Changes in the Cell Wall of Olive Fruit during Processing

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Olive fruits were sampled at various stages during their progress through the "California Black Ripe" process. Analysis of cell walls prepared from these fruits indicated that processing was accompanied by general solubilization of polysaccharides and that pectins and a noncellulosic glucan component were most clearly affected. Soluble polysaccharides accumulated in processing liquids. Analysis of these and of polysaccharides extracted from cell walls suggests that the polymer most extensively solubilized and eluted during processing is a relatively unbranched pectin.

INTRODUCTION

The two most widely used techniques for processing of olive fruits are the "California Black Ripe" and "Spanish" processes. Each involves holding large quantities of the fruit in brine, lye solution, and water, often with several changes of each solution. A crucial step in each processing approach is the lye treatment. As the NaOH penetrates the flesh, it hydrolyzes oleuropein, the "bitter" component of the flesh. Extensive washing with water or brine removes residual lye. The Spanish process includes a lactic fermentation step which can last from a few weeks to several months (Fernández Díez, 1985). This step is not part of the California Black Ripe process, which is usually complete within a week (Cruess, 1948).

Processing results in the removal of most water-soluble components of the fruit so that the primary quantitative constituents of the final product are oil and fiber. Size and texture of the processed fruits are also reduced during processing. Some of the loss of tissue firmness that is observed can be attributed to the fact that turgor is lost as cellular membranes are destroyed during the lye treatment. A portion of the decrease in texture and dry weight can also be attributed to the disruption/solubilization of cell walls that occurs during the several steeping steps (Floros et al., 1987). In this paper we characterize the changes in cell wall components that occur during the California Black Ripe process. While all noncellulosic components of the cell wall are solubilized during processing, the pectic constituents are most extensively disrupted. A preliminary analysis of acidic polymers from unprocessed and processed olives gives some information about the nature of the polysaccharides most affected during processing.

MATERIALS AND METHODS

Processing. Fruits of olive (*Olea europaea arolensis* var. Manzanilla) were harvested at the mature green stage. A portion of the fruit was sampled for cell wall analysis (below), and the remainder was placed in brine [3% (w/v) NaCl] to begin processing. The laboratory-scale processing started with approximately 10 kg of fruit in a 30-L container. The brine treatment continued for 4 days. Then a series of three 24-h treatments [4–6 h in lye [1% (w/v) NaOH] followed by 20–18 h in water] was begun. Aeration of the solutions was continuous. Fruits were sampled and sectioned periodically. The first lye treatment was stopped when the lye had penetrated the fruit epidermis. The second and third treatments were terminated when the lye had penetrated halfway through the flesh and reached the pit, respectively. After these treatments, the fruits were washed in water without aeration for several days (four changes of water/day) until the flesh was lye-free (tested with phenolphthalein solution).

Sampling. Samples of fruit were taken at several points during processing: after 1 and 4 days in brine, after each lye treatment and each, subsequent, water wash, and after the final wash. A total of 300 g of olives was taken at random with each sampling. Samples of the processing liquids (100 mL) were also taken when fruit were sampled. These samples were then neutralized with acetic acid (if necessary) and dialyzed (6000–8000 MW cutoff) for 3 days against distilled water (several changes/day). Dialyzed samples were then lyophilized and redissolved in a small volume of water for carbohydrate analysis (below).

Cell Wall Preparation. For each sample, quadruplicate 20-g samples of fruit flesh were cut from the pit, diced, and boiled in 95% ethanol for 20 min. Samples were then homogenized in the same ethanol at top speed in a Polytron for 60 s and then centrifuged at 1500*g* for 10 min. The supernatants were discarded and the pellets were resuspended three times in 80% ethanol, twice in petroleum ether, and twice in acetone. The last supernatants were colorless. The cell wall pellet was dried under an air stream and held in a vacuum oven (Campbell et al., 1990).

Carbohydrate Analysis. Noncellulosic neutral sugars, cellulose, and total uronic acids were quantified from the cell wall of each sample.

