Molecular Weight and Ionic Characteristics of Olive Cell Wall Polysaccharides during Processing

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The changes that occur during olive fruit (*Olea europaea arolensis*) processing (high pH treatment and fermentation) in the pectic fractions and hemicelluloses B of the cell wall have been studied. The amount of neutral arabinans in the cell wall and the composition of this fraction did not change, but there was a great decrease in the molecular weight [from higher than 400 000 in unprocessed fruit (UF) to 70 000 in processed fruit (PF)]. The water-soluble acidic polysaccharides almost disappeared, losing mainly their galacturonic acid-rich portions. The most important change that occurred in the oxalate-soluble rhamnogalacturonans was a decrease in their degree of esterification (from 75.44% and 35.42% for the peaks in UF to 28.42% for the peak in PF). Their composition and molecular weight were not greatly affected by processing. The molecular weights of the main polysaccharide components of the hemicelluloses B (xyloglucans, galactoglucomannans, and arabinoxylans) decreased greatly, although their sugar compositions remained almost stable.

Keywords: *Olive; cell wall; processing; polysaccharides; molecular weight; degree of esterification*

INTRODUCTION

Pectic polysaccharides are important determinants of fruit texture. In view of their physiological significance (their capacity to lower cholesterol levels and bind bile acids, polyvalent cations, and water, etc.), renewed attention has been focused on these compounds. Their effects on cholesterol levels and their binding capacity depend in part on the structure of their galacturonan components: degree of esterification (DE), insertion of rhamnose units, presence of neutral side chains, etc. (Goldberg et al., 1989). The other group of noncellulosic polysaccharides of the cell wall, the hemicelluloses, has a very important role in maintaining the wall structure, uniting pectins and cellulose. Small changes in their structure, therefore, even changes that affect only the molecular weight (as occur in fruit ripening and softening), inevitably produce losses in cell wall rigidity and, as a consequence, in fruit texture.

Olive fruits (*Olea europaea arolensis*) are very important in the economy and diet of Mediterranean countries, including Spain. Because of their bitterness, a technological process is needed to make them edible as "table olives" (Fernández-Díez, 1985). Changes in pectic polymers have been described in other processes such as canning (Chitarra et al., 1989), lactic fermentation (Howard and Buescher, 1990), and heat treatment (Feng et al., 1989), but very little is known about changes due to alkaline treatment. Furthermore, hemicelluloses have never been studied in relation to textural changes during vegetable processing. In a previous paper (Jiménez et al., 1994a), the "Californian black ripe olive" process and the alterations produced in the cell wall structure have been described.

In the present study, the structural changes in the pectic fractions (acidic and neutral) and hemicelluloses B arising from the "Spanish green olive" process have been studied. Knowledge of the nature of these changes would help in understanding the complex changes in firmness that take place during the processing of all plant products, especially olive fruits.

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MATERIALS AND METHODS

Olive Processing. Olive fruits (*O. europaea arolensis* var. Hojiblanca), harvested in the province of Seville (Spain), were processed as follows: the fruits were treated with a solution of 2−2.5% sodium hydroxide (lye) for around 7 h (the lye has to penetrate two-thirds of the distance to the pit). Olives were then subjected to a rapid wash, followed by another wash which lasted 14−16 h. The fruits were then placed in a solution of 10−11% sodium chloride (brine) that reached a concentration of around 7% at equilibrium. After the lactic fermentation, which gives the olives the typical "Spanish green olive" flavor, and a period of conservation in a more concentrated brine (around 10% at equilibrium), the olives were ready for packing.

Sampling. Around 500 g of olives was taken for both samples (unprocessed and processed fruit). These samples (UF and PF, respectively) were treated to isolate the cell wall material, the main pectic fractions (soluble in water and in oxalate), and hemicelluloses B.

Analytical Methods. Selvendran's method (1975) was used to isolate the cell wall material by treatments with sodium dodecyl sulfate and phenol/acetic acid/water. Cell wall material was depectinated by treatments with hot water and hot oxalate, and hemicelluloses B were extracted by sodium hydroxide (Jiménez et al., 1994a).

