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Rheological characterisation of juices obtained from transgenic pectate lyase-silenced strawberry fruits

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ABSTRACT

The present study is focused on the characteristics of juice made from transgenic strawberry fruits with a 90% reduction on pectate lyase mRNA expression. No differences of soluble solids, pH or solid volume fraction were found between control and transgenic juices. Total sugar content of the serum fraction was also similar but a slightly higher content of large molecular mass polyuronides was observed in transgenic juice. The solid fraction of transgenic juice contained larger particles than did the control. The dynamic shear analysis of the juices showed higher values of the storage (G') and loss (G'') moduli versus strain for the transgenic samples, with G' over G'' within the linear viscoelastic range (LVR). For both samples, G' and G'' increased with frequency, showing a weak-gel response, whereas complex viscosity (η^*) decreased with frequency, denoting a shear-thinning behaviour. Overall, the transgenic juices showed higher values of G', G'' and complex viscosity than did the control within the frequency range assayed and a more solid-like character. These results suggest that effects of pectate lyase-silencing in tissue integrity increased the content of large particles in juice, its viscoelastic properties being modified and its viscosity increased.

1. Introduction

Strawberries (*Fragaria x ananassa,* Duch.) are considered soft fruits, as classified by Bourne (1979). This group of fruits undergoes softening to a great extent at the end of its ripening stage, experiencing a dramatic drop in its firmness and leading eventually to a semi-liquid texture. Since strawberries retain optimum conditions for fresh consumption for a relatively short time (Manning, 1993), a large proportion of ripe and overripe fruits is used to obtain processed products.

The decay in strawberry fruit firmness, as a consequence of the softening process, involves some biochemical and tissue modifications, including the dissolution of the pectin-rich middle lamella of cortical parenchyma cells, which is in turn responsible for the separation and breakage of the adjacent cells (Brownleader et al., 1999). Such modification has been reported as the principal factor leading to softening (Perkins-Veazie, 1995). The histological changes occurring during ripening are caused by compositional and structural modifications of the fruit cell walls. The degradation of the cell wall components involves several enzymatic activities which mainly affect the matrix glycans and the pectin fraction. Although a depolymerization of hemicellulose occurs during strawberry ripening (Huber, 1984), the major biochemical changes in the strawberry fruit cell walls involve the pectin fraction. The proportion of water-soluble polyuronides in strawberry was found to increase from 30% in green fruit to 65% in ripe fruit (Huber, 1984), although total quantity of polyuronide residues (Huber, 1984; Knee, Sargent, & Osborne, 1977; Redgwell, McRae, Hallet, Fischer, Perry & Harker, 1997; Woodward, 1972) and polyuronide molecular size (Huber, 1984; Redgwell et al., 1997) do not seem to be significantly modified. Nevertheless, since pectins account for up to 60% of cell wall mass in many fruits (Redgwell et al., 1997), it is expected that the modifications of the polyuronides may have large effects on tissue texture. However, the relationship between the biochemical and histological modifications, and the softening of the fruits is very complex.

Regarding the enzymatic activities involved in the pectin degradation of the strawberry fruit cell walls, increases in the transcription levels of pectin methylesterase (PME) (Castillejo, de la Fuente, Iannetta, Botella, & Valpuesta, 2004), polygalacturonase (Redondo-Nevado, Moyano, Medina-Escobar, Caballero, & Muñoz-Blanco, 2001) and pectate lyase (PL) genes (Benitez-Burraco et al., 2003; Medina-Escobar, Cárdenas, Moyano, Caballero, & Muñoz-Blanco, 1997) have been reported. This indicates a possible role of these pectinases in the softening of the strawberry fruit.

The biotechnological alteration of several cell wall activities can modify, not only texture of fresh fruits, but also the rheological properties of the processed fruit products. In tomato, the processing of transgenic fruits exhibiting reduced levels of polygalacturonase

