JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 7465–7471 **7465**

Transgenic Overexpression of Expansin Influences Particle Size Distribution and Improves Viscosity of Tomato Juice and Paste

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Suppression of the expression of a ripening-related expansin gene, *LeExp1*, in tomato enhanced fruit firmness and overexpression of *LeExp1* resulted in increased fruit softening. Because of the incompletely understood relationship between fresh fruit texture and the consistency of processed products, we examined the effects of *LeExp1* overexpression on the processing characteristics of tomato fruit. As determined by Bostwick consistency and by controlled strain rheometry, juices and pastes prepared from transgenic tomatoes with suppressed *LeExp1* expression had a higher viscosity than preparations from control fruits. However, the viscosity of juice and paste prepared from fruit overexpressing *LeExp1* was significantly greater than products from controls or lines with reduced LeExp1. Bostwick consistency increased by 9% (juice) and 6% (paste) in lines with suppressed *LeExp1* expression but increased by 27.5% (juice) and 19.5% (paste) in lines overexpressing *LeExp1*, relative to controls. Determined by laser diffraction, the particles in juice and paste prepared from transgenic fruits with reduced *LeExp1* expression were smaller, and preparations from fruits overexpressing *LeExp1* had a size distribution indicating more large particles. Analysis of cell wall polysaccharides size indicated that LeExp1 overexpression enhanced depolymerization of water soluble pectins as well as tightly bound matrix glycans. LeExp1 overexpression may allow increased cell wall hydration, resulting in expanded particle size and increased viscosity of products. Because either *LeExp1* suppression or overexpression leads to improved consistency, the interactions that contribute to optimal product rheological properties are complex.

KEYWORDS: Expansinl; *Lycopersicon esculentum*; tomato juice; tomato paste; viscosity; cell wall polymer structure

INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill.) is a source of vitamin C (*1*), potassium, and antioxidants, mainly lycopene, in a nutritious human diet. Because of its overall contribution to nutrition and the volume of its cultivation, the tomato is an economically important fruit commodity worldwide (*2*, *3*). Tomatoes are consumed primarily in a processed form. Most processed tomato products are stored as concentrates until ready to use and then diluted to the desired consistency of the final products. Textural properties of the fruits are important contributors to the overall quality of fresh market tomato fruits and to the properties of products prepared from processing tomatoes (*4*).

After growth and cell expansion, tomato fruits ripen, undergoing changes in physiology and biochemistry that affect color, flavor, and texture (*5*, *6*). One of the most apparent changes during ripening is softening, which is associated with the dissolution of the middle lamella and structural changes in the networks of cellulose, cross-linking glycans (hemicelluloses), and pectins within the fruit cell wall. Cell wall-degrading and polysaccharide-modifying enzymes contribute to this disassembly process (*7*). Expansins, a class of cell wall proteins without known enzymatic activity, also are involved in cell wall disassembly during ripening (*8*). The proposed role of expansins in ripening is to loosen the associations between cross-linking polysaccharide networks in the wall and thus increase the accessibility of wall polymers to hydrolytic enzymes (*9*). In tomato, at least six expansin genes are expressed during fruit development and ripening (*10*, *11*). However, the product of the expansin gene, *LeExp1*, accumulates exclusively during tomato fruit ripening (*10*, *12*).

Because cell wall disassembly in ripening fruit contributes to fruit texture, modification of cell wall protein and enzymatic

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10.1021/jf0341666 CCC: $25.00
Published on Web 11/06/2003

activity during ripening can impact cell wall polysaccharide metabolism and influence texture. The development of transgenic tomato lines in which the expression of single or multiple genes has been altered has allowed the role of specific enzymes to be evaluated (*13*, *14*). Overexpression of the ripening-related *LeExp1* in tomato resulted in enhanced fruit softening (*8*, *15*−*17*). In contrast, suppression of expansin protein accumulation to about 3% of wild-type levels resulted in fruits with a firmer texture (*8*, *17*).

Because of the relationship between fresh fruit texture and the consistency of processed tomato products, fruits with altered textures would be expected to produce processed products with modified rheological properties. The role of suppression of *LeExp* and *LePG* gene expression on processing characteristics of tomato fruits has confirmed this expectation (*17*). Here, we examined the effects of overexpression of *LeExp1* on tomato fruit processing characteristics and identified unanticipated improvements of viscosity characteristics in these fruits.

MATERIALS AND METHODS

Fruit Material. All fruits came from control and transgenic lines of the tomato variety *Lycopersicon esculentum* Mill. cv. Ailsa Craig. Transgenic plants with suppressed expression of *LeExp1* (AC-Exp1) and plants overexpressing *LeExp1* (AC + Exp1) were generated as previously described (*8*). Control plants resulting from self-pollination of the primary transgenic plants had been identified by the lack of the transgenic construct. Plants were grown in a field trial in Davis, CA, in the summer season of 2001. Fruits were staged by date and color and were harvested manually at the red ripe stage.

