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Changes in Cell Wall Pectins Accompanying Tomato (*Lycopersicon esculentum* Mill.) Paste Manufacture

MONTAÑA CÁMARA HURTADO,[†] L. CARL GREVE,[‡] AND JOHN M. LABAVITCH*,[‡]

Morning Star Packing Company, Williams and Los Banos, California, and Department of Food Science and Technology and Department of Pomology, University of California, Davis, California 95616

The texture of processed tomato products is influenced by the size and solubility characteristics of soluble and particle-bound cell wall polysaccharides they contain. The acidic (pectin) polysaccharides are important contributors to texture because of their gel-forming capability and the fact that they can form aggregates. The present work describes the pectins in ripe tomato fruits and then follows changes in several classes of pectins as the fruits are subjected to hot break and the juice is subsequently concentrated to a 30 °Brix paste. Continued processing leads to progressive solubilization and depolymerization of polysaccharides so that the ionically and covalently bound materials that are the major pectin classes of ripe fruit are substantially reduced in amount with the concomitant increase in the more soluble water-soluble pectins of the paste product. Juice content of soluble solids (°Brix) rose steadily as water content was reduced during processing. Juice content of polymeric uronic acids (i.e., pectin) also rose with concentration, but to a lesser degree than the increase in soluble solids. This indicates that processing leads to almost complete pectin depolymerization and/or the alteration of uronic acid structures so that this assay could no longer detect them. It was concluded that reductions in heat input during processing would lead to pastes with greater pectin integrity and enhanced textural characteristics.

KEYWORDS: ***Lycopersicon esculentum*; pectin; texture; tomato paste**

INTRODUCTION

Over 9 million tons of tomatoes are processed annually in the United States, with California producing 85% of the total. More than 80% of U.S. tomato production is utilized in the manufacture of processed products such as tomato paste (*1*). Texture, particularly in relationship to flow characteristics, is among the important quality attributes of processed tomato products. Several authors (*1*−*4*) have reported that the most important factor in determining the viscosity and consistency of tomato paste and products derived from it is the high molar mass polymeric substances derived from cell wall polysaccharides during processing.

Pectins are common components in the cell walls of fruits that make important contributions to the texture of processed fruit products. Important in this is the tendency for pectins to form gels and influence the viscosity of solutions of higher molecular weight polymers. Pectin backbones are mainly composed of α-1,4-linked D-galacturonosyl (galactopyranosyluronic acid, GalUA) units, with various degrees of methyl esterification of the galacturonosyl residue carboxyl groups. Simple pectins are homogalacturonans, but the more complex rhamnogalacturonans (RGs) have backbones that contain α-1,2-linked D-rhamnopyranosyl residues as well as GalUA residues. The RGs are also branched polymers, bearing side chains containing arabinosyl and galactosyl residues as the major components as well as many other sugar species (*5*). In the middle lamella, pectins are thought to be associated with calcium ions, so removal of Ca^{2+} usually leads to cell separation. Food scientists have taken advantage of this structural characteristic by using additions of Ca^{2+}, often as $CaCl_2$, to increase the firmness of fresh and processed plant-derived foods (e.g., ref *6*). Chelator-insoluble pectic polysaccharides are usually highly branched and are probably cross-linked in the wall matrix via ester cross-links involving sugar residues and borate ions or phenolic residues (*5*, *7*). These pectins can be extracted with sodium carbonate (*8*). It is likely that all classes of cell wall pectins, as well as the other cell wall polysaccharides, make important contributions to the texture of tomato paste.

Plant biochemists and food scientists are interested in methods for measuring and characterizing pectin polymers in terms of their composition and molar mass distributions to understand their physiological and biochemical roles in plants and physicochemical functions in food (*9*). Although studies on tomato fruit, juice, and pulp have been reported, the importance of pectin polymer size distribution in tomato paste texture has not been reflected in the scientific literature. Luh et al. (*10*) reported

* Author to whom correspondence should be addressed [fax (530) 752-8502; e-mail jmlabavitch@ucdavis.edu].
[†] Permanent address: Departamento Nutrición y Bromatología II, Facultad de Farmacia, Universidad Complutense, Ciudad Universitaria, 28040 Madrid, Spain.
[‡] University of California.

