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Carbohydrate Polymers 68 (2007) 647–657
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

The combined effects of black oxidising table olive process and ripening on the cell wall polysaccharides of olive pulp

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Received 10 February 2006; received in revised form 28 July 2006; accepted 31 July 2006
Available online 26 September 2006

Abstract

Olive fruits harvested at cherry and black stages of ripening were processed according the table olive black oxidising processing and sampled after the three main steps: storage in brine, lye treatment and thermal treatment (final product). The results show that the storage in brine contributed positively to the stabilisation of cell wall polysaccharides of olive pulp as the amounts of main polysaccharides practically were maintained in both stages of ripening. The lye treatment introduced degradation of cell walls due to the generalised loss of pectic and hemicellulosic polysaccharides and cellulose, caused by the breakage of ester and hydrogen bonds. On the other hand, the lye treatment introduced shifts in the solubilisation of polysaccharides rendering them more difficult to extract by alkali solutions and enabling their retention in the cellulosic residue, which should contribute positively to cell wall firmness. The thermal treatment introduced degradation of cellulose and increased the solubilisation of polysaccharides with a higher extent in the black olives. This work showed that the differences on the cell wall polysaccharides between stages of ripening are magnified after processing and allowed to conclude that the stage of ripening of olive fruits is determinant for obtaining a final product with adequate texture properties for table olive consumption.

Keywords: Olive; Black oxidising process; Ripening; Californian process; Table olives; Cell wall polysaccharides; FT-IR; Negrinha do Douro

1. Introduction

The black oxidising or Californian processing is one of the three main methods of preparing table olives (Garrido Fernández, Fernández Díez, & Adams, 1997). The other two methods include the green olives according to the Spanish or Sevillian style and the naturally black olives in brine. In the black oxidising processing, the olives, mainly at the green and cherry stages of ripening, are stored in brine with 5–10% NaCl from 2 to 6 months, depending on the needs of production. The brine may be acidified to pH 4 with lactic and acetic acids and kept in anaerobic/aerobic conditions to prevent fermentation. To improve texture, calcium chloride could be added during this period. Once the fresh or stored fruits are sorted and occasionally graded, they are treated with a series of dilute sodium hydroxide solutions and exposed to air between treatments. The lye treatments are normally adjusted since the first one penetrates the skin while the remaining alkali solutions are permitted to penetrate the pulp progressively until the last one reaches the stone. After the lye treatments and oxidation, the olives are washed several times with water to remove most of the residual lye, reaching a final pH around 7, and are placed in 3–5% brine with ferrous gluconate or ferrous lactate to fix the black colour. The olives are then packed and sterilised (Garrido Fernández et al., 1997).

The olives harvested at different stages of ripening, i.e., green, cherry and, for certain varieties, black, are generally used in the black oxidising type of table olive preparation. Particularly, the olives of Negrinha do Douro variety can

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doi:10.1016/j.carbpol.2006.07.030

be processed from matured fruits at the green, cherry and black stages. Ripening-related changes of cell wall polysaccharides are characterised mainly by the increase in the solubilisation of pectic and hemicellulosic polysaccharides, the increase in the relative amount of arabinose in pectic polysaccharides, and the decrease in the degree of methylesterification of pectic polysaccharides (Mafra, 2002; Mafra, Barros, & Coimbra, 2006a; Mafra et al., 2001). Additionally to degrading processes, the synthesis of new polysaccharides was suggested to occur in Hojiblanca (Jiménez et al., 2001a, 2001b) and Negrinha do Douro varieties (Mafra et al., 2006b).

Furthermore, several studies concerning the effect of ripening on the cell wall polysaccharides of olives have reported that processing affects mostly the pectic polysaccharides, increasing their solubilisation (Coimbra, Waldron, Delgadillo, & Selvendran, 1996b; Jiménez Araujo, Labavitch, & Moreno, 1994; Sánchez-Romero, Guillén, Heredia, Jiménez, & Fernández-Bolãnos, 1998a) and leading to a decrease in tissue firmness (Jiménez, Guillén, Sánchez, Fernández-Bolãnos, & Heredia, 1995). Most studies on olive processing report to changes on olive cell wall polysaccharides related to the Sevillian style process (Coimbra et al., 1996b; Jiménez et al., 1995; Jiménez, Heredia, Guillén, & Fernández-Bolãnos, 1997; Jiménez, Sánchez-Romero, Guillén, Fernández-Bolãnos, & Heredia, 1998; Marsilio, Lanza, & De Angelis, 1996; Sánchez-Romero et al., 1998a, Sánchez-Romero, Guillén, Heredia, Jiménez, & Fernández-Bolãnos, 1998b), which includes a first step of lye treatment followed by a lactic fermentation in brine. Few studies concerning the effect of Californian processing on the cell walls were published. One preliminary study describes the changes as a result of Californian process of Manzanilla olives (Jiménez Araujo et al., 1994) and, more recently, a more detailed study describes the changes as a result of processing of Negrinha do Douro variety (Mafra et al., 2006a). The later work reported that the storage in brine promotes an increase in the amount of cell wall polysaccharides, suggesting their biosynthesis *de novo*; the lye treatment causes their degradation and also retention within the cell walls; and the thermal treatment of the olives increases the solubilisation of pectic polysaccharides and causes the loss of hemicellulosic polysaccharides and cellulose. However, this study describes only the changes related to the Californian processing of fruits harvested at the mature green stage of ripening. Thus, knowing the effect of ripening on the cell wall polysaccharides of unprocessed fruits and the effect of the main steps of Californian processing on the cell wall polysaccharides of green olives, it is now important to understand the changes that may occur during the processing of olives in more advanced stages of ripening.

The present work describes the changes in the cell wall polysaccharides of cherry and black olives of Negrinha do Douro variety after the three main steps of table olive black oxidising process and compares the results to the previous work describing the changes of processing of green olives (Mafra et al., 2006a).

2. Material and methods

2.1. Plant material

Olive fruits (*Olea europaea* L. var. Negrinha do Douro) with an average length of 2.0 cm and a diameter of 1.2 cm were provided by Maçarico Lda, Praia de Mira, Portugal. The olives at cherry (3.2 g pulp/fruit) and black (3.9 g pulp/fruit) stages of ripening were processed separately according to the Californian style, which consisted on three main steps:

Step 1, brine treatment: The olive fruits were left in brine (6% (w/v) NaCl, 0.10% (w/v) $CaCl_2$, 0.20% (w/v) lactic acid, 0.40% (w/v) acetic acid, pH 4) during 5 months.