Noncellulosic neutral sugars in cell walls and freeze-dried samples were derivatized to alditol acetates by hydrolysis in 2 N trifluoroacetic acid (TFA), reduction, and acetylation (Albersheim et al., 1967). The derivatives were identified by gas chromatography on a Perkin-Elmer chromatograph, fitted with a 30-m fused silica capillary column (DB-23, J&W Scientific). The oven temperature was 210 °C, and H_2 was used as carrier gas. The peaks identified by a flame ionization detector were integrated with a Perkin-Elmer Sigma 10 data system.

Cellulose was quantified from the TFA-insoluble residue by the anthrone colorimetric assay (Dische, 1962).

Uronic acids in freeze-dried redissolved samples were measured colorimetrically (Blumenkrantz and Asboe-Hansen, 1973). Cell walls were dissolved in sulfuric acid (Ahmed and Labavitch, 1977) for uronide analysis.

Processing-related changes in the more readily soluble uronide classes were followed by sequential extraction of cell walls in water and chelator solution. The samples were extracted twice with water and then twice with CDTA (40 mM CDTA in 20 mM sodium acetate buffer, pH 6.5), for 1 h, each step at room temperature. Uronic acids in extracts were quantified by colorimetric assay.

Cell Wall Fractionation/Extraction. Large samples of cell wall from unprocessed and fully processed fruits were sequentially extracted to provide roughly defined pectin, hemicellulose, and α-cellulose fractions.

1. Two 30-min extractions were performed in hot (80 °C) water. Extracts were collected by low-speed centrifugation (WSF).

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0021-8561/94/1442-1194$04.50/0

Table 1. Cell Wall Yield and Carbohydrate Composition of Unprocessed (U) and Processed (P) Olives[a]

	U	P
yield (% fresh wt)	3.92 ± 0.31	3.27 ± 0.18
total wall carbohydrate (% wall dry wt)	73.9	62.9
cellulose	24.88 ± 0.79	22.90 ± 0.60
uronic acid	26.56 ± 1.13	19.74 ± 0.62
noncellulosic NS	22.64 ± 0.43	20.21 ± 0.45
wall carbohydrate (by class, as % of total wall carbohydrate)		
cellulose	33.6 ± 1.07	36.4 ± 0.95
uronic acids	35.9 ± 1.53	31.4 ± 1.59
noncellulosic NS	30.6 ± 0.58	32.1 ± 0.71
rhamnose	2.04 ± 0.05 [6.71]	2.53 ± 0.08 [7.87]
fucose	0.19 ± 0.04 [0.62]	0.19 ± 0.01 [0.59]
arabinose	15.79 ± 0.34 [51.55]	16.18 ± 0.45 [50.37]
xylose	5.36 ± 0.24 [17.49]	6.41 ± 0.27 [19.44]
mannose	1.01 ± 0.02 [3.31]	0.89 ± 0.10 [2.8]
galactose	4.76 ± 0.08 [15.55]	4.86 ± 0.10 [15.14]
glucose	1.46 ± 0.07 [4.77]	1.18 ± 0.06 [3.66]

[a] ±SD. Values in brackets indicate the percent of the total noncellulosic neutral sugars represented by a given sugar.

2. Two 1-h extractions were performed in hot (80 °C) 1% (w/v) ammonium oxalate (pH 5.0) (Manzi et al., 1990). The insoluble residue was washed with oxalate and then water by resuspension/centrifugation (OSF).

3. One 4-h delignification treatment was performed in acetic acid/sodium chlorite at 80 °C (Brillouet and Mercier, 1981). Residue was washed in water.

4. One 24-h treatment was performed in 10% (w/v) NaOH, at room temperature and under N_2. The collected supernatant was neutralized with acetic acid and the resulting precipitate (HA) collected by centrifugation. The solution (HB) was dialyzed against several changes of water (as was the OSF) and freeze-dried (Siddiqui, 1990).

5. The water-washed insoluble residue was designated α-cellulose. Extracts were analyzed for carbohydrate as described above.