Noncellulosic neutral sugars were quantified by trifluoroacetic acid (TFA) hydrolysis (Ruiter and Burns, 1987), reduction, acetylation, and gas chromatography (Englyst and Cumming, 1984). The colorimetric assay of 3-phenylphenol (Blumenkrantz and Asboe-Hansen, 1973) was used for determination of uronic acids. Every determination was performed in duplicate (the coefficient of variation was less than 10%).

Ion Exchange Chromatography. The main pectic fractions (water-soluble and oxalate-soluble) and hemicelluloses B were fractionated on a column of QAE-Trysacryl M (IBF) (2.6 × 40 cm). The exchanger was equilibrated in 12.5 mM imidazole−HCl, pH 7. The samples were dissolved in the same buffer. The fractions were eluted with 200 mL of starting buffer, followed by 200 mL each of 125 and 550 mM imidazole−HCl, pH 7, at a rate of 40 mL/h. Ten milliliter fractions were collected and assayed for total sugars (Dubois et al., 1956). The different peaks isolated were dialyzed, freeze-dried, and assayed for uronic acids. Their neutral sugar composition was studied by GC (Englyst and Cumming, 1984).

Gel Filtration Chromatography. The molecular weights of the main peaks isolated by ion exchange chromatography were determined on a 2.6 × 70 cm column of Sephacryl S-300

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HR (Pharmacia Fine Chemicals, Uppsala, Sweden). The buffer used was 200 mM phosphate and 500 mM sodium chloride, pH 7. The samples ran at an ascendent flow rate of 40 mL/min. The peak elution was detected by a Waters refraction index detector (R404, Millipore), and the fraction volume was 5 mL. The eluted peaks were concentrated, desalted on PD-10 columns (Pharmacia), and freeze-dried. Uronic acids and neutral sugar composition were determined.

Determination of the Degree of Esterification. The samples were reduced with sodium borohydride (10 mg/mL) in 50% ethanol overnight. In this way, the de-esterified, but not the esterified, groups were reduced. The latter were quantified colorimetrically (Lurie et al., 1994).

RESULTS AND DISCUSSION

Three fractions of polysaccharides have been fractionated by ion exchange and gel filtration chromatography (water-soluble, oxalate-soluble, and hemicelluloses B), from both unprocessed and processed fruits. The glycosyl composition of the different peaks isolated and the degree of esterification of several of them are presented and discussed.

Changes in the Water-Soluble Fraction. The profiles obtained by ion exchange chromatography are presented in Figure 1; one neutral (N) and two acidic (A-1 and A-2) peaks have been isolated. As a result of processing, the size of the acidic peaks decreased in relation to that of the neutral one. Furthermore, in PF they eluted a little later than in UF. Table 1 shows the changes in yields and composition of the different peaks: in both samples, UF and PF, the neutral fraction was the major one, but in the latter the percentage was greater than 80% of the total polysaccharides recovered after ion exchange chromatography. In contrast, the acidic fractions decreased, each one representing less than 10% of the recovered material. The composition (neutral sugars and uronic acids) also changed as a result of processing (Table 1). Although the composition of the neutral fraction remained almost stable, the acidic ones were enriched in neutral sugars and depleted of uronic acids.

The neutral fraction consisted basically of arabinans in both the unprocessed and processed fruits (arabinose content > 90%). Their molecular weight distribution was determined by gel filtration chromatography using Sephacryl S300. The polysaccharides from the UF eluted at the void volume of the column (MW > 400 000), while the average molecular weight of those from the PF was estimated to be 70 000. It can be concluded, therefore, that during processing there is a marked decrease in the average molecular weight of these polysaccharides. It is unlikely that this decrease could take place during lye treatment since arabinans are fairly resistant to alkaline degradation. In contrast, they are very acid-sensitive, and it is very probable that the degradation occurs during fermentation, when the fruits are exposed to a low pH (pH <4) for a long period of time (2−3 months). The activity of exogenous enzymes could be another factor to take into account, although no arabinanase activities have been detected in the fruit or in the fermentation medium. The low molecular weight arabinans found in the PF could be the degradation products not only of the originally water-soluble polysaccharides but also of the other groups of polysaccharides in which they form side chains.

