Effect of Processing on Cell Wall Polysaccharides of Green Table Olives

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Cell wall material (CWM) was prepared from green olive pulp processed according to the "Sevillian Style". Polymers were solubilized from the CWM by the sequential extraction with *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, sodium salt (CDTA); Na_2CO_3; 1 M KOH; 4 M KOH; and 4 M KOH + borate to leave a cellulosic residue (CR). The polymers from the various extracts were fractionated and selected fractions were subjected to methylation analysis. This study showed that the softening of the olive pulp with the processing was due to a partial degradation of the pectic polysaccharides. Part of the degraded polymers were solubilized during the preparation of the CWM and the majority were solubilized after CDTA treatment. The CWM also showed coprecipitation with intracellular proteins and phenolic compounds that originated a material that was insoluble in aqueous solutions. The hemicellulosic polymers were not significantly affected by the processing.

Keywords: *Green olive pulp; cell walls; pectic polysaccharides; glucuronoxylans*

INTRODUCTION

The textural properties of plant tissues are largely dependent on the cell wall vigor (Van Buren, 1979). Softening of fruit and vegetable tissues is associated with changes in the chemistry of the cell wall polysaccharides. Depolymerization of pectic polysaccharides, particularly those of the middle lamella, often accompanies an increase in the ease of cell separation and/or wall weakening. In ripening fruits, dissolution of pectic polysaccharides occurs as a result of biochemical modification. Heat-processing, however, induces depolymerization usually through a mechanism of β-eliminative cleavage (Van Buren, 1979; Greve et al., 1994). Eliminative degradation of pectic polysaccharides is enhanced by higher levels of pectin-methyl esterification, and alkaline pH. Cell−cell adhesion is also modulated by the presence of Ca^{2+}. Removal of Ca^{2+} by chelating agents in the presence of salts of monovalent cations, such as NaCl, can also reduce tissue firmness (Van Buren, 1986; Parker and Waldron, 1995).

The processing of pickled green olives according to the "Sevillian Style" is widely used in Portugal and Spain. In this procedure, the fruits are collected in a mature, green stage (prior to ripening) and are treated anaerobically with a sodium hydroxide solution (lye) for several hours to remove the bitter glucoside oleuropein. The treatment is likely to inactivate all biochemical activity within the fruits. The strength of the alkali (~2% w/w) depends of the fruit size, temperature, and degree of maturation. Alkali treatment is terminated when two thirds of the pulp has been penetrated. The fruits are then washed several times with water and submitted to a lactic fermentation in a brine solution (7−10% NaCl) for 2−4 months. After fermentation, the green olives are packed for sale (Fernández-Díez, 1985).

Alkali treatment of the olive pulp removes the epicuticular waxes and causes the solubilization of the middle lamella (Lanza and Marsilio, 1994), thus facilitating cell separation and softening. This process is thought to involve dissolution of the pectic polysaccharides. However, it is also possible that changes in the solubility and structure of hemicellulosic polymers occur as a result of the alkali treatment and lactic fermentation. Indeed, studies on black olives processed by the "California Black Ripe" technique have shown that the storage in brine solution followed by treatment with aerated lye not only solubilized and degraded uronic acid−rich pectic polysaccharides but also increased the solubility of many other noncellulosic components (Araujo et al., 1994). Furthermore, the importance of hemicellulose in cell−cell adhesion has been highlighted in Chinese water chestnut (Parker and Waldron, 1995). This study was performed to provide information relating to changes in the chemistry of cell walls of olive pulp during processing by the "Sevillian Style".

MATERIALS AND METHODS

Plant Material. Olive (*Olea europaea* cv. Douro) fruits with an average length of 2.0 cm and diameter of 1.2 cm were harvested at the mature green stage and processed according to the "Sevillian Style" (Fernández-Díez, 1985) in a Portuguese olive industry. Olives were industrially processed. They were submitted to a 9-h anaerobic 2% NaOH treatment at room temperature, the alkali was removed with washings in water, and the olives were transferred to a brine solution (7−10% NaCl) and allowed to lactic ferment for 3 months at pH 4−5. Samples were collected before and after the overall treatment.

Preparation of the Cell Wall Material (CWM). Olive pulp CWM was prepared according to the method described by Coimbra et al. (1994). Olives (920 g) were stoned, and the pulp was frozen in liquid nitrogen. The tissue pieces of the pulp (750 g) were homogenized in 1 L of aq. 1.5% sodium dodecyl sulphate (SDS) containing 5 mM sodium metabisulfite ($Na_2S_2O_5$) with an Ultraturrax for 3 min. The homogenate was filtered through a nylon cloth. The residue was washed with aq. 0.5% SDS containing 3 mM $Na_2S_2O_5$, suspended in 1

