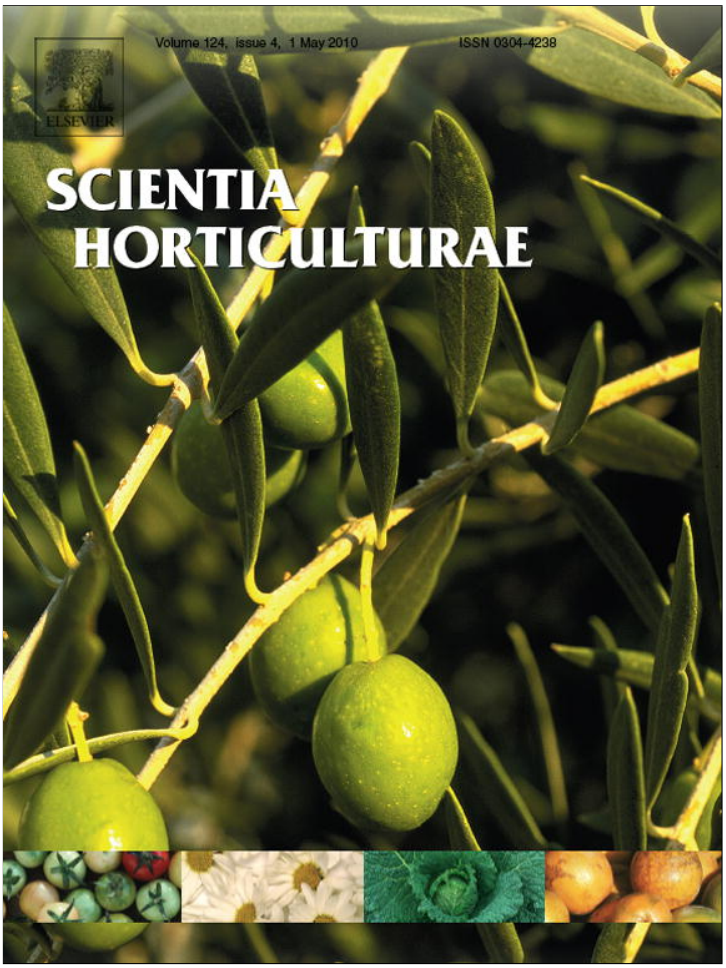

Scientia Horticulturae 124 (2010) 454–462

Contents lists available at ScienceDirect

Scientia Horticulturae

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Changes in cell wall polysaccharides and cell wall degrading enzymes during ripening of *Fragaria chiloensis* and *Fragaria* ×*ananassa* fruits

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ARTICLE INFO

Article history:
Received 11 November 2009
Received in revised form 7 January 2010
Accepted 5 February 2010

Keywords:
Strawberry species
Fruit softening
Cell wall polymers
Cell wall-modifying enzymes

ABSTRACT

Chilean strawberry (*Fragaria chiloensis*) is arising as a new fruit crop that can diversify the world supply of berries. Fruit softening is thought to be linked to an extensive modification of pectin and hemicellulosic cell wall fractions which in turn is determined to a great extent by the action of some cell wall-modifying enzymes. The objective of this work was to compare cell wall changes and the enzyme activities of pectin methylesterase (PME), polygalacturonase (PG), endoglucanase (EGase), α-arabinofuranosidase (AFase), β-galactosidase (βGal), and β-xylosidase (βXyl) between *F. chiloensis* and *Fragaria* ×*ananassa* (cv. Chandler) at three fruit developmental stages: large green (LG), turning (T) and ripe (R). A rapid decrease in fruit firmness between LG and T stages was observed in both species; nevertheless firmness reduction in *F. chiloensis* was steeper than in *F.* ×*ananassa*. This could be related to a faster loss of HCl-soluble polymers (HSP fraction) presented in *F. chiloensis* fruit. However, commercial *F.* ×*ananassa* showed a greater depolymerization of HSP polymers than *F. chiloensis*. With the exception of PME, the activity of all assayed enzymes was higher in *F. chiloensis* than in *F.* ×*ananassa* fruits. The role of PG and EGase seems to be central in both species: these enzymes are correlated to the decrease in the content of HSP and NaOH-soluble polymers (NSP fraction), respectively. In addition, activity levels of βGal and βXyl were significantly correlated with fruit firmness reduction in *F. chiloensis* and *F.* ×*ananassa*, respectively.

1. Introduction

Strawberry fruit has great importance in the global market of small fruits. The emergence of new strawberry species that can be developed into a commercial crop with novel and interesting traits is a great opportunity for the improvement of the berry market. Chilean strawberry (*Fragaria chiloensis* (L.) Mill.), the "mother" of *Fragaria* ×*ananassa* Duch., is emerging as an alternative plant material for this purpose. In this sense, this berry species is white-fruited and has attractive organoleptic characteristics like good flavor and aroma (Retamales et al., 2005). However, recent investigations in our group indicated that *F. chiloensis* fruit displayed a higher softening rate than that of *F.* ×*ananassa* cv. Chandler between the large green and the turning stages (Figueroa et al., 2008), which can become a drawback for postharvest life and fruit quality. These differences might be linked to some molecular events that determine fruit softening and texture.

The ripening-associated softening of fleshy fruit is mainly consequence of enzyme-mediated cell wall degradation (Brummell, 2006) although an important role for turgor pressure change and reactive oxygen species effects on cell wall disassembly are being considered to have an impact on this process (Saladié et al., 2007; Chen et al., 2008). In strawberry, cell wall changes associated to softening during ripening are mainly related to pectin solubilization and depolymerization rather than hemicelluloses degradation (Nishizawa et al., 2002; Koh and Melton, 2002; Rosli et al., 2004). The development and analysis of transgenic plants carrying antisense sequences to pectin and hemicellulosic degrading genes support this concept (Woolley et al., 2001; Santiago-Doménech et al., 2008; Quesada et al., 2009; Mercado et al., 2010). In addition, cellulose content seems to be small in strawberry fruit and remains unchanged during softening (Koh

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doi:10.1016/j.scienta.2010.02.003

et al., 1997). Thus, softening studies in strawberry fruit have been focused mainly on pectinolytic enzymes as pectin degradation has been proposed to affect fruit firmness (Jiménez-Bermúdez et al., 2002; Rosli et al., 2004; Figueroa et al., 2008; Santiago-Doménech et al., 2008; Villarreal et al., 2008; Quesada et al., 2009).

Pectinases, like polygalacturonase (PG), have received considerable attention due to their direct relation to strawberry fruit firmness. Transcript accumulation and activity of PG increase during fruit development of *F.* ×*ananassa* cultivars, having a direct relation to fruit firmness of different cultivars (Villarreal et al., 2008). On the other hand, some gene isoforms of pectin methylesterase (PME) have been related to fruit ripening in *F.* ×*ananassa* cv. Chandler (Castillejo et al., 2004) and it has been observed that ultraviolet-C (UV-C) irradiation, that delays strawberry (cv. Aroma) softening, reduces PME activity immediately after treatment (Pombo et al., 2009).

In strawberry fruit, hemicelluloses metabolism during ripening and its implication on softening is still uncertain. Interestingly, some authors have noted a low depolymerization degree (Rosli et al., 2004), while others have found considerable levels of modification of this cell wall fraction (Huber, 1984), as well as increasing levels of mRNAs associated to endoglucanse (EGase) gene during ripening (Harpster et al., 1998). In addition, the application of heat treatment in strawberry (cv. Selva) decreases fruit softening and causes lower levels of EGase gene expression and enzymatic activity (Martínez and Civello, 2008). Nonetheless, the reduction of EGase gene expression observed in antisense strawberry plants did not influence fruit firmness (Woolley et al., 2001; Palomer et al., 2006; Mercado et al., 2010).