Step 2, lye treatment: A first soak in lye during 4 h (2.25% (w/v) NaOH), which enables the hydrolysis of oleuropein and other bitter phenolic compounds and the darkening of olives due to the oxidation of polyphenols (Garrido Fernández et al., 1997), followed by washing with water, a second lye treatment reusing the alkali solution for 2 h, and a final wash until neutralisation (pH 7), with the addition of sodium benzoate for inhibition of yeasts and moulds.

Step 3, thermal treatment: Further to this treatment, the olives were stored in brine (pH 6) with 0.1% (w/w) ferrous lactate for colour fixation, and were thermally treated at 118 °C for 30 min.

Three sets of samples were collected after: brine (step 1), lye treatment (step 2) and thermal treatment (step 3), i.e., final product.

2.2. Preparation of cell wall material (CWM)

The CWM was prepared according to the method described by Coimbra, Delgadillo, Waldron, and Selvendran (1996a) with some changes to permit the use of the largest possible number of samples and to avoid the use of phenol reagent, as described by Mafra et al. (2001). The olive pulp was homogenised and triturated in a 1.5% sodium dodecylsulphate solution (SDS) and the resultant material was filtered and washed with 0.5% SDS solution containing 3 mmol L^{-1} sodium metabisulphite. The residue was washed with water, extracted with a solution of 1-propanol/acetic acid/water (PrAW 2:1:1 v/v/v), washed again with water and freeze-dried to give the CWM. All the extracts from CWM preparation were analysed separately, being the data condensed and named "SDS extracts".

2.3. Sequential extraction of CWM

The CWM was extracted according to the method described by Mafra et al. (2001). The CWM (10 g) was sequentially extracted with: (1) 0.5 M imidazole/HCl (pH 7.0), for 16 h at 20 °C; (2) 0.5 M imidazole/HCl (pH 7.0), for 2 h at 20 °C; (3) 50 mM Na_2CO_3 + 20 mM $NaBH_4$, for 16 h at 4 °C; (4) 50 mM Na_2CO_3 + 20 mM $NaBH_4$, for 2 h at 20 °C; (5) 0.5 M KOH + 20 mM $NaBH_4$, for 2 h at 4 °C;

(6) 1 M KOH + 20 mM $NaBH_4$, for 2 h at 4 °C; (7) 1 M KOH + 20 mM $NaBH_4$, for 2 h at 20 °C; (8) 4 M KOH + 20 mM $NaBH_4$, for 2 h at 20 °C and (9) 4 M KOH + 3.5% H_3BO_3 + 20 mM $NaBH_4$, for 2 h at 20 °C. The residue (cellulosic residue, CR) obtained after the alkali extractions was suspended in water, acidified (pH 5–6) and dialysed.

2.4. Carbohydrate analysis

Neutral sugars were released by Saeman hydrolysis (Selvendran, March, & Ring, 1979) and analysed as their alditol acetates by GLC (Blakeney, Harris, Henry, & Stone, 1983; Harris, Blakeney, Henry, & Stone, 1988) using a Carlo Erba 6000 chromatograph (Carlo Erba, Milan, Italy) with a split injector (split ratio 1:60) and a FID detector. A 30 m column DB-225 (J&W, USA) 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 program used was: 220 °C for 4 min, followed by 230 °C for 6.5 min with a rate of 25 °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 (HexA) were determined colorimetrically according to a modification of the method of Blumenkrantz and Asboe-Hansen (1973).

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

Cell wall polysaccharide composition estimates of the olive pulp were based on known figures for the different polysaccharides constituents of olive pulp cell walls for this variety, obtained by methylation analysis (Coimbra, Waldron, & Selvendran, 1994), ^{13}C NMR (Coimbra et al., 1996a), and FT-IR (Coimbra, Barros, Rutledge, & Delgadillo, 1999). All the extracts resultant from CWM preparation and sequential extraction were used for this calculation. The estimate of the pectic polysaccharides was calculated by the sum of HexA, Ara (arabinose), Gal (galactose) and Rha (rhamnose) present in all the extracts, with the correction for HexA because of the occurrence of glucuronoxylans in KOH extracts and CR, and of the Gal in xyloglucan-rich KOH extracts. Glucuronoxylans were estimated based on Xyl (xylose) and HexA amounts, with the correction for Xyl from xyloglucans and HexA from pectic polysaccharides. Xyloglucans were obtained by the sum of Glc (glucose) in non-cellulosic extracts (including Glc from CR after 1 M H_2SO_4 hydrolysis), the calculated amount of Xyl attributed to the xyloglucans, Fuc (fucose), and the contribution of Gal. Mannans were estimated according to the amount of Man (mannose). 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 in these extracts accounts for 60% of the total Ara. This proportion was assumed for the purpose of estimating the amount of Ara-rich glycoproteins and pectic polysaccharides. The amount of cellulose was estimated according to the Glc that remained after 1 M H_2SO_4 hydrolysis in CR.

2.5. Determination of the degree of methylesterification

The determination of the degree of methylesterification of pectic polysaccharides was based on the estimate of methanol content released by saponification (Waldron & Selvendran, 1990) as described by Barros et al. (2002).

2.6. FT-IR spectroscopy

FT-IR spectra of pectic polysaccharide extracts were acquired with a Golden-Gate single reflection ATR in a Bruker IFS-55 instrument (Bruker, Karlsruhe, Germany) at a resolution of 8 cm^{-1} and 128 co-added scans. Spectra for each sample were recorded, at least, in triplicate, in the absorbance mode from 4000 to 400 cm^{-1}. The spectra were transferred in the JCAMP-DX format (Rutledge & McIntyre, 1992) and analysed with a program developed in the Institut National Agronomique Paris-Grignon in collaboration with the University of Aveiro (Barros, 1999, ch. 1). The FT-IR spectral region used for both Principal Component Analysis (PCA) (Jolliffe, 1986) and Trimmed Object Projections (TOP) (Hove, Liang, & Kvalheim, 1995) was set to 1200–850 cm^{-1}. Previously to multivariate analysis the spectra were SNV (standard normal deviates) corrected, i.e., each spectrum was mean centred and divided by the standard deviation.