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the M_w of pectin acid from six cultivars of fresh tomatoes ranged from 26800 to 7870 Da. Analysis of the M_w of the pectins in the tomato pastes produced from these cultivars showed substantial decreases relative to the M_w in the fresh fruit. Chou and Kokini (*2*) used size exclusion chromatography (SEC) and found that tomato pectic substances were heterogeneous in size, with the majority of the polymers having an M_w of 2×10^5. Kokini and Chan (*3*) studied the conformation of tomato pectins in water/glycerol solutions as a function of degree of esterification. Porreta (*11*) evaluated the quality of tomato pulp by measuring physicochemical properties and their contribution to sensory attributes.

Our work extends the reports of others by following ripe tomatoes as they are processed into paste. We have characterized pectin fractions from tomato cell wall material (CWM) as isolated from ripe tomato fruits, hot break tomato juice, and juice at several points in the processing line (although only two are discussed herein) and in paste concentrates. We have shown that the amount of pectin decreases as processing continues and used SEC to show that this loss of pectin is accompanied by a steady and substantial decrease in pectin polymer size. Much of the pectin in the starting juice (combined serum and particulate fractions) is lost from the polymer fraction by the time that juice has been processed into paste.

MATERIALS AND METHODS

Samples. To evaluate the influence of processing conditions, samples from two tomato paste processing plants in two successive seasons (1997 and 1998) were studied. Tomatoes (*Lycopersicon esculentum* M.) of variety Heinz 8892 were used in the trials. The tests were designed to characterize the changes in pectin polymer solubility and structure that occurred as fruit processing progressed. Fresh fruit were sampled from sorting lines prior to entry into the hot breaks, and then hot break juice was sampled at selected points along the processing line after passage through 0.066 in. finishing screens. Sampling points were selected to represent several stages of juice concentration, including final pastes of ~30 °Brix, which were packed in sterile aluminum foil pouches prior to analysis.

Sampling Procedure. In 1997, one series of samples was collected at each site. In 1998, five series of samples were collected at each site. The timing of sample collection was set on the basis of estimates of the time it would take for the hot break juice from a given batch of fruit, sampled immediately prior to its entry into the hot break units, to reach the various sampling points. Our discussion of the progression of changes as processing continues is meant to represent the fate of the starting material for each sample series. Of course, there is no way to be certain that the flow of the specific starting material exactly matched the sampling times. The total time for each sampling series was ~3 h. Samples of fresh fruit are designated "step 1". "Steps 2, 3, 4, and 5" are, successively, hot break juice, juice concentrates (steps 3 and 4) representing approximately 3- and 5-fold concentrations of the original juice (Figures 1 and 4), and the final paste. Soluble solids (expressed as °Brix) were measured on the tomato juices, intermediate samples, and final paste by refractometry at 20 °C. Analysis of the hot break juice for polygalacturonase (PG) activity (*12*) indicated that the enzyme had been inactivated. Therefore, all samples of processed juice were treated as if they were free of autolytic capacity. They were chilled in an ice/water slurry immediately after collection and stored at 0 °C until further analysis.

Fruit samples were treated differently in 1997 and 1998. In 1997, juice was obtained from fresh tomatoes using a microwave hot break treatment. Fresh tomatoes (300 g) were halved and weighed. These were placed in a beaker and microwaved (Litton, model FS-14EVP, 1300 W) at full power for 6 min and then for 6 min at half power. The preparation was then cooled on ice for 45 min and reweighed. Water was added to give the initial weight. "Hot break" juice was then extracted using a 0.066 in. size finisher. In 1998, fruit were taken to the plant laboratory where ~100 g of fruit flesh was diced into 300 mL of 95% ethanol and homogenized in a blender. In either case, fruit homogenates were quickly chilled in an ice/water slurry and returned with the chilled processed juice samples to the UC Davis campus for further analysis (1−2.5 h of travel time, depending on location).

Cell Wall Preparation. CWM was prepared following a modification of the method of Huber and O'Donoguhue (*13*). Sixty grams of microwaved juice, hot break juice, or juice concentrate or final paste was homogenized in 200 mL of 95% ethanol using a Polytron (Brinkman Instrument Co., Westbury, NY), and the ethanol suspension was boiled for 30 min. The boiled preparations were then cooled and centrifuged at 4 °C for 15 min at 16800*g* in a model SLA 1000 rotor using a Sorvall model 5B refrigerated centrifuge. The solids were then suspended in 100 mL of Tris-buffered phenol (TBP) and stirred for 1 h to solubilize protein. The TBP preparations were then made 80% in ethanol and stored at −20 °C to allow polysaccharides to precipitate; protein remains soluble under these conditions. Insoluble material was recovered by filtration through GF/C glass fiber filters (Whatman, Maidstone, U.K.) and washed with ethanol. The pellet was suspended and stirred in chloroform/methanol (1:1) for 1 h, recovered by filtration, washed with acetone, dried in the fume hood overnight, and then dried in the vacuum oven at 50 °C for 48 h.