Alternatively, the study of enzyme activities during strawberry fruit ripening has been focused on neutral sugar-removing enzymes. This group of enzymes may act on side chains of pectic (or hemicellulosic) polysaccharides exposing the polymers to the action of the enzymes mentioned above. For example, β-galactosidase (βGal) gene expression and enzymatic activity have been detected during development and ripening of strawberry fruit (Trainotti et al., 2001). Transcripts of β-xylosidase (βXyl) gene accumulate earlier in a soft strawberry cultivar than in a firm one (Bustamante et al., 2006). α-Arabinofuranosidase (AFase), an enzyme that can remove arabinose from pectic side chains and hemicelluloses, has been detected in several fruits, including tomato (Sozzi et al., 2002). In the case of strawberry, three cDNAs have been obtained from fruit and the expression of AFase genes and total enzyme activity were characterized during ripening in different cultivars (Rosli et al., 2009). The AFase activity was considerably higher in the softest cultivar, Toyonoka, which suggests a possible relationship between this enzyme activity and the softening process in strawberry (Rosli et al., 2009).

The objective of the present research was to perform a comparative study between *F. chiloensis* and *F.* ×*ananassa* cv. Chandler in relation to changes in cell wall components and to the behavior of six cell wall-modifying enzymes (PG, PME, EGase, AFase, βGal and βXyl) in three fruit developmental stages.

2. Materials and methods

2.1. Plant material

Fruit of both strawberry species (*F. chiloensis* and *F.* ×*ananassa* cv. Chandler) of different ripening stages were harvested from plants grown in the same commercial field at Contulmo, Biobío Region, Chile (latitude 38°04′8.6″S; longitude 73°14′2.96″W). Fruit were classified into different developmental stages as previously reported by Figueroa et al. (2008), according to weight and color of the receptacle: large green fruit (LG); turning fruit (T); and ripe fruit (R). Firmness was measured using a digital force gauge pressure tester (model AD-4932-50N, AD, Taiwan) fitted with a 5 mm cylinder tip. Two measurements were performed in opposite equatorial sides of the berry; 30 berries per each developmental stage were analyzed. The mean was recorded and expressed as force (N) ± standard error (SE). After measurement of firmness, the peduncle and calyx of each fruit were removed, and the fruit cut longitudinally into two halves, frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Cell wall analysis

2.2.1. Isolation of cell wall material

Cell wall material was extracted according to Rosli et al. (2004). Three independent extractions for each fruit stage were prepared. In each extraction, approximately 10 g of frozen fruit were homogenized with 40 mL of boiling absolute ethanol and refluxed gently for 30 min in a magnetic stirrer. The homogenate was cooled and filtered, and the residue was washed three times with 15 mL of absolute ethanol. Then, the alcohol insoluble residue (AIR) was dried overnight at 37 °C in Petri dishes and finally weighted.

2.2.2. Extraction and quantification of cell wall fractions

Fractions of different cell wall components were obtained by sequential chemical extraction of the AIR, that allows its separation into several fractions: water extraction solubilizes loosely bound pectins (WSP fraction), whereas ethylenediaminetetraacetic acid (EDTA) and HCl extractions allow the enrichment of fractions with ionically- (ESP fraction) and covalently-bound pectins (HSP fraction), respectively (Rosli et al., 2004); NaOH solubilizes mainly hemicelluloses (NSP), while sulfuric acid removes cellulose (SSP).

Approximately 100 mg of AIR from each fruit stage was suspended in 100 mL of water and stirred overnight at room temperature. The homogenate was filtered and the solid washed three times with 10 mL of water. The filtrate and washings were pooled and designated as WSP. The residue was then extracted with 100 mL of 0.05 mol L^{-1} sodium acetate (pH 4.5) containing 0.04 mol L^{-1} EDTA for 4 h at room temperature with stirring. The homogenate was filtered and the solid washed three times with 10 mL of the same buffer. The filtrate and washings were pooled and designated as ESP. The ESP-insoluble pellet was then extracted with 100 mL of 0.05 mol L^{-1} HCl for 1 h at 100 °C with constant stirring. After cooling, the homogenate was filtered and the solid washed three times with 7 mL of 0.05 mol L^{-1} HCl. The filtrate and washings were pooled and designated as HSP. The pellet was then extracted with 100 mL of 4 mol L^{-1} NaOH for 8 h at room temperature with stirring. The homogenate was filtered and then washed three times with 5 mL of 4 mol L^{-1} NaOH. The filtrate and washings were pooled and designated as NaOH-soluble polymers (NSP). Finally, the remaining pellet was hydrolyzed with 30 mL of 66% (v/v) H_2SO_4 for 1 h at 37 °C with stirring. The homogenate obtained was designated as sulfuric acid-soluble polymers (SSP).

Uronic acid (UA) contents of WSP, ESP and HSP fractions were estimated by the *m*-hydroxydiphenyl method using galacturonic acid (GA) as standard (Blumenkrantz and Asboe-Hansen, 1973), and expressed as microgram of GA per mg of AIR. Neutral sugar (NS) contents were estimated by the anthrone method using glucose as standard (D'Amour et al., 1993), and expressed as microgram of glucose per mg of AIR. Quantification of NSP and SSP fractions were done after complete hydrolysis with 66% (v/v) H_2SO_4 and estimated as glucose by using the anthrone method.

2.2.3. Size exclusion chromatography

HSP fractions were dialyzed against distilled water during 16 h at 4 °C and then lyophilized. The HSP pellet was dissolved in 4 mL of buffer I (50 mmol L^{-1} sodium acetate/acetic acid (pH 5.0), 50 mmol L^{-1} EDTA, 50 mmol L^{-1} NaCl). WSP fractions were

directly lyophilized and then dissolved in 4 mL of buffer I. Concentrated extracts from WSP and HSP fractions were loaded onto a 98 cm × 1.3 cm column packed with Sepharose® CL-4B (Sigma) (fractionation range of dextrans: 3×10^4 to 5×10^6 g mol^{-1}) previously equilibrated with buffer I. The chromatographic separation was performed with buffer I at a flow rate of 12 mL h^{-1}. Fractions of 2 mL were collected and assayed for UA content as described above. The NSP fractions were initially neutralized with 12 mol L^{-1} HCl and then dialyzed against distilled water and lyophilized. The pellet was dissolved in 4 mL of buffer II (50 mmol L^{-1} sodium citrate (pH 5.5), 100 mmol L^{-1} NaCl). Concentrated NSP extracts were loaded onto a 98 cm × 1.3 cm column packed with Sepharose® CL-6B (Sigma) (fractionation range of dextrans: 1×10^4 to 1×10^6 g mol^{-1}) in the presence of buffer II, and eluted at a flow rate of 12 mL h^{-1}. Fractions of 2 mL were collected and the NS concentrations were estimated by the anthrone method.

2.3. Enzyme activity assays

For each enzymatic assay, 10 g of frozen strawberries from LG, T and R stages of each species were homogenized in an Omnimixer with 30 mL of the corresponding extraction buffer. Two extracts were prepared for each developmental stage of each species. Determinations of each enzyme activity were performed in duplicates.

