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Effect of ripening on texture, microstructure and cell wall polysaccharide composition of olive fruit (*Olea europaea*)

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Olive fruits at the green, cherry and black stages were used to investigate the structural and microstructural changes in tissues during ripening. Scanning electron microscopy (SEM) tissue fracture of green olives resulted in cell wall breakage of epicarp and mesocarp cells. Tissue fracture resulted in fewer broken cells in cherry than in green olives and even less in black olive tissues. Cell separation occurred in the middle lamella region in some of the cells of the cherry fruit and in most of the black olive cells. Solubilization and loss of pectic polysaccharides, mainly the arabinan moiety, and glucuronoxylans occurred in the green to cherry stages. The pulp cell wall constituent polysaccharides, pectic polysaccharides, cellulose, glucuronoxylans and xyloglucans, were degraded and/or solubilized at the cherry to black ripening stages. The resultant depolymerization of the pectic polymers, especially those of the middle lamella region, was consistent with the progressive cell separation at the different ripening stages by SEM. This showed that partial solubilization of pectic, hemicellulosic and cellulosic polysaccharides within the cell wall matrix weakened the cell wall structures, preventing the breaking of cells when the tissues were fractured.

Introduction

The olive fruit consists of an epicarp layer with a continuous well-developed cuticle, a mesocarp layer, which constitutes more than 80% of the fruit and a woody endocarp enclosing the embryo (kernel). Cellular structure influences table olive texture (Fernandez-Díez 1979, Jimenez et al. 1994). Texture changes result from the activity of cell wall degrading enzymes, which are at their maximum at the colour changing stage (Fernandez-Bolaños et al. 1995). Changes in cell wall polysaccharides play a major role in bringing about alterations in olive fruit texture during ripening. Olive pulp polysaccharides are composed mainly of pectic polysaccharides rich in arabinose, glucuronoxylans and cellulose, while xyloglucans, mannans and glycoproteins occur as minor components (Coimbra et al. 1994, 1995). During the early stages of ripening, when the olive turns from green to cherry, pectins become less branched (Huisman et al. 1996). However, the changes that occur in cell wall polysaccharides at the more mature black stage, when olives are suitable for processing, have not yet been studied. Black oxidized table olives can be obtained from fruits at different stages of ripeness depending on their texture characteristics. Olives from the Negrinha do Douro cultivar can be processed from matured fruits at the green, cherry or black stages. In this paper, scanning electron microscopy (SEM) was used to investigate the microstructural changes in tissues during olive ripening, and these results were related to texture characteristics of the olives and their cell wall polysaccharide composition.

Abbreviations – Ara, arabinose; CDTA, *trans*-1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid, sodium salt; CWM, cell wall material; CR, cellulosic residue; Fuc, fucose; Gal, galactose; GalA, galacturonic acid; Glc, glucose; GlcA, glucuronic acid; HexA, hexuronic acid; Man, mannose; PrAW, 1-propanol/acetic acid/water (2:1:1 v/v/v); Rha, rhamnose; SDS, sodium dodecyl sulphate; SEM, scanning electron microscopy; Xyl, xylose.

Materials and methods

Plant material

Olive (*Olea europaea* L. cv. Negrinha do Douro, 1997 harvest) fruits with an average length of 2.0 cm and a diameter of 1.2 cm were provided by Maçarico Lda, Praia de Mira, Portugal, at different stages of ripening: mature green (392 fruits kg^{-1}), changing colour (cherry, 356 fruits kg^{-1}) and mature black (262 fruits kg^{-1}). The time delay between the harvesting and the analysis of texture and microstructure was less than 1 week.

Fruit texture analysis

Ten uniformly sized fruit samples were used for the assessment of firmness using two instruments (Effegi, Alfonsine (RA), Italy): a pressure tester or durometer (Marsilio 1983) and a puncture tester or penetrometer (Brighigna et al. 1978–1980). The compression test measures were obtained by pressing each olive with a flat cylindrical plunger of 1 cm diameter, using a weight-force of 1 kg and a test time of 30 s. The plunger was electrically disengaged and allowed to fall straight down to make contact with the fruit, horizontally positioned on the table. Values are reported as millimetres of deformation (ratio of decrease in the fruit-pulp height to the initial height). The puncture tester measured the force (N) required to push a probe of 2 mm diameter into 5 mm of flesh. The fruit mesocarp was cut around the equator and positioned vertically. Four readings for each fruit were made at the same distance from the skin (Fig. 1).

SEM

Fresh tissue blocks (approximately $3.0 \times 3.0 \times 1.5$ mm sizes) of 5 replicate samples were fractured revealing the epicarp and mesocarp in longitudinal view. The fracture face was cut away from the rest of the slice and fixed with 3% glutaraldehyde in 0.05 *M* cacodylate buffer (pH 7.2). The samples were washed with the same buffer, dehydrated in alcohol series, then transferred to 100% acetone before drying at the critical point. The dry tissues were then mounted, fracture surface upwards onto aluminium stubs and coated with gold (25 nm thick) in a Sputter Coater. Representative specimens were examined with a Philips XL 20 SEM and then photographed.

