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Effect of black oxidising table olive process on the cell wall polysaccharides of olive pulp (*Olea europaea* L. var. Negrinha do Douro)

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Abstract

Olive fruits of Negrinha do Douro variety were sampled after the three main steps of black oxidising processing: storage in brine, lye treatment and thermal treatment (final product). The analysis of cell wall polysaccharides showed that brine storage increased the amount of pectic polysaccharides, glucuronoxylans and cellulose in olive fruit. Those increases suggest the biosynthesis of new polysaccharides during the long storage in brine. The lye treatment had two effects: it caused degradation and loss of polysaccharides and, on the other hand, it increased their retention in the cell walls. The breakage of ester and hydrogen bonds should have been responsible for their degradation. The retention could be due to the ionisation of hydroxyl groups of cell wall polysaccharides, preventing the diffusion of negatively charged pectic polysaccharides. The thermal treatment caused mainly the loss of hemicellulosic polysaccharides and cellulose. The increased solubilisation of pectic polysaccharides observed in the final product should have been the result of dissolution of pectic polysaccharides involved in the cellular adhesion, which should result in a decreased firmness of the flesh. The application of FT-IR/multivariate analysis to the cell wall material allowed the distinction of olives before and after the lye treatment, attributed mainly to the changes in pectic polysaccharides.

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

1. Introduction

The olive fruit (*Olea europaea* L.) is a drupe, similar to other drupes or stoned fruits. However, the olive fruit differ from all other drupes in its chemical composition due to the relatively low sugar content (2–5% versus around 12% for other drupes), high oil content (20–30% versus 1–2% for other drupes), and its characteristic strong bitter taste caused by oleuropein (Garrido Fernández, Fernández Díez, & Adams, 1997). Because of those characteristics, the olive fruits are not edible without treatment, but they are the raw material for a number of products, particularly olive oil and table olives. Table olives are included in the pickled products, which are defined as those products whose preparation and preservation are carried out by a combination of salting, fermentation and/or acidification. The natural bitterness of the fruit can be eliminated, or at least reduced, by processing to make the product acceptable for human consumption. The processing is also responsible for softening of the tissue, which is desirable if the raw product is too hard, but is a problem when olives become too soft. The black oxidising or Californian processing is one of the mostly used industrial methods of preparing table olives. According to this type of processing, the olives, mostly in the green and cherry stages of ripening, are stored in brine with 5–10% NaCl from two to six 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 diluted 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 lies 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 to reach a final pH around 7 and placed in 3–5% brine with ferrous gluconate or ferrous lactate to maintain the black colour. The olives are then packed and thermally treated (Garrido Fernández et al., 1997).

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One of the main purposes of alkali treatment is to hydrolyse and to remove oleuropein, the molecule responsible for the bitter taste (Brenes & de Castro, 1998). However, the action of alkali reagent is complex as it affects the skin permeability causing the loss of numerous components, such as soluble sugars, organic acids, etc (Garrido Fernández et al., 1997). The sodium hydroxide solution dissolves the epicuticular waxy coating increasing the diffusion of components through the skin and affecting the pectic polysaccharides and cellular integrity (Marsilio, Lanza & De Angelis, 1996). Several studies have reported that processing affects mostly the pectic polysaccharides, increasing their solubilisation (Araujo, Labavitch, & Moreno, 1994; Coimbra, Waldron, Delgadillo, & Selvendran, 1996a; Sánchez-Romero, Guillén, Heredia, Jiménez, & Fernández-Bolãnos, 1998a) and leading to a decrease of tissue firmness (Jiménez, Guillén, Fernández-Bolaños, & Heredia, 1995). Only one preliminary study reports changes in the cell wall polysaccharides as a result of Californian process of Manzanilla olives (Araujo et al., 1994). Most of other studies report to changes on olive cell wall polysaccharides related to the Sevillian style process (Coimbra et al., 1996a; Jiménez et al., 1995; Jiménez, Heredia, Guillén, & Fernández-Bolaños, 1997; Jiménez, Sánchez, Guillén, Fernández-Bolaños, & Heredia, 1998; Marsilio et al., 1996; Sánchez-Romero et al., 1998a,b), which includes a first step of lye treatment followed by a lactic fermentation in brine.