2.3.1. Pectin methylesterase (PME) activity

PME activity was assayed based on the spectrophotometric procedure of Hagerman and Austin (1986). The enzyme extract was obtained by homogenizing fruit tissue with a solution containing 1 mol L^{-1} NaCl and 1% (w/v) PVPP under stirring at 4 °C for 4 h, and then centrifuged at 11,000 × *g* for 30 min. The pH of supernatant was adjusted to 7.5 and used to determine PME activity. The following reaction mixture was prepared in a total volume of 1000 μL: 0.1% (w/v) citrus pectin (Sigma), 0.0015% (w/v) bromothymol blue, 3 mmol L^{-1} K_3PO_4 (pH 7.5), 100 μL of enzymatic extract. The mixture was incubated at 37 °C and changes in absorbance were monitored at 620 nm for 5 min. As blank reactions, all mixture components were included except for the enzymatic extract which was replaced with 100 μL of 1 mol L^{-1} NaCl. PME activity was expressed as nmol galacturonic acid g^{-1} min^{-1}.

2.3.2. Polygalacturonase (PG) and endo-1,4-β-glucanase (EGase) activities

PG and EGase were extracted with buffer A (0.05 mol L^{-1} sodium acetate/acetic acid (pH 6.0)) containing 1% (w/v) PVPP. The mixture was centrifuged at 11,000 × *g* for 30 min at 4 °C. The pellet was resuspended in 30 mL buffer A and then centrifuged at 11,000 × *g* for 30 min at 4 °C; this step was repeated once. The final pellet was resuspended in buffer A containing 1 mol L^{-1} NaCl under stirring at 4 °C for 4 h, and then centrifuged at 11,000 × *g* for 30 min. The supernatant obtained was employed for EGase assay. In the case of PG, the supernatant was previously dialyzed overnight against buffer A at 4 °C.

The method to measure total PG activity was adapted from Villarreal et al. (2008). The activity was assayed in a mixture of 1400 μL total volume containing 0.3% (w/v) polygalacturonic acid (Sigma) in buffer A and 700 μL of enzymatic extract. The reaction mixture was incubated at 37 °C, aliquots of 300 μL were removed at different times for up to 23 h, frozen with liquid nitrogen and stored at −20 °C until quantification. In blank tubes, enzymatic extract was replaced by buffer A. Reducing ends generated during the reaction were measured using 2-cyanoacetamide according to Gross (1982). Total PG activity was expressed as nmol galacturonic acid g^{-1} h^{-1}.

The EGase activity was determined according to Vicente et al. (2005). The activity assay was performed in a total volume of 2000 μL in a mixture consisting of 2% (w/v) carboxymethylcellulose (Fluka) in buffer A and 1500 μL of enzymatic extract. The mixture was incubated at 37 °C, aliquots of 400 μL were taken at different times, frozen with liquid nitrogen and stored at −20 °C until quantification. In blank tubes, enzymatic extract was replaced with buffer A containing 1 mol L^{-1} NaCl. Reducing sugars were determined using dinitrosalicylic acid (DNS) method according to Miller (1959). EGase activity was expressed as nmol glucose g^{-1} h^{-1}.

2.3.3. α-Arabinofuranosidase (AFase), β-galactosidase (βGal) and β-xylosidase (βXyl) activities

For AFase activity detection, the fruit homogenate was obtained as described by Rosli et al. (2009) in extraction buffer A plus 1 mol L^{-1} NaCl, 1% (w/v) PVPP, 0.05% (v/v) Triton X-100 under stirring at 4 °C for 4 h, and then centrifuged at 11,000 × *g* for 30 min. In the case of βGal and βXyl, the homogenates were obtained in buffer A containing 1 mol L^{-1} NaCl and 1% (w/v) PVPP under stirring at 4 °C for 3 h and then centrifuged at 11,000 × *g* for 30 min. The supernatants were used to determine each enzyme activity by using the corresponding *p*-nitrophenyl derivates.

The activity of AFase (Rosli et al., 2009) was measured in a reaction mixture of 550 μL total volume consisting of 3 mmol L^{-1} *p*-nitrophenyl α-L-arabinofuranoside (Sigma) in 0.150 mol L^{-1} buffer citrate (pH 4.5), and 250 μL of enzymatic extract. The mixture was incubated at 37 °C, aliquots of 130 μL were taken at different times, frozen with liquid nitrogen and stored at −20 °C until quantification. In blank tubes, enzymatic extract was replaced by 0.150 mol L^{-1} buffer citrate (pH 4.5). To measure βGal activity (Trainotti et al., 2001), the following reaction mixture in 600 μL total volume was prepared: 30 mmol L^{-1} *p*-nitrophenyl β-D-galactopyranoside (Sigma) in buffer 0.05 mol L^{-1} sodium acetate/acetic acid (pH 4.5), and 360 μL of enzymatic extract. The mixture was incubated at 37 °C, aliquots of 150 μL were removed at different times and the reaction was stopped by adding 500 μL 0.4 mol L^{-1} Na_2CO_3. As blank, 360 μL of buffer 0.05 mol L^{-1} sodium acetate/acetic acid (pH 4.5) was used instead of enzymatic extract. βXyl activity assay (Martínez et al., 2004) was performed in a mixture of 600 μL total volume consisting in 10 mmol L^{-1} 4-nitrophenyl β-D-xylopyranoside (Sigma) in buffer A, and 300 μL of enzymatic extract. The mixture was incubated at 55 °C, aliquots of 135 μL were taken at different times, frozen with liquid nitrogen and stored at −20 °C until quantification. For blank tubes, buffer A was used instead of enzymatic extract.

The amount of *p*-nitrophenol released in each reaction was determined by measuring the optical density at 405 nm. A calibration curve was prepared with *p*-nitrophenol. Activity was expressed as nmol *p*-nitrophenol g^{-1} min^{-1} and h^{-1}.

2.4. Statistical analysis

Cell wall isolations and fractionation were done three times for each developmental stage, NS and UA measurements and enzymatic activities were done in duplicates. Exclusion chromatographies were performed twice and measurements were done in duplicates. Data of weight, firmness, AIR, cell wall polysaccharides and enzymatic assays were analyzed by analysis of variance (ANOVA) using SPSS v.14 (SPSS Inc.) software and means were compared by a least significant difference (LSD) test at a significance level of 0.05. Pearson linear correlations were performed using Texasoft WINKS SDA (6th Ed., Cedar Hill, TX, USA) software.

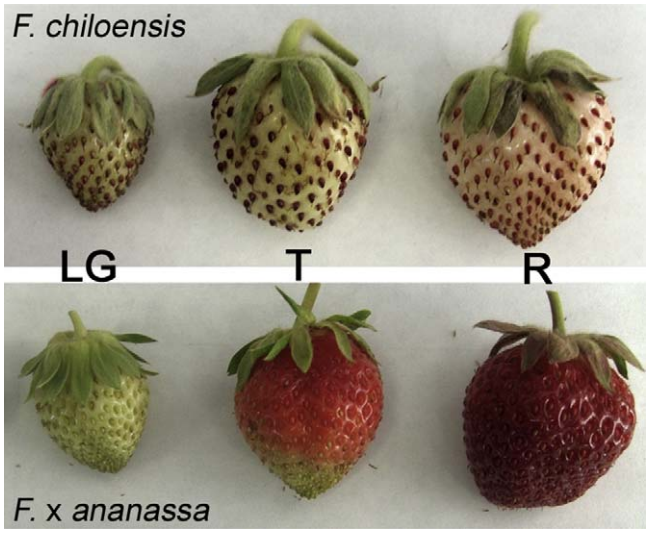

Fig. 1. Fruit developmental stages of strawberry species used in this research. LG, large green; T, turning; R, ripe. Classification was made according to Figueroa et al. (2008).