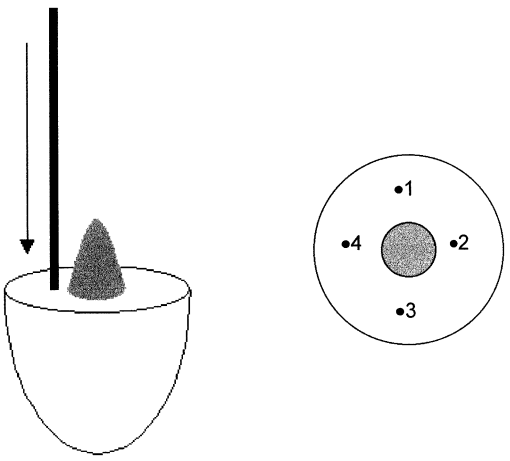

Fig. 1. Olive pulp puncture test.

Preparation of cell wall material (CWM)

The CWM was prepared according to the method described by Coimbra et al. (1996) with some changes to permit the use of the largest possible number of samples and to avoid the use of phenol reagent. Fresh olives (500 g) were destoned on arrival at the laboratory on the day of harvesting, frozen in liquid nitrogen and stored at -20°C until required (1 week, 1 month and 2 months, for black, green and cherry, respectively). The frozen olive pulps were homogenized and triturated in 1.5% sodium dodecylsulphate solution (SDS) containing 750 ml of 5 m*M* sodium metabisulphite. The homogenized material was filtered through a polyester cloth and washed twice in 650 ml 0.5% SDS solution containing 3 m*M* of sodium metabisulphite. The residues were washed twice with 500 ml distilled water, centrifuged (24400 *g*, 20 min, 4°C) and filtered (Whatman GF/C). The material was then extracted with a solution of 1-propanol/acetic acid/water (PrAW 2:1:1 v/v/v), twice (800 + 500 ml). The residue was washed 3 times in 500 ml distilled water and then the hydrated suspension was frozen and freeze-dried, to give the CWM. All extracts obtained were dialysed, concentrated under reduced pressure, frozen and freeze-dried for further analysis.

Sequential extraction of CWM

The CWM was extracted according to the method described by Coimbra et al. (1996) using imidazole 0.5 *M*, pH 7 instead of CDTA, as proposed by Mort et al. (1991). The CWM (10 g) was sequentially extracted with: (1) 0.5 *M* imidazole/HCl (pH 7.0), using 750 ml of solution for 16 h at 20°C; (2) 0.5 *M* imidazole/HCl pH (7.0), using 700 ml of solution for 2 h at 20°C; (3) 50 m*M* Na_2CO_3 + 20 m*M* $NaBH_4$, 750 ml, for 16 h at 4°C; (4) 50 m*M* Na_2CO_3 + 20 m*M* $NaBH_4$, 700 ml, for 2 h at 20°C; (5) 0.5 *M* KOH + 20 m*M* $NaBH_4$, 500 ml, for 2 h at 4°C; (6) 1 *M* KOH + 20 m*M* $NaBH_4$, 500 ml, for 2 h at 4°C; (7) 1 *M* KOH + 20 m*M* $NaBH_4$, 500 ml, for 2 h at 20°C; (8) 4 *M* KOH + 20 m*M* $NaBH_4$, 500 ml, for 2 h at 20°C and (9) 4 *M* KOH + 3.5% H_3BO_3 + 20 m*M* $NaBH_4$, 500 ml, for 2 h at 20°C. The KOH extractions were carried out with O_2 free solutions under N_2. After each extraction, the solubilized polymers were separated from the insoluble residue by centrifugation (24400 *g* for 10 min) followed by a filtration of the supernatant through a glass fibre filter (Whatman GF/C). The Na_2CO_3 and KOH extracts were neutralized to pH 5–6 with glacial acetic acid prior to dialysis. The residue (Cellulosic Residue) obtained after the alkali extractions was suspended in water, acidified (pH 5–6) and dialysed. The supernatant from the dialysis of the cellulosic residue was collected separately from the residue by centrifugation and filtration. After dialysis, all extracts were concentrated under reduced pressure, frozen and freeze-dried.

Table 1. Compression test deformation (durometer) and puncture test force (penetrometer) of green, cherry and black olives. Each value is the mean of 10 determinations ± SD.

Ripening stage	Deformation (mm)	Force (N)
Green	1.09 ± 0.65	3.61 ± 0.38
Cherry	2.68 ± 0.44	1.11 ± 0.20
Black	7.52 ± 0.35	0.25 ± 0.09

Carbohydrate analysis

Neutral sugars were released by Saeman hydrolysis (Selvendran et al. 1979) and analysed as their alditol acetates by GLC (Blakeney et al. 1983, Harris et al. 1988) using a Carlo Erba 6000 with a split injector (split ratio 1:60) and a FID detector. A 30 m column DB-225 (J&W) with i.d. 0.25 mm and 0.15 μm film thickness was used. The injector and detector temperatures were 220 and 230°C, respectively. The oven temperature programme used was: 220°C for 5 min, followed by 230°C for 6 min with a rate of 20°C min^{-1}. The flow rate of the carrier gas (H_2) was set at 1 ml min^{-1} at 220°C.

Cellulosic glucose was calculated as the difference between the content found with and without Saeman 72% H_2SO_4 pre-hydrolysis. Hexuronic acids were determined colorimetrically as described by Coimbra et al. (1996).

The hydrolysis of all samples was done in duplicate and each one was injected twice. Results with less than 5% variability in the major component cell wall sugars were obtained. A third analysis was done for the few samples with higher variability.