More recently, the textural changes of Hojiblanca olives which underwent the Californian process have been reported (Georget, Smith, Waldron, & Rejano, 2003). These authors used the approach of separating the epicarp (skin) from the mesocarp (flesh) and demonstrated that the strength of the epicarp decreased significantly with processing, whereas the flesh became stronger after storage in brine and treatment with lye. Most treatments resulted in reduced strength and stiffness of the skin. However, brine storage enhanced the strength of the flesh, the lye treatment enhanced the stiffness and the strength of the flesh, and the heat treatment resulted in a decreased strength. To understand the mechanical changes of olive flesh, which underwent the Californian process, the knowledge of cell wall modifications is an important achievement.

The present work is a contribution to the elucidation of chemical changes that occur at the level of cell wall polysaccharides of olive pulp after the three main steps of table olive black oxidising process.

2. Material and methods

2.1. Plant material

Olive fruits (*O. europaea* L. var. Negrinha do Douro) with an average length of 2.0 cm and a diameter of 1.2 cm, from 1998 harvest, were provided by Maçarico Lda, Praia de Mira, Portugal. The olives at a green mature stage were processed according to the Californian style, which consisted on three main steps.

Step 1 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 a first soak in lye during 4 h (2.25% (w/v) NaOH), which enables the hydrolysis of oleuropein 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 of the washing water (pH 7). Sodium benzoate was added for inhibition of yeast and moulds growth.

Step 3 further to this treatment, the olives were stored in brine (pH 6) with 0.1% (w/w) ferrous lactate for colour fixation, and 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 (1996b) 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, Lanza, Reis, Marsilio, Campestre and Angelis (2001).

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 imidazole, Na_2CO_3, and KOH solutions of increasing strength to leave a final residue (cellulosic residue, CR).

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 with a split injector (split ratio 1:60) and a FID detector. A 30 m column DB-225 (J and 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 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 & Asboe-Hansen (1973).

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.

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., 1996b), 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 reached by the addition 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, Mafra, Ferreira, Cardoso, Reis and Lopes da Silva (2002). The saponification of samples was done in duplicate and each one was injected twice (GLC-FID). Results with less than 5% variability were obtained. A third analysis was done for the few samples with higher variability.

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 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). The FT-IR spectral region used for principal component analysis (PCA) was 1200–850 cm^{-1}. In order to minimize the effect of baseline shifts and other factors that may interfere with the multivariate analysis, the spectra were SNV corrected (standard normal deviates).

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 olives at a green stage of ripening. The study focuses the changes related to the main steps of processing: after brine (step 1), after lye treatment (step 2) and final product after thermal treatment (step 3). The results of raw olives at the green mature stage here presented for comparative purposes were published in Mafra et al. in press.

3.1. Cell wall material composition

The yield of CWM changed from 4.2% in raw olives, to 4.8% after brine (step 1), 4.4% after lye (step 2) and 4.3% in final product after thermal treatment (step 3). These variations indicated a higher retention of polymeric material after brine attributed to the presence of calcium and sodium ions, as was observed in the processing of olives according to the Sevillian style (Jiménez et al., 1995; 1997). Black oxidised olives of Manzanilla variety showed a decrease of CWM from 3.9% for raw to 3.3% for processed olives (Araujo et al., 1994). However, the brining step of those olives did not last more than 4 days, which was not probably enough time to promote the increase of the polymeric material, as was observed in olives of Douro variety after 5 months in brine. The calculation of yield on a dry pulp weight basis shows an increase from 15% in raw olives to 18% after brine, and to 21% after lye and in the final product, which makes clear the solubilisation of non-polymeric material along processing.