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activity yielded juices with increased viscosity when compared to those made out of non-transgenic fruits (Errington, Tucker, & Mitchell, 1998). Further rheological characterisation showed higher values and time-dependence of storage modulus (G') for these transgenic lines, suggesting the formation of an elastic network within the sample (Errington et al., 1998). Additionally, some studies on the effect of suppression and overexpression of an expansin gene (LeExp1) in tomato showed modifications of particle size distribution, polymer size and viscosity of the solid fraction of transgenic juices and pastes (Kalamaki, Harpster, Palys, Labavitch, Reid & Brummell, 2003). In strawberry, Jiménez-Bermúdez et al. (2002) reported that the reduction of the expression of a pectate lyase gene in ripe fruit, by means of antisense technology, reduced fruit softening. Recently, Sesmero, Quesada, and Mercado (2007) prepared strawberry jams with these anti-pectate lyase fruits, and demonstrated that transgenic berries resisted the cooking process better than did non-modified fruits. Additionally, the fragments of berries remaining in the jam were firmer in the transgenic material. It was concluded that the genetic modification affected the pectin metabolism of the strawberry fruits and gave rise to an improved texture of the jam. Besides jam, juice is one of the most important strawberry-derived products. In the present study, we have characterised some rheological and biochemical characteristics of juices made out of transgenic anti-pectate lyase strawberry fruits. As observed in jams, the rheological behaviour of the juices was modified as a result of pectate lyase silencing.

2. Materials and methods

2.1. Plant material

Transgenic strawberry (*Fragaria x ananassa*, Duch.) plants, cv. Chandler, containing an antisense sequence of the strawberry pectate lyase gene *plC*, were previously obtained and characterised by Jiménez-Bermúdez et al. (2002). Among them, the transgenic line Apel14 was selected for juice rheological characterisation and compared with a non-transgenic control line of cv. Chandler. At the full-ripened stage (i.e., when the whole surface of the fruit is red-coloured), this line is 90% silenced at the level of mRNA compared to the control (Jiménez-Bermúdez et al., 2002) and it showed a consistent phenotype with firmer fruits at harvest than the control during several growing seasons. Further characterisation of fruits of line Apel14, processed as jam, has also been reported recently (Sesmero et al., 2007). Strawberry plants were grown in a greenhouse and fruits collected between May and June. Fruits were harvested at full-ripened stage, transported to the laboratory, frozen in liquid nitrogen and stored at −28 °C until used.

2.2. Strawberry juice processing

Approximately 150 g of frozen strawberry fruits, with an average weight of 12.7 g, were processed as juices. Fruit surfaces were wiped with a cellulose paper in order to remove any trace of ice. Then, they were weighed, rapidly put on a metallic net on top of a container and incubated in a closed chamber at 30 °C for 15–16 h with some wet cotton on the side in order to avoid water loss. Then, the strawberries were blended with a commercial blender, using a grating, which excludes epidermis and seeds from the resulting juice. The drip was added to the juice through a cooking sieve, in order to avoid pouring the rest of tissues. Juices were centrifuged at 2000 rpm for 90 s to separate the bubbles, the bulk of which were removed with a spoon. Then, they were mixed again by gently shaking, yielding a homogeneous solution without any visible bubbles. This solution was subjected to rheological analysis. At least three juice replicates of both transgenic and control fruits were performed.

For serum and solid phase separation, 25 ml of juice were centrifuged at 2100*g* for 20 min at 20 °C. Then, the serum was collected by vacuum-filtration, using GF/A glass microfibre filters (Whatmann, UK). The volume of the solid fraction (VSF) was calculated as a percentage of total juice volume.

2.3. Rheological and viscosity characterisation of juice

The small amplitude oscillatory shear (SAOS) tests were carried out using a Bohlin C-VOR Rheometer (Malvern Instruments Ltd., UK) at 25 °C, with a plate–plate geometry and a gap of 1 mm. The edge of the probe was covered with liquid paraffin and a plastic cap to preserve samples from dehydration. The strain amplitude sweep (i.e., the dependence of the shear moduli G' and G'' on the deformation of the fluid) was analysed and the linear viscoelastic ranges (LVR) of the juices were determined. Then, the frequency sweep tests were carried out at 0.005 strain over the range 0.02–3.35 Hz, and the shear moduli (G' and G'') and the complex viscosity (η^*) dependence over the frequency (ω) was determined. In a different experiment, time sweep tests (1 Hz frequency, 0.005 strain, 23 min) were performed to analyse the stability of the samples. G' is a measure of the energy stored in the material or the solid-like behaviour, G'' is a measure of the energy lost as viscous dissipation or the liquid-like behaviour, and η^* is a measure of the resistance to flow. Results of the rheological analysis are averages of at least three measurements from independent juices.