3. Results

3.1. Changes in fruit firmness and cell wall content

Fruit of both species were classified into three developmental stages according to previous classification criteria (Figueroa et al., 2008) (Fig. 1). Fruit weight of both strawberry species increased significantly throughout development, although *F. ×ananassa*'s fruit were significantly heavier than *F. chiloensis* fruit at all stages (Fig. 2A). Both species have similar firmness values at the initial stage of development (LG stage), and also at the end of ripening (R stage); a rapid decrease in firmness was observed after LG stage (Fig. 2B). The main firmness reduction occurred between the LG and T stages in both species, nevertheless a greater reduction was recorded in *F. chiloensis* than in *F. ×ananassa*. Total AIR content expressed per g of fruit, which reflects the abundance of cell wall components, decreased markedly during fruit development in both species (Fig. 2C). Total AIR content was higher in *F. chiloensis* than in *F. ×ananassa* at all developmental stages.

3.2. Changes in pectin and hemicellulosic fractions

A clear increase in the UA content was observed in the WSP fraction as fruit ripening progresses in both species; a great increment between the LG and T stages was recorded (Fig. 3A), and a significant correlation was found between WSP content and fruit firmness reduction in both species (Table 1). Regarding the ESP fraction, the content of UA was lower than that of the corresponding WSP or HSP fractions, and it remained relatively stable during fruit development in both species (Fig. 3B and Table 1). The abundance of UA in the HSP fraction decreased during ripening of both species (Fig. 3C), with a significant correlation with fruit softening (Table 1). Nevertheless, a greater reduction was observed between LG and T stages in *F. chiloensis* than in *F. ×ananassa*, with 70% and 25% reduction respectively. The content of NS in the NSP fraction decreased constantly during fruit ripening in both species (Fig. 3E), with a significant linear correlation coefficient between fruit firmness and NSP content (Table 1). SSP fractions remained constant in *F. chiloensis* but decrease in *F. ×ananassa* throughout ripening (Fig. 3D). However, no significant correlation with fruit firmness was found in both species (Table 1).

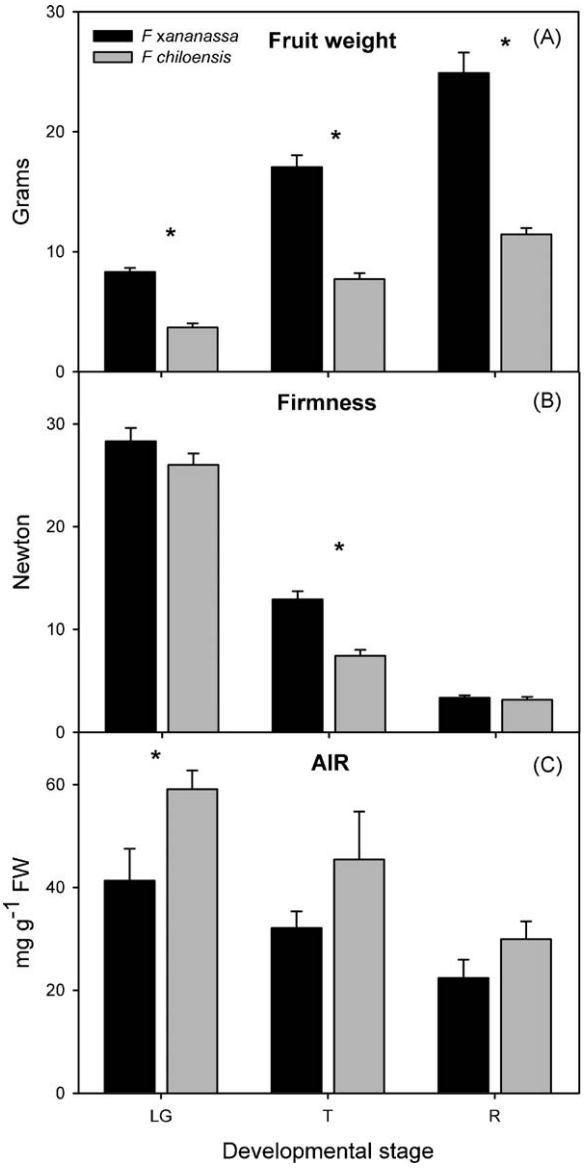

Fig. 2. Changes in fruit weight (A), firmness (B) and cell wall material content (alcohol insoluble residues, AIR) (C) in the different developmental stages defined as in Fig. 1. Each firmness and AIR data corresponds to the mean (±SE) of thirty fruit and of three independent cell wall extractions, respectively. Different asterisks indicate significant differences between species at the same stage ($P < 0.05$).

In complement to compositional changes in cell wall polysaccharides, the molecular size of the polymers was also analyzed by means of size exclusion chromatography (Fig. 4). Small changes in size in the WSP fraction were registered between the LG and R stages in *F. chiloensis*, indicating that some depolymerization of WSP fraction is taking place during ripening of the fruit (Fig. 4A). No changes in polymers size were observed in WSP fraction in *F. ×ananassa*. A wider distribution in size was observed in HSP polymers compared to that of WSP polymers. In addition, changes in size of HSP polymers displayed more dramatic changes than in WSP fraction in both species. *F. chiloensis*'s HSP fraction had a slight depolymerization during transition from LG to R stages, however a clear shift towards lower molecular mass was observed in *F. ×ananassa*. On the other hand, chromatographic analysis performed in *F. chiloensis* indicates a slight reduction in the molecular mass of hemicellulosic polymers, and no changes in size in *F. ×ananassa* (Fig. 4B).

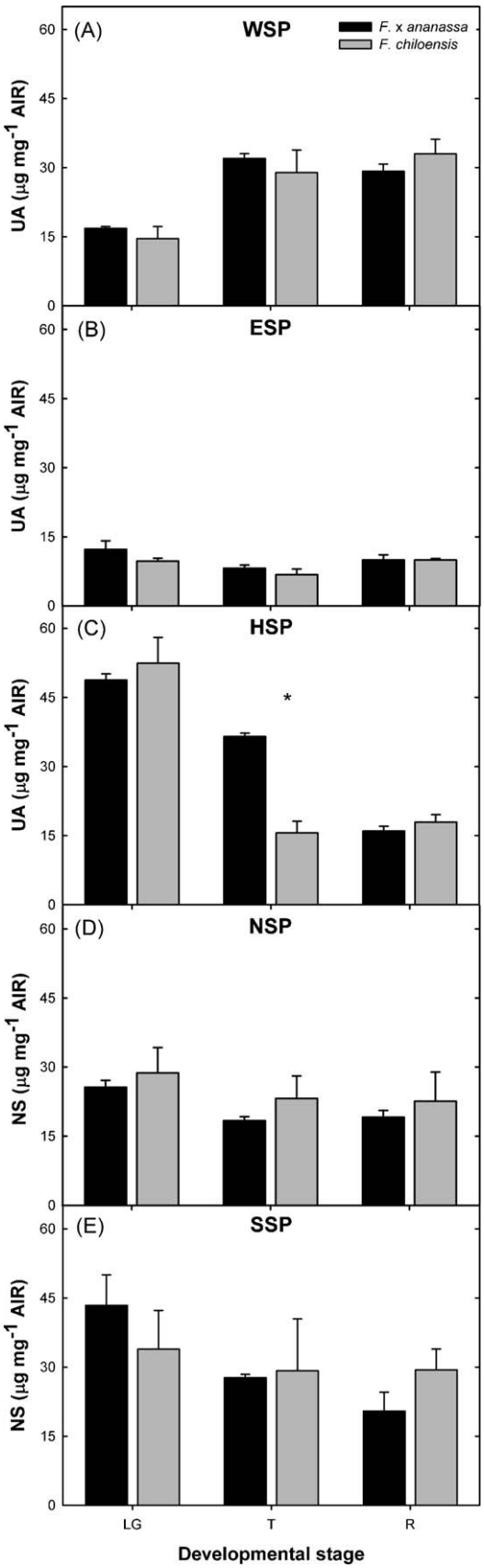

Fig. 3. Changes in content of uronic acids (A–C) and neutral sugars (D and E) in different cell wall fractions extracted from developing strawberry fruit. Developmental stages defined as in Fig. 1. Each WSP (water-soluble polymers), ESP (EDTA-soluble polymers), HSP (HCl-soluble polymers), NSP (NaOH-soluble polymers) and SSP (sulfuric acid-soluble polymers) data corresponds to the mean (±SE) of three independent cell wall extractions. Different asterisks indicate significant differences between species at the same stage ($P < 0.05$).