Determination of the degree of esterification

Determination of the degree of esterification of pectic polysaccharides was based on the estimate of methanol content released by saponification (Waldron and Selvendran 1990). The sample (5 mg) was dispersed in water (2 ml) and sonicated for 10 min; 1-propanol (0.4 ml of 0.5 g l^{-1} stock solution) was added as internal standard. The polysaccharides were saponified with 0.8 ml of 2 *M* NaOH for 1 h at 20°C. The sample suspension was neutralized by the addition of 0.8 ml of 2 *M* HCl and the resultant solution was filtered through a glass fibre filter (Whatman GF/C) and a nylon membrane filter NL16 0.2 μm (Schleicher & Schuell). The filtrate was injected (0.2 μl) in a gas chromatograph HP 5890 series II with a purged packed injector and a FID detector. The injector and detector temperatures were 180 and 220°C, respectively. A column DB-Wax (30 m length, 0.53 mm i.d. and 1.0 μm film thickness) was used with N_2 as carrier and a temperature programme between 50 (1 min) and 140°C, with a rate of 15°C min^{-1}.

Protein analysis

The protein content was obtained by a colorimetric determination of total Kjeldahl nitrogen (Willis et al. 1996). The samples (50 mg) were digested with 2 ml of concentrated H_2SO_4, 10 mg of selenium and 0.350 g of K_2SO_4. The cool solution was diluted to 100 ml with distilled water. To 0.2 ml of the digested and diluted sample were added 4 ml of salicylate reagent (32 g anhydrous sodium salicylate, 40 g of trisodium phosphate and 0.5 g of sodium nitroprusside in 1 l of water) and 1 ml of hypochlorite reagent (50 ml of commercially available bleach containing 5% sodium hypochlorite in 1 l). The solution was stirred, allowed to react for a minimum of 12 min, and the absorbance was measured at 685 nm. Calibration was performed using ammonium chloride as the standard. The conversion factor used was 6.25.

Moisture content

The moisture content of the olive pulp was determined in triplicate by oven drying 5 g of crushed olive pulp at 102 ± 2°C for 4 h, plus 2 h to confirm weight stabilization.

Results and discussion

Fruit texture analysis

Green olives were the least deformable and the most difficult to penetrate whereas black olives were the most deformable and the easiest to penetrate (Table 1). Both puncture and compression tests showed tissue softening induced by ripening.

SEM

Fractured epicarp and mesocarp in longitudinal view of green (Fig. 2a,b), cherry (Fig. 2c–e) and black (Fig. 2f,g) olive fruit pulps were examined by SEM. In green olives, the thin-walled parenchyma cells were uniform and tightly packed (Fig. 2b). Tissue fracture involved cell walls breaking, both in epicarp and mesocarp (Fig. 2a), but cell separation at the middle lamella level was not observed. The fracture surface of cherry olives (Fig. 2c) consisted of two regions: (1) one with 400–500 μm containing broken cell walls and some separated cells (Fig. 2d) and (2) a second one containing intact cell walls (Fig. 2e). The region with broken cell walls extended from the olive epidermis as far as the eighth-ninth layer of mesocarp. In black olives, the region of broken cell walls was smaller (100–200 μm) and involved only the epicarp and the first layers of the mesocarp (hypodermis and, sometimes, the following first layer) (Fig. 2f). The other cells showed rounded outlines and were divided along the middle lamella, showing cell separation (Fig. 2g).

During ripening, progressive disruption of the middle lamella parenchyma cells was observed (Fig. 3). According to Waldron et al. (1997), cell separation consists mainly in the dissolution of middle lamella pectic polysaccharides. Their solubilization is the result of the activity of the pectinmethylesterase and polygalacturonase (Langley et al. 1994). The texture of edible fruits and vegetables is strongly influenced by the chemical, physical and structural properties of the plant cell walls, which through adhesion provide mechanical strength and protection (Brett and Waldron 1996, Harker et al. 1997). To correlate the results of the fruit

texture analysis and SEM with the chemical composition of cell walls, the CWM was prepared for each stage of ripening and the polysaccharides were sequentially extracted and analysed.

Cell wall analysis

The yield of CWM in relation to the fresh pulp weight decreased from 3.5% for green olives, to 3.1% for cherry and 2.3% for black (Table 2). The value obtained for the green olives was slightly lower than the value of 3.7% previously reported (Coimbra et al. 1994). The moisture content was 77% in green and cherry and 61% in black olives. Similar moisture values were reported for the Spanish varieties Ascolano, Manzanilla and Sevillano at the black stage (Agar et al. 1998).