The glycosidic composition of CWM showed an increase of the relative proportion of Ara and Xyl after brine, with the concomitant decrease of Glc and HexA (Table 1). In the final product, emphasis is given to the decrease of HexA and the increase of Glc. The decrease of the relative proportion of HexA was also noticed in olives of Manzanilla variety after black oxidising processing (Araujo et al., 1994) and in olives of Douro variety processed according to the Sevillian style (Coimbra et al., 1996a). The glycosidic composition of SDS extracts indicates that they contain mainly pectic polysaccharides rich in Ara. The increase of Ara proportion together with the decrease of HexA in the final product suggests a decrease of linear main chain of GalA of pectic polysaccharides obtained in the final product.

The results of the CWM on a dry pulp basis (Fig. 1(a)) show an overall increase of sugars after brine, attributed mainly to Ara, Xyl and Gal and, to a smaller extent, to Glc and HexA. After alkali treatment, the values remained approximately the same, but after thermal treatment, i.e. in the final product, there was a decrease of HexA.

Table 1
Sugar composition of purified SDS extracts and cell wall material of olive pulp

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											
	Raw[b]	8.8	3	–	33	3	1	10	12	38	252
	Brine	6.0	1	–	33	6	2	11	7	41	257
	NaOH	7.4	1	–	33	5	Tr	9	10	42	66
	Final	5.1	3	–	52	3	Tr	12	5	24	233
CWM											
	Raw[b]	42.5	Tr	–	25	14	3	4	3	21	416
	Brine	47.6	Tr	–	29	18	2	5	29	17	644
	NaOH	43.7	Tr	–	28	17	2	3	28	21	533
	Final	43.3	Tr	–	29	18	2	3	33	14	533

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. in press.

The higher solubilisation in the SDS extracts (Fig. 1(b)) was observed in the raw olives, while the smaller one was noticed after lye treatment, indicating a more difficult removal of pectic polysaccharides after step 2. The drastic increase of solubilisation in the final product indicates that the thermal treatment and/or new equilibrium in brine turned pectic polysaccharides more soluble in SDS solutions, reaching a level similar to the one found in raw olives, although with higher Ara and lower HexA relative proportions (Table 1, Fig. 1(b)).

The overall presentation of cell wall material composition per fruit (Fig. 2) emphasises the changes due to the main steps of processing, once the presentation on a dry pulp basis does not account for the increased concentration of polymers caused by the release of intracellular compounds. After brine, it can be noticed a significant increase of total sugars, indicative of a general increase of pectic and hemicellulosic polysaccharides. When the olives were placed in a brine solution containing 6% of NaCl and 0.1% of $CaCl_2$ at pH 4, the presence of divalent cations such as calcium, and monovalent cations such as sodium might have contributed for the electrostatic stabilisation of cell walls (Jiménez et al., 1997). Ca^{2+} is known to improve texture of vegetable tissues because of the formation of complexes between the pectic polysaccharides (Brett & Waldron, 1996). The low pH might reduce the dissociation of carboxylate groups by decreasing of electrostatic repulsion (Van Buren, 1979). Hence, the amount of cations present in brine should have contributed for a higher retention of pectic polysaccharides in the cell wall matrix, which was noticed in the SDS extracts. However, this is not enough to justify the great increase of cell wall sugars. The fact that the olives remained in brine during a long period (5 months), and because it is still a living tissue, the results suggest that synthesis *de novo* might have occurred on those conditions.