Tomato Juice and Paste Manufacture. The surfaces of the fruit (ca. 1 kg, approximately 50 fruits per line) were wiped with a moist towel to remove dust, and fruits were weighed and chopped in quarters. Autolysis by endogenous wall-degrading enzymes was allowed for 15 min at room temperature. Chopped fruits were then subjected to a microwave hot break in a Litton commercial microwave oven operating at 1194 W (Litton Microwave Cooking Products, Memphis, TN). Heating was performed at full power for 6 min followed by heating at half power for an additional 6 min and then rapid cooling on ice. To compensate for evaporative water loss during heating, deionized water was added to the samples. Juice was extracted using a benchtop finisher with a screen size of 0.033 in. (0.84 mm). A portion of the juice was retained, and the rest was concentrated to paste using a scraped surface bench scale concentrator under vacuum (740 mmHg). Paste samples had a concentration of approximately 30° Brix. Refractive index measurements were made on serum filtered though a tissue paper onto the surface of a RFM 80 digital refractometer (Bellingham & Stanley Ltd., Kent, England). Sodium azide (0.02%) was added to juice and paste samples to retard spoilage, and samples were stored at 4 °C until evaluation. Samples were diluted to 5° Brix by addition of deionized water and equilibrated at 4 °C for 24 h before evaluation.

Rheological Characterization. Bostwick consistency measurements were made of juices and pastes at 5° Brix. The distance traveled by the juice front in 30 s was measured at 20 °C. Triplicate measurements were performed for each sample.

The rheological properties of the juice and diluted paste at 5° Brix were evaluated using the Haake Rotovisco 20 (Haake Buchler Instruments, Saddle Brook, NJ) controlled strain rate rheometer fitted with an M5/MVII sensor system. Shear stress was measured in response to increasing shear rate after equilibration at 20 ± 0.1 °C. Shear rate increased linearly from 4.5 to 450 s^{-1} in 3 min, and 250 data points were acquired. Rheograms were fitted to a power law model (*18*) using the computer software program KaleidaGraph, version 3.09 (Synergy Software, Reading, PA).

Particle Size Measurements. The size distributions of tomato particles in juice and diluted paste were determined using a Coulter LS-230c Laser Diffraction Particle Size Analyzer (Beckman Coulter, Hialeah, FL). The instrument converts diffraction patterns into particle size distributions with 117 classes from 0.04 to 2000 mm based on the Fraunhofer theory (*19*). Because samples required dilution, a sucrose solution of 5° Brix was prepared and used as the carrying medium for particle evaluations. The sucrose solution was allowed to circulate at 48% power in the analyzer followed by laser beam alignment and calculation of offsets. Tomato sample was added to the circulating sucrose solution to an obscuration value of 8−12% and equilibrated for 5 min to reach equilibrium swelling, and particle sizes were evaluated for 60 s.

Sequential Extraction of Cell Wall Fractions. Fractions of the fruit cell walls were extracted sequentially from the juice and paste preparations. From each juice or diluted paste preparation, 40 mL was centrifuged (20 min, 5900*g*) and the pelletted material was extracted twice for 5 h with 25 mL of water containing 0.02% NaN_3. After it was centrifuged (20 min, 5900*g*), the soluble aqueous extracts were pooled and extensively dialyzed against water, and the combined extract was identified as the water extractable cell wall fraction. The insoluble residue from the water extraction was subsequently extracted twice with 25 mL of 50 mM CDTA, 50 mM sodium acetate 0.02% NaN_3 (for 8 and 24 h), and once with 25 mL of water with 0.02% NaN_3 (for 24 h). After it was centrifuged, pooled, and extensively dialyzed against water, the combined extracts were identified as the CDTA extractable cell wall fraction. To obtain the carbonate soluble cell wall fraction, the pellet remaining after the CDTA extraction steps was extracted twice with 25 mL of 50 mM Na_2CO_3, 20 mM $NaBH_4$ with 0.02% NaN_3 for 12 h followed by a 12 h extraction with 25 mL of water with 0.02% NaN_3. These centrifuged, pooled, and dialyzed fractions were the carbonate extractable fractions. The insoluble material remaining from this extraction was then extracted twice with 1 N KOH, 0.1% $NaBH_4$, and 0.02% NaN_3 for 24 h (1 N KOH extract), pooled, centrifuged, and dialyzed. The resulting insoluble pellet was extracted once with 4 N KOH, 0.1% $NaBH_4$, and 0.02% NaN_3 for 48 h (4 N KOH extract), and the supernatant was dialyzed. In each extraction step, the insoluble material was pelleted at 5900*g* for 20 min. The carbonate and 1 and 4 N KOH extracts were neutralized with glacial acetic acid before dialysis. Each of the pooled extracts was dialyzed (molecular mass cutoff, 6−8000 Da) for 60 h with water changes every 12 h. Because these preparations were not dehydrated by organic precipitation or lyophilization, it was assumed that they were hydrated as in the source juice and paste preparations.