2.4. Particle size distribution analysis

Particle size analysis of the solid phases was performed with the Mastersizer S laser diffraction device (Malvern Instruments Ltd., UK). Approximately 1 g of solid fraction was suspended in 10 ml of a solution of sucrose (5° brix). Then the sample was added to the dispersion unit, which was filled with the same medium, until an obscuration value below 20% was reached. The new dispersion was allowed to reach the swelling equilibrium for 5 min, and the measurement was finally performed. The *Fraunhoffer* model was chosen to analyse the data, since it is a valid model for tomato products (Getchell & Schlimme, 1985; Kalamaki et al., 2003). This method converts the diffraction patterns into 64 different size classes (from 0.058 μm to 878 μm). The particle size distribution plot and the volume moment mean (also named as De Brouckere mean diameter) were obtained. Results are averages of at least 10 independent replicates.

2.5. Pectin analysis on serum

The uronic acid and sugar contents of serum samples were measured, following the procedure described by Blumenkrantz and Asboe-Hansen (1973) and Dubois, Gilles, Hamilton, Rebers, and Smith (1956), respectively. Analysis of total pectin and sugar contents were performed in three independent juices. Further characterisation of the pectic polymers present in serum was performed by size exclusion chromatography (SEC). Similar volumes of serum samples from three independent juices were mixed and heated at 95 °C for 3 min to minimize pectin-degrading enzyme activities, then immediately frozen in liquid nitrogen and stored at −28 °C prior to further processing. Serum samples were dialysed for 2 d against deionized water, using a 7000 MW cut-off dialysis membrane (Snakeskin, Pierce, USA). After dialysis, sera were centrifuged at 23000*g* for 15 min at 4 °C; the supernatants were recovered and freeze-dried. Finally, fractions of 8 mg of freeze-dried serum samples belonging to control and transgenic line were dissolved in 1 ml of 0.2 M amonium acetate buffer, pH 5.0 (elution buffer),

and loaded onto the column (1.5 × 40 cm) containing Sepharose CL-2B as stationary medium. The rate of elution was fixed at 14 ml/h and fractions of 1 ml were collected. Then, uronic acid content was measured in every fraction, following the procedure of Blumenkrantz and Asboe-Hansen (1973).

3. Results

3.1. Physicochemical characterisation of juices

The volumes of juice per fresh fruit weight were similar in the control and Apel14 line, with a mean value of 74.3 ± 3 ml/100 g. Table 1 summarizes the main features of juices and sera for both genotypes. The values obtained for soluble solids, pH and solid volume fractions of juices were similar in the control and Apel14 line, as also were total sugar contents of the two sera. However, the amount of pectins was slightly lower in the transgenic serum than in the control line, although this was not statistically significant. Elution profiles of the pectic polymers present in the serum are shown in Fig. 1. In both genotypes, a significant amount of the polymers showed average molecular sizes higher than 2000 kDa, with a prominent peak at an elution volume of 14 ml, close to the void volume of the Sepharose CL–2B column (i.e., 20 MDa). The transgenic line showed a lower amount of medium and low-size pectins whereas, interestingly, the major peak was displaced towards lower elution volumes, indicating that this serum was enriched in larger polyuronide chains.

3.2. Small amplitude oscillatory shear (SAOS) analysis of juices

The amplitude sweep analysis at 1 Hz showed two different regimes for G' and G'' (Fig. 2). At low deformation values, the shear moduli were parallel to each other, i.e., independent of strain. At higher strain values, G' and G'' decreased, with G' showing a more dramatic decay. The range at which the shear moduli are independent of strain is the LVR, the maximum strain limit being around 0.006 for both juices. Therefore, a strain value of 0.005 was chosen for subsequent frequency sweep analysis, since it was within the LVR for both transgenic and control juices. Furthermore, it is noteworthy that, for the control juice, $G' = G''$ within the LVR, whereas, for the Apel14 juice, $G' > G''$. This would indicate a more elastic or solid-like character of this juice, possibly as result of a structure formation (Sharoba, Senge, El Mansy, Bahlol, & Blochwitz, 2005). In accordance with this result, time sweep tests, performed at 1 Hz and 0.005 strain, showed that the tangent of the phase angle was significantly lower in the transgenic juices (Fig. 3A). Moreover, for the Apel14 juice the G' and G'' values were nearly one order of magnitude higher than those found for the control (Fig. 2), denoting a higher η^* for the former, since:

$$\eta^* = \frac{\sqrt{(G')^2 + (G'')^2}}{\omega} \quad (1)$$

The frequency sweep analysis at 0.005 deformation showed an increase in the magnitudes of G' and G'' with increase in ω for the control and transgenic juices (Fig. 4A). Pooled data were subjected to the power-law regression, as proposed by Rao and Cooley (1992), following the next equations:

$$G' = K'(\omega)^{n'} \rightarrow \log G' = \log K' + n' \log \omega \quad (2)$$

$$G'' = K''(\omega)^{n''} \rightarrow \log G'' = \log K'' + n'' \log \omega \quad (3)$$

The results of the average curves are shown in Fig. 4B. For both moduli, a positive dependence on the frequency was observed, the average slopes found being 0.33 ± 0.05 and 0.44 ± 0.04 for n' and n'', respectively. This dependence, and the K' values higher than K'' values, as shown in the Figure, indicate that the structure of the juice falls into the category of weak gel (Clark & Ross-Murphy, 1987; Ross-Murphy, 1984).

As mentioned above, η^* was higher for the transgenic Apel14 juice than for the control, such difference being nearly constant over the frequency range assayed (Fig. 5). Additional SAOS experiments at 1 Hz and 0.005 strain gave similar results (Fig. 3B). However, both samples have, in common, an evident shear-thinning behaviour, since the η^* exhibited a negative dependence on ω (Fig. 5).

Table 1
Characteristics of juice and serum obtained using control and transgenic Apel14 ripe fruits. Values are means ± SD of three independent juices per line.

		Control	Apel14
Juice	pH	3.56 ± 0.05	3.64 ± 0.08
	Soluble solids (°brix)	7.8 ± 0.9	7.8 ± 2.1
	Volume of the solid fraction (%)	45.6 ± 3.1	42.9 ± 9.3
Serum	Total sugars (mg g^{-1} fw)	45.1 ± 15.4	46.3 ± 11.4
	Polyuronides (mg g^{-1} fw)	2.2 ± 1.2	1.9 ± 1.4

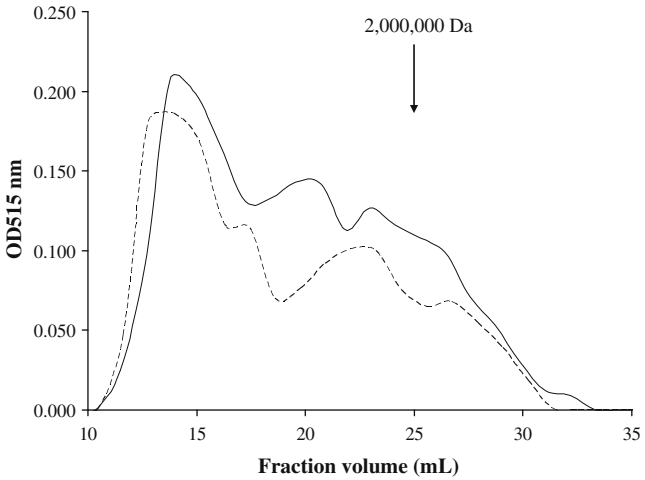

Fig. 1. Size exclusion chromatography profiles on Sepharose CL–2B of serum samples obtained from control (solid line) and Apel14 (dashed line) juice. Column fractions were assayed for polyuronide content. The elution volume for the blue dextran standard (2000 kDa) is shown in the Figure.

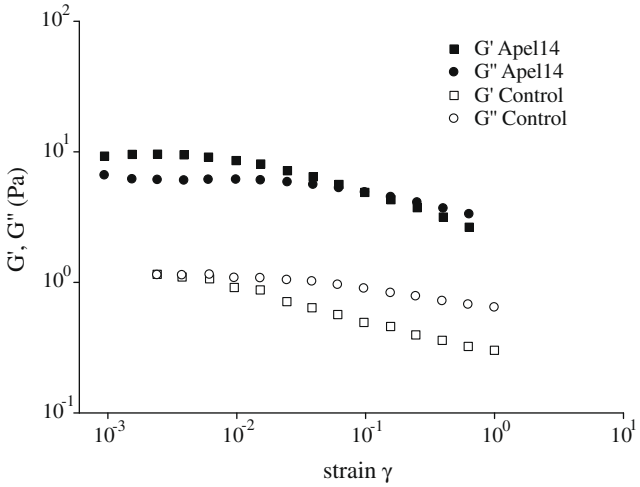

Fig. 2. Amplitude sweeps of representative samples for control and transgenic strawberry juices. G' and G'' curves are shown as a function of the deformation (strain).