Table 1
Pearson linear correlations between average fruit firmness and cell wall fractions (CWFs) and enzymes (CWEs).

CWF	*F. ×ananassa*		*F. chiloensis*	
	Coefficient (*r*)	*P*-value	Coefficient (*r*)	*P*-value
Correlation between firmness and CWFs				
WSP	−0.823	0.006**	−0.835	0.005**
ESP	0.446	0.229	0.376	0.463
HSP	0.956	0.001**	0.930	0.001**
NSP	0.770	0.015*	0.838	0.005**
SSP	0.314	0.410	0.327	0.390
CWE	***F. ×ananassa***		***F. chiloensis***	
	Coefficient (*r*)	*P*-value	Coefficient (*r*)	*P*-value
Correlation between firmness and CWEs				
PME	−0.228	0.664	−0.626	0.183
PG	−0.818	0.047*	0.809	0.051
EGase	−0.797	0.058	−0.818	0.047*
AFase	−0.688	0.131	0.733	0.097
βGal	−0.775	0.071	−0.905	0.013*
βXyl	−0.990	0.001**	−0.628	0.181

Abbreviations of cell wall fractions and enzymes as described in Section 2.
* Correlation was significant at the 0.05 level.
** Correlation was significant at the 0.01 level.

3.3. Patterns of enzyme activities

With the aim to associate the important cell wall changes observed with some enzyme activities we compared the activity of six representative enzymes in both strawberry species. The activity of the cell wall degrading enzymes analyzed displays particular patterns throughout the ripening of both strawberry species. With the exception of PME, *F. chiloensis* fruit presents higher levels of activity than *F. ×ananassa* in all the enzymes assayed (Fig. 5).

PME activity displays a similar pattern during ripening in both species: activity was a medium level at LG and R stages, and a high level of activity was recorded at T stage. The activity was not correlated to fruit firmness evolution in both species (Table 1). However PME activity in *F. ×ananassa* was higher than in *F. chiloensis* at all stages (Fig. 5A). The analysis of PG activity revealed contrasting patterns between both species, with higher levels of activity in *F. chiloensis* than in *F. ×ananassa* at all stages. While the level of PG activity decreases as softening progresses in *F. chiloensis* (Table 1), the contrary was observed in *F. ×ananassa* in which a progressive increment was observed (Fig. 5B), showing a significant correlation with fruit firmness (Table 1). Although patterns of activity changes were different, PG activity levels in *F. chiloensis* were clearly higher than those of *F. ×ananassa*. EGase activity increased during ripening in both species, nevertheless, higher activity levels were observed in *F. chiloensis* fruit than in *F. ×ananassa* (Fig. 5C), with a significant correlation to fruit softening (Table 1).

AFase activity in *F. chiloensis* decreased during the transition from LG to T stages, and remains constant until R stage, while in *F. ×ananassa* AFase activity was low at LG and T stages but it doubles during the transition from T to R stage (Fig. 5D). Only at LG stage a higher level of AFase activity was recorded in *F. chiloensis* than in *F. ×ananassa*. βGal and βXyl activities increased during softening in both species (Fig. 5E and F): βGal significantly correlated with fruit softening in *F. chiloensis*, and βXyl in *F. ×ananassa*'s fruit (Table 1).

4. Discussion

As *F. chiloensis* fruit displays a larger decrease in firmness during transition from LG to T stages than *F. ×ananassa*, we decided to

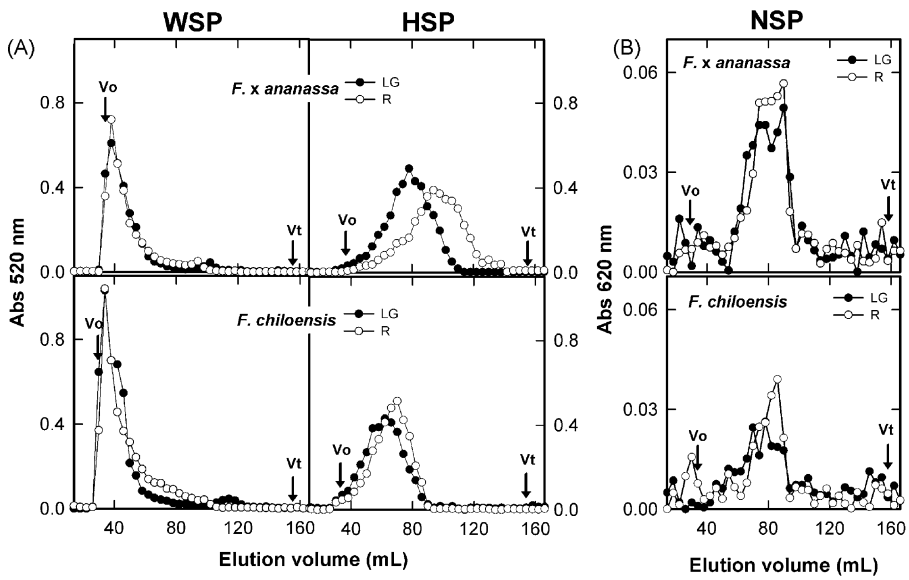

Fig. 4. Gel filtration chromatography on Sepharose® CL-4B of WSP and HSP fractions (A) on Sepharose® CL-6B of NSP fraction (B) from LG and R strawberry fruit stages. Developmental stages defined as in Fig. 1. Data represent the mean of two chromatographic runs. Vo, void volume; Vt, total volume.