Pectin Extraction. Samples (300 mg) of dried CWM were then sequentially extracted (*8*) with aqueous extractants to give three "pectin" fractions. Each extraction involved stirring the CWM or insoluble residue from the previous step in 100 mL of extractant (below) for 16 h at room temperature in the presence of 0.02% sodium azide to retard microbial growth. After extraction, the suspension was centrifuged for 5 min at 15000*g* in a model SLA 1000 rotor using a Sorvall model 5B refrigerated centrifuge (4 °C). The supernatant was decanted and saved. The insoluble residue was resuspended in 100 mL of water and recentrifuged to wash the residue. This supernatatant was combined with the extract. Pelleted residues were then subjected to the next extraction step, and the supernatants were dialyzed against several changes of distilled water at 4 °C using dialysis tubing with a 6000−8000 MW cutoff (Spectropor). Extractants used were water (to give the WS fraction), 50 mM CDTA in 50 mM sodium acetate (pH 6.0) (to give the "ionically bound" pectin fraction), and 50 mM Na_2CO_3 containing 20 mM sodioum borohydride (to give the "covalently bound" pectin fraction).

Extract Analysis. The uronic acid contents of the various extracts, the starting CWM, and the residue remaining after the carbonate extraction were measured colorimetrically (*14*, *15*). The distributions of the polymer sizes of the various pectins in each of the extracts were determined using SEC on Toyopearl HW-55. Approximately 1 mg of uronic acid from each sample was dissolved in 1 mL of 0.3 M ammonium acetate (pH 5.2) and applied to the column for the analysis. The column (3.0 × 38 cm; bed volume of 268 mL) was eluted with ammonium acetate buffer at a flow rate of 2.0 mL/min. Fractions of 3.2 mL were collected and assayed for uronic acid.

In 1998 all five WS samples from each of the two sites were carried through the analysis of pectin polymer size. All WS samples had essentially identical pectin size distributions, indicating (to a degree) that fruit with identical pectin characteristics were being used in both locations on the days that samples were collected. Because of this, only three of the five sample series from each location were subjected to the complete pectin analysis.

RESULTS AND DISCUSSION

Figure 1 shows the changes in soluble solids (°Brix) of the two paste series (one each from the two locations) for 1997 as paste manufacturing proceeds. Soluble solids of fruit samples measured around 5 °Brix and, as expected, the soluble solids levels increased during dehydration steps, with final pastes showing a concentration between 30 and 35 °Brix.

As expected, the yields of CWM as well as the cell wall-associated uronic acid (UA) content increased during processing as a function of the fresh weight (fruit samples) or juice/concentrate weight as the dehydration/concentration depicted in Figure 1 continued. On the basis of the increase in soluble solids

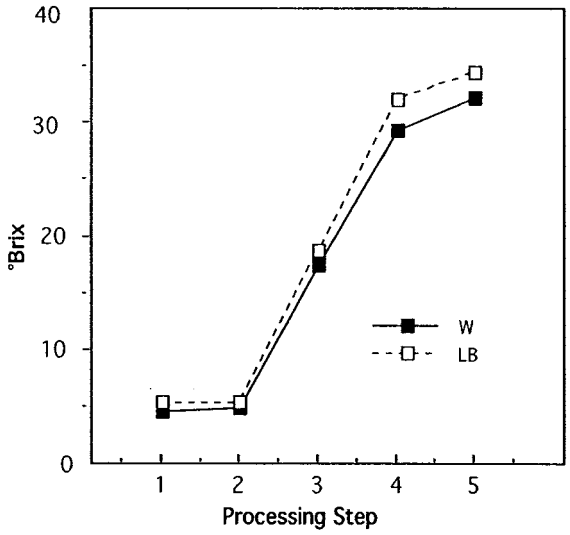

Figure 1. Changes in tomato juice soluble solids concentration (°Brix) over the course of paste processing. Samples were collected at processing plants of Morning Star Packing Co. at Williams and Los Banos, CA (W and LB, respectively). One series of samples was collected at each plant in 1997. Processing step 1 is fresh, ripe tomatoes; step 2 is hot break juice; steps 3 and 4 represent intermediate stages of water removal; and step 5 is the final paste concentrate.