Separation of Polysaccharides Using Size Exclusion Chromatography (SEC). The polysaccharide polymers in the water, CDTA, and carbonate extracts were separated by size using a Sepharose CL-2B SEC column. Approximately 1 mg of uronic acid equivalents from each extract was loaded on the column in a volume of 1.3 mL. Samples were eluted with 0.2 M ammonium acetate (pH 5). The ammonium acetate was allowed to evaporate before the sugar content of the fractions was measured. Approximately 1 mg of neutral sugar equivalents in 1 mL from the 1 N KOH and the 4 N KOH extracts was separated using a Sepharose CL-6B SEC eluted with 0.1 M NaOH. Fractions of 2.0 mL were collected from each separation at a flow rate of 1 mL/3.5 min. The void volume (V_o) was approximately 50 mL, and the total volume (V_t) was approximately 140 mL. Neutral sugar determinations of Sepharose CL-2B and CL-6B column fractions were performed by the phenol sulfuric acid method (*20*). The uronic acid content was measured as described by Blumenkrantz and Asboe-Hansen (*21*).

RESULTS

Bostwick consistency was measured for the paste and juice samples, which were all diluted to 5° Brix before measurement. Using AC-Exp1 fruits, diluted juice had a 9% decrease and diluted paste had a 6% decrease in the Bostwick values as compared to juice and paste from control fruits (AC) (**Figure 1**). Surprisingly, products from AC + Exp1 fruits had larger decreases in Bostwick values; diluted juice decreased 27.5% and diluted paste decreased 19.5% as compared to preparations from control fruits. The decreased Bostwick values of the juice from the AC + Exp1 line were statistically significant as

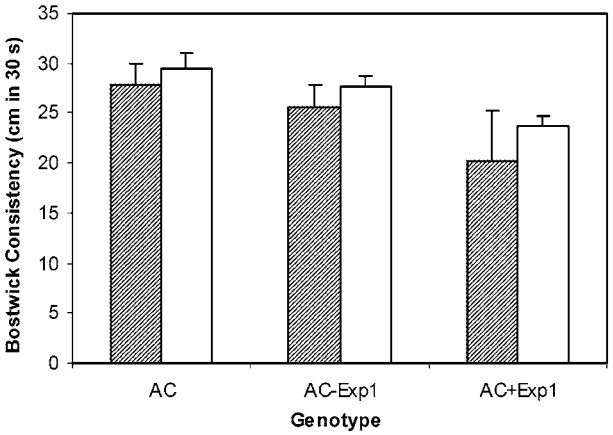

Figure 1. Bostwick consistency in juice (hatched bars) and paste (clear bars) diluted to 5° Brix. The average distance traveled by the sample fronts of diluted pastes and juices in a Bostwick consistometer in 30 s was measured at 20 °C. Data are averages of three sets of juices and pastes prepared from fruits harvested at different times (measurements were performed in triplicate for each set of samples). Error bars extend one standard deviation above the mean.

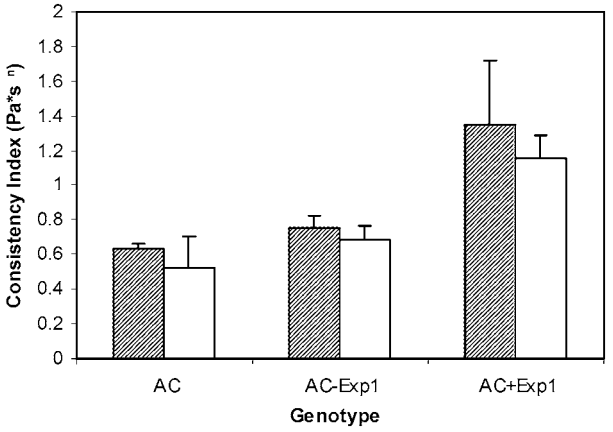

Figure 2. Consistency index, *K*, in juice (hatched bars) and paste (clear bars) evaluated after dilution to 5° Brix. Rheological profiles for ascending shear rates obtained with the Rotovisco 20 control strain rate rheometer were fitted to a power law model. Data are averages of two determinations per sample. Three juice and paste samples prepared from fruits harvested at different times were evaluated.