Arabinans were also isolated from UF in the OSF, but in this case they disappeared as a result of the processing (Figure 2; Table 2). The total percentage that

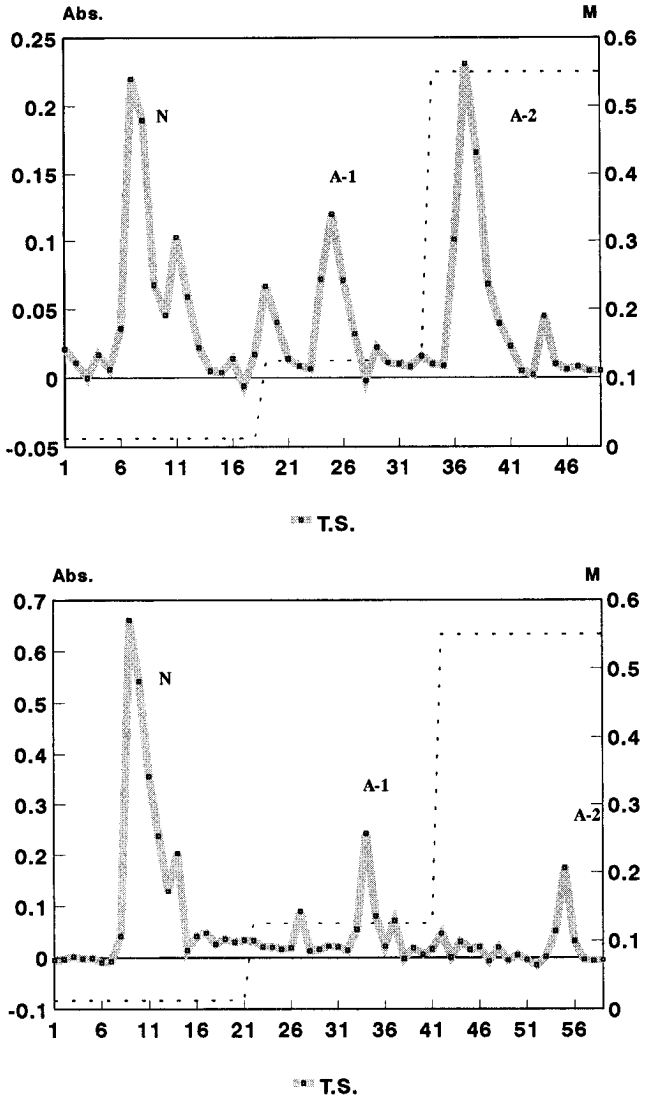

Figure 1. Elution profiles of water-soluble pectins (WSF) in ion exchange chromatography: upper, unprocessed fruit; lower, processed fruit. The dotted line corresponds to the molar concentration of the buffer. N, neutral fraction; A-1, A-2, acidic fractions.

neutral arabinans represent in olive cell wall has been calculated as 1.52% (including the small neutral peak isolated from the oxalate-soluble fraction) in UF and 1.57% in PF. Even though they are water-soluble and undergo a very marked decrease in MW, these polysaccharides remain in the cell wall structure and are not solubilized into the treatment liquids during processing.

In contrast, the acidic polysaccharides showed greater variations in their total sugar molar fraction but not in the neutral sugar one, which only showed minor changes (Table 1). These variations are more evident when the ratios Rha/UA (degree of branching) and Rha/Ara (length of side chains) are examined. Both ratios were the same in the two acidic peaks isolated from UF. Nevertheless, after processing, the structure of the rhamnogalacturonans/homogalacturonans, which are the main components of these fractions, showed some alterations: Rha/UA and Rha/Ara decreased greatly in A-1 and A-2. If both acidic peaks are considered together, it is possible to summarize these changes: these polysaccharides represent 0.87% (0.25% of neutral sugars and 0.62% of uronic acids) in the cell walls from UF and 0.34% (0.25% of neutral sugars and 0.09% of uronic acids) in those from PF. Therefore, uronic acids

Table 1. Composition of the Fractions Isolated from Water-Soluble Pectins (WSF)[a]