Ion-Exchange Chromatography. Water- and oxalate-soluble fractions from cell walls were fractioned on a column of QAE Sephadex A-25 (30.0 × 1.6 cm). The exchanger was equilibrated in 125 mM imidazole hydrochloride, pH 7. The samples were dissolved in the same buffer. The fractions were eluted with 50 mL of starting buffer followed by 50 mL, each, of 550 and 800 mM imidazole hydrochloride, pH 7. Fractions of 4 mL were collected, and aliquots were assayed for uronic acid (Blumenkrantz and Asboe-Hansen, 1973) and neutral sugars (Dische, 1962).

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that the yield of cell wall material decreased substantially with processing. On a fresh weight basis, fruit contain 17% less cell wall after processing. Measurement of the carbohydrate in these walls accounts for 74% (unprocessed olive) to 63% (processed fruit) of the crude cell wall. The implication is that processing removes wall carbohydrate to a greater extent than other (unidentified) materials isolated with the wall. When the carbohydrate in the isolated cell wall is analyzed, it becomes clear that the processing solutions remove more pectic material (based on uronic acid content, excluding consideration of the neutral rhamnosyl, arabinosyl, and galactosyl residues often associated with pectic polysaccharides, which change little) than hemicellulose and cellulose (Table 1). While the proportion of the wall carbohydrate represented by uronic acid decreased with processing, the cellulosic and noncellulosic neutral sugar fractions increased slightly. The individual sugars in the noncellulosic (TFA-hydrolyzable) neutral fractions of these walls changed little (in a relative sense) with processing. A possible exception to this is the noncellulosic glucose which, while representing less than 5% of the residues in the fraction, decreased by about 24% during processing.

That there is a selectivity of extraction that favors uronic acid (i.e., pectin) and, perhaps, the small amount of noncellulosic glucan is supported by analysis of the processing liquids (Table 2). Our processing included an initial steeping in brine followed by three cycles of lye treatment, each followed by a water wash. A fixed volume of liquid (100 mL) was sampled at each step, dialyzed against water, lyophilized, redissolved in water, and assayed. While values for carbohydrates solubilized in each step/cycle can be compared to one another (each sample representing a relatively constant ratio of liquid/olives), they cannot be readily related to the amounts of cell wall (fresh weight basis) reported in Table 1. Furthermore, if the treatment caused cleavage of polysaccharides to relatively low molecular weight species, some of these would have been lost during dialysis. Also lost during dialysis would be most of the di- and monosaccharide sugars in soluble pools of the fresh fruit. These have been reported to include glucose (most abundant), fructose, mannitol, and sucrose (Guillen et al., 1992). These may not have been entirely removed during dialysis, however, and, because our GC analysis would have converted glucose (free, and from hydrolyzed sucrose) to glucitol hexaacetate, would be included in the glucose values in Table 2. Fructose would also have been converted to an equal mixture of glucitol and mannitol hexaacetates. The TFA treatment tends to destroy fructose, however, and so it would have made little contribution to the glucose and mannose reported.

A preliminary methylation analysis of the polysaccharides present in the WSF of fresh fruit (data not presented) indicated the presence of a substantial amount of 3- and 4-linked glucosyl residues. These were not found in the WSF of processed fruit, suggesting that a water-soluble mixed-linkage β-glucan is rapidly eluted from the olives. This, too, would have contributed to the glucose reported in Table 2.

While the brine treatment lasted for a total of 5 days,

Table 2. Amount (Milligrams per 100 mL) of Carbohydrate (±SD) Accumulated in Processing Liquids[a]

	brine	1st lye cycle	2nd lye cycle	3rd lye cycle
Rha	0.22 ± 0.006 (5.4)	0.62 ± 0.02 (4.0)	0.45 ± 0.02 (5.5)	0.68 ± 0.01 (7.4)
Fuc	0.05 ± 0.002 (1.2)	0.04 ± 0.004 (0.3)	0.04 ± 0.005 (0.5)	0.06 ± 0.002 (0.7)
Ara	0.12 ± 0.01 (2.9)	0.78 ± 0.06 (5.1)	0.62 ± 0.04 (7.6)	0.96 ± 0.06 (10.5)
Xyl	0.29 ± 0.008 (7.1)	0.43 ± 0.03 (2.8)	0.34 ± 0.01 (4.2)	0.77 ± 0.02 (8.4)
Man	0.48 ± 0.01 (11.7)	0.51 ± 0.02 (3.3)	0.39 ± 0.009 (4.8)	0.98 ± 0.07 (10.7)
Gal	0.27 ± 0.03 (6.6)	0.64 ± 0.02 (4.2)	0.53 ± 0.03 (6.5)	0.55 ± 0.03 (6.0)
Glc	2.05 ± 0.14 (49.9)	6.36 ± 0.20 (41.3)	3.74 ± 0.10 (45.8)	3.15 ± 0.12 (34.5)
UA	0.63 ± 0.01 (15.3)	6.01 ± 0.30 (39.1)	2.06 ± 0.16 (25.2)	1.99 ± 0.14 (21.8)
total	4.11 ± 0.20	15.39 ± 0.74	8.17 ± 0.51	9.14 ± 0.60