3. Results and discussion

The results described in the present work concern the study of changes in the cell wall polysaccharides of olive pulp along table olive black oxidising processing of fruits at cherry and black stages of ripening. The study focuses the changes related to the main steps of processing: after brine (step 1), after lye (step 2) and final product after thermal treatment (step 3). The study concerning the effect of black oxidising processing on the cell wall polysaccharides of green olives was described by Mafra et al. (2006a). The results of raw olives at the cherry and black stages here presented for comparative purposes were described by Mafra et al. (2006b).

3.1. Cell wall material composition

The yield of CWM of cherry olives changed from 4.1% in raw olives, to 3.9% after brine (step 1), 3.6% after lye (step 2) and 4.3% in final product after thermal treatment (step 3) (Table 1). In black olives, the yield changed from 3.3% in raw olives, to 3.6% after step 1, 3.1% after step 2 and 3.6% after step 3 (Table 2). Comparing these values with those obtained for green olives (4.2%, 4.8%, 4.4% and 4.3%) (Mafra et al., 2006a), it can be noted a

decrease of polymeric material after step 2 and an increase of polymeric material after step 3, common in the three stages of ripening. The breakage of linkages, which should have increased the solubilisation of polymeric material during alkali treatment, might have been responsible for the decrease in polymeric material, while the equilibrium in brine of final product might have contributed to the stabilisation of cell walls (Ng & Waldron, 1997; Van Buren, 1979). The yield on a dry pulp weight basis shows an increase in polymeric material along processing of both cherry (15% for raw and step 1, 18% and 21%, respectively, for steps 2 and 3) and black (12, 13, 15 and 16%, respectively, for raw olives and for steps 1, 2 and 3) olives, as was observed in green olives. That fact indicates the consistent release of non-polymeric material along processing of olives in the three stages of ripening.

The sugar composition of CWM of cherry (Table 1) and black olives (Table 2) shows, in general, an increase in the relative proportion of Ara with the processing, as previously observed for green olives (Mafra et al., 2006a). Glucose (Glc) proportion decreased along processing of black olives, mainly in the final product. The composition of SDS extracts, collected during the preparation of the CWM, indicates that they contain mainly pectic polysaccharides rich in Ara. The increase in Ara proportion together with the decrease in HexA along processing of cherry (Table 1) and black olives (Table 2), suggests the loss of GalA from pectic polysaccharides, which is consistent with the results obtained for green olives.

The variations of sugars in CWM on a dry pulp weight basis show an increase in the concentration of the main sugars along the processing of both cherry (Fig. 1(a)) and black olives (Fig. 1(c)). While in green olives that increase was observed only after brine storage (Mafra et al., 2006a), in cherry and black olives that occurred gradually along processing.

The total sugars solubilised during CWM preparation showed similar variations for cherry (Fig. 1(b)) and black olives (Fig. 1(d)), where the most consistent finding was the decrease after lye treatment, also noted in green olives (Mafra et al., 2006a). The brine storage also decreased the solubilisation of polysaccharides in green and cherry olives, although to a smaller extent than lye treatment. In black olives it can be observed an increase in solubilisation of HexA, Ara and Gal, probably due to

Table 1
Sugar composition of purified SDS extracts and cell wall material of olive pulp at cherry stage of ripening

Fraction	Processing stage	Yield[a] (g kg^{-1})	Cell wall sugars (mol%)								Total sugars (mg g^{-1})
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
SDS extracts											
	Raw[b]	10.9	5	–	30	5	2	10	15	33	254
	Brine	6.5	1	–	39	2	–	2	6	50	303
	NaOH	4.3	5	–	48	3	–	12	11	20	170
	Final	5.1	4	–	53	5	tr	11	7	19	229
CWM											
	Raw[b]	41.3	tr	–	25	16	3	3	34	19	376
	Brine	39.3	tr	–	27	17	2	2	31	21	446
	NaOH	35.5	1	–	31	16	2	2	31	18	487
	Final	42.6	tr	–	28	16	2	1	32	21	440

tr, Trace amount.
[a] Yield is expressed in g of dry weight material per kg of fresh weight olive pulp.
[b] Data from Mafra et al. (2006b).

Table 2
Sugar composition of purified SDS extracts and cell wall material of olive pulp at black stage of ripening

Fraction	Processing stage	Yield[a] (g kg^{-1})	Cell wall sugars (mol%)								Total sugars (mg g^{-1})
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
SDS extracts											
	Raw[b]	11.4	6	–	22	3	1	8	23	37	230
	Brine	8.2	4	–	34	4	tr	10	12	36	364
	NaOH	4.8	3	–	41	4	–	15	15	22	118
	Final	3.9	3	tr	57	4	tr	12	6	17	320
CWM											
	Raw[b]	33.0	1	–	25	14	3	4	35	18	435
	Brine	36.1	1	tr	29	16	2	2	33	17	461
	NaOH	30.8	tr	tr	30	16	2	2	31	18	503
	Final	36.5	tr	tr	33	13	2	2	27	24	538

tr, Trace amount.
[a] Yield is expressed in g of dry weight material per kg of fresh weight olive pulp.
[b] Data from Mafra et al. (2006b).