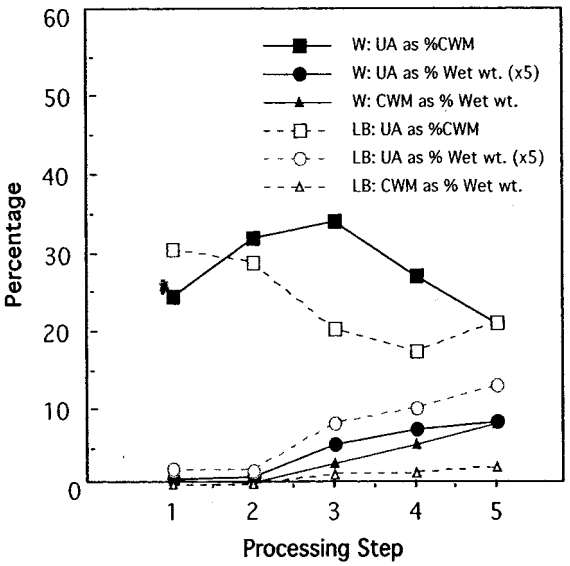

Figure 2. General description of the alcohol-insoluble cell wall materials (CWM) prepared from fresh tomato fruits or juice samples collected at steps along the processing line (1997 data). Data for %CWM indicate the dry weight of the alcohol-insoluble CWM as a percentage of 300 g of fresh fruit or 60 g of juice or paste samples. Data for %UA indicate the recovery of UA in CWM expressed as a percentage of sample "wet" weights. Data for UA%CWM indicate the percentage of CWM dry weight that is uronic acid. **Note**: The values for UA as a percentage of wet weight have been multiplied by 5 to better fit the *y*-axis scale.

content, the concentration of the original fruit juice was 6-fold or greater during paste manufacture. However, although the concentration of AIS increased to about the same extent, the concentration of UA increased only 3−4-fold and, consequently, the proportion of CWM weight that was represented by UA decreased, particularly in the latter stages of dehydration (Figure 2). The colorimetric procedure used measures both serum-soluble and particle-bound UA, both of which had been collected with the AIS because of the ethanol precipitation step. The

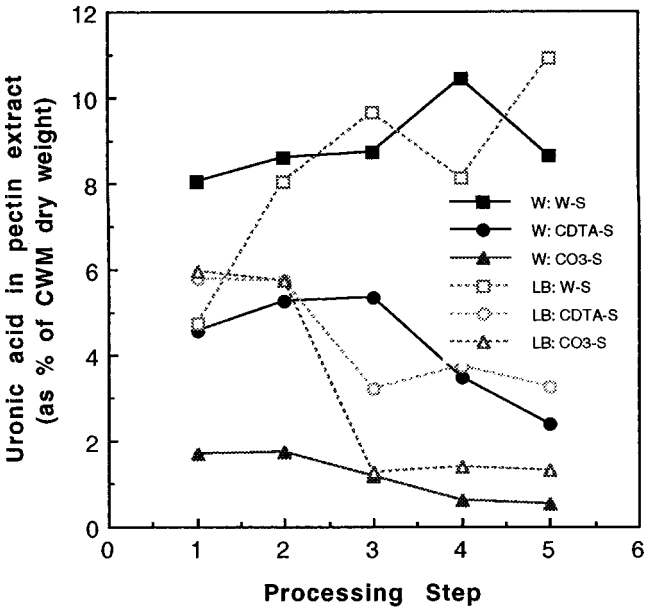

Figure 3. Distribution of uronic acids among the water-soluble, CDTA-soluble, and Na_2CO_3-soluble pectin extracts from tomato fruits, juice samples, and pastes (1997 data). Data (expressed as %CWM dry weight) are calculated to reflect the amount of uronic acid extracted by each solvent. **Note**: Some CWM uronic acid is not accounted for because it remains insoluble after the three extraction steps.

decrease in UA, therefore, represents either a chemical alteration of the UA molecules, a depolymerization of pectins to products that are soluble in the ~80% ethanol used for homogenization, conversion to oligomeric products that are lost when pectin extracts are dialyzed, or some combination of these possibilities.