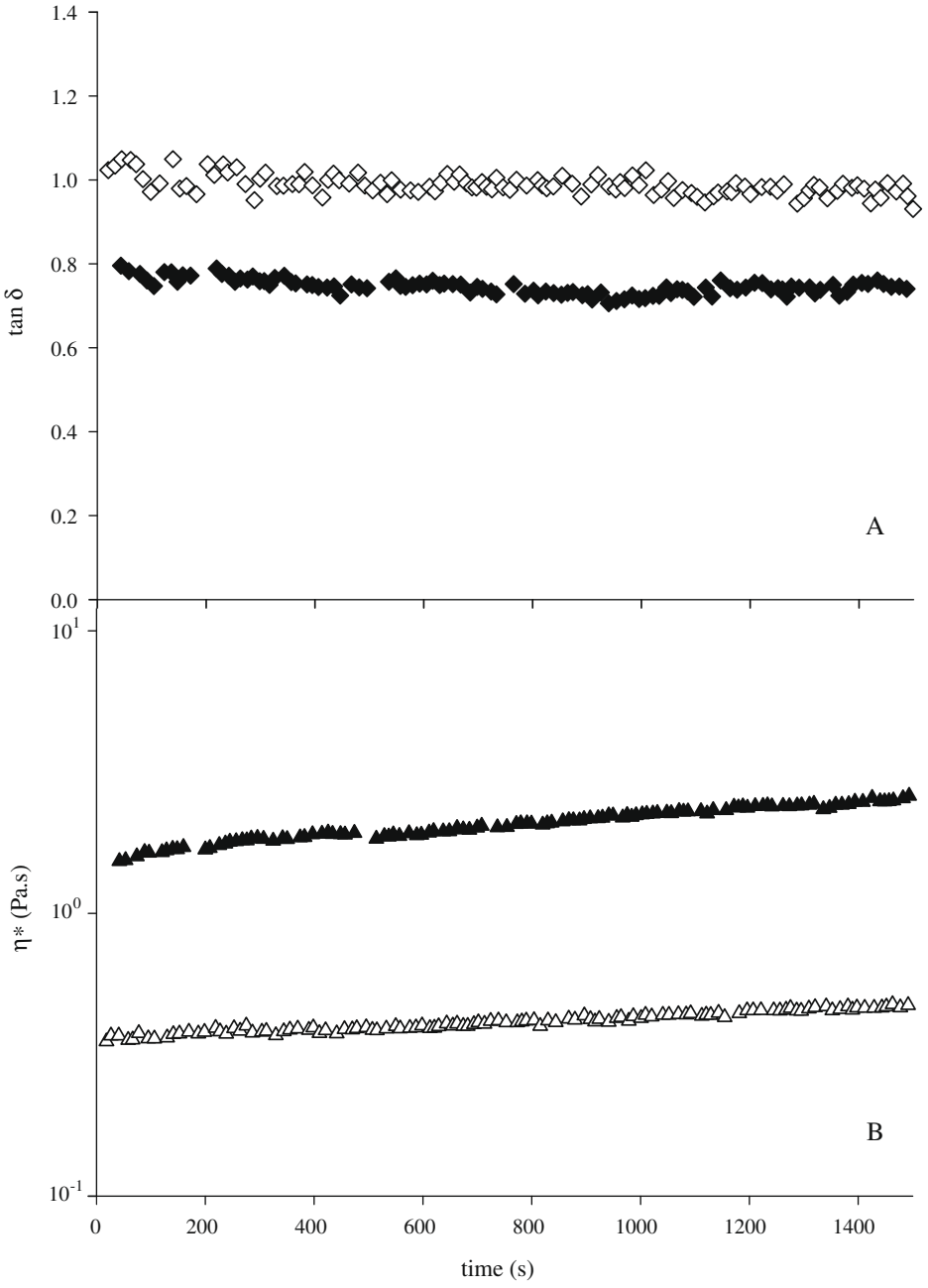

Fig. 3. Time sweep analysis of control and transgenic juices at fixed frequency (1 Hz) and strain (0.005). (A) Plot of the tangent of the phase angle in control (open symbols) and transgenic (filled symbols) strawberry juices. (B) Plot of η^* in control (open triangles) and transgenic (filled triangles) strawberry juices. Curves are averages of at least three independent juices per line.