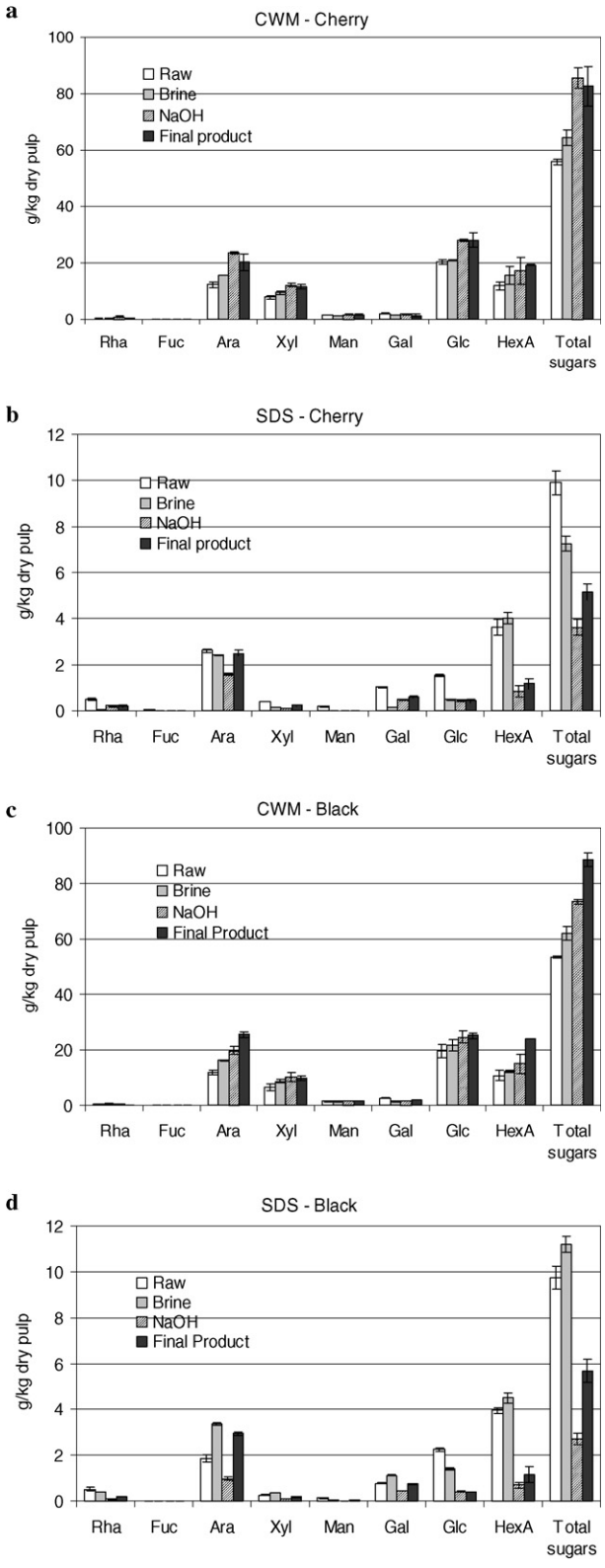

Fig. 1. Cell wall sugars of cherry and black olives along processing expressed as g per kg dry pulp: (a) CWM of cherry olives; (b) SDS extracts of cherry olives; (c) CWM of black olives; (d) SDS extracts of black olives.

the more advanced stage of ripening, which is known to cause a general increase in solubilisation of pectic polysaccharides (Mafra, 2002; Mafra et al., 2006b). Between steps 2 and 3, the solubilisation of pectic polysaccharides increased in cherry and black olives, although to a smaller extent than the obtained for green olives.

The overall presentation of CWM composition per fruit (Fig. 2(a) and (b)) emphasises the changes due to the main steps of processing, as the presentation of the results on a dry pulp basis does not account for the increased concentration of polymers attributed to the release of intracellular compounds. In the cherry olives, the variations were very small (Fig. 2(a)), with a small increase in cell wall polysaccharides after brine (11%), mainly pectic polysaccharides due to the increases in Ara and HexA. In the black olives, there was also a small increase in sugars after brine (14%) also due to the pectic polysaccharides (Fig. 2(b)); however, after alkali treatment the values were reduced (20%) due to the loss of pectic polysaccharides and Glc. In the final product of black olives the total sugars increased 15%.

The comparison of data for the three stages of ripening indicates a consistent increase of pectic polysaccharides as a result of brine storage. That increase was much higher for green olives (42%) (Mafra et al., 2006a), where the higher biosynthetic and/or lower hydrolytic activities might have been the responsible factor(s). After alkali treatment, the cherry olives did not show a significant variation, in opposition to green and black olives. The oxidation with NaOH is applied in cycles whose duration depends on the desired penetration of the reagent in each cycle (Garrido Fernández et al., 1997). The extent of pen-

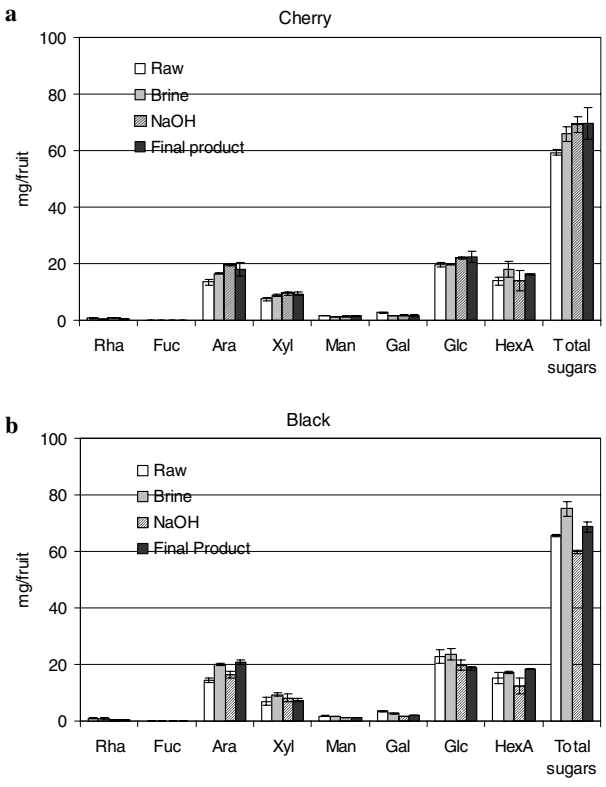

Fig. 2. Cell wall sugars of CWM and SDS extracts of cherry (a) and black olives (b) along processing expressed as mg per fruit.

etration depends on the size and stage of ripening of fruits, as smaller and greener fruits offer higher resistance to penetration. In the present work, the alkali treatment was applied in two cycles, where the first should reach 3/4 of pulp penetration (about 4 h) and the second one should penetrate completely until the stone (about 2 h). This fact increased the duration of each alkali treatment in green olives and, as a consequence, it might have introduced higher cell wall degradation than in the cherry and black stages. Because black olives were the riper, they might have been more susceptible to alkali treatment than cherry olives, although the alkali treatment was milder.