Glc (34 mol%), Ara (23 mol%), HexA (21 mol%) and Xyl (16 mol%) were the major sugars present in the CWM of green olive pulp (Table 2). Gal, Man and Rha occurred only in small amounts. These results differed slightly from those previously published for this variety (Coimbra et al. 1994), where higher relative amounts of HexA (26 mol%) and Ara

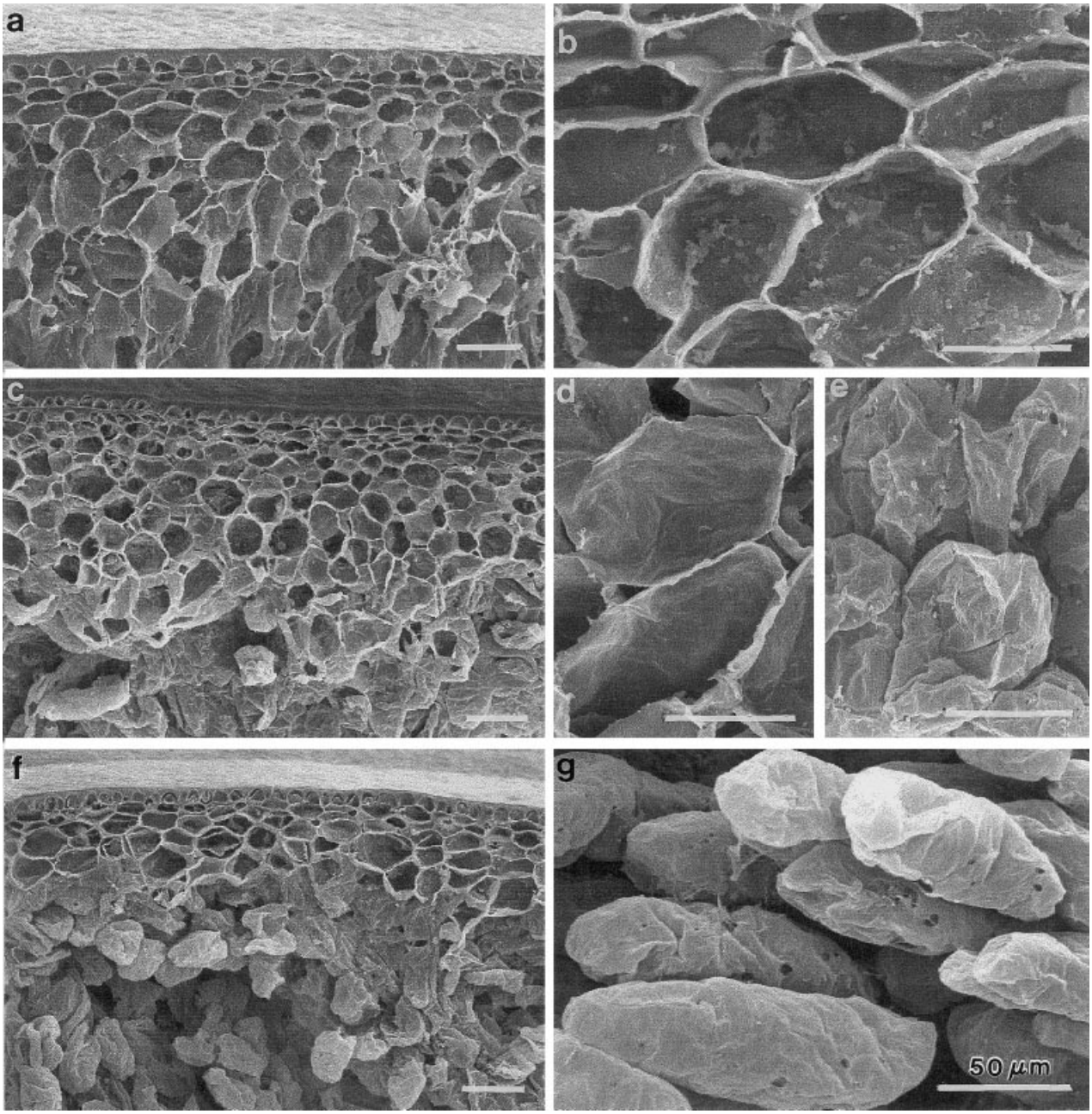

Fig. 2. SEM photographs of broken surface olives. (a) Green olives, overview, showing tissues fracturing through the cells. (b) Details of green olive parenchyma cells tightly packed (cell adhesion). (c) Cherry olives, overview, showing the beginning of cell separation. (d,e) Details of cherry olive parenchyma cells. (f) Black olives, overview, showing tissues mainly fracturing along the middle lamellae. (g) Details of the black olive parenchyma showing the dissolution of pectic polysaccharides (cell separation).