[a] Values in parentheses indicate the percentage of total solubilized carbohydrate represented by each component. Data for each of the lye cycles represent a composite of the lye solution and subsequent water washes.

Table 3. Water- and Chelator-Soluble Uronic Acids in Cell Walls from Olives Sampled at Different Stages during Processing[a]

processing stage	water-soluble uronide	CDTA-soluble uronide	total water- and CDTA-soluble uronide	water-soluble as % of total
1. fresh fruit	9.58 ± 0.28	2.08 ± 0.20	11.66	85.8
2. brine (post 24 h)	6.68 ± 0.19	3.42 ± 0.13	10.1	66.1
3. brine (post 4 days)	6.05 ± 0.09	4.14 ± 0.20	10.19	59.4
4. post lye 1	6.68 ± 0.33	5.45 ± 0.21	12.13	55.1
5. post lye 1, wash	5.21 ± 0.33	4.67 ± 0.32	9.88	52.7
6. post lye 2	2.24 ± 0.09	3.91 ± 0.74	6.15	36.4
7. post lye 2, wash	1.80 ± 0.11	2.70 ± 0.19	4.5	40.0
8. post lye 3	2.44 ± 0.18	3.60 ± 0.21	6.04	40.4
9. post lye 3, wash	1.27 ± 0.08	5.77 ± 0.36	7.04	18.0
10. post final wash	2.04 ± 0.08	6.30 ± 0.16	8.34	24.5

[a] Data are expressed as a percentage of cell wall weight (±SD).

Table 4. Distribution of Individual Noncellulosic Neutral Sugars and Uronic Acids in the Cell Wall Fractions from Unprocessed (U) and Processed (P) Olives[a]

	WSF		OSF		HA		HB		α-cell	
	U	P	U	P	U	P	U	P	U	P
Rha	6.17 ± 1.13	2.54 ± 0.22	3.49 ± 0.20	8.21 ± 0.25	0.51 ± 0.02	0.56 ± 0.06	2.23 ± 0.22	1.53 ± 0.15	4.69 ± 0.33	3.74 ± 0.11
Fuc	0.64 ± 0.15	0.22 ± 0.02	0.40 ± 0.06	0.53 ± 0.01	t	t	0.26 ± 0.02	0.30 ± 0.01	0.21 ± 0.02	0.22 ± 0.001
Ara	29.89 ± 1.20	25.75 ± 1.21	18.40 ± 0.87	49.98 ± 2.30	1.25 ± 0.10	0.96 ± 0.10	22.71 ± 1.20	17.15 ± 0.76	30.96 ± 2.60	23.29 ± 1.54
Xyl	3.35 ± 0.36	2.84 ± 0.01	0.74 ± 0.01	0.53 ± 0.05	77.37 ± 7.26	80.47 ± 1.72	22.21 ± 1.70	22.95 ± 0.84	3.55 ± 0.21	8.66 ± 0.66
Man	3.24 ± 0.41	0.68 ± 0.05	1.75 ± 0.06	0.31 ± 0.01	0.39 ± 0.02	0.60 ± 0.04	10.46 ± 0.64	13.47 ± 0.49	0.35 ± 0.02	0.44 ± 0.03
Gal	23.87 ± 2.10	22.76 ± 0.75	6.58 ± 0.30	7.32 ± 0.37	0.56 ± 0.01	1.22 ± 0.10	9.99 ± 0.62	10.40 ± 0.41	5.66 ± 0.69	4.95 ± 0.28
Glc	5.76 ± 0.37	1.99 ± 0.14	2.45 ± 0.33	0.65 ± 0.003	1.00 ± 0.05	0.52 ± 0.06	22.83 ± 1.23	29.38 ± 1.18	10.94 ± 0.40	15.49 ± 0.54
UA	27.07 ± 1.35	42.19 ± 1.33	66.12 ± 2.15	32.44 ± 0.61	18.85 ± 0.78	15.57 ± 1.22	9.30 ± 0.93	4.79 ± 0.89	43.72 ± 3.86	43.19 ± 0.92