3.2. FT-IR analysis of CWM of olive pulp in different steps of processing

The comparative analysis of CWM of olives in the three stages of ripening and along the steps of processing was performed by means of PCA and TOP based on the FT-IR spectra. Fig. 3 presents the analysis by TOP, as this was more informative than PCA. The application of TOP allowed the

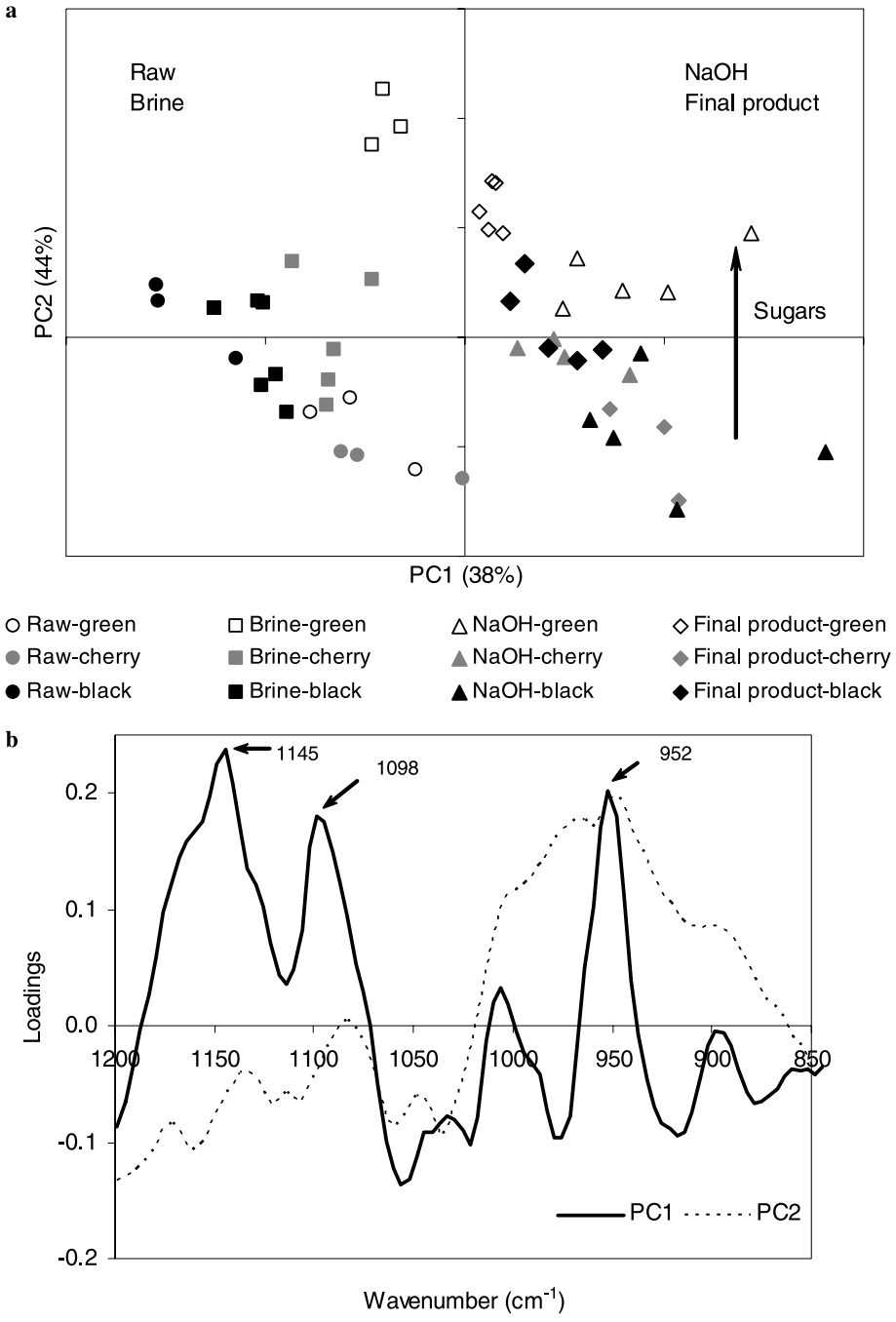

Fig. 3. TOP of the FT-IR spectra of the CWM of olives along processing and in the three stages of ripening: (a) scores scatter plot (PC1 vs. PC2) (axes cross each other at origin); (b) loadings plot.

distinction along PC1 axis of all the raw samples and after brine from those after lye treatment and final product, i.e., before and after lye treatment (Fig. 3(a)). The loadings plot shows that the positive side of PC1 axis is related to the absorbances at the wavenumbers of 1145, 1098 and 952 cm^{-1} (Fig. 3(b)), ascribed to the pectic polysaccharides (Coimbra et al., 1999). This finding highlights the distinction between two types of olives by the structural differences in pectic polysaccharides. The samples before the lye treatment with esterified polysaccharides and the samples after lye with de-esterified polysaccharides, characterised by the band located at 952 cm^{-1}, as suggested by Mafra et al. (2006a). The PC2 axis allows the separation according to the sugar concentration, as the samples with lower sugars (raw-cherry, 38%) are on the negative side, whereas the more concentrated ones (brine-green, 64%) are on the positive side of the axis.

3.3. Fractionation of CWM

The CWM polysaccharides of cherry and black olives 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 the sugar composition are shown in Tables 3 and 4, respectively, for cherry and black olives after the three steps of processing. Each one of the nine extracts was analysed separately, but the data were condensed in five major extracts: (1) imidazole + Na_2CO_3; (2) 0.5 M KOH; (3) 1 M KOH; (4) 4 M KOH; and (5) cellulosic residue. For comparative purposes the results of raw olives at cherry and black stages, published by Mafra et al. (2006b), were included in Tables 3 and 4, respectively.

The yield of polymeric material obtained in imidazole and Na_2CO_3 extracts of CWM of cherry olives changed from 8.4% in raw olives to 6.6% after brine, 3.5% after lye and 8.1% in the final product (Table 3). In the black olives the same yield ranged from 11.1% in raw to 8.1% after brine, 3.3% after lye and 3.9% in the final product (Table 4). These variations indicated a small decrease in solubilisation of polymeric material with imidazole and carbonate solutions in olives after brine and at both stages of ripening, whereas after lye the amount of material solubilised decreased drastically in cherry (47%) and black olives (60%). As observed for green olives, the lower recovery of polymeric material with imidazole and Na_2CO_3 solutions obtained after lye is consistent with the low solubilisation also observed in the SDS extracts. In the final product the solubilisation of cell wall polysaccharides with imidazole and carbonate solutions increased more than twice in cherry and 18% in black olives.