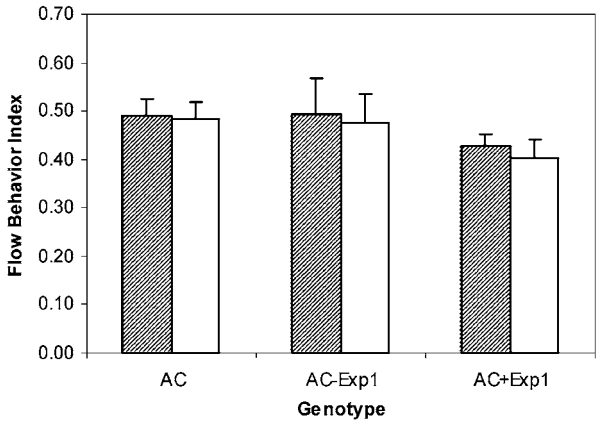

Figure 3. Flow behavior index, *n* (dimensionless), of juice (hatched bars) and paste (clear bars) from AC, AC-Exp1, and AC + Exp1 fruits.

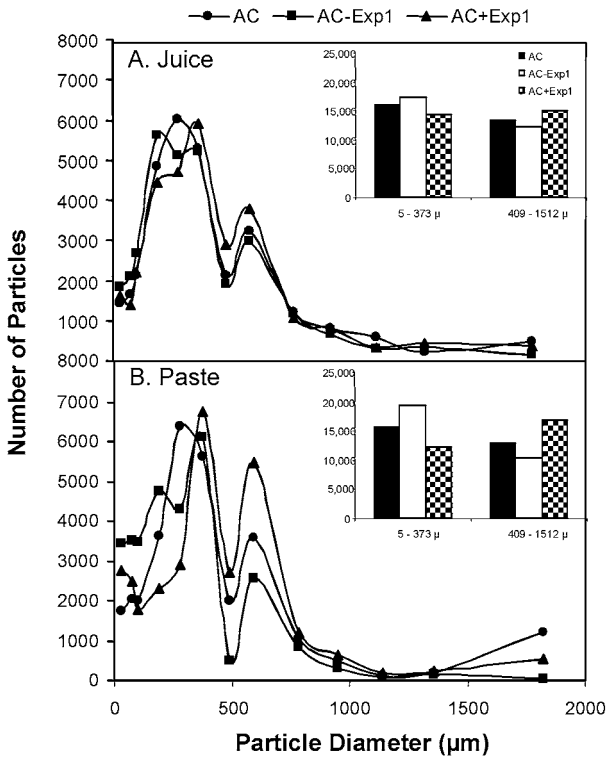

Figure 4. Particle size distribution by laser diffraction in juice (**A**) and paste (**B**) from AC (●), AC-Exp1 (■), and AC + Exp1 (▲) fruits. Distributions are averages of three determinations per genotype. Insert plots indicate the total number of particles in indicated size classes.

compared to the juice from control fruits (Tukey, $p < 0.1$), but the differences were not significant when the pastes were compared.

An alternative method of measuring viscosity utilized a Rotovisco-20 controlled strain rheometer. Shear stress vs shear rate data were fitted to a power law model. The consistency index, *K* (**Figure 2**), and the flow behavior index, *n* (**Figure 3**), were calculated as averages of three preparations of juice and paste from fruits harvested at different times with duplicate measurements for each sample. Juice and paste from AC-Exp1 and AC + Exp1 fruits had higher consistency indices than products from the control fruits, with the AC + Exp1 fruit values more than double those of control fruits. *K* values are higher for material from AC-Exp1 and from AC + Exp1 fruits indicating greater viscosities. Two way analysis of variance (ANOVA) revealed that there was a significant difference ($p < 10^{-4}$) in the consistency indices between genotypes. There was no significant influence ($p = 0.67$) of concentration level (juice vs paste) on consistency index and the interaction of genotype and concentration level also was not significant ($p = 0.8$, Tukey's studentized range method). The consistency index of juice prepared from the AC + Exp1 fruits was significantly different from *K* values for AC control ($p < 0.01$) and AC-Exp1 juices ($p < 0.05$). In diluted paste, a single factor ANOVA also showed a significant difference in *K* values among genotypes ($p = 0.0013$). Pairwise comparisons revealed the same differences in diluted paste as in juice for fruits from AC + Exp1. Values of the flow behavior index indicate a shear thinning behavior for both juices and diluted pastes, with the juice and paste from AC + Exp1 fruits exhibiting a lower average flow behavior index as compared to the materials from either the AC or the AC-Exp1 fruit. This reduced flow behavior