The 30% decrease of total sugars per fruit (Fig. 2) after lye treatment evidenced the degradation of polysaccharides. When the fruits are subjected to several cycles of alkali treatment (until complete penetration of solution in pulp), it occurs

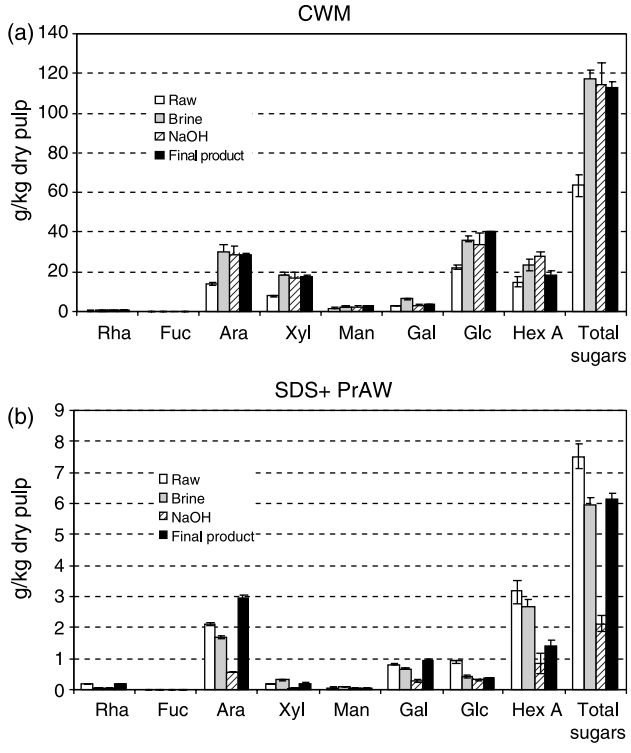

Fig. 1. Cell wall sugars of raw olives, after brine storage, after lye treatment and final product expressed in g per kg dry pulp: (a) CWM; (b) SDS and PrAW extracts.

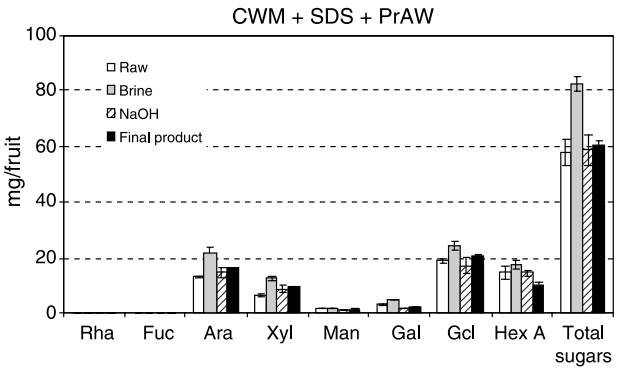

Fig. 2. Cell wall sugars of CWM and SDS + PrAW extracts of raw olives, after brine storage, after lye treatment and final product expressed in mg per fruit.

the dissolution the epicuticular waxy coating of olives, which allows the removal of water soluble components (Marsilio et al., 1996). The hydrolysis of links leading to the removal of oleuropein occurs during this step, including the ester bonds with the de-esterification of pectic polysaccharides and hydrogen bonds. Alkali may also promote β-elimination of esterified pectic polysaccharides with concomitant formation of shorter polymers. The low amount of polysaccharides in SDS solutions after lye treatment (Fig. 1(b)) might have been the result of loss of degraded polysaccharides, and the ionisation of hydroxyl and carboxylate groups due to the high pH increased the possibility of Ca^{2+} bridges between the pectic polysaccharides allowing them to remain in the cell walls. Araujo et al. (1994) verified that the processing of black oxidised olives was accomplished by a general solubilisation of polysaccharides, where the pectic polysaccharides and the non-cellulosic glucans were the compounds most affected. Marsilio et al. (1996) observed the loss of cellular cohesion with lye treatment resulting in the softening of middle lamella and consequent loss of texture.