The sugar composition of imidazole and Na_2CO_3 extracts indicates the presence of pectic polysaccharides rich in Ara (Tables 3 and 4). The calculation of the molar

Table 3
Sugar composition of fractions of cell wall material of olive pulp at cherry stage of ripening obtained by sequential extraction with aqueous solvents

Fraction	Processing stage	Yield[a] (%)	Cell wall sugars (mol%)								Total sugars (mg g^{-1})
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
Imidazole + Na_2CO_3											
	Raw[b]	8.4	2	tr	40	2	tr	5	2	49	732
	Brine	6.6	tr	–	32	tr	tr	2	1	65	749
	NaOH	3.5	1	–	40	1	–	1	3	53	602
	Final	8.1	1	–	51	1	–	2	1	44	875
0.5 M KOH											
	Raw[b]	1.9	tr	–	20	37	2	7	23	11	656
	Brine	1.9	tr	tr	9	54	2	3	17	14	621
	NaOH	1.1	tr	–	6	39	3	4	40	8	687
	Final	2.8	tr	tr	15	41	3	6	23	12	930
1 M KOH											
	Raw[b]	4.6	tr	1	19	31	6	8	25	10	498
	Brine	5.2	tr	tr	13	41	7	7	24	7	461
	NaOH	5.0	tr	tr	10	44	7	5	25	8	726
	Final	4.2	tr	tr	20	32	9	6	19	15	645
4 M KOH											
	Raw[b]	7.3	1	tr	14	26	16	9	20	14	291
	Brine	15.7	tr	tr	49	15	3	4	6	23	377
	NaOH	10.5	1	tr	39	9	5	3	18	25	497
	Final	7.6	tr	tr	38	12	6	5	9	29	506
CR											
	Raw[b]	59.5	tr	–	24	16	1	3	43	12	545
	Brine	53.6	tr	tr	25	19	1	1	40	13	613
	NaOH	67.6	tr	tr	35	14	1	tr	35	14	505
	Final	41.8	tr	–	25	18	1	tr	42	13	506

tr, Trace amount; CR, cellulosic residue.
[a] Yield is expressed in percentage of CWM.
[b] Data from Mafra et al. (2006b).

Table 4
Sugar composition of fractions of cell wall material of olive pulp at black stage of ripening obtained by sequential extraction with aqueous solvents

Fraction	Processing stage	Yield[a] (%)	Cell wall sugars (mol%)								Total sugars (mg g^{-1})
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
Imidazole + Na_2CO_3											
	Raw[b]	11.1	1	tr	40	1	1	5	2	49	675
	Brine	8.1	1	–	45	tr	tr	2	2	51	733
	NaOH	3.3	1	–	37	3	tr	1	2	56	589
	Final	3.9	1	–	54	1	–	2	1	41	816
0.5 M KOH											
	Raw[b]	1.9	tr	tr	15	42	1	7	25	10	790
	Brine	2.6	1	tr	13	44	2	5	28	7	833
	NaOH	1.9	tr	tr	10	48	3	6	27	6	821
	Final	1.9	tr	tr	21	40	1	5	18	15	950
1 M KOH											
	Raw[b]	6.2	tr	tr	18	34	5	8	26	8	567
	Brine	5.9	tr	tr	13	37	8	7	27	9	566
	NaOH	3.9	1	tr	7	40	11	8	29	5	648
	Final	3.2	tr	tr	18	49	1	5	18	9	253
4 M KOH											
	Raw[b]	10.5	tr	tr	17	15	20	11	27	10	360
	Brine	9.7	1	tr	45	15	8	6	10	17	417
	NaOH	5.4	tr	tr	27	17	14	7	21	13	391
	Final	9.5	tr	tr	26	32	7	5	12	18	127
CR											
	Raw[b]	57.6	tr	–	26	15	1	4	44	9	658
	Brine	56.3	tr	–	29	16	1	1	39	14	524
	NaOH	67.7	tr	tr	33	9	1	1	34	21	518
	Final	38.8	tr	tr	30	13	1	1	36	18	610

tr, Trace amount; CR, cellulosic residue.
[a] Yield is expressed in percentage of CWM.
[b] Data from Mafra et al. (2006b).

ratio HexA/Ara for cherry (1.2, 2.0, 1.3 and 0.83 for raw, and steps 1, 2 and 3, respectively) and black olives (1.2, 1.1, 1.5 and 0.76) highlights the great decrease of HexA in the final product, also patent in the green olives (2.2, 2.3, 2.0 and 1.3) (Mafra et al., 2006a). The variation of the degree of methylesterification (DE) of imidazole extracts with the brine storage and ripening is presented in Fig. 4. In the first extracts with imidazole solutions (IMID-1) the DE decreased after brine 20%, 40% and 30%, respectively, for green, cherry and black olives. These results point out to a decrease trend of DE, which might be related to the activity of pectinmethyl esterase during brine. The lower decrease in DE of green olives might be attributed to the lower activity of pectinmethyl esterase at this stage of ripening, which agrees with the increasing activity of this enzyme with the ripening stage (Mafra et al., 2006b).

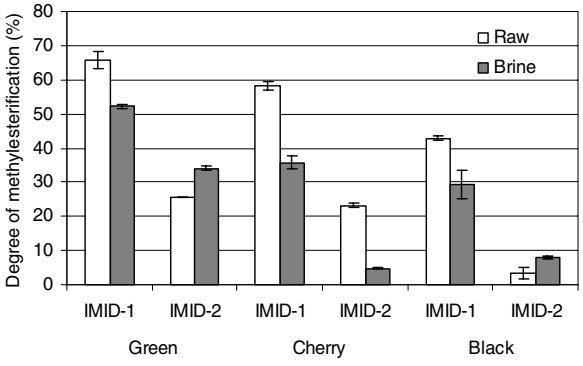

Fig. 4. Variation of the degree of methylesterification of imidazole extracts with the storage in brine and with ripening.

The yield of cell wall sugars of cherry olives obtained with 0.5 M KOH solutions decreased after lye treatment, increasing about three times in the final product ($1.9\% \times 0.656\,g\,g^{-1} = 1.2\%$ for raw and after brine, 0.76% after lye and 2.6% in the final product). The sugar composition shows that after brine there was a decrease in the solubilisation of polysaccharides rich in Ara and Glc residues (Table 3). The lye treatment decreased the proportions of Xyl, HexA and Ara, but in the final product all these sugars increased in these extracts.