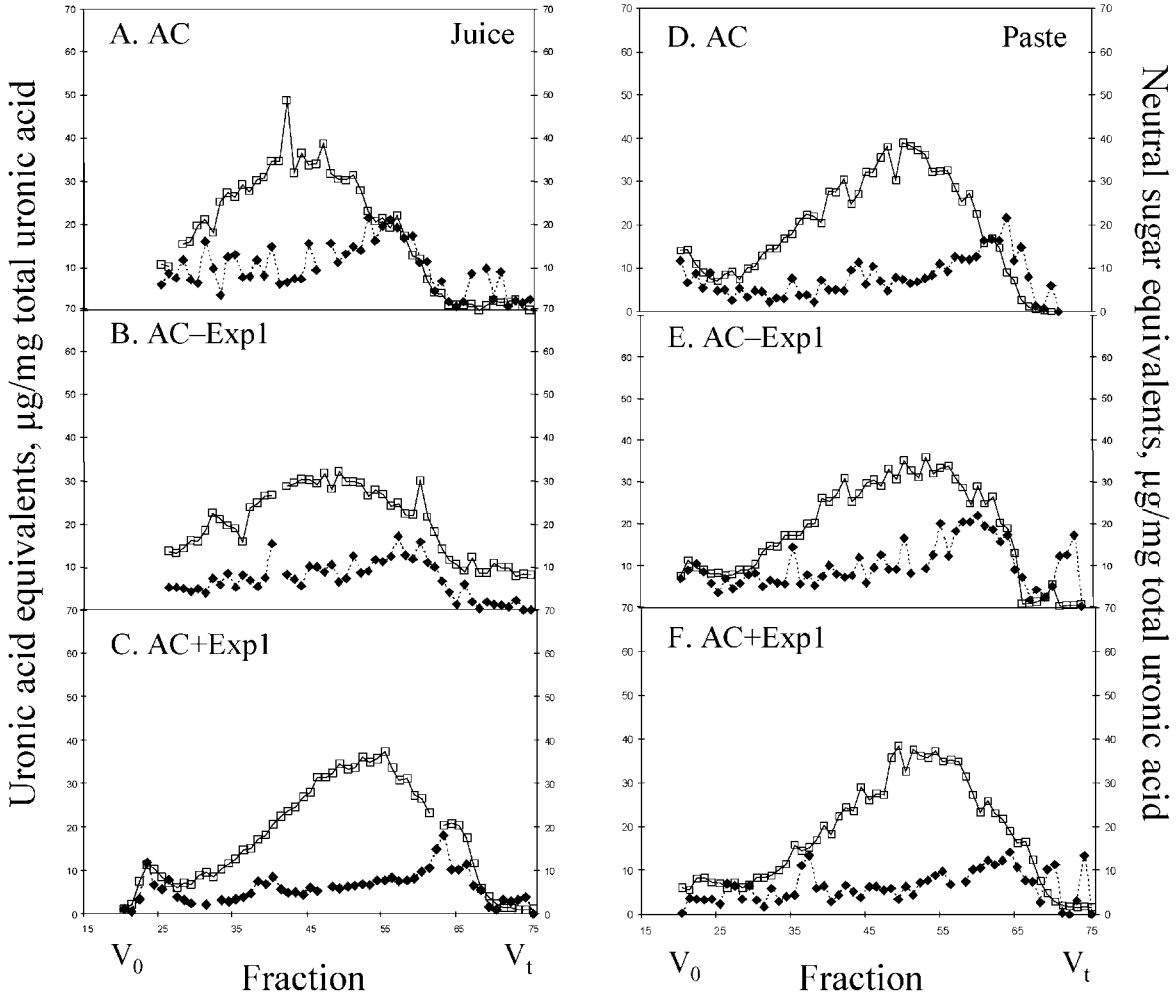

Figure 5. Size distribution profiles of water soluble polysaccharides in juice and paste from AC, AC-Exp1, and AC + Exp1 fruits. Values are standardized for the total amount of polysaccharides applied to the size exclusion column, and the values of the neutral sugars are corrected for the amount of uronic acid present; uronic acid (□) and neutral sugar (◆).

index indicates an increased shear thinning character in both juices and pastes from AC + Exp1 fruit. The difference in *n* is statistically significant in paste preparations as compared to those from AC or AC-Exp1 fruit (Tukey, $p < 0.05$).