After thermal treatment of final product, there was no significant variation of total sugars (Fig. 2), which indicates minimal degradation of polysaccharides during this step. The increased solubilisation of pectic polysaccharides in SDS solutions, shown by the increase of HexA, Ara, Gal, and Rha (Fig. 1(b)), might be attributed to the dissolution of pectic polysaccharides involved in the cellular adhesion. The degradation process by β-elimination is favoured by the neutral pH at high temperatures, but it should not be likely to occur as the pectic polysaccharides were de-esterified at this step (Ng & Waldron, 1997; Van Buren, 1979). Lecain, Ng, Parker, Smith, and Waldron (1999) verified that the thermal treatment under pressure of onion increased the softening of tissues by involving cellular separation, which was accomplished by general dissolution and de-polymerisation of cell wall polysaccharides.

3.2. 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 the sugar composition of olives in the three steps of processing are shown in Table 2. 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) CR. For comparative purposes the results of raw olives at the green mature stage, published by Mafra et al. in press, were included in Table 2.

The yield of polymeric material obtained with imidazole and Na_2CO_3 solutions changed from 9.0% in raw olives to 10.4% after brine, decreasing drastically after lye (1.8%), and showing a small recovery in the final product (3.8%) (Table 2).

Table 2
Sugar composition of fractions of cell wall material of olive pulp 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]	9.0	1	–	29	2	1	4	2	63	705
	Brine	10.4	Tr	–	28	1	Tr	4	2	65	801
	NaOH	1.8	Tr	–	31	2	–	2	2	62	527
	Final	3.8	Tr	–	42	1	–	2	1	55	694
0.5 M KOH											
	Raw[b]	2.0	Tr	Tr	14	41	2	7	24	12	645
	Brine	1.8	Tr	Tr	13	47	1	3	24	12	505
	NaOH	1.2	Tr	Tr	8	53	3	5	23	7	821
	Final	1.6	Tr	Tr	20	40	1	4	13	22	808
1 M KOH											
	Raw[b]	5.6	Tr	1	16	33	7	9	26	8	479
	Brine	3.8	Tr	Tr	17	36	7	9	24	7	637
	NaOH	5.0	Tr	Tr	9	43	9	8	27	5	676
	Final	4.6	Tr	Tr	15	48	5	6	19	6	599
4 M KOH											
	Raw[b]	7.9	1	Tr	25	11	16	10	20	16	296
	Brine	5.7	Tr	Tr	29	15	16	12	20	8	687
	NaOH	6.0	1	Tr	25	20	13	8	19	14	426
	Final	6.0	Tr	–	15	28	15	8	20	13	395
CR											
	Raw[b]	59.6	1	–	28	14	1	4	40	14	534
	Brine	61.7	Tr	–	31	19	1	5	33	13	720
	NaOH	85.3	Tr	–	35	13	1	2	30	18	574
	Final	71.9	1	–	36	16	1	2	26	19	650

Tr, trace amount; CR, cellulosic residue.
[a] Yield is expressed in percentage of CWM.
[b] Data from Mafra et al. in press.