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L of the same solution and, to break the clusters of cells, was ball-milled for 8 h and centrifuged. The residue was washed with distilled water and centrifuged. The residue was then stirred overnight in phenol:HOAc:H_2O (PAW, 2:1:1, w/v/v, 2 L) at 20 °C, centrifuged, and stirred again for 2 h in PAW (1 L). The residue was collected, washed with distilled water, dispersed in water, dialyzed, and stored as a frozen suspension in water at −20 °C. A freeze-dried aliquot indicated the yield to be 24 g (3.2 g/100 g of pulp tissue). During the cell wall preparation, aliquots (∼20%) of the 1.5% SDS and PAW extraction media were taken and dialyzed. The 0.5% SDS extract was totally dialyzed. Because a considerable amount of oil remained in the dialyzed material, SDS and PAW extracts were submitted to 2:1 (v/v) $CHCl_3$:MeOH extractions and then freeze-dried. All fractions were submitted to sugar analysis.

Sequential Extraction of CWM. CWM was extracted by adaptation of the method proposed for the sequential extraction of CWM from parenchymatous tissues (Selvendran and O'Neill, 1987). CWM (15 g) was sequentially extracted with (*i*) 50 mM *trans*-1,2-cyclohexane-diamine-*N*,*N*,*N*′,*N*′-tetraacetate (CDTA, Na salt, 1.5 L), pH 6.5 at 20 °C for 6 h (CDTA-1); (*ii*) 50 mM CDTA (1.5 L), pH 6.5 at 20 °C for 2 h (CDTA-2); (*iii*) 50 mM Na_2CO_3 + 20 mM $NaBH_4$ (1.5 L) at 1 °C for 16 h (Na_2CO_3-1); (*iv*) 50 mM Na_2CO_3 + 20 mM $NaBH_4$ (1.5 L) at 20 °C for 3 h (Na_2CO_3-2); (*v*) 1 M KOH + 20 mM $NaBH_4$ (1 L) at 1 °C for 2 h; (*vi*) 1 M KOH + 20 mM $NaBH_4$ (1 L) at 20 °C for 2 h; (*vii*) 4 M KOH + 20 mM $NaBH_4$ (1 L) at 20 °C for 2 h; and (*viii*) 4 M KOH + 3.5% H_3BO_3 + 20 mM $NaBH_4$ (1 L) at 20 °C for 2 h. The alkali extractions were carried out with O_2-free solutions under argon. After each extraction, solubilized polymers were separated from the insoluble residue by centrifugation (CDTA and Na_2CO_3 extracts) or by filtration through a G1 glass sinter funnel (KOH extracts). All extracts were filtered through Whatman glass fiber filters type e (GF/C) and dialyzed exhaustively; Na_2CO_3 and KOH extracts were acidified to pH 5 with HOAc prior to dialysis. Precipitates formed during dialysis of alkali extracts were collected separately. The cellulose-rich residue (CR) remaining after the final alkali extraction (4 M KOH + borate) was suspended in water (300 mL), and the solution was acidified to pH 5 and dialyzed. The supernatant of the CR dialysis was collected separately by centrifugation from the CR1 residue. All extracts collected after dialysis were concentrated and stored as frozen suspensions at −20 °C, and a small amount of each extract was freeze-dried.

Graded Precipitation with Ethanol. The polymers were dissolved in water and precipitated by the addition of ethanol, the concentration of which was increased in steps between 10 and 20% (Selvendran and King, 1989). Each mixture was placed at 4 °C for 1 h, and the precipitate was collected by centrifugation. To remove the ethanol completely, each precipitate was dissolved in water and rotary evaporated. The fractions were stored as frozen suspensions at −20 °C. A small amount of each fraction was freeze-dried for sugar analysis.

Ion-Exchange Chromatography. Some of the ethanol-precipitated fractions were suspended in water and stirred at 20 °C. The insoluble residues were removed by centrifugation. To the supernatant solution was added potassium-phosphate buffer (pH 6.5) to a final concentration of 50 mM and 1 mg of material/mL of buffer. Each solution was passed through a column of DEAE- Trisacryl M phosphate form (Redgwell and Selvendran, 1986) 1 mL of ion exchanger/7.5 μmol of uronic acid present, at 10 mL/h. The fractions were eluted sequentially with the same volume of buffer and buffer containing 0.125, 0.25, 0.5, and 1 M NaCl. Fractions (3 mL) were collected and aliquots (20 μL) were assayed for carbohydrate by the phenol−H_2SO_4 method (Dubois et al., 1956). The appropriate fractions were combined, dialyzed, concentrated, and stored at −20 °C. A small amount of each fraction was freeze-dried.