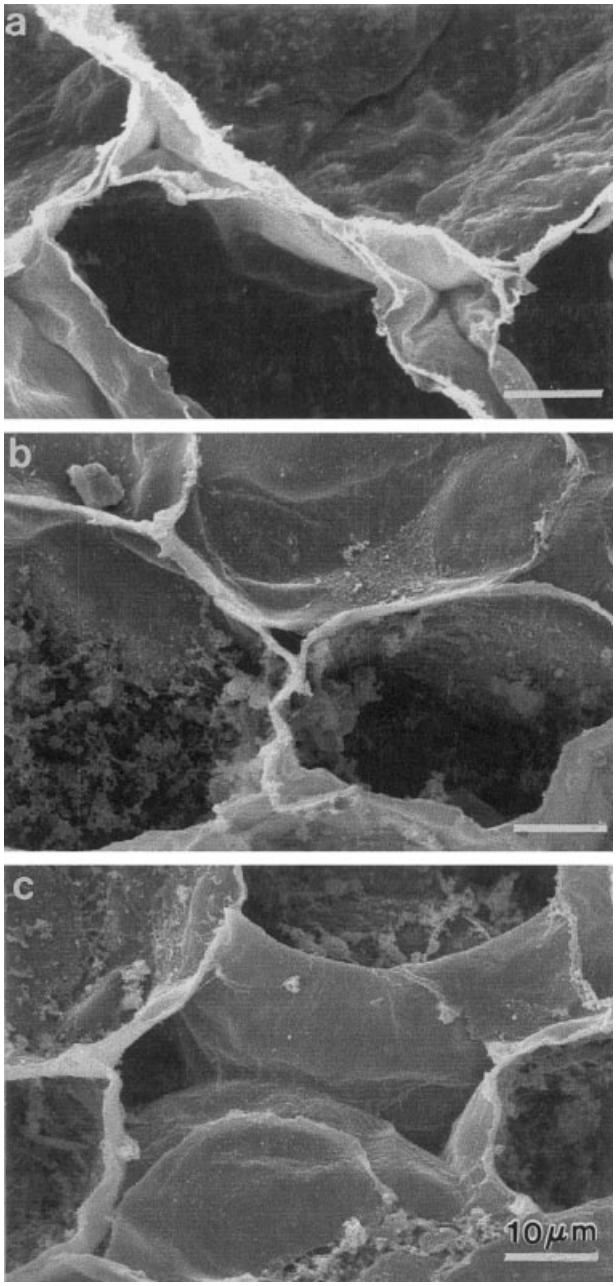

Fig. 3. Progressive disruption of the middle lamella parenchyma cells during ripening: (a) green olives; (b) cherry olives and (c) black olives.

(27 mol%), and lower amounts of Glc (29 mol%) and Xyl (12 mol%) were found. Ripening was associated with a decrease in the relative amount of HexA and Ara, and an increase in the relative amount of Glc (Table 2). The total sugars of cell wall polysaccharides present in CWM, on a dry pulp weight basis, decreased 7% from the green to the cherry stage and 51% from the cherry to the black stage (Fig. 4a). A decrease in Xyl and Glc was observed only from the cherry to the black stages, 54 and 50%, respectively. The slight increase in Xyl from green to cherry may be a result of the continued development of sclereids in the early stages of ripening, as occurs in pears (Martin-Cabrejas et al. 1994). The amount of HexA and Ara showed a continuous decrease from the green to the black stage. For both sugar residues, the decrease was more pronounced from cherry to black: 17 and 19%, respectively, from green to cherry, and 59 and 48%, respectively, from cherry to black.

Most of the polysaccharides removed from olive pulp during the preparation of CWM was collected in the SDS extracts. Treatment with a solution of PrAW allowed the removal of non-carbohydrate polymeric material. During CWM preparation, 2% of the polysaccharides of green olive pulp were solubilized, 8% in cherry, and 21% in black (Table 2). The sugar composition of SDS extracts revealed an increase in the relative amount of Rha with ripening and also a slight increase in Ara and Glc, with concomitant decrease in HexA and Xyl. The change in the sugar composition of these extracts may indicate that, with ripening, pectic polysaccharides and glucans were solubilized. In addition, ripe olives solubilized more Ara-rich pectic polysaccharides. These results were more apparent when the sugar composition was expressed in terms of dry weight pulp basis (Fig. 4b). The amount of Glc solubilized was not followed by an increase in Xyl, indicating that these glucans were not of the xyloglucan type.

The degree of methyl esterification (DM) of pectic polysaccharides present in CWM (Table 2) decreased progressively from 71% in green to 51% in cherry and 35% in black olives. However, the DM of the pectic polysaccharides in SDS extracts showed a slight increase, from 58% in green to 62% in cherry and 70% in black. Similar decreases have been reported for the DM in other fruits such as tomato (Koch and Nevins 1989), kiwifruit (Redgwell et al. 1990), nectarines (Dawson et al. 1992) and grape berries (Nunan et al. 1998). These results suggest the occurrence of de-esterification of CWM, possibly caused by an increase in the activity of pectinmethylesterase with ripening (Fernandez-Bolaños et al. 1995). The DM of SDS soluble polymers increased slightly with ripening, indicating solubilization of esterified pectic polysaccharides.

The protein present in CWM accounted for 5.4% in green, 8.0% in cherry and 6.3% in black. The values were of the same order as those previously reported for this olive variety (Coimbra et al. 1994).

Fractionation of CWM

The CWM polysaccharides were sequentially extracted with aqueous solutions of imidazole, Na_2CO_3 and KOH of increasing strength to leave a final cellulose-rich residue. The amount of polymeric material and polysaccharides present in the different extracts and their sugar composition are shown in Table 3 for green, cherry and black olives. The CWM from each olive pulp was extracted through 9 different solutions and each extract was analysed separately. The data were condensed and described as 5 major extracts: (1) imidazole + Na_2CO_3; (2) 0.5 *M* KOH; (3) 1 *M* KOH; (4) 4 *M* KOH; and (5) cellulosic residue.

Imidazole and Na_2CO_3 solutions extracted mainly pectic polysaccharides, as inferred by the high amount of HexA and Ara. Gal occurred in olive pulp cell wall polysaccha-

Table 2. Sugar composition of purified SDS extracts and cell wall material of olive pulp. Yield is expressed in g dry weight material kg^{-1} fresh weight olive pulp. The values in parentheses give the per cent of carbohydrate content of the fractions. DM, degree of methylesterification; tr, trace amount.

