruit during refrigerated storage. Postharvest Biol. Technol. 25, 59–71.

Vicente, A.R., Repice, B., Civello, P.M., Martínez, G.A., Chaves, A.R., Sozzi, G.O., 2004. Quality preservation of fresh boysenberry (*Rubus hybrid*) with UV-C light and heat treatments. J. Hort. Sci. Biotech. 79, 246–251.

Woodward, J.R., 1972. Physical and chemical changes in developing strawberry fruits. J. Sci. Food Agric. 23, 465–473.

Woolf, A.B., MacRae, E.A., Spooner, K.J., Redgwell, R.J., 1997. Changes to physical properties of the cell wall and polyuronides in response to heat treatment of "Fuyu" persimmon that alleviate chilling injury. J. Am. Soc. Hort. Sci. 122, 698–702.

Woolley, L.C., James, D.J., Manning, K., 2001. Purification and properties of an endo-β-1,4-glucanase from strawberry and down-regulation of the corresponding gene, *cel*. Planta 214, 11–21.

Yoshida, O., Nakagawa, H., Ogura, N., Sato, T., 1984. Effect of heat treatment on the development of polygalacturonase activity in tomato fruit during ripening. Plant Cell Physiol. 25, 505–509.