The sugar composition indicates the presence of pectic polysaccharides rich in Ara whose ratio HexA/Ara did not change much from the raw product (2.2) to the one after brine (2.3), but decreased after lye treatment (2.0) and especially in the final product (1.3). The slight increase of polysaccharide extraction after brine agrees with the results of CWM composition, suggesting the biosynthesis of polysaccharides. However, the increased solubility was noted at the level of Na_2CO_3 extracts, and not imidazole (results not shown), indicating the need of stronger reagents to solubilise pectic polysaccharides. The values of degree of methylesterification of pectic polysaccharides obtained with imidazole solutions changed from $58.4 \pm 2.5\%$ in raw to $46.4 \pm 0.9\%$ in olives after brine, which suggests that pectinmethyl esterase was also active during brine storage. The low recovery of polymeric material with imidazole and Na_2CO_3 observed after lye treatment is consistent with the one observed in SDS extracts. With the breakage of ester linkages caused by the alkali, it was expected to obtain a lower yield of pectic polysaccharides with Na_2CO_3 solutions. The de-esterification of pectic polysaccharides increased their ability to form Ca^{2+} bridges decreasing, as a consequence, their extractability. However, this fact should have increased the amount of material extracted with imidazole solutions, whose role is to quelate Ca^{2+} and so, to solubilise the pectic polysaccharides attached by Ca^{2+} bridges. This occurred after lye treatment of green olives processed according to Sevillian style (Coimbra et al., 1996a; Sánchez-Romero et al., 1998a). Anyway, the high increase of pectic polysaccharides strongly linked to the cell wall matrix is evidenced by the high increase of yield of CR (Table 2). The pectic polysaccharides that have been solubilised during dialysis of CR accounted 4.3% for raw olives, 6.1% after brine, 11.4% after lye treatment and 5.3% after thermal treatment. The thermal treatment and new equilibrium in brine doubled the amount of pectic polysaccharides extracted in relation to the previous step of lye treatment (Table 2), as was also observed in the SDS extracts. The decrease of Hex/Ara proportion suggests the degradation of pectic polysaccharides by preferential removal of the GalA residues, which is consistent with changes obtained after black oxidising processing of olives of Manzanilla variety (Araujo et al., 1994). These results could explain the increase in the strength of the pulp observed after brine and lye treatment of olives of Hojiblanca variety (Georget et al., 2003), where pectic polysaccharides seem to be strongly linked to the cell wall matrix, while the decrease in the strength after heat treatment could be related to the increased solubilisation of pectic polysaccharides observed in SDS, imidazole and Na_2CO_3 extracts.

The partially depectinated CWM was extracted with KOH solutions of increasing strength until obtaining a cellulosic residue. The yield of cell wall sugars extracted with 0.5 M KOH did not change much with processing (the value was about 1% from raw ($2.0\% \times 0.645$ g/g) to final product ($1.6\% \times 0.808$ g/g)), where Xyl and Glc were the main sugars, indicating sugar composition characteristic of the presence of xylans and xyloglucans (Table 2). The 1 M KOH extractions showed a higher yield of polymeric material as well as polysaccharides, where the highest yield of sugars (3.4%) was observed after lye treatment. Their sugar composition indicated also the presence of xylans and xyloglucans. The main sugars obtained with 4 M KOH extractions were Ara, Glc, Xyl and Man, suggesting the presence of glycoproteins rich in Ara as the amount of Ara was not followed by HexA, and probably xyloglucans and glucomannans (Coimbra et al., 1994). The highest yield of cell wall sugars was obtained after brine treatment (3.9%).

The yield of cell wall sugars that remained in the cellulosic residue increased 40% after brine, 10% between brine storage and lye treatment and decreased 5% in the final product in relation to the lye treatment. The increase noticed after brine was attributed to an increased retention and probably formation of pectic and hemicellulosic polysaccharides, as Ara and Xyl proportions increased and Glc decreased (Table 2). After lye treatment, the increase of polysaccharides was due mainly to the retention of pectic polysaccharides as both proportions of Ara and HexA increased. In the final product the proportions of Ara, Xyl and HexA increased due to the decrease of cellulosic glucose (data not shown), which suggests the degradation cellulosic microfibrils as a consequence of thermal treatment. Similar results were obtained during processing of olives of Manzanilla according to the Sevillian style (Sánchez-Romero et al., 1998b).