Copper Precipitation of Xylans. Copper precipitation was carried out on the precipitate from the 1 M KOH 1 °C fraction by the method described by Coimbra et al. (1994). The material (300 mg) was dispersed in 50 mL of 1 M NaOH. To the solution was added copper acetate (7%, w/v; 10 mL) and the mixture was stirred for 3 h at room temperature, when a blue precipitate was obtained. This precipitate was isolated by centrifugation and washed with distilled water. The precipitate was dissolved in a solution of 5% HCl in ethanol (50 mL) to give a pale-yellow solution. This solution, on addition of water (100 mL), gave a white precipitate that was collected by centrifugation, neutralized, and freeze-dried (180 mg).

pH Fractionation. The foregoing purified xylan (KCu, 75 mg) was dispersed in 6 mL of 1 M NaOH containing 20 mM $NaBH_4$, and a small amount of insoluble material (1 mg) was removed by centrifugation. The pH of the supernatant solution was lowered by gradual addition of 1 M HCl until a precipitate (53 mg) was obtained at pH 12, which was removed by centrifugation (K12). When the pH reached 5, another precipitate was obtained (19 mg) and was removed by centrifugation (K5). The supernatant solution at pH 5 was concentrated (3 mg), and the supernatant solution and precipitates were dialyzed and freeze-dried.

Carbohydrate and Linkage Analysis. Neutral sugars were released by Saeman hydrolysis (Selvendran et al., 1979) and analyzed as their alditol acetates by gas-liquid chromatography (GLC; Blakeney et al., 1983; Harris et al., 1988). Uronic acids (Ur.A) were determined colorimetrically by a modification (Selvendran et al., 1979; Selvendran et al., 1989) of the method of Blumenkrantz and Asboe-Hansen (1973). Polysaccharides were methylated by a modification of the Hakomori method (Hakomori, 1964) as described by Ring and Selvendran (1978) and Waldron and Selvendran (1990), and then converted into partially methylated alditol acetates that were separated by GLC on an OV-225 column and characterized by GLC/MS, using the molar response factors of Sweet et al. (1975). Prior to methylation, the CDTA-soluble fraction (C30) was de-esterified according to the procedure of Aspinall et al. (1970) as described by Coimbra et al. (1994). The methylated fractions (2−5 mg) were carboxyl-reduced by a modification of the method described by Lindberg and Löngren (1978) as follows. The methylated fraction was heated in a sealed tube with a mixture of $LiAlD_4$ (20 mg) in THF (1 mL) at 60 °C for 4 h. The excess of reagent was then destroyed with ethanol (2−3 drops) and water (2−3 drops), and the pH of the mixture was adjusted to neutrality with 2 M H_3PO_4. The reduced polymer was isolated by filtration, washed thoroughly with $CHCl_3$:MeOH (2:1, v/v), evaporated to dryness, hydrolyzed, reduced, and acetylated as previously described (Ring and Selvendran, 1978).

RESULTS AND DISCUSSION

Isolation of CWM. In a previous study (Coimbra et al., 1994), cell wall polysaccharides from unprocessed olive pulp were isolated and characterized. The results of this previous study were compared in the present paper with those of the alkali-treated and fermented green olive. The sugar compositions of the various polymers solubilized, of the purified CWM, and of the total processed and unprocessed pulps are shown in Table 1 . The yields of the SDS and PAW extracts and of the CWM from processed and unprocessed olive pulp are compared in parts a and b of Figure 1.

During the preparation of the CWM, 17.6% of cell wall sugars were solubilized, which represented double of the amount solubilized from the unprocessed olives all the material was solubilized by the SDS solutions; and PAW treatment did not solubilize significant amounts of material from the cell wall. The material of the SDS extracts that remained soluble in water after dialysis were rich in arabinose (Ara; possibly of pectic origin) and galactose (possibly from glycoproteins rich in arabinogalactans; Coimbra, 1993). The insoluble material was rich in arabinose and uronic acid and had a sugar composition characteristic of pectic polysaccharides.

The solubilization of polymers rich in Ara and Ur.A during the preparation of the CWM suggested that the

Table 1. Sugar Composition of Purified Cell Wall Material of Processed Green Olive Pulp, of Material Solubilized during Purification, and of the Total Processed and Unprocessed Pulps

		cell wall sugars (mol%)								
fraction	yield[a] (g/kg)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Ur.A	total sugar[b] (μg/mg)
1.5% SDS										
soluble	1.3 (3.2%)	3	1	59	5	1	20	5	6	503
precipitate[d]	9.1 (4.4%)	4	14	33	6	4	8	13	18	100
0.5% SDS										
soluble	0.4 (1.0%)	2	t[c]	46	23	t	16	2	11	502
precipitate[d]	4.7 (9.0%)	3	t	42	1	t	4	3	47	397
PAW	< 0.1									
purified CWM	32.0 (82.4%)	1	t	25	15	3		31	20	533
total pulp										
processed	47.5	1	t	28	13	3	6	27	22	
unprocessed[e]	46.5	1	t	27	12	2	4	28	26	

[a] Yield is expressed in g/kg fresh weight of olive pulp; the values in parentheses give the carbohydrate content (%) of the fractions. [b] Values are expressed as μg of "anhydrosugar"/mg dry polymer. [c] t, trace. [d] Material that precipitated on dialysis. [e] Values from Coimbra et al. (1994).

cell walls of processed olive pulp were partially degraded, resulting in more easy solubilization of these polymers. The CWM sugar composition showed a decrease in the Ur.A content (26 to 20 mol%) as well as a slight decrease in the Ara content (27 to 25 mol%), with a concomitant increase of glucose (29 to 31 mol%) and xylose (12 to 15 mol%). The yield of CWM was 3.2% on a fresh weight basis (3.7% for unprocessed olives, Figure 1a), and the amount of sugars present in the CWM was 82.4% of total sugars (90.8% for unprocessed; Figure 1b). In processed "California Black Ripe" olives, a similar decrease in the yield of CWM and a decrease in the proportion of Ur.A was observed (Araujo et al., 1994).

