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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),