The 0.5 M KOH extracts of black olives showed an increase in sugars (yield) after brine and a decrease after lye (1.5%, 2.2%, 1.6% and 1.8%, respectively, for raw, after brine, after lye and final product) (Table 4). The small increases in Xyl and Glc were responsible for the increase in sugars after brine, whereas the decrease in all main sugars contributed to the decrease after lye. Comparing the 0.5 M KOH extracts in the three stages of ripening, it can be pointed out that after the lye treatment the extraction of polysaccharides, mainly of pectic origin (smaller amounts of HexA and Ara), was reduced.

The 1 M KOH extractions of cherry olives showed a higher yield of polysaccharides after lye (2.3%, 2.4%, 3.6% and 2.7%, respectively, for raw, and after steps 1, 2 and 3) due to the solubilisation of xylans and xyloglucans, inferred by the amounts of Xyl and Glc (Table 3). This finding occurred also in the 1 M KOH extracts of green olives (Mafra et al., 2006a), indicating a shift of solubilisation of polysaccharides to higher concentrations of KOH. In the black olives, the 1 M KOH extractions show a decrease in polysaccharides from brine (3.3%) to lye (2.5%) and, especially, after thermal treatment (0.81%) (Table 4). This fact points out to some degradation of polysaccharides more evidenced in the riper olives.

The 4 M KOH extractions of cherry olives show a great increase in polysaccharides between raw (2.1%) and brine storage (5.9%) attributed to the increase in pectic polysaccharides, inferred by the high of amounts HexA and Ara, and possibly also glycoproteins rich in Ara (Coimbra et al., 1994) (Table 3). This fact was also evident in the green olives (Mafra et al., 2006a), suggesting a shift in the solubilisation of pectic polysaccharides to more concentrated KOH solutions as a result of brine storage. In the black olives, that finding was not as clear, as the variation of polysaccharides between raw (3.8%) and brine storage (4.0%) was negligible. However, the glycosidic composition shows an increase in pectic polysaccharides and possibly glycoproteins rich in Ara, inferred by the increased proportions of Ara and HexA (Table 4).

The variation of polymeric material obtained in the cellulosic residue along the processing of green (Mafra et al., 2006a), cherry (Table 3) and black olives (Table 4) showed a similar trend. The retention of polymeric material with the lye treatment was evident and common to all stages of ripening. The thermal treatment introduced an increase in solubilisation and/or degradation of polymeric material, which was progressively higher along ripening. The sugar composition of cellulosic residues of cherry olives indicates a high retention of pectic polysaccharides, inferred by the amounts of HexA and Ara, and hemicellulosic polysaccharides, namely, glucuronoxylans, inferred by the amounts of Xyl and occurrence of HexA (Table 3) not accompanied by non-cellulosic Glc (data not shown). The proportions of those sugars were equivalent for cherry and black olives (Table 4), which indicates similar retention of pectic and hemicellulosic polysaccharides. However, in green olives, the amounts of Ara, HexA and Xyl were higher, indicating that the retention of pectic polysaccharides and glucuronoxylans in the cellulosic residue was higher for the greener olives (Mafra et al., 2006a).

3.4. Changes in the cell wall polysaccharides

The estimates of cell wall polysaccharides were done using all the data from the extracts resultant from CWM preparation and sequential extraction, as described in Section 2.

The results of polysaccharide composition on a fruit basis for cherry and black olives after brine, lye and thermal treatments are presented in Table 5. For comparative purposes, the polysaccharide composition of raw samples at both stages of ripening, published by Mafra et al. (2006b), were also included. In the cherry olives it can be noted that after brine there was no change in the amount of pectic polysaccharides per fruit, whereas glucuronoxylans, galacturonans and Ara-rich glycoproteins increased, and xyloglucans and cellulose decreased. After lye treatment of cherry olives, there was a generalised loss of all cell wall polysaccharides, with the exception of xyloglucans and arabinans. The black olives show a slight increase of pectic polysaccharides after brine. The lye treatment of black olives introduced a decrease of 30% in the pectic polysaccharides, 41% in the glucuronoxylans and 43% in the xyloglucans. The amount of cellulose decreased progressively along the processing of both cherry and black olives.

The comparison of these results with those obtained for green olives (Mafra et al., 2006a) indicates that the brine storage promotes a generalised increase of polysaccharides only in green olives. The non detected increase in cherry and black olives might be the result of higher activities of cell wall degrading enzymes at these advanced stages of

Table 5
Olive pulp cell wall polysaccharide composition of cherry and black olives at different stages of processing[a]

	Cherry				Black			
	Raw[b]	Brine	NaOH	Final	Raw[b]	Brine	NaOH	Final
Pectic polysaccharides	33	33	31	30	37	38	27	23
Galacturonan	(13)	(15)	(11)	(12)	(14)	(16)	(11)	(8)
Arabinan	(16)	(16)	(18)	(16)	(18)	(19)	(14)	(13)
Glucuronoxylan	9	12	9	9	11	10	6	6
Xyloglucan	6	4	6	5	9	7	4	5
Mannan	1	1	1	1	2	1	1	1
Ara-rich glycoprotein	tr	2	1	1	tr	1	tr	tr
Cellulose	20	18	17	14	22	17	14	10
Total polysaccharides	69	70	65	60	80	74	52	46

tr, Trace amount. Values in parenthesis are part of pectic polysaccharides.
[a] Values expressed as mg per fruit.
[b] Data from Mafra et al. (2006b).

ripening. The high increase of polysaccharides in green olives after brine might also be attributed to higher biosynthetic than hydrolytic activities.

The lye treatment promoted a higher decrease in pectic polysaccharides of green olives than cherry, reaching similar amounts of polysaccharides. That might be explained by the need of longer lye treatment of green olives, once they offer a higher resistance to the penetration of alkali (Garrido Fernández et al., 1997). The black olives showed the greatest loss of polysaccharides after lye treatment, probably due to the more advanced stage of ripening, as the oxidation in alkali should be shorter at this stage.