On a sugar basis, the wall components solubilized with 1.5% SDS were 1.56 g of sugar/kg of pulp [(1.3 g/kg * 503 μg/mg + 9.1 g/kg * 100 μg/mg)/1000] and with 0.5% SDS were 2.07 g of sugar/kg of pulp. These results show that during the preparation of the CWM, 3.63 g of sugar/kg of pulp were solubilized and 17.1 g of sugar/kg of pulp were retained in the CWM (Table 1 and Figure 1c). Previous results for the preparation of the CWM from unprocessed olive pulp showed 2.34 g of sugar solubilized/kg of unprocessed olive pulp (0.73 g/kg in 1.5% SDS, 0.89 g/kg in 0.5% SDS, and 0.72 g/kg in PAW); 23.0 g of sugar/kg of pulp were retained in the CWM (Figure 1c). The overall mass of sugars in the pulp (calculated by the addition of the mass of the sugars solubilized in each extract to the mass of sugars in the CWM) was 20.7 g of sugars/kg of pulp for the processed olive. This value was 4.6 g smaller than that found for the unprocessed olive (Figure 1c). The amount/kg of pulp for all major sugars was decreased compared with the unprocessed pulp (Figure 1d). Except for the Ur.A that showed a 15% decrease, the relative molar percentages were the same (Table 1, bottom). Laboratory scale experiments showed that a 3−5% swelling of the pulp occurred when the olive was treated with 2% NaOH solutions and, at the same time, a 6−7% loss of the overall sugars was detected. Loss of 1% of the overall sugars was observed when the olives were stored for 20 days in a brine solution. The swelling effect and the diffusion of the cell wall constituent sugars appears to be the reason for the detected loss of sugars reported in Figure 1c. A loss of material due to the diffusion of the cell wall constituent sugars to the medium was also observed in the "California Black Ripe" olives during the various phases of the processing (Araujo et al., 1994).

To investigate the origin of the changes in extractability, the CWM was sequentially fractionated.

Fractionation of CWM. The CWM was sequentially extracted with aqueous inorganic solvents to leave a cellulose-rich residue (CR). On neutralization of CR, a pectic polysaccharide was released into the supernatant solution, leaving the residue CR1. The amounts of polymers solubilized by the various extractants and their sugar composition are shown in Table 2.

The CDTA extractions solubilized those pectic substances held in the walls by Ca^{2+}. The first extraction with CDTA (CDTA-1) solubilized 20.0% of the CWM; in contrast, the second CDTA extraction (CDTA-2) and the two sodium carbonate extractions, together, solubilized only 7.0% of CWM. The first CDTA extraction solubilized the majority of the pectic material present in the CWM, which was in contrast to what occurred with unprocessed CWM because a large amount of material was solubilized with Na_2CO_3 (Figure 1e,f). All the pectic extracts from the processed olive pulp were rich in arabinose and showed a Ur.A:Ara ratio between 1:2 and 1:3.5. These results are in contrast with those reported for the sugar composition of the CDTA extracts from unprocessed olive pulp where the Ur.A:Ara ratios were 8:1 and 3:1, respectively, for CDTA-1 and CDTA-2. The difference in the Ur.A:Ara ratios between processed and unprocessed material showed that the composition of the CDTA (and Na_2CO_3 extracts) from processed olives was comparable to the composition of the Na_2CO_3 extractions from the unprocessed olives (Ur.A:Ara = 1:1). These polymers were solubilized by a chelating agent, so it can be inferred that the pectic polymers in processed olive cell walls were associated essentially by calcium bridges. Because the fruits were alkali-treated before being fermented, a carbonate treatment was not necessary to solubilize the bulk of the pectic polymers. Similar solubility changes involving wall pectic polysaccharides were observed in the "California Black Ripe" olives (Araujo et al., 1994), confirming that the alkali treatment produces an effect similar to the carbonate treatment used in the extraction of the cell wall polymers.