[a] Values are the percentages of the total noncellulosic carbohydrate in a given fraction represented by the individual components (±SD). The α-cellulose samples for U and P olives also contain 71.1% and 78.1% cellulose, by weight.

more carbohydrate was washed from the fruit in each of the 1-day lye/water cycles than by the brine. Examination of hand-sectioned fruits sampled at each processing step indicated that the lye penetrated to a slightly greater depth at each stage, reaching the pit in the third cycle (data not shown). A decreasing volume of fruit flesh was, thus, exposed to the lye for the first time with successive cycles. Furthermore, polysaccharides solubilized closer to the pit would be less likely to be washed into the steeping solution during the water wash. Table 2 shows that the predominant nondialyzable sugar accumulating in the processing solutions at all stages was glucose. Loss of glucose from the wall was, proportionally, a bit greater than changes in other neutral residues (Table 1). All of the other noncellulosic sugars are represented in the solutions described in Table 2. Xylose and mannose, sugars that increase in relative amount in more lignified tissues, increase a bit in the final lye cycle. It is clear, however, that pectic components and glucan are the most abundant polymers recovered from the processing liquids. Uronic acids are the second most abundant carbohydrate in the solutions, and arabinose, galactose, and rhamnose are also significant components. In addition to galacturonic acid, rhamnose is likely to be a constituent of pectin polymer backbones. Furthermore, a pectin that is relatively enriched in rhamnose will tend to be more highly branched (McNeil et al., 1984). Thus, the fact that the uronic acid/rhamnose ratio of solubilized polymers decreases with successive lye cycles suggests that more linear polymers are the first pectins removed (Feng et al., 1989). Whether this indicates that linear pectins predominate in the outer flesh or are more readily solubilized is not clear. The uronic acid/rhamnose ratio in residual cell walls (Table 1) falls with processing. This also suggests a preferential removal of unbranched pectins.

The data in Table 2 make clear that polymeric material (glucan and pectin) is being washed from the fruit and that the proportions of the different pectins eluted change as processing continues. This pattern is roughly analogous to the changes in pectin solubility that accompany fruit ripening. In tomato, for instance, pectin solubility increases and the ratio of water-soluble to chelator-soluble pectins falls as fruits soften (Campbell et al., 1990). These solubility changes involve all classes of wall pectins, however, because as ripening continues, more tightly bound (i.e., Na_2CO_3-soluble) pectins are digested and converted into more soluble species (Carrington et al., 1993). Similar changes apparently occur in olives, although enzyme action, at least in the California Black Ripe process, is not likely to be involved. The data for water- and chelator (CDTA)-soluble uronic acid in walls of olives processed to various extents are reported in Table 3. The uronic acid contents of walls from unprocessed and fully processed olives are approximately 27% and 20% of cell wall weight, respectively (Table 1). Thus, the sum of the water- and CDTA-soluble uronic acids in unprocessed and processed olives (Table 3) represents 42–44% of the wall uronic acid. While this proportion of the total is similar before and after processing (even though pectin is being washed from the fruit), the amount of these pectins that is water-soluble decreases steadily (Table 3). The pectins that remain behind with the fruit are less soluble. The patterns of change in these pectin fractions are not steady, however. Table 3 shows that the cell wall contents of both water- and CDTA-soluble pectins fall during the lye treatments. The lye, analogous to the Na_2CO_3 used in typical pectin extraction protocols (Selvendran and O'Neill, 1987), is a more efficient solvent than the water or CDTA and probably elutes portions of these two fractions as well as more tightly bound uronide. As the last lye is washed away (Table 3, samples 9 and 10) some solubilized pectin apparently can form (re-form) chelator-soluble associations with the remaining cell wall.