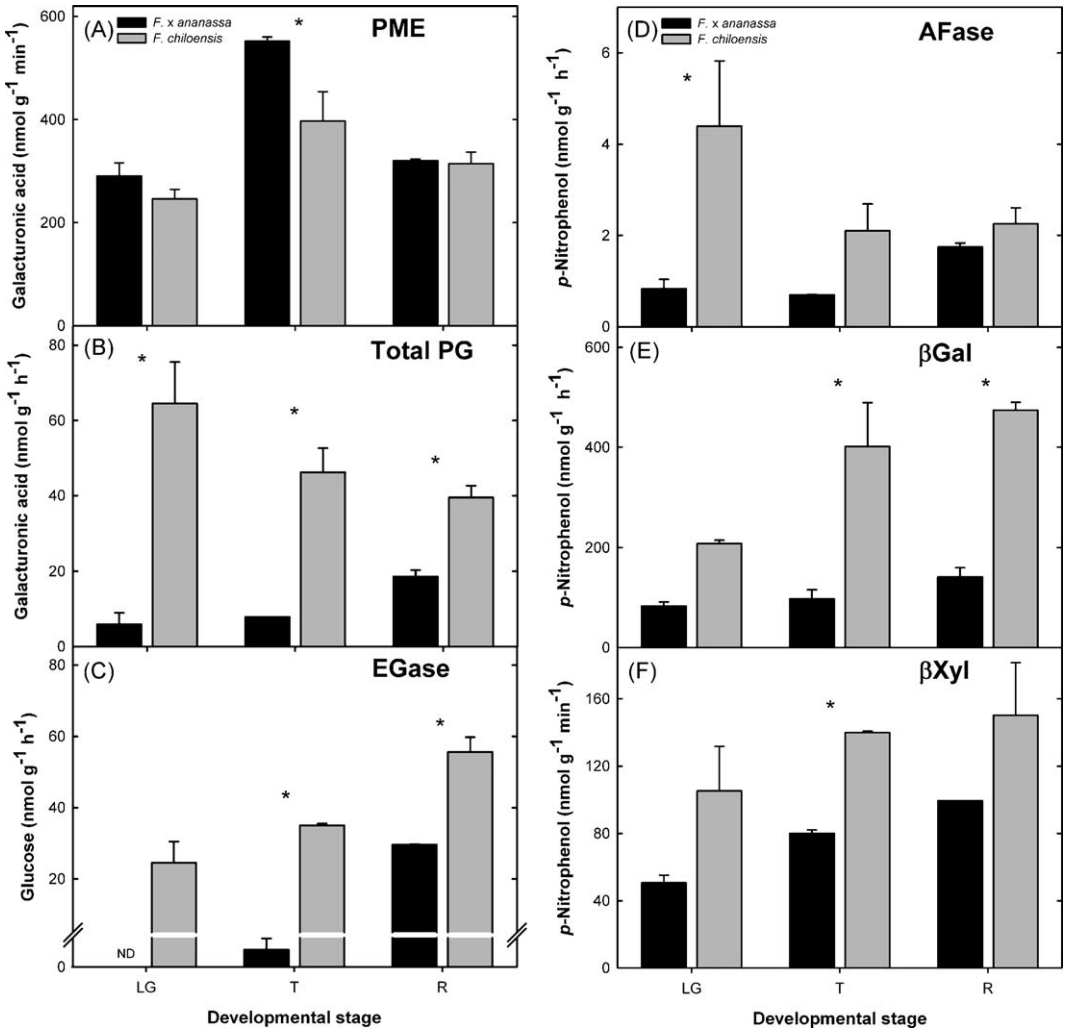

Fig. 5. Patterns of pectin methylesterase (A), total polygalacturonase (B), endo-1,4-β-glucanase (C), α-arabinofuranosidase (D), β-galactosidase (E) and β-xylosidase (F) enzymatic activities assayed during fruit development and ripening of *F. chiloensis* and *F. ×ananassa* cv. Chandler. Enzyme activity assays as described in Section 2. Stages analyzed included: LG, T and R. Values represent the mean ± SE and asterisks indicate significant differences between species at the same stage ($P < 0.05$). In (C), ND, not detected.

investigate if this difference could be related to changes in cell wall components. As fruit grows, a reduction in fruit firmness takes place which is accompanied by a decrease in total AIR content in both species (Fig. 2). Nevertheless, the faster reduction in firmness observed in *F. chiloensis*'s fruit was not followed by a greater reduction in total AIR content. Similar results have been observed in different strawberry cultivars (Huber, 1984; Rosli et al., 2004). In addition, firmness differences in firm (Camarosa) and soft (Toyonoka) strawberry cultivars were not followed by AIR differences (Rosli et al., 2004). These results suggest that changes in AIR content do not influence the fast softening rate observed in the Chilean strawberry fruit.

During development and ripening of both strawberry species under study the most significant changes in cell wall components were observed in pectin-rich fractions, especially in the HSP fraction, rather than in the hemicellulosic fraction (Fig. 3D). This could indicate that softening of strawberry fruit is closely related to catabolism of covalently bound pectins rather than ionically bound pectins or hemicelluloses. The reduction in UA content is verified in the HSP fraction, and at the same time the UA content in the WSP fraction accumulates in both species. This strongly suggests that WSP polyuronides are derived from the HSP fraction rather than ESP, as ESP pectin fraction remained at low levels and relatively stable during fruit development. As previously proposed (Nishizawa et al., 2002) the lower amount of ionically- and covalently-bound pectins and the concomitant higher water soluble pectin content found at the ripe stage in *F. chiloensis* fruit could explain the softer cortical tissue of the species. Nevertheless, this observation is based in measurements performed at the ripe stage. Koh and Melton (2002) described a reduction in the UA content of both the chelator and Na_2CO_3 soluble pectins at late stages of development of *F. ×ananassa* (cv. Yolo), as it has been observed previously by Knee et al. (1977) in which at least half of the polyuronide in ripening strawberry cell walls was weakly bound.

Previous works done in strawberry fruit reported the absence of depolymerization in WSP fraction during ripening of Dover and Pajaro *F. ×ananassa* cultivars (Huber, 1984; Redgwell et al., 1997a), but a slight depolymerization was detected in Toyonoka, a fast softening rate cultivar (Nogata et al., 1996; Rosli et al., 2004). In our case, we found in *F. chiloensis*, the softest species in this study, a similar depolymerization pattern of the WSP fraction to that described for Toyonoka, and no depolymerization in *F. ×ananassa* cv. Chandler coincident with Pajaro cultivar (Rosli et al., 2004). On the other hand, we found for the HSP fraction a marked shift towards lower molecular mass polymers during softening of *F. ×ananassa* cv. Chandler, coincident with the reports made for Toyonoka (Rosli et al., 2004). In *F. chiloensis* a small reduction in molecular mass of the HSP fraction polymers was observed between LG and R stages. Furthermore, ripe fruit HSP polymer sizes are larger than those recorded for Chandler cultivar, even at the LG stage. These data suggest that depolymerization is reduced in *F. chiloensis*, although an important reduction in the content of HSP fraction is taking place.

In general, with the exception of PME, the activity levels found in *F. chiloensis* fruit were higher than those of *F. ×ananassa*. PME activity reached a maximum level at T stage in both species, being higher in *F. ×ananassa* than in *F. chiloensis*. The enzymatic profile is similar to the pattern described by Barnes and Patchett (1976). A close relationship between PME and softening of strawberry fruit seems to exist since it has been reported that UV-C irradiation delays softening, being PME activity reduced immediately after treatment (Pombo et al., 2009). According to Draye and Van Custem (2008) a strong and early PME activity measured during ripening of *F. ×ananassa* cv. Elsanta fruit is compatible with an increment in acidic pectin content and consequently with the action of pectolytic enzymes. In this sense, the fast reduction in UA content in the HSP fraction of *F. chiloensis* fruit between LG and T stages could be associated with the high PG activity observed at the initial stages of ripening (Fig. 5B), and to the early and high expression rate of PG transcripts recorded during ripening of *F. chiloensis* fruit (Figueroa et al., 2008). PG could have a key role in pectin degradation since the enzymatic activity is closely related to strawberry softening (Villarreal et al., 2008; Quesada et al., 2009). In addition, several authors suggested that the reduction in the content of covalently bound pectins might be due to PG activity and also that high PG activity was associated with softer strawberry cultivars (Rosli et al., 2004; Villarreal et al., 2008). Thus, the high level of PG activity observed in *F. chiloensis* fruit at the LG stage (Fig. 5B) could be related to its marked decrease in the content of HSP, but unexpectedly, not related to its depolymerization degree. Probably, the high PG activity detected in *F. chiloensis* is due in a great extent to an exo-activity since the method does not discriminate between both exo- and endo-activities. Therefore, PG in this species seems to be more related with pectin solubilization rather than depolymerization, although this hypothesis needs to be corroborated.