Fraction	Ripening stage	Yield ($g\ kg^{-1}$)	Cell wall sugars (mol%)								Total sugars ($mg\ g^{-1}$)	DM (%)
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA		
SDS												
	Green	2.1 (2.3)	2	tr	17	5	1	12	7	57	474	58.1
	Cherry	3.1 (8.2)	4	tr	23	3	tr	9	8	53	479	61.6
	Black	7.9 (21.0)	12	tr	26	1	tr	6	12	44	480	69.9
CWM												
	Green	34.7 (96.8)	2	–	23	16	2	3	34	21	515	71.3
	Cherry	30.9 (91.2)	4	–	20	18	2	2	36	19	539	50.5
	Black	22.6 (77.5)	3	tr	21	17	2	4	37	16	607	35.2

rides mostly as a minor component (Coimbra et al. 1994). The relative amount of polymeric material present in these extracts decreased 6% from green to cherry and 18% from cherry to black. The pectic polymers became richer in Ara while HexA content decreased. The ratio HexA/Ara was 4.5 in green, 2.4 in cherry and 0.5 in black. Similar results were obtained in kiwifruit CDTA and Na_2CO_3 extracts (Redgwell et al. 1990), where, with ripening, a relative increase in Ara, Gal and Rha, and a decrease in HexA was revealed. The sugar content of imidazole + carbonate extracts, as a CWM basis, confirmed the decrease in HexA and the increase in Ara (Fig. 5a). From the green to cherry stages, the major changes in the sugar composition were because of the loss of HexA; from the cherry to black stages the loss of HexA was followed by an increase in Ara. These results showed a variation in the content of branched pectic polysaccharides, suggesting a preferential loss of the backbone GalA residues with the ripening process. The decrease in the total amount of pectic polymers in these fractions, as well as the results obtained in the SDS extracts previously described, showed

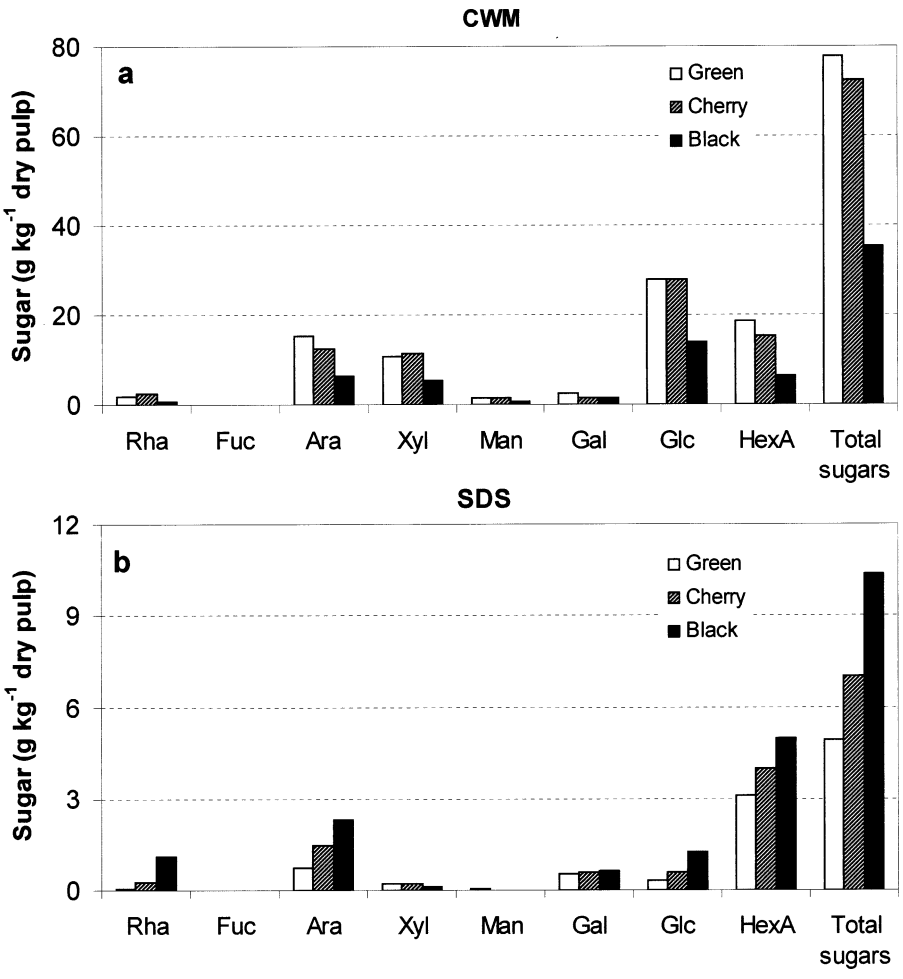

Fig. 4. Concentration of cell wall sugars in green, cherry and black olives: (a) CWM and (b) SDS extract.

Table 3. Sugar composition of fractions of CWM of olive pulp obtained by sequential extraction with aqueous solvents. tr, trace amount.