Large samples of cell walls from unprocessed and processed olives were extracted with a series of solvents to remove pectin-enriched material (water- and oxalate-soluble fractions; WSF and OSF, respectively) and fractions enriched in hemicellulosic polysaccharides [sodium hydroxide-soluble material; subsequently neutralized to precipitate hemicellulose A (HA) and leave hemicellulose

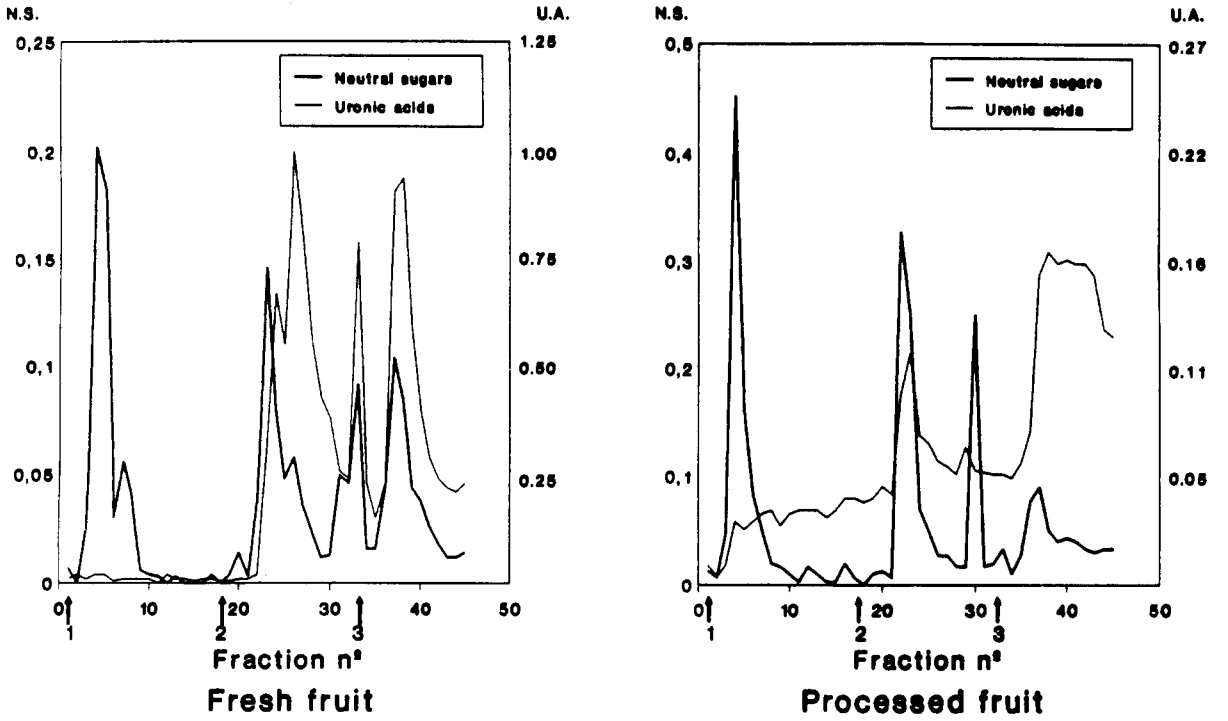

Figure 1. Fractionation of WSF from unprocessed (fresh) (a) and processed (b) fruit by ion-exchange chromatography. Arrows numbered from 1 to 3 indicate points at which elution with 125 mM (1), 550 mM (2), and 800 mM (3) imidazole hydrochloride buffer was begun. N.S. values on the y axis are A_{620} for the anthrone assay. U.A. values are A_{520} for the m-phenylphenol assay.