Distributions of particle diameters in juice and diluted paste were averaged over three replicates for each genotype (**Figure 4**). All distributions were normalized to include the same number of particles. As compared to juice from the control AC, juice from the AC-Exp1 line had a greater number of small particles (5−373 μm size range) and fewer large diameter particles (409−1512 μm size range). In contrast, juice from the AC + Exp line had a greater number of large diameter particles (409−1512 μm size range) and fewer smaller size particles as compared with juice from control fruit. In paste from control fruit, particle diameters were shifted slightly to smaller sizes as compared to juice. Pastes from AC-Exp1 fruit had 12% more of the smaller size particles (5−373 μm size range) as compared to AC-Exp1 juices. However, in the pastes of AC + Exp1, there was a 14% increase in the number of particles in the 409−1512 μm size range with the peak at 594 μm having about 44% more particles in paste than in juice.

To assess the structure of cell wall polysaccharides that might contribute to some of the changes observed in processed fruit particle size, the size distribution profiles of cell wall fractions isolated directly, without dehydration, from juices and pastes were assayed by SEC as illustrated in **Figures 5**−**7**. Water soluble pectin eluted as a broad peak at around fraction 45 in control juice and paste samples and exhibited a similar elution pattern in AC-Exp1 juice. The water soluble pectin elution profile was shifted in the AC + Exp1 juice and paste, indicating enhanced pectin depolymerization in juice and paste from AC + Exp1 fruits. Polysaccharides in the CDTA soluble fraction elute as two peaks, one near the void volume of the column, at fraction 24, and one near fraction 55. Very similar elution profiles were observed for juice and paste from control AC, AC-Exp1, and AC + Exp1 fruits indicating that the chelator-bound pectins were not affected significantly by the suppression or overexpression of expansin.

Tightly bound matrix glycans soluble in 4 N KOH were also assayed and found to elute as a broad peak between fractions 50 and 65 (**Figure 7**). The elution profiles of the AC control and AC-Exp1 juice and paste samples were very similar, but the elution profiles for juice and paste from the AC + Exp1 fruits indicated a shift to lower molecular weight. Because the higher molecular weight peak is attributed mostly to xyloglucan, the shift to lower molecular weights suggests an increase in xyloglucan depolymerization. Similar findings have been reported for this cell wall fraction isolated from fresh AC + Exp1 fruits (*8*).