Although the final contents of CWM in pastes as well as the UA contents of the CWM in the two manufacturing locations are similar, the patterns of change leading to the final values are different, perhaps reflecting differences in the details of the processing operation in the two locales. The distributions of UA in the different pectin fractions of the CWM from these pastes are shown in Figure 3. The general pattern shared by the two locations is a tendency for the WS pectin content to increase with processing while the contents of ionically bound and covalently bound pectins (CDTA- and Na_2CO_3-soluble UA, respectively) decrease. The specific patterns of change throughout processing differ somewhat in the two locations. Considering the total pectin population obtained (WSF + CDTA + Na_2CO_3), the proportions of WS pectins vary from 50 to 70% in the product manufactured at the Williams (W) plant and from 25 to 70% in the product from the Los Banos (LB) plant. These values are reflected in the progressive decreases in concentrations of the other pectin fractions from the W and LB products. Whereas the WS pectin in the fruit sample likely represents pectins that have been solubilized from cell walls during the ripening process, the WS material in processed juice samples represents both the initially soluble material and that pectin which is solubilized from the more tightly cell wall-bound fractions during paste manufacture. Presumably most of the pectin in the WS fraction from the final pastes represents pectins that would be soluble in the paste serum fraction. However, because there is a net decrease in UA content, it is clear that the undefined chemical changes in pectins that lead to their solubilization also must, ultimately, bring changes that rather completely depolymerize the pectins and/or alter the UA chemistry. Chou and Kokini (*2*) reported that pectins are partially solubilized when plant tissues are softened by heating,

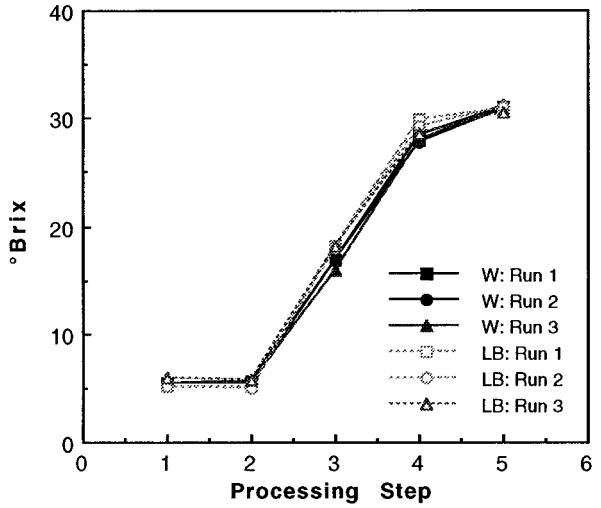

Figure 4. Changes in tomato juice soluble solids concentration (°Brix) over the course of paste processing (1998 data). Samples were collected at processing plants in Williams (W) and Los Banos (LB), CA. Five series of samples were collected at each location, and data from three series from each location are reported.

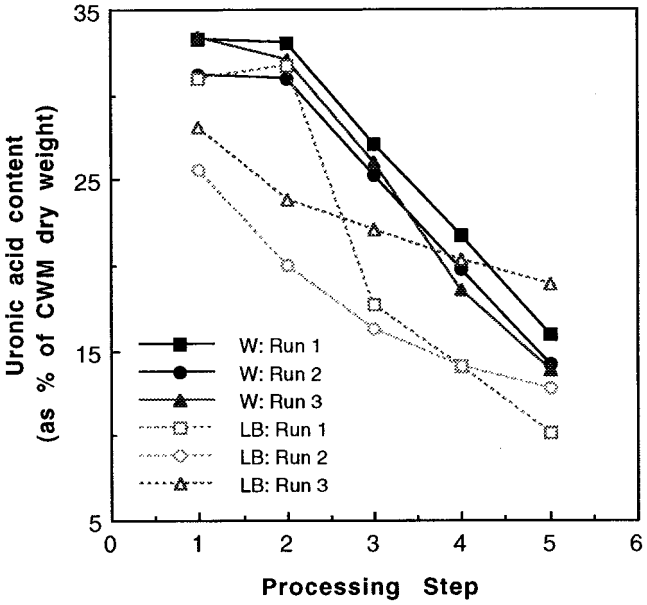

Figure 5. Uronic acid content of CWM prepared from fresh fruit, juice, and paste samples collected in 1998. Data are the uronic acid contents (expressed as a percentage of CWM dry weight) for three sample series each from the Williams and Los Banos plants.