In the case of *F. ×ananassa* cv. Chandler, PG activity increased during ripening, in agreement with data described in Toyonoka and Camarosa cultivars (Villarreal et al., 2008). In the case of *F. ×ananassa* the greater pectin depolymerization and consequently later softening, could be related to the action of both PG and pectate lyase (PL) enzymes, which can act in a coordinated way on homogalacturonan disassembly. We reported a higher PL transcript levels during ripening of Chandler cultivar than those of *F. chiloensis* (Figueroa et al., 2008). In agreement with this, strawberry plants (Chandler cultivar) with antisense expression of a PL gene produced firmer fruits than controls showing a reduction in pectin solubility and depolymerization (Santiago-Doménech et al., 2008), decreased intercellular spaces and a higher degree of cell to cell adhesion (Youssef et al., 2009).

In addition to pectin solubilization, the loss of neutral sugars from side chains of pectins seems to be a common mechanism that accompanies softening in the majority of fruit species (Gross and Sams, 1984). Strawberry fruit loses about 30% of neutral sugars during ripening, being the decrease of arabinose (Ara) and galactose (Gal) particularly high (Gross and Sams, 1984). One isoform of βGal gene has been related to the softening process in *F. ×ananassa* and a considerable enzymatic activity level has been detected in white and pink fruit (Trainotti et al., 2001). In *F. chiloensis*, the high level of βGal activity found at the end of ripening, could be related to an active deglycosylation when approaching the R stage. In contrast, enzymatic activity found in *F. ×ananassa* fruit was lower than that of *F. chiloensis*, however βGal activity increases from T to R stage, in accordance to the loss of Gal reported at the end of ripening (Knee et al., 1977; Redgwell et al., 1997b). Regarding Ara, it decreases sharply during ripening of *F. ×ananassa* (Koh and Melton, 2002) and *F. chiloensis* fruit (Nishizawa et al., 2002). The AFase enzymatic activity in *F. ×ananassa* cv. Chandler was very low at LG stage and increases during ripening, which coincides with the profile reported in other strawberry cultivars (Rosli et al., 2009). Instead, in *F. chiloensis* the AFase activity is high at LG stage and then decreases, although it reached similar levels than those found in the commercial strawberry at R stage. These two neutral sugar-removing enzymes could be associated to pectin degradation, since side chains of pectins are composed mainly of neutral sugars, being Ara and Gal the most abundant in strawberry fruit (Koh and Melton, 2002; Nishizawa et al., 2002). In the case of *F. chiloensis* the high levels of activity showed by both enzymes during ripening could be related to the higher degree of HSP fraction loss observed in this species.

Regarding hemicelluloses, some reports indicate little changes in the molecular mass of the polymers during ripening of fruits

such as tomato and melon (Tong and Gross, 1988; Rose et al., 1998), while others indicate that some fruits soften without any detectable depolymerization of xyloglucan or other matrix glycans (Goulao and Oliveira, 2008). Our results indicate that the slight depolymerization observed during softening of *F. chiloensis* and the significant correlation between NSP content with firmness in both species, suggests some participation of hemicellulose degrading enzymes, such as EGase and βXyl, previously detected in *F. ×ananassa* fruit (Abeles and Takeda, 1990; Martínez et al., 2004). There is enough evidence that mRNA and protein levels along with EGase enzymatic activity increased during ripening of strawberry fruit (Harpster et al., 1998; Palomer et al., 2004). In this sense, the high levels of enzymatic activities in *F. chiloensis* could be participating in some hemicellulose depolymerization. However, hemicellulose depolymerization caused by EGase would not be the main factor affecting strawberry softening, since suppression of EGase genes does not prevent fruit softening (Woolley et al., 2001; Palomer et al., 2006; Mercado et al., 2010). Because of its high activity in *F. chiloensis* fruit and other cultivars of *F. ×ananassa* (Harpster et al., 1998; Trainotti et al., 1999), EGase might contribute in the hydrolysis of noncrystalline regions of cellulose (Llop-Tous et al., 1999; Woolley et al., 2001).

Xylose (Xyl) and glucose (Glc) are important neutral sugar components of xyloglucans, which are associated to cellulose microfibrills, and their content decreases in parallel to the reduction of xyloglucan molecular size (Huber, 1984; Rose et al., 1998). High levels of Xyl and Glc were found in the hemicellulosic fractions of commercial strawberry (Gross and Sams, 1984; Huber, 1984; Nogata et al., 1996) and Chilean strawberry (Nishizawa et al., 2002). On the other hand, strawberry cell walls have a high Xyl/Glc ratio in the hemicellulosic fraction (Huber, 1984; Koh and Melton, 2002). This suggests the presence of polymers containing Xyl like xylan and xylogalacturonan that could be targets of βXyl action. The activity of βXyl increased in both species during fruit ripening suggesting some participation of this enzyme in hemicelluloses degradation.

Finally, the enzymes EGase and βXyl, possibly in coordination with other cell wall-modifying proteins such as expansins, could be involved in the progressive disassembling of the cellulose–hemicellulose network. In this sense, our group has recently studied the gene expression profile of five expansin genes during fruit development of *F. chiloensis* (Figueroa et al., 2009). The expression of expansins 2 and 5 concomitant to the decline of fruit firmness may indicate a possible role in the process of cell wall loosening.

5. Conclusions

The role of PG and EGase in the softening process of *Fragaria* species seems to be central: both enzymes are correlated to the decrease in the content of HSP and NSP, respectively. On the other hand, activity levels of βGal and βXyl were significant correlated with fruit firmness reduction in *F. chiloensis* and *F. ×ananassa*, respectively. In addition, our findings support the idea that softening of *F. chiloensis* fruit is probably related to pectin solubilization at the early stages of ripening rather than pectin depolymerization. Further research is needed to better understand how these differences in pectin catabolism are produced.

Acknowledgements

This work has been partly funded by grants from Fundación Andes (C-13855/12), PBCT Project Anillo de Ciencia y Tecnología (ACT-41), "Proyecto de Colaboración Internacional CONICYT-SECYT 189/2005", Fondecyt Project 1050987, ANPCYT, PICT 2006 No 01140.