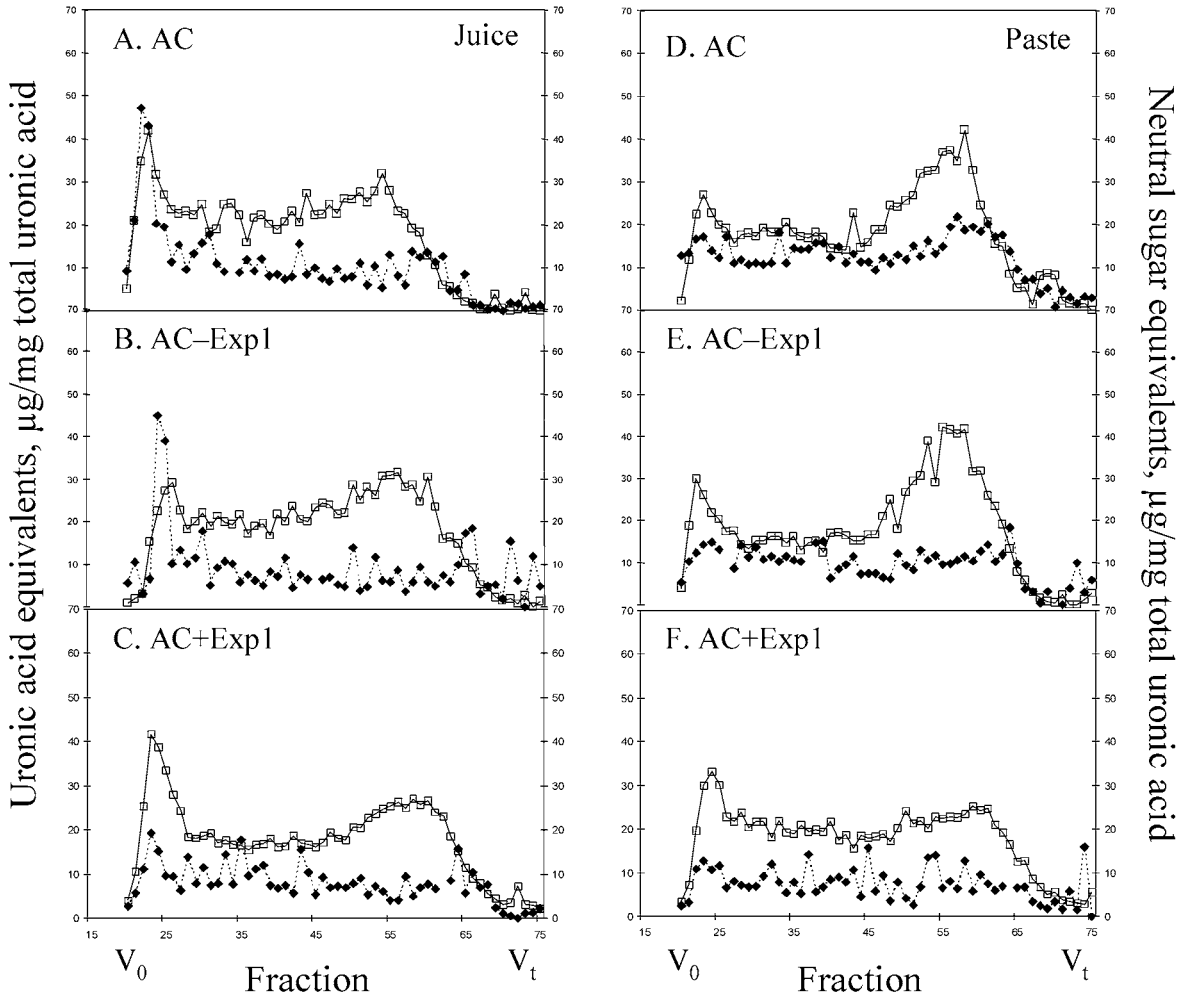

Figure 6. Size distribution profiles of chelator-bound polysaccharides in juice and paste from AC, AC-Exp1, and AC + Exp1 fruits. Values are standardized for the amount of polysaccharides applied to the size exclusion column. and the values of the neutral sugars are corrected for the amount of uronic acid present; uronic acid (□) and neutral sugar (◆).

DISCUSSION

Suppression of *LeExp1*, a ripening-related tomato fruit expansin gene, has been shown previously to contribute to decreased fruit softness during ripening, and overexpression of *LeExp1* contributes to accelerated fruit softening (*8*). Because of the incompletely characterized relationship between fresh fruit texture and consistency of processed tomato products, we examined the effects of overexpression of the *LeExp1* gene on processing characteristics of tomato juice and paste.

Consistency of tomato juice and concentrates is influenced by two factors: the viscosity of the serum fraction and the properties of the suspended particles (*22*, *23*). The serum fraction viscosity is largely attributed to the amount and size of soluble pectins (*24*), and particles are composed of intact cells, ruptured cells, aggregated cell wall polymers, and cell wall fragments (*25*). In addition to particle size, properties of the suspended particles that influence consistency include the swelling characteristics and the degree of deformability of the particles (*26*). In diluted preparations, particles are completely swollen and viscosity is proportional to the volume fraction of the particles. At higher concentrations, the suspended particles exhibit a yield stress threshold and the deformability of the particles starts to affect consistency.

Finisher screen size influences the average and the range of particle sizes. A shift in particle size distribution to smaller diameters has been shown to increase viscosity (*23*). This effect may result from the larger number of smaller particles with increased surface and a consequent increase in interparticle interactions and serum−particle interactions (*27*). Here, we have shown that suppression of *LeExp1* expression resulted in a small increase in the number of small particles and this increase may account for the small increase in paste viscosity. In contrast, overexpression of *LeExp1* resulted in an increase in the number of large particles but had a greater effect on increased paste viscosity. This increased viscosity may result from greater swelling of particles or the accumulation of irregularly shaped particles, which exert a greater influence in the flow behavior of tomato juices because they provide a higher resistance to flow (*28*−*30*). Yoo and Rao (*31*) report that concentrates with a wide range of particle sizes had higher apparent viscosities than concentrates with very tight particle size distributions; concentrates from the fruit overexpressing *LeExp1* exhibit a more polydisperse particle size distribution.

The contribution of expansin to cell wall disassembly during ripening has been proposed to be that of altering the availability of cell wall polysaccharides to depolymerization by other enzymes (*8*, *9*). Swelling and deformability of particles depend on the degree of polymer cross-linking and polymer−solvent interactions. Analysis of the pectin and hemicellulose polymer size in juice and paste cell wall fractions indicated that