The Na_2CO_3-2 extract showed 16% xylose. This sugar is a component of the hemicellulosic material (Coimbra et al., 1994), so it is probable that the processing also affected the solubilization of this type of wall material. The 1 M KOH 1 °C and the 4 M KOH extractions were the treatments that solubilized the major amounts of hemicellulosic polysaccharides (Table 2 and Figure 1e). This result suggests that the polymers usually solubilized by the 1 M KOH 20 °C and 4 M KOH + borate extractions from unprocessed olive pulp were solubilized in the previous treatments, showing an increase in

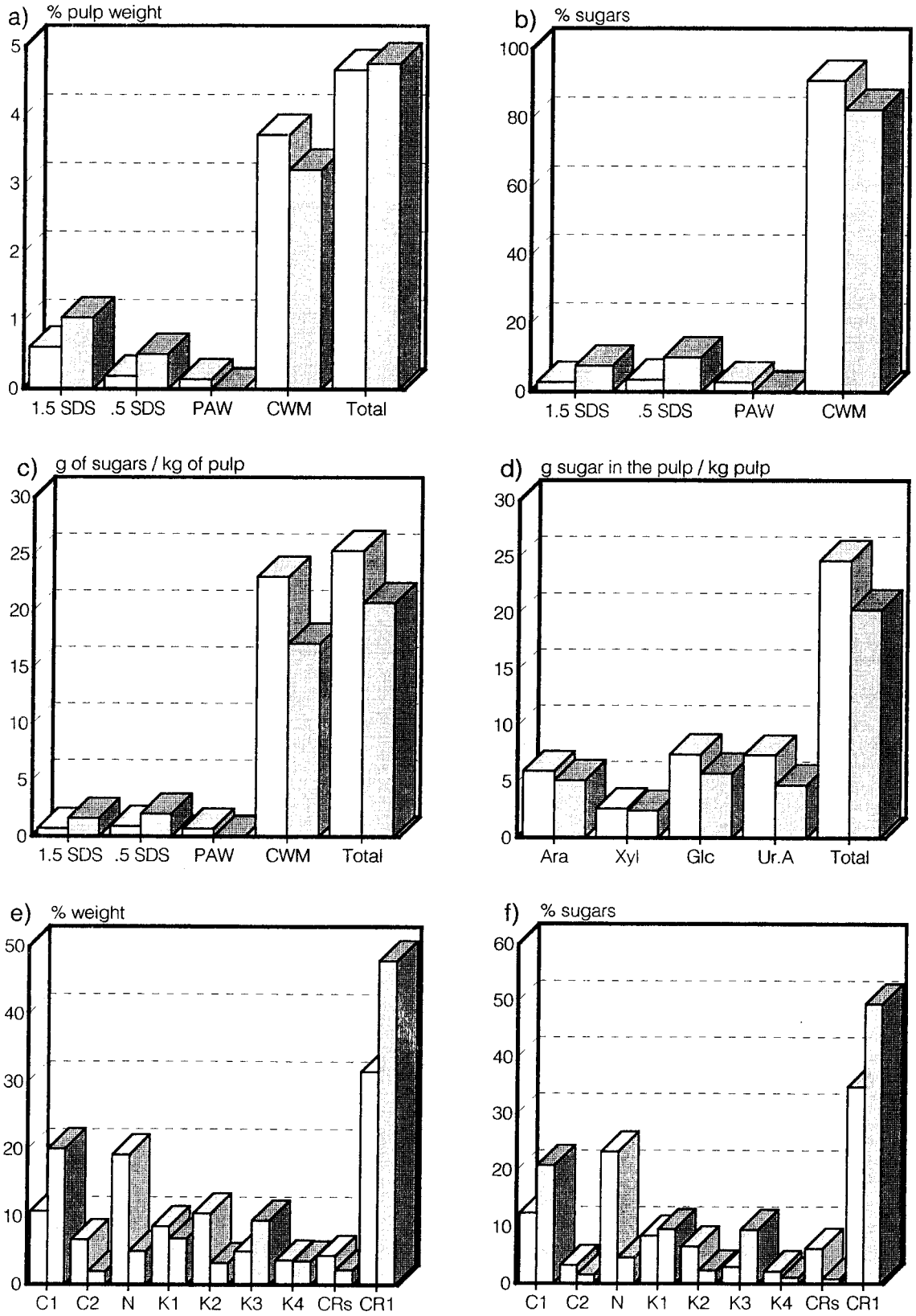

Figure 1. Comparison of the differences in several parameters between processed and unprocessed olive pulp cell walls: (□) unprocessed; (■) processed; (a) percentage of the yield, as percentage of the pulp weight, in the SDS and PAW extracts and in CWM; (b) percentage of the yield, as percentage of sugars, in the SDS and PAW extracts and in CWM; (c) mass of the sugars/kg of pulp present in the SDS and PAW extracts and in CWM; (d) amounts of the different sugars/kg of pulp present in the cell walls of olive pulps; (e) percentage of material extracted from the olive pulps CWM with the various aqueous solutions; (f) percentage of sugars extracted from the olive pulps CWM with the various aqueous solutions. Fresh olive pulp was investigated in a previous paper (Coimbra et al., 1994); total values are given in the figures for comparative purposes.

solubilization with processing. The same observations were made in California Black Ripe olives where a shift of the wall polymers to a more readily extracted status was observed (Araujo et al., 1994).