	N		A-1		A-2	
	UF	PF	UF	PF	UF	PF
yield	55.34	82.20	21.69	8.36	22.97	9.44
total carboh	60.05	63.13	33.56	53.65	32.70	50.24
Rha	0.1 [0.1]	0.2 [0.2]	4 [11]	13 [14]	4 [11]	15 [21]
Fuc	0.2 [0.2]	0.1 [0.1]	0.9 [2]	0.6 [0.7]	0.5 [1]	0.6 [0.8]
Ara	94 [96]	97 [98]	28 [76]	64 [71]	26 [72]	49 [68]
Xyl	0.6 [0.6]	0.3 [0.3]	1 [3]	5 [5]	1 [3]	0.7 [3]
Man	0.8 [0.8]	0.1 [0.1]	ND	ND	0.6 [2]	ND
Gal	0.8 [0.8]	0.5 [0.5]	2 [5]	7 [8]	2 [6]	6 [8]
Glc	2 [2]	0.5 [0.5]	1 [3]	1 [1]	2 [6]	0.8 [1]
UA	0.5	0.6	62	9	63	27
NS/UA	124/1	102/1	1/2.5	1/0.15	1/2.4	1/0.59
Rha/UA			1/15.5	1/0.69	1/15.7	1/1.8
Rha/Ara			1/7	1/4.9	1/6.5	1/3.3

[a] Upper, yield (% of the total weight recovered by ion exchange) and total carbohydrates (% in weight of the total weight of the fraction); middle, sugar composition [molar fraction (the data in brackets represent the molar fraction of the neutral sugars; ND, nondetected)]; lower NS/UA total composition ratio, Rha/UA degree of branching, and Rha/Ara side chain length.

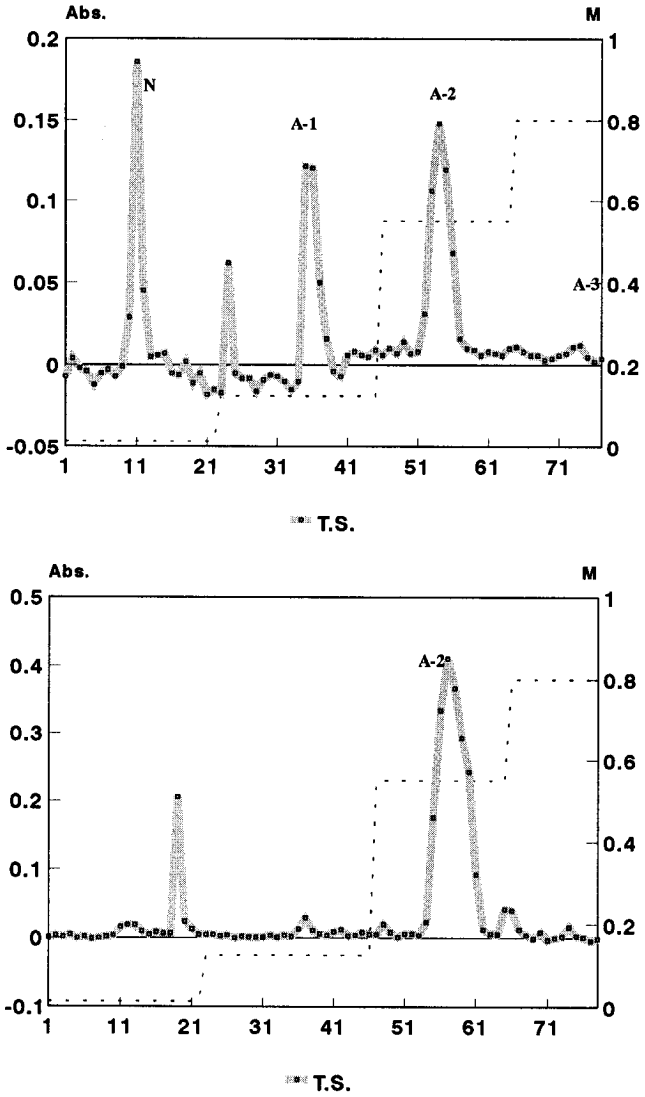

Figure 2. Elution profiles of oxalate-soluble pectins (OSF) in ion exchange chromatography: upper, unprocessed fruit; lower, processed fruit. The dotted line corresponds to the molar concentration of the buffer. N, neutral fraction; A-1−A-3, acidic fractions.