3.3. Particle size distribution within juice

Particle size distribution (PSD) and volume moment mean (*D* [4,3]) of the juice solid fraction was analysed within the size range 0.05–878 μm. The Apel14 line exhibited a higher proportion of larger particles than did the control line (Fig. 6). Similar results were previously found by Sesmero et al. (2007), who demonstrated that, when processed into jam, the pectate lyase-silencing reduced tissue disintegration of the strawberry fruits and enhanced the firmness of the berries after the cooking process.

The volume moment mean, which represents the 'centre of gravity' of the volumetric size distribution (Rawle, 2007), confirmed a significant displacement of the distribution to higher volume values in the case of the Apel14 line (Fig. 6). The solid fraction of the Apel14 transgenic juice seems to be enriched with particles of larger volume compared to the control.

4. Discussion

In this study, we have processed strawberry fruits into juices and analysed their rheological behaviour. Some studies on the steady-shear rheological characterisation of strawberry processed into jam have been done (Carbonell, Costell, & Duran, 1991; Costell, Carbonell, & Duran, 1993; Grigelmo-Miguel & Martin-Belloso, 1999). However, the dynamic-shear characterisation of the strawberry juice has not so far been studied. The results of the frequency sweep analysis showed that the strawberry juice is within the category of weak gel (Ross-Murphy, 1984; Clark & Ross-