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

The comparative analysis of CWM of raw olives and along the three main steps of processing was performed by means of FT-IR and principal component analysis (PCA) (Fig. 3). The scores scatter plot of FT-IR spectra (Fig. 3(a)) along PC1 axis is partially related to the amount of sugars as raw olives in the positive side have the lowest sugar concentration and the olives after brine storage in the negative side have the highest concentration, while those after lye and final product have intermediate values (Table 1). The scores scatter plot of FT-IR spectra along PC2 axis allows the distinction of olives in fresh and after brine storage from those after lye treatment and final product. The loadings plot shows that the negative side of PC2 is related to the absorbance at the wavenumbers of 1145, 1095 and 952 cm^{-1} (Fig. 3(b)), which are attributed to GalA (Coimbra et al., 1999). The positive side of PC2 axis shows a band between 1060 and 1040 cm^{-1} attributed to hemicellulosic polysaccharides. The PC2 axis distinguishes olives before and after lye treatment as related to pectic and hemicellulosic polysaccharides. This distinction might be attributed to the de-esterification of pectic polysaccharides caused by the lye treatment. Although that variation is known to alter the absorbances at 1750 and 1630 cm^{-1} attributed to ester and carboxylate groups, respectively (Coimbra et al., 1999), the methylesterification degree of pectic polysaccharides is also related to the region of 1200–850 cm^{-1} (Barros et al., 2002). According to Coimbra et al. (1999), the pectic polysaccharides extracted with CDTA, Na_2CO_3 and soluble in water after

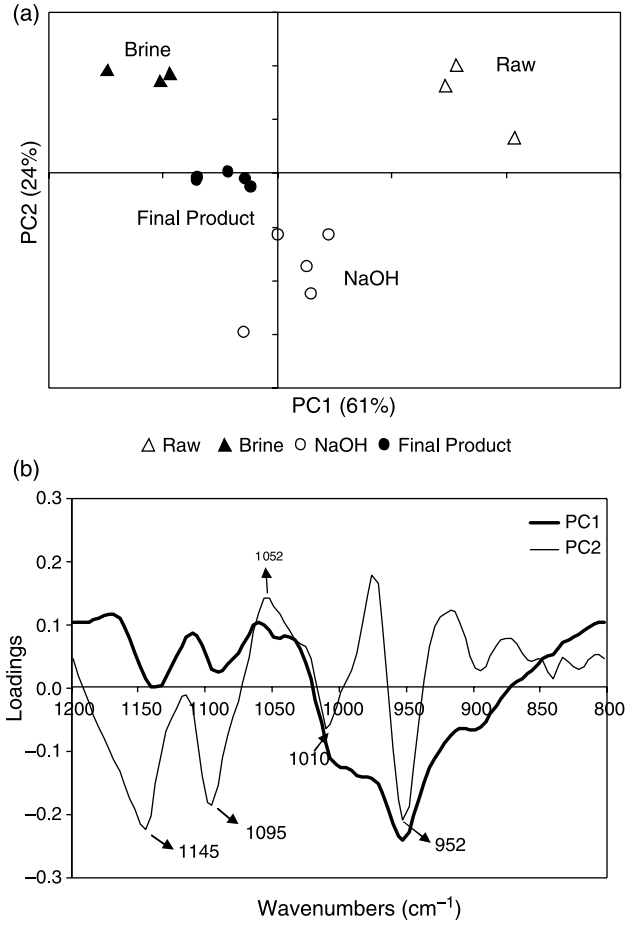

Fig. 3. PCA of the FT-IR spectra of the CWM of raw olives, after brine storage, after lye treatment and final product: (a) scores scatter plot (PC1 versus PC2) (axes cross each other at origin); (b) loadings plot.

dialysis of CR (sn-CR) are related to the absorbance bands at 1145, 1014, 984 and 952 cm^{-1}, but those extracted with CDTA (esterified) do not include the band at 952 cm^{-1}. Once the Na_2CO_3 and sn-CR extracts were de-esterified, probably 952 cm^{-1} is characteristic of de-esterified pectic polysaccharides. According to Barros et al. (2002), the band at 952 cm^{-1} is negatively related to those at 1746 and 1603 cm^{-1}, and so to the degree of methylesterification. Furthermore, the loadings plot of CWM of raw olives at different stages of ripening does not show any band at 952 cm^{-1} (Mafra et al. in press).