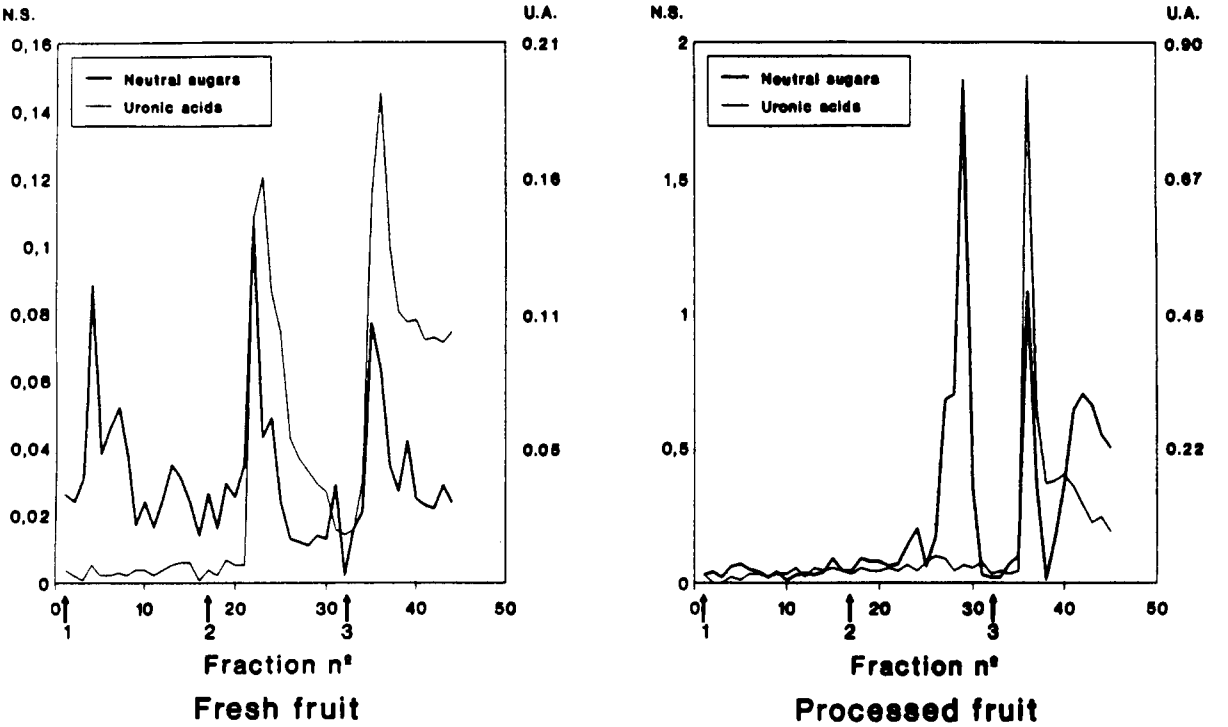

Figure 2. Fractionation of OSF from unprocessed (fresh) (a) and processed (b) fruit by ion-exchange chromatography. Arrows numbered from 1 to 3 indicate points at which elution with 125 mM (1), 550 mM (2), and 800 mM (3) imidazole hydrochloride buffer was begun.

B (HB) in solution]. The material that remained insoluble after base extraction (α-cellulose) contained significant amounts (up to 30%) of recovered carbohydrate as TFA-hydrolyzable (i.e., noncellulosic) neutral sugars and uronic acids (Dusterhöft and Voragen, 1991; Koller et al., 1991) as well as cellulose. The carbohydrate composition of each of these fractions is presented in Table 4.

WSF and OSF are the main pectin-containing fractions, although it is clear that they are not exclusively pectinaceous. For instance, the fact that there is a significant relative loss of glucose from the WSF during processing suggests that it is the source of the glucan that accumulates in processing liquids. If we assume that the pectic polymers in these fractions are roughly equated to their content of uronic acids, arabinose, galactose, and rhamnose (McNeil et al., 1984), then it is clear that the pectin polymer distributions change during processing. This is because processing treatments solubilize wall polymers and shift them to a more readily extracted status. For instance, while the pectins of the WSF contain approximately equal

amounts of arabinose and galactose, the OSF pectins are richer in arabinose. As processing goes to completion the uronic acid:arabinose ratio of the OSF goes from 3.6:1 to 0.6:1. Neither the WSF nor OSF represents a single polymer species (see below). Therefore, this change in sugar content indicates a significant shift in solubility of pectins. For the OSF this shift presumably includes a loss of uronic acid (and galactose)-rich pectins and an accumulation of initially less soluble pectins from other wall fractions. For instance, the uronic acid-rich α-cellulose fraction of unprocessed fruits has a rhamnose: arabinose ratio of 0.16:1, very similar to that (0.15:1) of the OSF from processed fruit. Perhaps the arabinose-rich material in the OSF is derived from the α-cellulose of unprocessed olives.