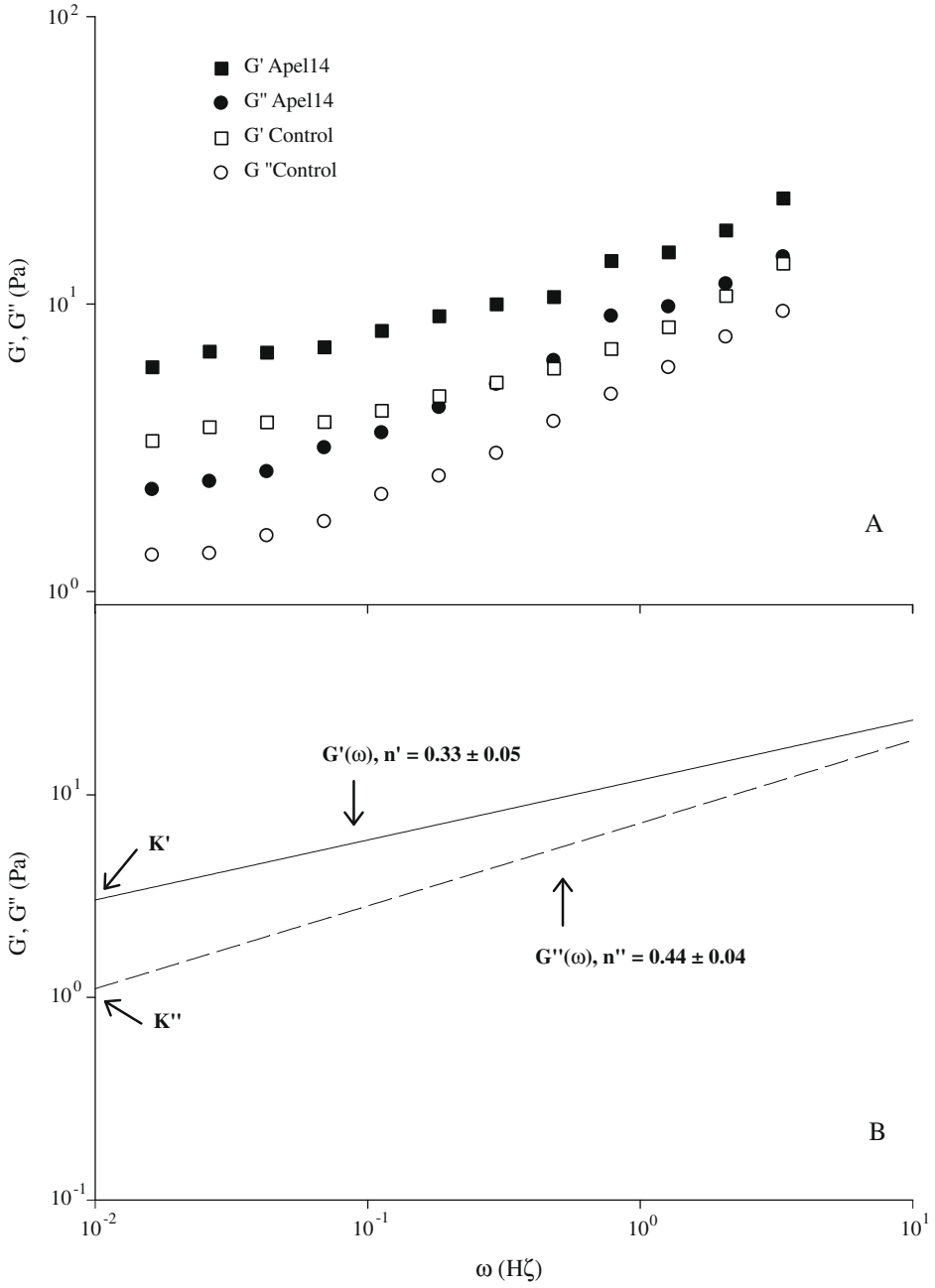

Fig. 4. Mechanical spectra of the strawberry juices. (A) Plot of G' (squares) and G'' (circles) versus frequency (ω), in control (open symbols) and transgenic (filled symbols) strawberry juices, at 0.005 strain and 25 °C. Curves are averages of at least three independent juices per line. (B) Power-law regression curves obtained with pooled data of control and Apel14 lines.

Murphy, 1987). Similar results have been obtained for several food products, such as tomato paste (Rao & Cooley, 1992) and rice flour dispersions (Chun & Yoo, 2004; Yoo, 2006), and also for systems composed of polysaccharide mixtures of xanthan and locust bean gum (Doublier & Cuvelier, 1996). These kinds of fluids may present a three-dimensional network, in which the polymer chains interact by means of low-energy bonds and cooperative effects along relatively extended junction zones (Doublier & Cuvelier, 1996), leading to an increase in the amount of stored energy (G') within the fluid (Errington et al., 1998).

Furthermore, the structure of the juice must be influenced by qualitative differences of the polymers present in the strawberry juice. Among them, pectin is an important candidate affecting such a structure, since it is one of the more abundant polymer compounds present in fruits (Redgwell et al., 1997). Therefore, differences in the pectic metabolism of the fruits may lead to structural and thus rheological changes in the juice.

The transgenic strawberry line, Apel14, employed in this study, has an altered pectin metabolism as a consequence of the pectate lyase gene antisense inhibition. A previous molecular analysis, performed in ripened fruits, showed a significant reduction in *plC* gene expression, the steady state level of this transcript being estimated by Northern blot analysis, as 10% of the level found in control fruits (Jiménez-Bermúdez et al., 2002). Silencing of *plC* resulted in fruits with firmer texture than the controls. This phenotype was observed at the time of harvest of ripened fruit (Jiménez-Bermúdez et al., 2002) and also in fruits processed into jam (Sesmero et al., 2007).

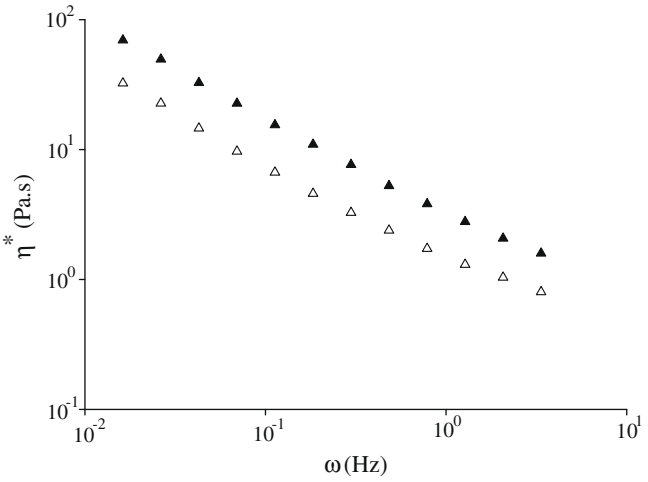

Fig. 5. Plot of η^* versus frequency of control (open triangles) and transgenic (filled triangles) strawberry juices, at 0.005 strain and 25 °C. Curves are averages of at least three independent juices per line.