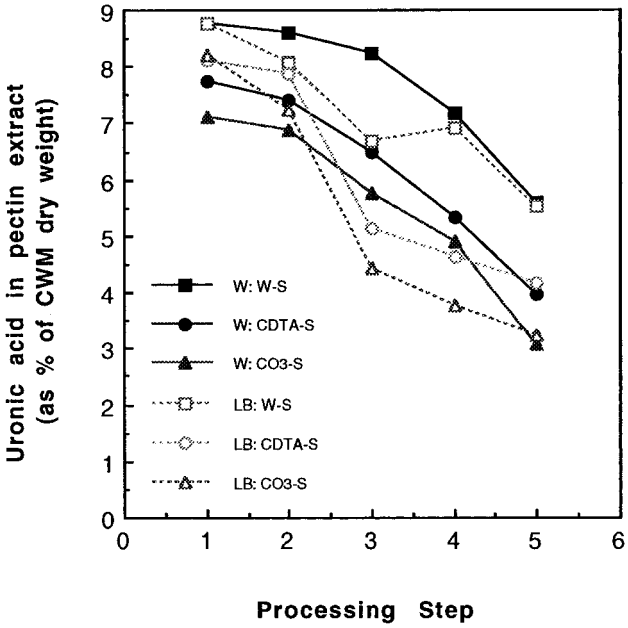

Figure 6. Distribution of uronic acids among the water-soluble, CDTA-soluble, and Na_2CO_3-soluble pectin extracts from tomato fruits, juice samples, and pastes (1998 data). Data (expressed as %CWM dry weight) are calculated to reflect the amount of uronic acid extracted by each solvent. Values shown are the means of the separate values for three sample series collected at the Williams and Los Banos processing plants. **Note**: Some CWM uronic acid remains insoluble after the three extraction steps.

mainly by β-elimination of internal cellular pectin. This is in agreement with the conclusions of Greve et al. (*16*) in their work on cooking-related changes in carrot pectins.

The different patterns of pectin change seen in the two plants may also be due to differences in the quality of the fruit being processed. Substantial differences were found in the CWM composition of the fresh tomatoes used in the two plants in the 1997 season. The lower content of the Na_2CO_3 fraction in fresh tomatoes used at the W plant correlates with a higher proportion of WS pectin in the fresh fruit. This may be explained by the higher level of bruising damage on the fruit going into the hot breaks. We (Labavitch, Mitcham, Greve, and Cámara Hurtado, unpublished material) have observed a considerable pectin solubilization, presumably due to PG action (*17*), in tomatoes following rough handling. At the W plant, samples appeared to be more affected by the dehydration process than at the LB plant. Samples that changed early in the process (i.e., hot break) remained stable thereafter, perhaps because the presumed bruising-related changes have consequences vis-à-vis pectin solubility and integrity similar to those caused by fruit processing. Therefore, despite starting with very different qualities of fresh fruit in terms of pectin content, the final characteristics of pastes from both plants are very similar. Thus, the management of the processing itself [i.e., amount of fresh fruit used per weight unit of paste produced, heat input (°C × time), etc.] can override differences in fruit quality, although there may also be a resulting difference in cost per unit.

The aim for our work in 1998 was to separate effects on paste characteristics that were caused by differences in fruit quality from differences in the details of processing at the same two plants. For that reason, five series of samples were taken at each plant. After a preliminary analysis of WS pectins extracted from the CWM from the fruit samples of each series (to ensure uniformity of starting material), three of the five series from each location were selected for full analysis. Changes in °Brix with processing (Figure 4) are similar to those reported (Figure 1) for the same two locations in 1997.