Fraction	Ripening stage	Yield (%)	Cell wall sugars (mol%)								Total sugars (mg g^{-1})
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
Imidazole + Na_2CO_3											
	Green	10.7	1	tr	17	1	tr	5	1	76	820
	Cherry	10.1	1	tr	27	1	1	3	1	65	634
	Black	8.3	1	tr	60	1	tr	6	2	29	775
0.5 M KOH											
	Green	1.9	1	tr	40	20	tr	4	6	29	660
	Cherry	1.5	tr	1	23	37	1	5	17	17	833
	Black	5.3	tr	tr	9	66	tr	3	8	14	786
1 M KOH											
	Green	6.4	1	tr	14	40	4	7	19	15	342
	Cherry	4.2	tr	1	16	29	5	9	30	12	495
	Black	7.8	1	tr	14	51	2	5	14	11	543
4 M KOH											
	Green	1.8	1	tr	16	16	17	11	30	11	566
	Cherry	5.9	tr	tr	13	21	18	11	29	11	455
	Black	8.1	1	tr	24	26	10	8	17	14	419
Cellulosic residue											
	Green	64.3	tr	–	28	24	1	2	31	13	515
	Cherry	55.6	tr	tr	18	21	1	2	43	14	660
	Black	43.1	1	tr	20	8	2	3	57	9	805

that the branched pectic polymers tend, with ripening, to be more soluble in aqueous solutions.

The neutralization of the KOH solutions gave a large amount of precipitated polymeric material that was removed from the supernatant solutions. The sugar composition of the soluble and precipitated material was condensed in 'KOH soluble' and 'KOH precipitate' fractions (Fig. 5b,c). The amount of polysaccharides present in KOH soluble fractions increased with ripening from 3.9% in green to 5.4% in cherry and 7.1% in black. This variation was because of the increase in the amount of Xyl, Ara and, to a lesser extent, HexA (Fig. 5b). The KOH precipitates of green and cherry olives had low sugar content (12 and 14%, respectively); however, black olives contained 38% of polymeric sugars. This variation in the sugar content of black olives was mainly because of the high increase in Xyl (Fig. 5c).

The amount of polysaccharides recovered in the 0.5 *M* KOH extract (calculated by multiplying the yield in polymeric material shown in Table 3 by the total sugars in the fraction as a g g^{-1} basis) was similar for green and cherry olives (1.3 and 1.4% of CWM weight) and was higher in black (4.2%). The sugar composition of green olives showed that this extract contained pectic polysaccharides, glucuronoxylans and xyloglucans. The presence of pectic polysaccharides was inferred by the occurrence of Ara and HexA. The presence of Xyl in relatively higher amounts than Glc was diagnostic of xylans. Olive pulp xylans occur as glucuronoxylans (Gil-Serrano et al. 1986, Coimbra et al. 1994), as shown by the proportion of HexA. The presence of xyloglucans was inferred by the occurrence of Glc, Gal and traces of Fuc, beyond Xyl (Gil-Serrano and Tejero-Mateo 1988, Coimbra et al. 1995). From the green to cherry stages, the relative amount of pectic polysaccharides decreased while xyloglucans and glucuronoxylans increased. The relative amount of glucuronoxylans showed a great increase from the cherry to black stages.

Polysaccharides recovered in the 1 *M* KOH extracts were similar for the green and cherry stages (2.2 and 2.1% of CWM weight) but were higher in the black (4.2%). Green olives contained glucuronoxylans, together with some xyloglucans and pectic polysaccharides. At the cherry stage the olives contained proportionately more xyloglucans and fewer glucuronoxylans than at the green stage but the proportion of glucuronoxylans increased from the cherry to black stages.

The amount of polysaccharides recovered in the 4 *M* KOH extracts was 1.0% of CWM weight in green, 2.7% in cherry and 3.4% in black. Green olives contain a heterogeneous mixture of polymers such as xyloglucans, glucuronoxylans, (gluco)mannans and, possibly, Ara-rich glycoproteins (Coimbra et al. 1994). Green and cherry olives had similar proportions in the different polysaccharides, but in black olives the proportions of xyloglucans and (gluco)mannans decreased as glucuronoxylans and Ara-rich polymers increased.

The amount of polysaccharides that remained in the cellulosic residue (CR) was 33% of CWM weight in green, 37% in cherry and 35% in black olives. The proportion of Glc in CR was lowest in green olives and highest in black ones. The non-cellulosic Glc accounted for 21% of total Glc in green CR, and 12% in cherry and black. From green to cherry, the most noticeable decrease in sugar was observed for Ara, possibly pectic polysaccharides rich in Ara. From cherry to black, the major decrease was observed for Xyl and HexA, which indicated predominant solubilization of glucuronoxylans. Despite the easy solubilization of non-cellulosic polysaccharides with ripening, the CR of black olives still contained Ara-rich polymers, possibly of pectic origin, and, as shown by Coimbra et al. (1994), some glycoproteins rich in Ara.