are the only components that are lost from this fraction during processing (by solubilization in the treatment liquids and/or by extraction in other steps of the fractionation). Neutral sugars remain at the same level

Table 2. Composition of Fractions Isolated from Oxalate-Soluble Pectins (OSF)[a]

	N	A-1	A-2		A-3
	UF	UF	UF	PF	UF
yield	5.47	26.27	66.45	100.00	1.81
total carboh	48.22	89.98	98.69	112.91	25.52
Rha	0.2 [0.2]	2 [12]	4 [15]	2 [7]	1 [6]
Fuc	0.2 [0.2]	0.3 [2]	0.3 [1]	0.4 [2]	1 [6]
Ara	88 [88]	11 [65]	18 [69]	20 [74]	9 [56]
Xyl	0.9 [0.9]	0.8 [5]	0.3 [1]	0.8 [3]	ND
Man	2 [2]	0.5 [3]	0.3 [1]	1 [4]	1 [6]
Gal	4 [4]	1 [6]	2 [8]	2 [7]	2 [12]
Glc	4 [4]	0.8 [5]	0.6 [2]	0.5 [2]	1 [6]
UA	ND	83	74	73	84
NS/UA		1/6.7	1/4.6	1/4.4	1/6.9
Rha/UA		1/41.5	1/18.5	1/24.3	1/84
Rha/Ara		1/5.5	1/4.5	1/10	1/9

[a] Upper, yield (% of the total weight recovered by ion exchange) and total carbohydrates (% in weight of the total weight of the fraction); middle, sugar composition [molar fraction (the data in brackets represent the molar fraction of the neutral sugars; ND, nondetected)]; lower, NS/UA total composition ratio, Rha/UA degree of branching, and Rha/Ara side chain length.

as in UF. It is fairly well demonstrated that the high DE of these polysaccharides (Jiménez et al., 1994b) permits a process of β-eliminative cleavage on the chain (Sajjaanantakul et al., 1989). These results allow us to conclude that as a result of processing these polysaccharides from UF (with high DE) lose the "smooth" regions (homogalacturonans) of their molecules, composed of galacturonic acid, and are enriched in "hairy" regions (rhamnogalacturonans), where the side chains are located. Furthermore, the length of these side chains (composed of arabinose and, in a lower proportion, by galactose) decreases but to a lesser degree than the main chain.

Changes in the Oxalate-Soluble Fraction. By ion exchange chromatography (Figure 2), neutral and acidic peaks have been isolated, their composition being shown in Table 2. It is noteworthy that the neutral arabinans (N in the figure) disappeared as a result of processing and that the acidic polysaccharides (A-1−A-3) did not show the same kind of variations as those of the water-soluble fraction. In this case, these polysaccharides did not lose their smooth regions (homogalacturonans), the ratio Rha/UA remaining almost stable. The value of this ratio for the only peak isolated from PF is the average value for the main peaks, A-1 and A-2 from UF

(A-3 was not taken into account because of its low yield), although the length of the side chains increased. To explain this fact, it is necessary to bear in mind that the yield of this fraction increased greatly because of processing, mainly during lye treatment (Jiménez et al., 1995). A greater amount of rhamnogalacturonans/homogalacturonans was, therefore, extracted from PF by ammonium oxalate. These newly extracted pectins could be those which are stabilized in the cell wall structure by ester or phenolic linkages and, consequently, they can only be solubilized by treatment in acidic medium (delignification). If this solubilization does not occur, they cannot be released from UF by any of the fractionation steps. In PF they are easily extracted by ammonium oxalate: the ester or phenolic linkages are broken during the lye treatment and its high pH produces de-esterification of the new molecules solubilized. This would explain why the OSF increased because of lye treatment and why its degree of esterification decreased greatly. An increase in OSF has also been observed in pickled cucumbers (Howard and Buescher, 1990), this being caused by enzymatic activity as well as low pH values. The new pectic polymers that are extracted have a similar degree of branching but may have longer side chains, since the average Rha/Ara ratio from PF was higher than those from UF.