The data in Figure 5 show that the trend of a decreasing UA content for CWM prepared at points along the processing line that was seen in 1997 (Figure 2) was a feature of all three 1998 series from each location. The patterns for both locations were more similar to the pattern for the LB plant in 1997, but the two plants still showed differences. Nevertheless, the final UA contents of the pastes were similar. These differences in pattern of pectin change were also seen following fractionation of the CWM (Figure 6). Because there appeared to be no difference in initial fruit quality in 1998 (i.e., initial pectin solubility and integrity characteristics), the variation in the patterns of pectin change can be only explained in terms of differences in

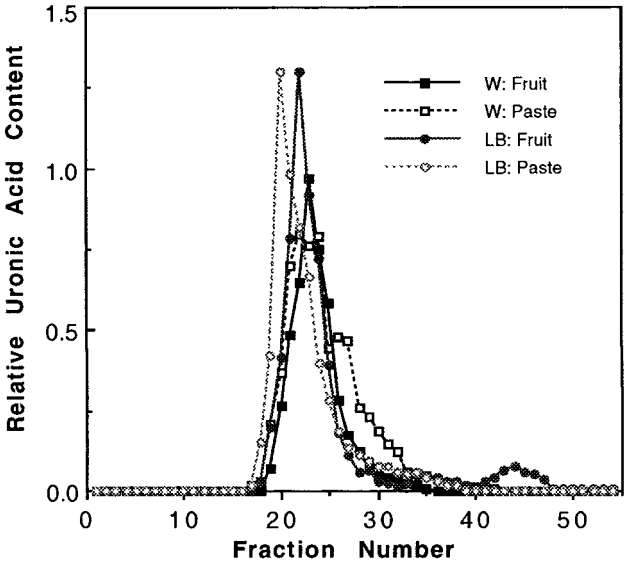

Figure 7. Molecular weight distributions of water-soluble pectin preparations collected from ripe fruits and paste concentrates manufactured at the Williams and Los Banos processing plants. The distributions of the polymer sizes of pectins in each of the extracts were determined using SEC. Approximately 1 mg of uronic acid from each sample was dissolved in 1 mL of 0.3 M ammoniun acetate (pH 5.2) and applied to a Toyopearl HW-55 column (3.0 × 38 cm; bed volume of 268 mL) that was eluted with ammonium acetate buffer at a flow rate of 2.0 mL/min. Fractions of 3.2 mL were collected and assayed for uronic acid. The values shown for each sample and location are the means of the uronic acid contents (expressed as A_{520}, which is measured in the assay) of the corresponding fractions from the separate SEC runs of the extracts from all three paste runs analyzed at each location. Calibration with a set of polydisperse dextran standards (Sigma Chemical Co., St. Louis, MO) determined the column void at fraction 19; peaks of dextrans with average molecular weights of 2000000 and 503000 eluted together. Peaks of smaller dextrans, with average molecular weights of 150000, 74000, and 39000 eluted at fractions 25, 31, and 35, respectively. The fully included volume for the column was at fraction 52.

processing details (i.e., heat and mechanical treatment during fruit processing to paste).

The pectins in each of the extracts were subjected to SEC to better understand the changes that lead to increased pectin solubility and absolute pectin loss during processing. Figures 7−9 show the mean values of the size distributions of the three classes of pectins in the three series of samples collected from each plant in 1998. The Toyopearl gel used has a nominal void size of 500 kDa for globular proteins. The largest peak of UA for each of the series shown is near the void volume. Of course, because the pectins are expected to be more extended than globular proteins, the sizes of the largest pectins may be somewhat smaller than 500 kDa. The CDTA- and Na_2CO_3-soluble pectins are shown in Figures 8 and 9, respectively. In each case, the corresponding extract from the paste has smaller pectins than those in the extract from the fruit, suggesting that an aspect of processing, presumably continuing heat input, brings about depolymerization. The data in Figure 6 indicate that the amounts of these pectin fractions decrease with processing. Therefore, it is easy to conclude that the depolymerization depicted in Figures 8 and 9 leads to a shift to greater solubility that transfers the affected pectins to the WS fraction. However, analysis of the WS−pectin size distributions (Figure 7) does not show this. The WS pectins from paste samples do not show an accumulation of larger polymers, although distribu-

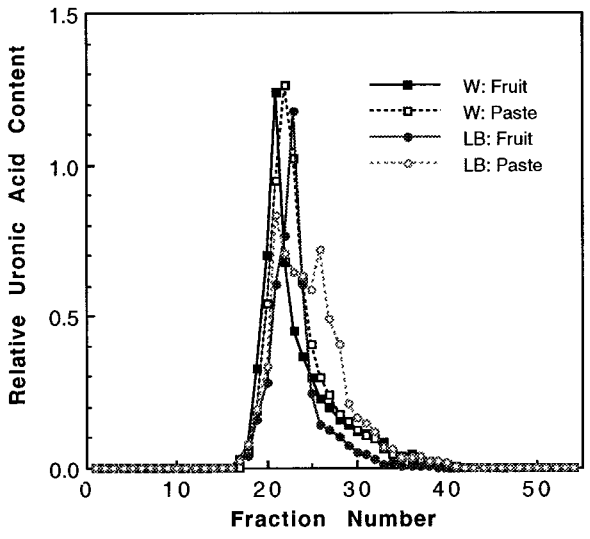

Figure 8. Molecular weight distributions of CDTA-soluble pectin preparations collected from ripe fruits and paste concentrates manufactured at the two locations. Chromatographic and sampling details were as in Figure 7.