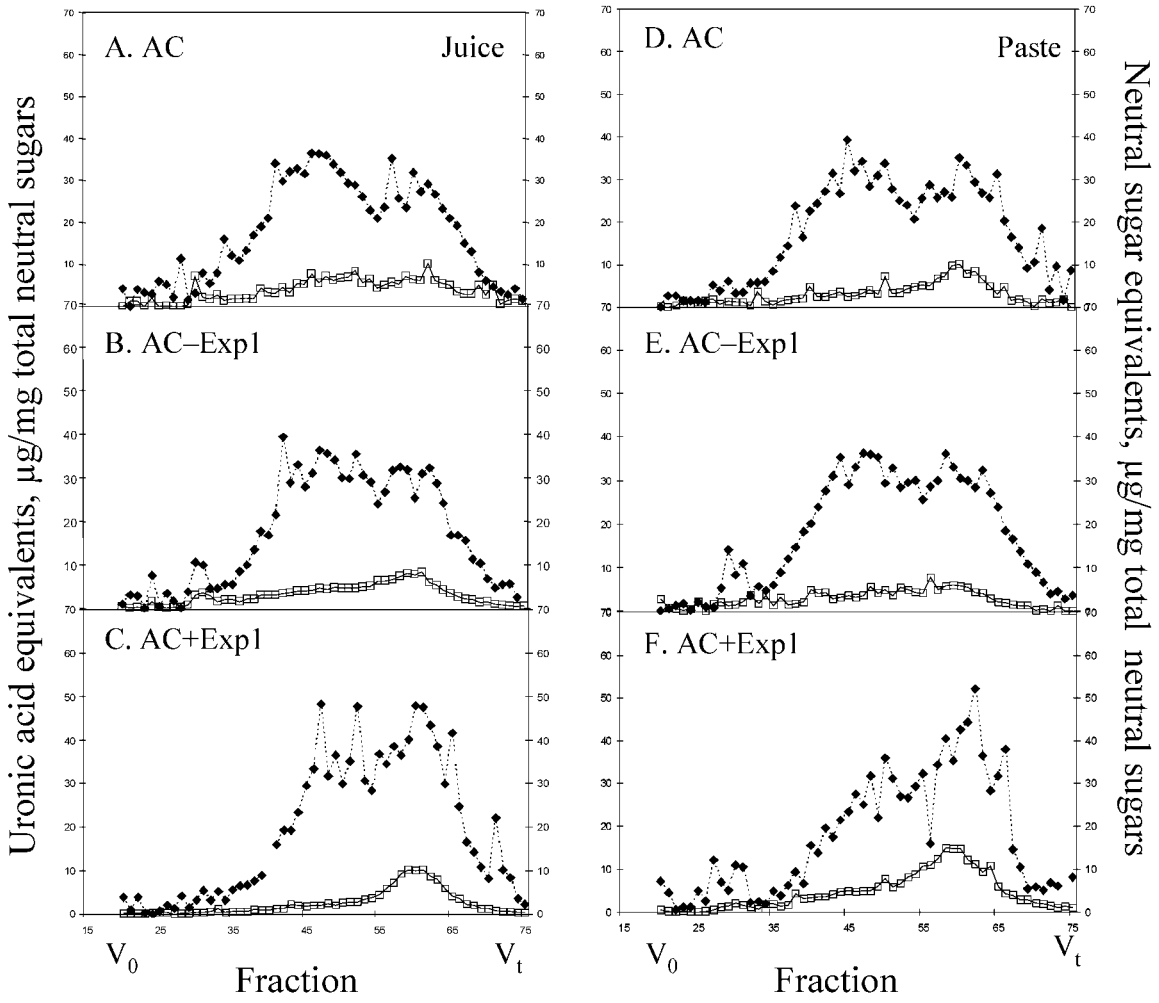

Figure 7. Size distribution profiles of 4 N alkaline-solubilized polysaccharides in juice and paste from AC, AC-Exp1, and AC + Exp1 fruits. Values are standardized for the amount of polysaccharides applied to the size exclusion column, and the values of the neutral sugars are corrected for the amount of uronic acid present; uronic acid (□) and neutral sugar (◆).

overexpression of *LeExp1* resulted in lower molecular weights of water soluble pectins and of tightly bound matrix glycans. These cell wall polymer modifications may reflect the increased accessibility of lytic enzymes to cell wall polymer substrates that may be a consequence of the greater swelling of the cell wall polymer matrix caused by the overexpression of *LeExp1*. Alternatively, these changes in cell wall polymer structure may reflect reduced cell wall polymer cross-linking and this reduced cross-linking may be the cause of greater swelling of the cell wall polymer matrix. In either case, the effect of increased levels of LeExp1 on cell wall polymer structure, either directly or indirectly, is likely to influence the swelling and/or aggregation of juice and paste particles, which in turn influence processed product viscosity.

While tomato product viscosity can be altered by regulation of the processing conditions, genetic manipulation of the endogenous functions that alter cell wall polymer cross-linking may provide raw material that requires less processing to achieve the desired product viscosity. Energy savings and improved fresh qualities of the products may be consequences of lower temperatures or reduced time of processing. An assessment of the optimal processing conditions for fruit with altered *LeExp1* expression will resolve the commercial relevance of this genetic modification.

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Received for review February 20, 2003. Revised manuscript received August 13, 2003. Accepted August 22, 2003. The California League of Food Processors supported portions of this work.

JF0341666