Although there were no changes in composition, the DE was the characteristic of these polysaccharides most affected by high pH values, and the changes in this parameter support the explanations given above: peaks A-1 and A-2, the most important in UF, had DEs of 75.44% and 35.42%, respectively (Jiménez et al., 1994b), while that of the polymers isolated from PF was 28.43%. This represents a marked decrease. The DE of a pectin is a very important characteristic, affecting cell wall structure and fruit firmness. At low DE values the pectic macromolecules suffer conformational changes: they uncoil from their rigid, native, molecular configuration to form an elastic, linear one, which presents strong repulsive forces if its acidic groups are not neutralized (Rees et al., 1982). In pickled cucumbers the critical DE has been studied (Hudson and Buescher, 1986): a DE lower than 12.3% produces the most marked losses in texture. The final DE in processed olive fruit was 28.43%. Thus, if the processing conditions were stronger or if the DE decreased further, the fruits might lose their firmness. This would lead to their being rejected by consumers and could present problems during the industrial manipulation of the fruits (stoning, stuffing, and packing processes).

The molecular weight was another characteristic studied that did not change very much during processing: around 360 000 in UF and 400 000 in PF. Ben-Shalom et al. (1992), working with blanched carrots, found similar results: pectins of high molecular weight were isolated from blanched tissue, suggesting that interchanges of pectic fractions occurred in the cell wall and that newly degraded pectins appeared in this fraction because of their low DE.

To conclude, it can be said that, besides the great increase in the yield of this fraction, the decrease in the DE was the most noteworthy change. Both of these observations have important implications for fruit firmness during processing. Jiménez et al. (1995) observed a marked decrease in texture during lye treatment and washing and a marked increase in this parameter after soaking the fruits in brine. The polymers with a low degree of esterification are those which are mainly

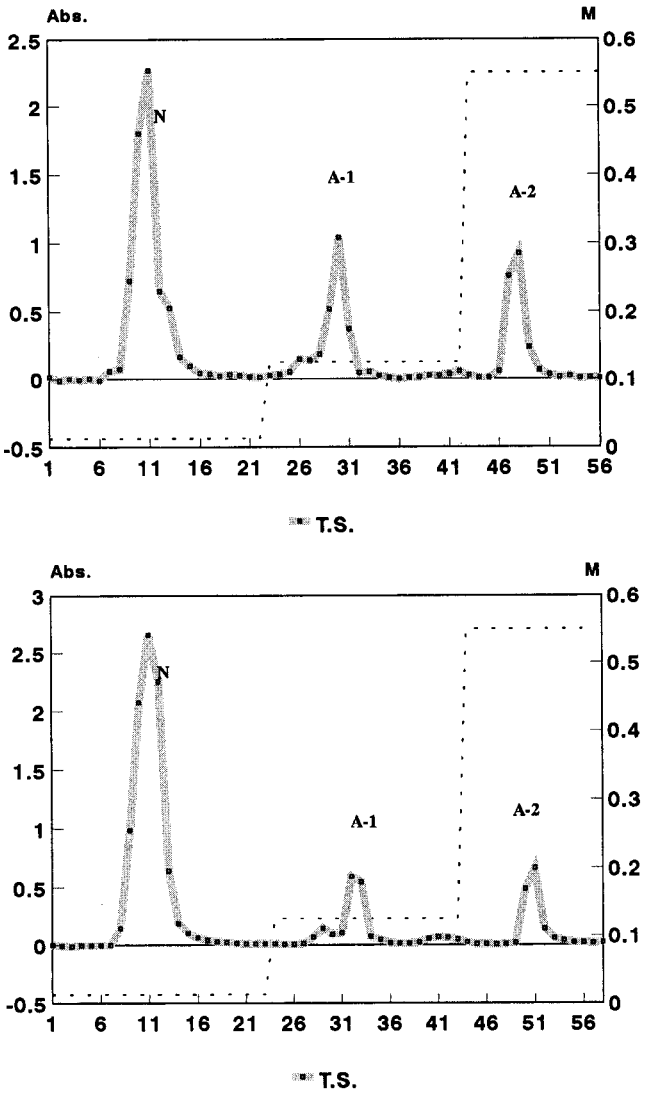

Figure 3. Elution profiles of hemicelluloses B (HB) in ion exchange chromatography: upper, unprocessed fruit; lower, processed fruit. The dotted line corresponds to the molar concentration of the buffer. N, neutral fraction; A-1, A-2, acidic fractions.