Table 2. Sugar Composition of Fractions of Cell Wall Material of Processed Green Olive Pulp Obtained by Sequential Extractions with Aqueous Solvents

		cell wall sugars (mol%)								
fraction	recovery (%)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Ur.A	total sugar[a] (μg/mg)
CDTA-1 (C1)	20.0	2	-	62	t[b]	1	4	t	31	621
CDTA-2 (C2)	1.9	3	-	67	1	t	6	4	19	500
Na_2CO_3-1 (N1)	3.9	3	-	64	4	t	5	1	23	593
Na_2CO_3-2 (N2)	1.2	3	-	52	16	2	7	2	18	360
1 M KOH 1 °C (K1)										
supt.[c]	3.6	1	t	4	59	3	4	13	16	846
ppt.[d]	3.1	1	-	1	82	1	1	3	13	856
1 M KOH 20 °C (K2)										
supt.	1.8	1	t	8	52	4	5	20	10	560
ppt.	1.4	1	-	3	74	1	1	6	14	268
4 M KOH (K3)										
supt.	7.4	t	t	7	22	22	12	33	4	700
ppt.	2.1	1	t	4	61	7	3	14	10	230
4 M KOH + borate (K4)										
supt.	1.4	1	t	15	23	15	10	26	10	391
ppt.	2.1	2	-	22	26	5	3	25	17	51
CR										
supt. (CRs)	2.2	2	-	50	1	-	6	8	33	227
CR1	47.9	1	-	18	7	1	2	63	8	614

[a] Values are expressed as μg of "anhydrosugar"/mg dry polymer. [b] t, trace. [c] Material isolated from the supernatant solution. [d] Material that precipitated on dialysis.

Table 3. Sugar Composition of Fractions Gradually Precipitated with Ethanol from the Material Solubilized with 0.5% SDS during the Preparation of the CWM, and from CDTA-1 Soluble Extract after Ethanol Precipitation Followed by Anion-Exchange Chromatography

		cell wall sugars (mol%)								
fraction	recovery (%)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Ur.A	total sugar[a] (μg/mg)
CDTA-1										
insol. res.[b]	23.2	3	t[c]	50	1	t	4	2	40	657
EtOH 15%	7.8	2	t	45	1	-	4	t	48	627
EtOH 30% (C30)	61.1	3	t	57	1	t	4	1	35	692
EtOH 50%	6.4	3	t	52	1	2	4	3	35	742
EtOH 80% (C80)	12.2	3	t	57	1	1	4	2	32	736
0.125 M NaCl	8.6									
0.25 M NaCl	65.7	4	-	39	t	-	3	1	53	946
0.5 M NaCl	5.7	6	-	35	t	t	2	4	53	592
EtOH 80% supt.[d]	5.6	3	t	63	1	t	4	2	27	807
SDS 0.5%										
Insol. res.	31.2	3	1	39	20	1	18	4	14	280
EtOH 40%	20.6	t	t	7	59	t	14	2	18	744
EtOH 60%	2.4	5	1	31	27	t	21	2	13	679
EtOH 80%	34.8	4	t	62	4	t	17	3	10	670
EtOH 80% supt.	11.5	2	t	89	1	1	1	2	4	475

[a] Values are expressed as μg of "anhydrosugar"/mg dry polymer. [b] Material that became insoluble upon thawing. [c] t, trace. [d] Supernatant solution.

The residue remaining after 4 M KOH + borate extraction was neutralized and dialyzed. The polymeric material isolated from the supernatant solution (CRs), which accounted for 2.2% of the CWM, was rich in pectic polysaccharides, as shown by the levels of Ara and Ur.A. A similar type of material was previously isolated from unprocessed olive pulp, which accounted for 4.3% of CWM and was shown to be very rich in sugars (94%), which contrasted with the low sugar content found in this fraction (23%). CR1 contained 47.9% of the CWM and the Ur.A content (8 mol%) was less than that found in the cellulosic residue from unprocessed olive pulp (14 mol%). The relative amount of sugars present in CR1 was higher in the processed olives (Figure 1f), which suggests that the processing also caused the insolubilization of material that remained associated in the cellulosic matrix even after the extraction treatments. This material, which had a green color and remained insoluble even after a 72% and 1 M H_2SO_4 treatment, seemed to prevent the extraction of part of the glycosidic polymers. The presence of lignin-like material was previously reported to occur in the olive pulp cell walls (Coimbra et al., 1994; Coimbra et al., 1996).

To gain further information on the origin of the process-related changes, selected extracts were fractionated.

Fractionation of the Pectic Extracts. To assess the extent of degradation of the pectic polysaccharides, the polymers present in CDTA-1 and in SDS 0.5% extracts (prior to the preparation of the CWM) were subjected to graded precipitation with ethanol, and selected fractions were further resolved by anion-exchange chromatography (Table 3).