The clearest processing-related changes in wall constituents appeared to be related to the pectic materials in WSF and OSF (Hudson and Buescher, 1986; Plat et al., 1991). Accordingly, these fractions were submitted to analysis by anion-exchange chromatography. Polymers with increasing charge density are eluted in later fractions. Because the presence of methyl-esterified pectin carboxyl groups will reduce charge density, more highly esterified polymers will tend to elute in earlier fractions. We presume that the relative degree of esterification should be less in pectins of processed olives because of the long exposure to saponifying (alkaline) conditions.

For both fresh and processed olives, the WSF (Figure 1) was separated into four neutral sugar-containing (i.e., anthrone-positive) fractions. In each case a substantial peak of neutral sugar containing very little uronic acid showed little interaction with the column. The 550 mM imidazole hydrochloride buffer step eluted two neutral sugar-containing fractions. The relative uronic acid content of these was less in the WSF from processed fruit. A single peak of carbohydrate was tightly bound by the column and eluted with 800 mM buffer. The uronic acid content of this fraction was relatively high, particularly in the material from processed fruit.

The data for ion-exchange separation of the OSF (Figure 2) reflect the overall compositional analysis of this fraction presented in Table 4. No neutral sugar-rich, nonbound fraction was found in the OSF from processed fruits. For each fruit wall preparation a single major peak was eluted with 550 mM buffer. The material derived from processed fruit was eluted a bit later from the column (perhaps suggesting a lower degree of esterification) and had a lower relative uronic acid content. We suggest that this represents the more highly branched pectin whose presence in processed fruit is suggested by the sugar composition data of Table 4. A single uronic acid-rich peak is eluted with 800 mM imidazole hydrochloride for each OSF sample. The sample from processed fruit also presented a more tightly bound fraction that had a lower relative uronic acid content.

When olive fruits are processed, a substantial amount of cell wall polysaccharide is solubilized. While all noncellulosic components appear to be involved, the pectic polysaccharides appear to be most directly affected. Analysis of pectins extracted from cell walls *in vitro* suggests that processing has its greatest impact on neutral sugar-poor (i.e., less branched) polymers. The most important pectic polymer solubilized may be represented by the peak of uronic acid of the WSF fraction of processed fruit that is eluted with 800 mM buffer. This leaves behind a pectin fraction that is relatively uronic acid-poor, probably represented by the first major peak seen in the ion-exchange analysis of the OSF from processed fruit.

Although the pectins are quantitatively the wall components most clearly affected by processing (Table 1), in relative terms wall glucan is greatly affected. Furthermore, our analysis of polymeric (i.e., nondialyzable) material in processing liquids (Table 2) shows an accumulation of more glucose than uronic acid in spite of the fact that there is 25 times as much uronic acid as noncellulosic glucose in the walls of unprocessed olives. Cellulosic glucose could be solubilized and contribute to this accumulation, but this is not supported by the data because cellulose content is fairly constant (Table 1). It is more likely that the processing, marked by steeping of fruits in aerated lye solution, both solubilizes and degrades the uronide-rich pectins so that they are not retained during dialysis. Fishman et al. (1989) have reported an apparent depolymerization or disaggregation of pectins when they are dialyzed against 0.05 M NaCl. This phenomenon could also explain the apparent loss of olive cell wall uronide from processing liquids.

ABBREVIATIONS USED

CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid; HA, hemicellulose A; HB, hemicellulose B; OSF, 1% sodium oxalate-soluble cell wall fraction; WSF, water-soluble cell wall fraction; TFA, trifluoroacetic acid.

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Received for review January 26, 1994. Accepted February 10, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1994.