References

Abeles, F.B., Takeda, F., 1990. Cellulase activity and ethylene in ripening strawberry and apple fruit. Sci. Hortic. 42, 269–275.
Barnes, M.F., Patchett, B.J., 1976. Cell wall degrading enzymes and the softening of senescent strawberry fruit. J. Food Sci. 41, 1392–1395.
Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54, 484–489.
Brummell, D.A., 2006. Cell wall disassembly in ripening fruit. Funct. Plant Biol. 33, 103–119.
Bustamante, C.A., Rosli, H.G., Añón, M.C., Civello, P.M., Martínez, G.A., 2006. β-Xylosidase in strawberry fruit: isolation of a full-length gene and analysis of its expression and enzymatic activity in cultivars with contrasting firmness. Plant Sci. 171, 467–504.
Castillejo, C., de la Fuente, J.I., Iannetta, P., Botella, M.A., Valpuesta, V., 2004. Pectin esterase gene family in strawberry fruit: study of *FaPE1*, a ripening-specific isoform. J. Exp. Bot. 55, 908–918.
Chen, G., Duan, X., Shi, J., Lu, W., Luo, Y., Jiang, W., Jiang, Y., 2008. Effects of reactive oxygen species on cellular wall disassembly of banana fruit during ripening. Food Chem. 109, 319–324.
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.
Draye, M., Van Custem, P., 2008. Pectin methylesterases induce an abrupt increase of acidic pectin during strawberry fruit ripening. J. Plant Physiol. 165, 1152–1160.
Figueroa, C.R., Pimentel, P., Gaete-Eastman, C., Moya, M., Herrera, R., Caligari, P.D.S., Moya-León, M.A., 2008. Softening rate of the Chilean strawberry (*Fragaria chiloensis*) fruit reflects the expression of polygalacturonase and pectate lyase genes. Postharvest Biol. Technol. 49, 210–220.
Figueroa, C.R., Pimentel, P., Dotto, M.C., Civello, P.M., Martínez, G.A., Herrera, R., Moya-León, M.A., 2009. Expression of five expansin genes during softening of *Fragaria chiloensis* fruit. Effect of auxin treatment. Postharvest Biol. Technol. 53, 51–57.
Goulao, L.F., Oliveira, C.M., 2008. Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends Food Sci. Technol. 19, 4–25.
Gross, K.C., 1982. A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. Hortscience 17, 933–934.
Gross, K.C., Sams, C.E., 1984. Changes in cell wall neutral sugar composition during fruit ripening: a species survey. Phytochemistry 23, 2457–2461.
Hagerman, A.E., Austin, P.J., 1986. Continuous spectrophotometric assay for plant pectin methyl esterase. J. Agric. Food Chem. 34, 440–444.
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. Plant Physiol. 118, 1307–1316.
Huber, D.J., 1984. Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. J. Food Sci. 47, 1310–1315.
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.
Knee, M., Sargent, J.A., Osborne, D.J., 1977. Cell wall metabolism in developing strawberry fruits. J. Exp. Bot. 28, 377–396.
Koh, T.H., Melton, L.D., 2002. Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues. Postharvest Biol. Technol. 26, 23–33.
Koh, T.H., Melton, L.D., Newman, R.H., 1997. Solid-state ^{13}C NMR characterization of cell walls of ripening strawberries. Can. J. Bot. 75, 1957–1964.
Llop-Tous, I., Dominguez-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.
Martínez, G.A., Civello, P.M., 2008. Effect of heat treatments on gene expression and enzyme activities associated to cell wall degradation in strawberry fruit. Postharvest Biol. Technol. 49, 38–45.
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. Biochem. 42, 89–96.
Mercado, J.A., Trainotti, L., Jiménez-Bermúdez, L., Santiago-Doménech, N., Posé, S., Donolli, R., Barceló, M., Casadoro, G., Pliego-Alfaro, F., Quesada, M.A., 2010. Evaluation of the role of the endo-β-(1,4)-glucanase gene FaEG3 in strawberry fruit softening. Postharvest Biol. Technol. 55, 8–14.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.
Nishizawa, T., Nagasawa, S., Retamales, J.B., Lavín, A., Motomura, Y., 2002. Comparison of cell wall components between *Fragaria ×ananassa* and *Fragaria chiloensis* grown in Chile. J. Hortic. Sci. Biotechnol. 77, 404–410.
Nogata, Y., Yoza, K., Kusumoto, K., Ohta, H., 1996. Changes in molecular weight and carbohydrate composition and hemicellulose during ripening in strawberry fruit. In: Visser, J., Voragen, A.G.J. (Eds.), Pectins and Pectinases. Elsevier, Amsterdam, pp. 591–596.
Palomer, X., Domínguez-Puigjaner, E., Vendrell, M., Llop-Tous, I., 2004. Study of strawberry cel1 endo-β-(1,4)-glucanase protein accumulation and characterization of its in vitro activity by heterologous expression in *Pichia pastoris*. Plant Sci. 167, 509–518.
Palomer, X., Llop-Tous, I., Vendrell, M., Krens, F.A., Schaart, J.G., Boone, M.J., van der Valk, H., Salentijn, E.M.J., 2006. Antisense down-regulation of strawberry endo-β-(1,4)-glucanase genes does not prevent fruit softening during ripening. Plant Sci. 171, 640–646.

Pombo, M.A., Dotto, M.C., Martínez, G.A., Civello, P.M., 2009. UV-C irradiation delays strawberry fruit softening and modifies the expression of genes involved in cell wall degradation. Postharvest Biol. Technol. 51, 141–148.

Quesada, M.A., Blanco-Portales, R., Posé, S., García-Gago, J.A., Jiménez-Bermúdez, S., Muñoz-Serrano, A., Caballero, J.L., Pliego-Alfaro, F., Mercado, J.A., Muñoz-Blanco, J., 2009. Antisense down-regulation of the FaPG1 gene reveals an unexpected central role for polygalacturonase in strawberry fruit softening. Plant Physiol. 150, 1022–1032.

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

Redgwell, R.J., Fischer, M., Kendal, E., MacRae, E.A., 1997b. Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. Planta 203, 174–181.

Retamales, J.B., Caligari, P.D.S., Carrasco, B., Saud, G., 2005. Current status of the Chilean native strawberry (*Fragaria chiloensis* L. (Duch.)) and the research needs to convert the species into a commercial crop. Hortscience 40, 1633–1634.

Rose, J.K.C., Hadfield, K.A., Labavitch, J.M., Bennett, A.B., 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol. 117, 345–361.

Rosli, H.G., Civello, P.M., Martínez, G.A., 2004. Changes in cell wall composition of three *Fragaria* × *ananassa* cultivars with different softening rate during ripening. Plant Physiol. Biochem. 42, 823–831.

Rosli, H.G., Civello, P.M., Martínez, G.A., 2009. α-L-Arabinofuranosidase from strawberry fruit: cloning of three cDNAs, characterization of their expression and analysis of enzymatic activity in cultivars with contrasting firmness. Plant Physiol. Biochem. 47, 272–281.

Saladié, M., Matas, A.J., Isaacson, T., Jenks, M.A., Goodwin, S.M., Niklas, K.J., Xiaolin, R., Labavitch, J.M., Shackel, K.A., Fernie, A.R., Lytovchenko, A., O'Neill, M.A., Watkins, C.B., Rose, J.K.C., 2007. A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiol. 144, 1012–1028.

Santiago-Doménech, N., Jiménez-Bermúdez, S., Matas, A.J., Rose, J.K.C., Muñoz-Blanco, J., Mercado, J.A., Quesada, M.A., 2008. Antisense inhibition of a pectate lyase gene supports a role for pectin depolymerization in strawberry fruit softening. J. Exp. Bot. 59, 2769–2779.

Sozzi, G.O., Greve, C., Prody, G.A., Labavitch, J.M., 2002. Gibberellic acid, synthetic auxins, and ethylene differentially modulate α-L-arabinofuranosidase activities in antisense 1-aminocyclopropane-1-carboxylic acid synthase tomato pericarp discs. Plant Physiol. 129, 1330–1340.

Tong, C.B.S., Gross, K.C., 1988. Glycosyl-linkage composition of tomato fruit cell wall hemicellulose fractions during ripening. Physiol. Plant. 74, 365–370.

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.

Vicente, A.R., Costa, M.L., Martínez, G.A., Chaves, A.R., Civello, P.M., 2005. Effect of heat treatments on cell wall degradation and softening in strawberry fruit. Postharvest Biol. Technol. 38, 213–222.

Villarreal, N.M., Rosli, H.G., Martínez, G.A., Civello, P.M., 2008. Polygalacturonase activity and expression of related genes during ripening of strawberry cultivars with contrasting fruit firmness. Postharvest Biol. Technol. 47, 141–150.

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, *cel1*. Planta 214, 11–21.

Youssef, S.M., Jiménez-Bermúdez, S., Bellido, M.L., Martín-Pizarro, C., Barceló, M., Abdal-Aziz, S.A., Caballero, J.L., López-Aranda, J.M., Pliego-Alfaro, F., Muñoz, J., Quesada, M.A., Mercado, J.A., 2009. Fruit yield and quality of strawberry plants transformed with a fruit specific pectate lyase gene. Sci. Hortic. 119, 120–125.