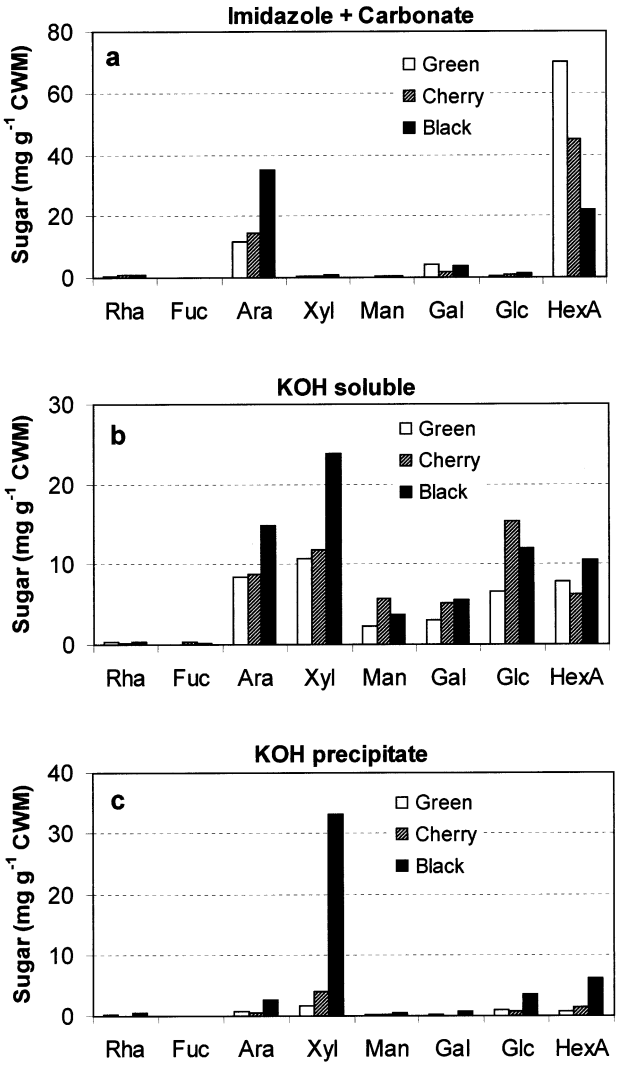

Fig. 5. Sugar composition (mg g^{-1} CWM) of CWM extracts for green, cherry and black olives. (a) Imidazole + carbonate extracts; (b) material soluble in KOH and water; (c) material soluble in KOH but insoluble in water.

Changes in cell wall polysaccharides with ripening

Cell wall polysaccharide composition estimates of the pulps of green, cherry and black olives were based on known figures for the different polymer components of olive pulp cell wall polysaccharides for this variety (Table 4), which were obtained by exhaustive fractionation of cell wall extracts and subsequent analysis by methylation analysis (Coimbra et al. 1994, 1995), ^{13}C NMR (Coimbra et al. 1996) and FT-IR/chemometrics (Coimbra et al. 1999). The estimate of the pectic polysaccharides on a g kg^{-1} dry pulp weight basis, was reached by the addition of HexA, Ara, Gal and Rha present in all the extracts, with correction for HexA because of the occurrence of glucuronoxylans in KOH extracts and CR, and of Gal in xyloglucan-rich KOH extracts. Glucuronoxylans were estimated on the basis of the amounts of Xyl and HexA, with correction for Xyl from xyloglucans and HexA from pectins. Xyloglucans were estimated calculating the sum of Glc in non-cellulosic extracts, the calculated amount of Xyl attributed to the xyloglucan, Fuc, and the contribution of Gal. Mannans were estimated according to the amount of Man. The Ara present in the 4 *M* KOH extracts belonged either to pectic polysaccharides or Ara-rich glycoproteins. According to Coimbra et al. (1994), the amount of Ara from glycoproteins accounts for 60% of total Ara. This proportion was assumed for the purpose of estimating the amount of Ara-rich glycoproteins and pectic polysaccharides in these extracts. The amount of cellulose was estimated according to the Glc that remained after the 1 *M* sulphuric acid hydrolysis, both for CR and CWM.

A loss (19%) of pectic polysaccharides was observed between the green and cherry stages because of the degradation of the galacturonan moiety (18%) as well as the arabinan side chains (26%). Glucuronoxylans were also reduced by 14%. However, no changes were observed in xyloglucan and cellulose. Higher solubilization was observed in all polymers between the cherry and black stages. Pectic polysaccharides, glucuronoxylans, cellulose and xyloglucans decreased 37, 58, 49 and 40%, respectively. In pectic polysaccharides the decrease was more pronounced in the galacturonan (49%) than the arabinan (29%) moiety.

Conclusions

Texture, SEM and cell wall analysis of olive pulp were carried out at 3 stages of ripening. From green to cherry and black stages the tissue became soft. In green olives, the observation by SEM of tissue fracture at the level of epicarp and mesocarp cells revealed cell wall breakage and a lack of separation between cells. This result contrasted with the observation of a certain proportion of cell separation in the middle lamella region in cherry olives and of a large proportion of cell separation in black olives. Cell wall analysis showed solubilization and loss of both pectic and hemicellulosic polymers within the cell wall matrix. When the colour changed from green to cherry, these changes affected the arabinan side chains of the pectic polysaccharides, the galacturonan backbone, and the glucuronoxylans. In the later ripening stage, when the colour changed from cherry to black, all olive pulp cell wall constituent polysaccharides became degraded and/or solubilized. The resultant depoly-

Table 4. Olive pulp cell wall polysaccharide composition at different stages of ripening (g kg^{-1} dry pulp). tr, trace amount.

	Green	Cherry	Black
Pectic polysaccharides	40	32	20
Galacturonan	(20)	(16)	(8)
Arabinan	(16)	(12)	(9)
Glucuronoxylan	15	12	5
Xyloglucan	8	8	5
Mannan	1	1	1
Ara-rich glycoprotein	tr	tr	tr
Cellulose	24	24	12
Total polysaccharides	87	77	43

merization of the pectic polymers, especially those of the middle lamella region, seemed to be related to the progressive cell separation visible at the different ripening stages by SEM. It seems possible that this partial solubilization of pectic, hemicellulosic and cellulosic polysaccharides within the cell wall matrix, which softened the cell wall structure, prevented the breaking of cells when the tissues were fractured.

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