The CDTA-1 extract yielded a considerable amount of precipitate upon thawing. The graded ethanol fractionation gave five fractions rich in Ara and Ur.A. The fractions that precipitate with higher concentrations of ethanol were less rich in Ur.A. The fraction that precipitated with 80% ethanol, one of the richest in Ara, was fractionated by anion-exchange chromatography, and the majority of the material was retained in the column and was eluted with 0.25 M NaCl. This behavior indicated that the neutral sugars were linked to the Ur.A and the polymers were probably deesterified.

When the material solubilized by the SDS 0.5% solution, which was rich in Ara and galactose, was

Table 4. Sugar Composition of Fractions from 1 and 4 M KOH

fraction	recovery (%)	cell wall sugars (mol%)								total sugar[a] (μg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Ur.A	
1 M KOH 1 °C ppt.										
$Cu(OAc)_2$ ppt. (KCu)	60.0	1	-	t[b]	88	1	-	t	10	773
NaOH insol. res.	1.3									
pH 12 ppt. (K12)	70.7	1	t	1	85	1	1	2	9	873
pH 5 ppt. (K5)	25.3	t	t	1	88	1	t	2	8	671
pH 5 supt.[c]	4.0	1	1	5	59	7	3	13	11	855
1 M KOH 20 °C supt.										
Insol. residue[d]	14.8	1	1	12	32	6	5	40	3	405
EtOH 50%	47.1	1	1	8	43	8	7	26	6	670
buffer[e]	47.5	t	1	11	44	6	7	27	4	750
EtOH 80%	29.4	1	t	11	51	4	6	21	6	787
EtOH 80% supt.	6.9	5	4	27	20	10	5	29	-	48
4 M KOH supt.										
Insol. residue[d]	16.2	1	t	10	32	15	8	31	3	505
EtOH 50%	41.3	t	t	6	18	25	13	36	2	937
buffer[e]	65.3	t	t	7	17	27	13	35	1	825
EtOH 80% supt.	9.3	3	t	38	16	10	10	14	9	251

[a] Values are expressed as μg of "anhydrosugar"/mg dry polymer. [b] t, trace. [c] pH 5 supernatant solution. [d] Material that became insoluble upon thawing. [e] Material not retained by the anion-exchange column.

thawed, it gave a large insoluble residue that was removed by centrifugation. The supernatant was submitted to ethanol precipitations and gave four fractions. Fractions less soluble in ethanol (40 and 60% EtOH) were rich in xylose and galactose and also contained Ur.A and Ara; the fractions more soluble in ethanol were rich in Ara. Fraction "EtOH 80% supt." contained 89 mol% of Ara and seemed to be an "arabinan". A small amount of a similar "arabinan" was isolated from unprocessed olive pulp after hot water extraction (Coimbra, 1993). A nondegradative extraction of pectic polymers prevented the appearance of "arabinans" (Coimbra et al., 1994); therefore, the occurrence of an "arabinan" shows that it is a product of degraded pectic polysaccharides. From olive pulp, as from other parenchymatous tissues, it was not possible to isolate arabinans unless degradative processes were used. It is possible that this type of polymer was also present in the 1.5% SDS soluble extracts, as they were very rich in Ara.

Fractionation of the Hemicellulosic Extracts. To assess the differences in the fractionation profile of the hemicellulosic polysaccharides, the insoluble material from the 1 M KOH 1 °C extract was dissolved in 1 M NaOH and was precipitated with copper acetate/HCl. The white precipitate obtained (KCu) was rich in xylose (Xyl) and Ur.A (Table 4, top); the Xyl:Ur.A ratio was 9:1. The precipitate was resuspended in 1 M NaOH and the material that became insoluble was removed by centrifugation. To the supernatant, HCl was added with a gradual decrease of pH. A precipitate was collected at pH 12 (K12) and another at pH 5 (K5). Fractions K12 and K5 were very rich in Xyl and also contained Ur.A. This glycosidic composition is characteristic of glucuronoxylan (Coimbra et al., 1994).

The polymers present in 1 M KOH 20 °C and 4 M KOH extracts were subjected to graded precipitation with ethanol and the major fractions were further submitted to anion-exchange chromatography (Table 4). From the 1 M KOH 20 °C extract, three fractions were obtained by graded precipitation with ethanol. The major fraction was rich in Xyl and glucose, in a ratio of 2:1. The neutral fraction obtained by anion-exchange chromatography had the same sugar composition, which indicates the presence of a xyloglucan as inferred by the amount of glucose, Xyl, and galactose; the high percentage of Xyl showed that a xylan is also present. Closely associated acidic xylans and xyloglucans, possibly in covalent association, were recently reported in olive pulp (Coimbra et al., 1995). From the 4 M KOH extract,an insoluble fraction and two more fractions were obtained by graded ethanol precipitation. The major fraction, precipitated with 50% ethanol, was rich in glucose, mannose, Xyl, and galactose, probably as a mixture of cell wall polysaccharides. This mixture was maintained in the neutral fraction obtained by anion-exchange chromatography, which showed the same sugar composition.