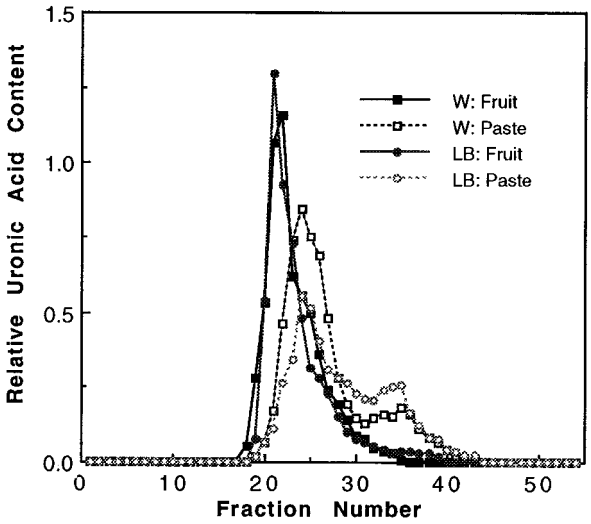

Figure 9. Molecular weight distributions of Na_2CO_3-soluble pectin preparations collected from ripe fruits and paste concentrates manufactured at the two locations. Chromatographic and sampling details were as in Figure 7.

tions of paste pectin sizes are different from those of fruit. Presumably the processing effects on integrity of the two less soluble pectin fractions also affect the WS fraction so that it does not represent a "final repository" of solubilized pectin. Only the fruit and paste samples are included in Figures 7−9 to make it easier to see differences. Analysis of the pectins from the intermediate samples (2−4; not shown) generally shows trends intermediate to those depicted for paste pectins.

There is an absolute loss of pectin (polymeric uronide) during the processing of tomato fruit to paste. The data of this paper do not provide a complete accounting of the loss because we have not accounted for material that was small enough to remain soluble in 80% ethanol. We have also not made a strict assessment of the processing-related transfer of pectins from a particle-bound to a serum-soluble form, because it is not clear that the WS pectins we have isolated from the CWM after it is prepared in ethanol are identical to the serum fractions of processed juices and pastes which have not been subjected to alcohol precipitation. Work to make this assessment is under-

way. Nevertheless, it is clear that processing, probably heat input, has a substantial impact on the pectins of tomato paste. Although the temperature of the juice may have fluctuated somewhat during processing, particularly during the primary concentration steps, the temperature of the juice immediately after hot break was 92 °C, and the temperatures of our samples at the time of their collection were in the range of 89−95 °C over the entire course of processing, a period of ∼3 h. The specific nature of the reactions that contribute to pectin solubilization and depolymerization is not clear. Krall and McFeeters (*18*) have reported that nonenzymatic pectin hydrolysis and β-elimination are quite slow, even at elevated temperatures. On the basis of their observations, we anticipate that most of the pectin breakdown in juice samples was via β-elimination. They reported that β-elimination predominates over hydrolysis at pH values >3.5, and hot break juice and paste concentrates had pH values in the range of 4.4 ± 0.1 (measured at room temperature). We did not identify oligomeric pectin breakdown products or attempt to describe the mechanism of breakdown.

Our crude CWM contains all pectin polysaccharides that are alcohol insoluble, whether they were soluble or wall/particle-bound in intact fruit or hot break juice. The data in Figure 5 indicate that the amount of alcohol-precipitable pectin represented 33% of the weight of the CWM from hot break juice and only 16% of the CWM weight in the final paste. Thus, <50% of the higher molecular weight pectin of the original fruit homogenates remained after processing. Because the amounts and sizes of these polymers (in both serum and particulate fractions of the paste) will influence the textural (i.e., flow) characteristics of the final paste, it is clear that a modification of processing technology to reduce heat input (i.e., energy use) could have a substantial impact on product quality as well as production costs.

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