In the final product, the increase in pectic polysaccharides and glucuronoxylans of green olives should be attributed to the stabilisation of cell walls promoted by the new equilibrium in brine. This effect tended to be less obvious with the increasing stage of ripening. The decrease of cellulose in the final product was evident in the three stages of ripening, with an increasing extent with ripening stage, probably due to a higher degradation during the thermal treatment of riper olives.

The comparison of cell wall polysaccharide contents of olives in the three stages of ripening and after the main steps of processing by means of PCA is presented in Fig. 5. A clear distinction between the raw samples and their respective final products can be observed along PC1 axis (Fig. 5(a)) due to the loss of galacturonans in the processed samples, which introduces an enrichment in arabinans, and also to the loss of cellulose in the final product caused by the sterilisation step (Fig. 5(b)). The distinction between the samples after brine and the samples after lye treatment can be observed along PC2 axis (Fig. 5(a)) and was mainly the result of the loss of galacturonans as a consequence of lye treatment (Fig. 5(b)).

4. Concluding remarks

The storage in brine contributed positively to the stabilisation of cell wall polysaccharides of olives in the three

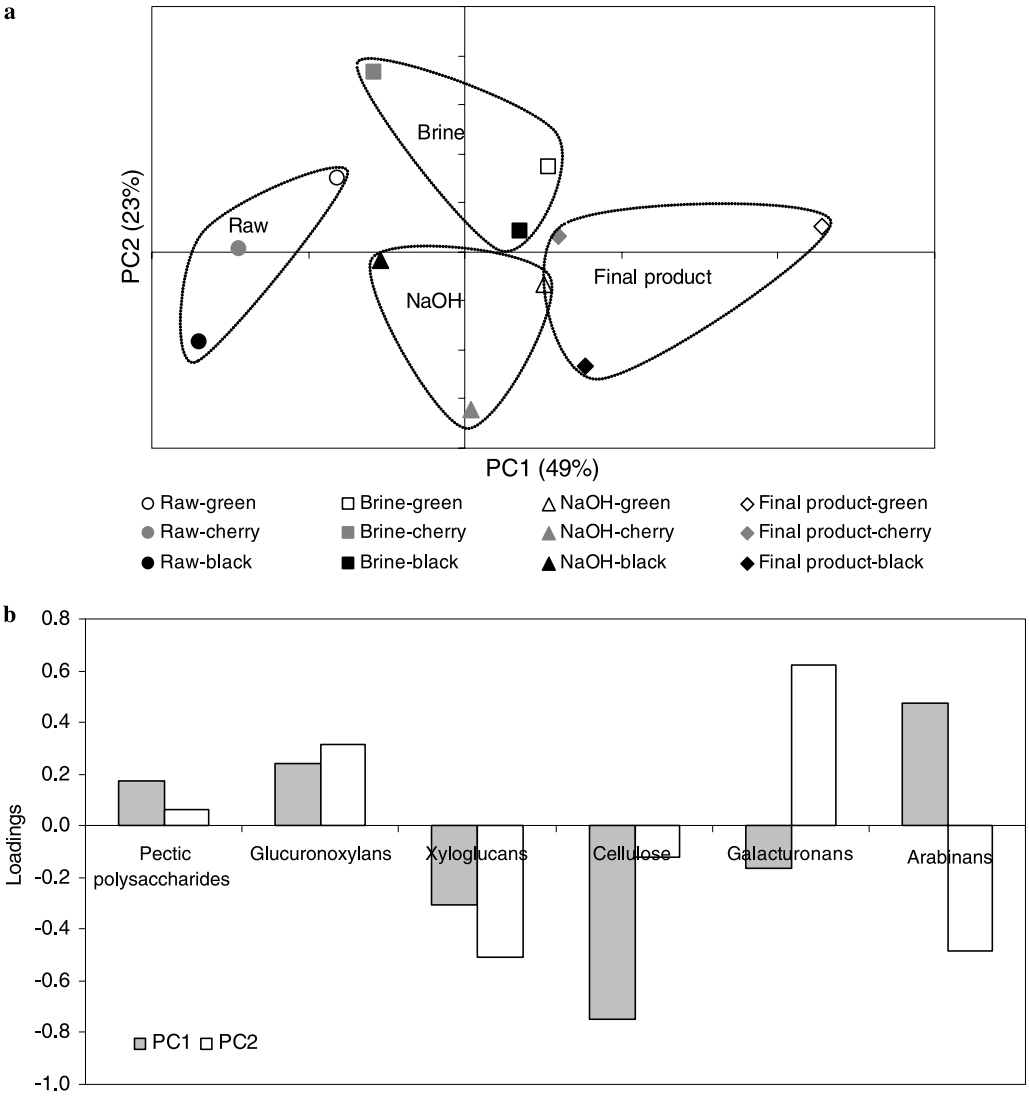

Fig. 5. PCA of contents (mg/fruit) of pectic polysaccharides, glucuronoxylans, xyloglucans, cellulose, galacturonans and arabinans of olives along processing and in the three stages of ripening: (a) scores scatter plot (PC1 vs. PC2) (axes cross each other at origin); (b) loadings plot.

stages of ripening, conferred by Na^+ and Ca^{2+}. The lye treatment introduced degradation of cell walls due to generalised loss of pectic and hemicellulosic polysaccharides, and cellulose, caused by the breakage of ester and hydrogen bonds. The shifts in solubilisation towards more concentrated solutions and the retention in the cellulosic residue contributed to the attenuation of losses and to the increased stiffness and strength of flesh, as observed in olives of Hojiblanca variety (Georget, Smith, Waldron, & Rejano, 2003). In the final product, the stage of ripening was determinant as the thermal treatment introduced higher losses and solubilisation of polysaccharides in the black olives. That should contribute to the decreased strength of flesh as occurred in olives of Hojiblanca variety (Georget et al., 2003).

The results of changes of cell wall polysaccharides along processing and ripening of olives allowed concluding that the differences between the stages of ripening of raw samples were magnified after black oxidising processing.

Acknowledgements

The authors are grateful for the financial support of the EU Project OLITEXT FAIR CT97-3053. I. Mafra is grateful to FCT for the PhD grant SFRH/BD/1245/2000. The authors also like to remember the memory of Professor Giorgio Bianchi, from Pescara, Italy, due to the contribution with his expertise and skills to the OLITEXT Project.

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