Glycosidic Linkage Analysis. Fractions C30, from the CDTA-1 extract, and KCu, K12, and K5 from the 1 M KOH 1 °C extract, were methylated, and the carboxyl groups were reduced with $LiAlD_4$; the results are shown in Table 5. The methylation analysis is quantitative for the neutral sugars present; however, the percentage of reduced Ur.A is not.

The methylation of C30 showed that the majority of the galacturonic acid residues were (1→4)-linked; C2- and C3-branched residues as well as terminally linked GalA residues were also detected. The relative proportions of the variously linked major Ara residues (T-Ara*f*: 3-Ara*f*:5-Ara*f*:3,5- Ara*f*) were similar those of the pectic material from the unprocessed pulp (Coimbra et al., 1994).

The methylation of KCu confirmed that the xylosyl residues were glucuronoxylans. The glucuronoxylans analyzed showed a degree of polymerization estimated by methylation analysis of 230 residues (as inferred from the ratio of total xylosyl residues to terminally linked xylosyl residues) and a percentage of branching points of 10% [as inferred from the ratio of (1→2,4)-linked xylosyl residues to total xylosyl residues]. These values are very similar to those reported for the unprocessed olive (Coimbra et al., 1994). Small amounts of (1→3)-linked rhamnosyl residues were detected. These residues were reported to occur in glucuronoxylans in association with (1→2)-linked galacturonic acid residues at the reducing end of glucuronoxylans (Johansson and Samuelson, 1977; Andersson et al., 1983).

General Discussion. The major structural modification of the cellular matrix with processing of pickled green olives according to the "Sevillian Style" was due to the degradation of the pectic polysaccharides. This modification was reflected both in the pectic polysaccharides associated with Ca^{2+} and in the pectic polysaccharides solubilized with alkali solutions and could be

Table 5. Glycosyl Linkage Composition of Selected Cell Wall Polysaccharides from CDTA and 1 M KOH Extracts (Values Expressed as Relative mol %)

	CDTA	1 M KOH		
linkage	C30	KCu	K12	K5
2-Rha*p*	2.1			
3-Rha*p*	0.2	0.4	0.5	0.5
2,3-Rha*p*	0.4			
2,4-Rha*p*	2.1			
T-Ara*f*	11.9	0.2	0.6	0.2
2-Ara*f*	0.6			
3-Ara*f*	7.1			
5-Ara*f*	17.9			
3,5-Ara*f*	21.2			
arabinitol	1.4			
T-Xyl*p*		0.4	0.6	0.4
4-Xyl*p*	0.5	79.4	83.3	83.1
2,4-Xyl*p*	1.5	12.6	9.3	9.7
xylitol		1.9	0.4	0.6
T-Gal*p*	0.8			
4-Gal*p*	1.2			
galactitol	1.1			
T-Gal*p*A	0.7			
2-Gal*p*A		0.4		
4-Gal*p*A	22.0			
2,4-Gal*p*A	3.2			
3,4-Gal*p*A	3.0			
T-Glc*p*		0.6	0.7	0.7
4-Glc*p*	0.3			
2,4-Glc*p*	0.4			
glucitol	0.4	0.2	1.0	0.2
T-Glc*p*A		3.6	3.6	4.6
3-Glc*p*A		0.3		

the principal factor in the alteration of olive pulp texture with processing. The preparation of the CWM solubilized a significant amount of pectic material; in addition, some pectic polysaccharides that were extracted from the CWM of unprocessed fruit with Na_2CO_3 solutions were only associated by calcium bridges, and were solubilized by the CDTA solutions. The changes observed in the extractability of the pectic polysaccharides were not reflected significantly in the structure of the "arabinan moiety" of the pectic polysaccharides but, possibly, only in the depolymerization of the rhamnogalacturonan backbone that is known to occur in the presence of alkali solutions, with concomitant loss of Ara short chains. The glucuronoxylans from processed olive pulp do not show any degradation. The fractions rich in xyloglucans were solubilized with alkali solutions with lower concentration than that observed for unprocessed olives. As was verified with unprocessed fruits, it was not possible to isolate a pure xyloglucan from processed olive pulp with the usual procedures; xyloglucans usually occur associated with xylans. However, it is probable that small amounts of isolated xyloglucans could be obtained from pickled green olive pulp, as was reported by Gil-Serrano et al. (1988). The phenolic material present in these pickled olives may have prevented the solubilization of the glycosidic polymers.

ABREVIATIONS USED

CDTA, *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, sodium salt; CR, cellulosic residue; CWM, cell wall material; PAW, phenol−HOAc−H_2O; SDS, sodium dodecyl sulfate; Ur.A, uronic acid; Ara, arabinose; Xyl, xylose; Rha, rhamnose; Fuc, fucose; Man, mannose; Gal, galactose; Glc, glucose.

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