3.4. Changes in cell wall polysaccharides with processing

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 olives after brine, lye and thermal treatments are presented in Table 3. For comparative purposes, the polysaccharide composition of raw olives at the green mature stage, published by Mafra et al. in press, were also included. The estimates of total polysaccharides obtained from sequential extraction of CWM showed a great consistency comparing with the total cell

Table 3
Olive pulp cell wall polysaccharide composition of olives at different stages of processing

	Raw[a]	Brine	NaOH	Final
Pectic polysaccharides	32	40	31	35
Galacturonan	(14)	(15)	(11)	(12)
Arabinan	(14)	(20)	(18)	(20)
Glucuronoxylan	7	12	10	11
Xyloglucan	5	5	4	3
Mannan	1	2	1	1
Ara-rich glycoprotein	Tr	1	Tr	Tr
Cellulose	16	19	16	14
Total polysaccharides	62	77	63	64

Values expressed as mg per fruit. Tr, trace amount. Values in parenthesis are part of pectic polysaccharides.
[a] Data from Mafra et al. in press.

wall sugars from CWM (Fig. 2). Thus, the amount of polysaccharides per fruit after brine was about 80 mg, while for raw fruit and other steps of processing was around 60 mg.

After brine storage, the pectic polysaccharides, the glucuronoxylans and the cellulose contributed to the increase in cell wall polysaccharides of about 20, 70 and 15%, respectively. The alkali treatment has lead to a generalised decrease of cell wall polysaccharides (about 20%), suggesting their degradation. After thermal treatment, there was a small recovery of pectic polysaccharides (12%) and glucuronoxylans (15%), probably due to the stabilisation of negative charges promoted by pH neutralisation, sodium cations (Jiménez et al., 1997) and to the addition of iron divalent cations, which in addition to its effect upon colour, might play a role similar to calcium ions, as suggested by Garrido, Garcia, Brenes, and Romero (1995). However, the same was not observed in xyloglucans and cellulose, which decreased 21 and 17%, respectively.

4. Concluding remarks

The effect along the black oxidising processing on the cell wall polysaccharides of green olives showed that brine storage increased the amount of pectic polysaccharides, glucuronoxylans and cellulose. The maintenance of cell wall polysaccharides in NaCl solutions containing $CaCl_2$ might be the result of charge stabilisation conferred by Na^+ and Ca^{2+}, and mainly the ability of Ca^{2+} to form complexes with pectins (Jiménez et al., 1997), allowing them to form gels (Cardoso, Coimbra, & Lopes da Silva, 2003). The increase of polysaccharides can be attributed, possibly, to the biosynthesis of new polysaccharides during the long storage period. This step should contribute to an increased strength of the pulp, as observed in olives of Hojiblanca variety (Georget et al., 2003).

The alkali treatment had two effects: it caused degradation and loss of polysaccharides and, on the other hand, it increased their retention in the cell walls. The degradation should be attributed to the breakage of ester and hydrogen bonds. The retention could be due to the ionisation of the hydroxyl groups of cellulose that could prevent the diffusion of negatively

charged pectic polysaccharides enmeshed within the swollen cellulose matrix, attenuating their loss. This step should contribute to the increased stiffness and strength of flesh, as observed in olives of Hojiblanca variety (Georget et al., 2003).

The final equilibrium in brine and thermal treatment caused mainly the loss of hemicellulosic polysaccharides (except glucuronoxylans) and cellulose. The increased solubilisation of pectic polysaccharides observed in the final product might be attributed to the dissolution of pectic polysaccharides involved in the cellular adhesion, which should result in a decreased strength of flesh, as observed in olives of Hojiblanca variety (Georget et al., 2003).

FT-IR/multivariate analysis is a useful tool for the distinction of CWM of olives in the main steps of black oxidising processing, allowing the distinction of those before and after lye treatment attributed mainly to the changes in pectic polysaccharides.

The present study demonstrated that the knowledge of cell wall modifications is a major achievement for understanding the textural changes that occur in olive pulp along the black oxidising process.

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