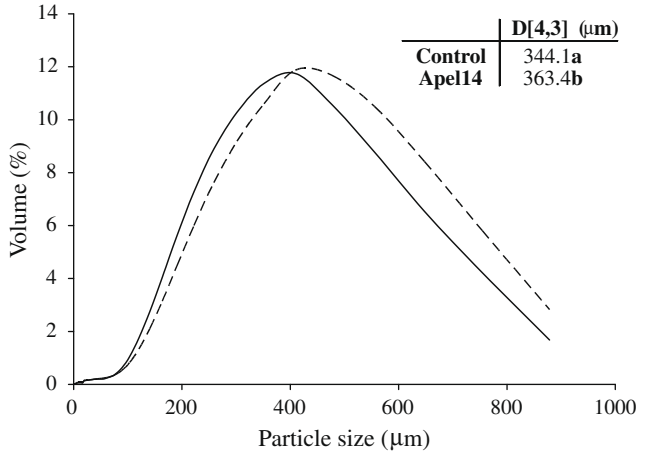

Fig. 6. Particle size distribution of the solid fraction for control (solid line) and Apel14 (dashed line) strawberry juice. Insert table shows the volume moment mean, also referred to as the DeBrouckere mean diameter (*D*[4.3]). Mean separation by *T*2 Tamhane test, $P = 0.05$).

As pointed out by Kalamaki et al. (2003), fruits with altered texture would be expected to yield processed products with modified rheological properties. Thus, differences in fresh fruit texture caused by the antisense inhibition of the pectate lyase gene may lead to modifications of the rheological properties of the processed strawberry products. As mentioned above, Sesmero et al. (2007) showed that jams prepared with anti-pectate lyase fruits contained more berries, which resisted the cooking process better than did the control fruits and, consequently, exhibited a higher firmness. Similarly, in the present work, a different rheological behaviour was found for the strawberry juices made out of transgenic anti-pectate lyase fruits when compared to the control. The Apel14 juices showed a higher elastic character than did the control, as well as a higher complex viscosity. These differences between control and transgenic juices could be caused by changes in the properties of serum and/or the suspended particles that constitute juices (Den Ouden & van Vliet, 1997; Kalamaki et al., 2003). However, preliminary experiments showed that, in strawberry juice, the viscosity of the serum was negligible compared to the overall viscosity of the juice (data not shown), indicating that the rheological differences between genotypes were mainly related to the properties of the solid fraction. A similar result was reported for tomato concentrates, where the contribution of the dispersed solids (pulp) was much greater than that of the serum (Hayes, Smith, & Morris, 1998). Additionally, complex viscosity seems to be influenced by particle sizes, as stated for processed products of tomato by Rao and Cooley (1992), Sánchez, Valencia, Gallegos, Ciruelos, and Latorre (2002) and Valencia, Sanchez, Ciruelos, Latorre, Franco and Gallegos (2002). We have found that the solid phase of Apel14 juices contains a higher proportion of large particles than does the control juices. Therefore, we propose that the higher elasticity of the transgenic juice is correlated with the increase on the volume of the dispersed solids.

During ripening, inhibition of the pectate lyase gene modifies pectin metabolism and cell wall disassembly (Jiménez-Bermúdez et al., 2002). The detachment of polyuronide chains from the cell walls of the fruit tissues during ripening may have been reduced in the case of transgenic fruits, giving rise to an enrichment in large particles in transgenic juices. This would be in agreement with the lower content of pectic polymers and the higher average molecular size of these polymers found in the serum of the transgenic line. Microscopy sections of ripe Apel fruits showed a higher degree of cell-to-cell adhesion and a decrease of intercellular spaces when compared with control fruits (Santiago-Doménech et al., 2008). Therefore, it seems plausible that the reduction of cell separation during ripening in transgenic fruits could be linked to the higher amount of large particles in juice, causing the above-mentioned increase in viscosity.

In conclusion, pectate lyase gene silencing in strawberry fruits modified the viscosity and viscoelastic properties of the juices, probably due to an increment in the amount of solid particles of large size in the transgenic juices. This characteristic of the transgenic juices is likely related to the higher integrity of the cell wall in these fruits which is in accordance with the lower amount of pectin and the higher size of pectin polymers found in transgenic serum samples.

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