stabilized in the cell wall by ionic bonds with calcium. Thus, an increase in their amount or in their ionic charge could produce a substantial recovery of the firmness lost by the strong treatment with sodium hydroxide, providing that positive ions (calcium, sodium, or even low pH values) were available in the medium to neutralize these newly created charges.

Changes in Hemicelluloses B. The elution profiles of this fraction during ion exchange (Figure 3) showed only a slight decrease in the yield of acidic fractions and a delay in their elution. This could indicate a decrease in their molecular weight, as will be discussed below.

No marked changes occurred in the sugar composition (Table 3) as a result of processing. In the absence of any further analysis the conclusion can be drawn that the same polysaccharides are present in PF as in UF (Jiménez et al., 1994b). In the neutral fraction the polysaccharides are mainly xyloglucans and glucomannans, and in the first and second acidic fractions they are mainly arabinoxylans and arabinans.

Gel filtration allowed a more detailed analysis of the fractions. In Table 4, the results obtained from the neutral fraction of PF are presented. The decrease in the molecular weight of these polysaccharides is par-

Table 3. Composition of Fractions Isolated from Hemicelluloses B (HB)[a]

	N		A-1		A-2	
	UF	PF	UF	PF	UF	PF
yield	28.60	24.14	19.41	11.11	17.53	16.19
total carboh	89.15	90.67	69.08	70.37	34.94	33.62
Rha	0.3 [0.3]	0.5 [0.5]	1 [1]	1 [1]	6 [7]	3 [3]
Fuc	0.5 [0.5]	0.5 [0.5]	0.2 [0.2]	0.3 [0.3]	0.6 [0.7]	0.3 [0.3]
Ara	9 [9]	8 [8]	12 [12]	15 [15]	44 [49]	49 [51]
Xyl	21 [21]	21 [21]	61 [63]	57 [58]	23 [25]	29 [30]
Man	17 [17]	18 [18]	4 [4]	5 [5]	2 [2]	2 [2]
Gal	12 [12]	12 [12]	5 [5]	6 [6]	7 [8]	6 [6]
Glc	40 [40]	40[40]	1 [1]	1 [1]	2 [2]	0.8 [1]
UA	0.3	0.2	3	2	10	4

[a] Upper, yield (% of the total weight recovered by ion exchange) and total carbohydrates (% in weight of the total weight of the fraction); lower, sugar composition [molar fraction (the data in brackets represent the molar fraction of the neutral sugars)].

Table 4. Molecular Weight and Composition (as Molar Fraction) of the Five Peaks Isolated from the Neutral Fraction (N) of the Hemicelluloses B of Processed Fruit[a]

	1	2	3	4	5
MW	170 000	75 000	40 000	20 000	10 000
Rha	1 [1]	0.7 [0.7]	0.6 [0.6]	ND	ND
Fuc	ND	ND	ND	0.2 [0.2]	ND
Ara	9 [9]	7 [8]	6 [6]	6 [6]	3 [3]
Xyl	29 [30]	20 [21]	14 [14]	8 [8]	6 [6]
Man	2 [2]	14 [15]	23 [24]	30 [30]	36 [38]
Gal	13 [13]	16 [17]	17 [18]	17 [17]	16 [17]
Glc	43 [44]	40 [43]	37 [38]	38 [38]	35 [36]
UA	2	6	3	1	4

a The data in brackets represent the molar fraction of the neutral sugars. ND, nondetected.

Table 5. Molecular Weight and Composition (as Molar Fraction) of the Five Peaks Isolated from the First Acidic Fraction (A-1) of the Hemicelluloses B of Processed Fruit[a]

	1	2	3	4	5
MW	>400 000	130 000	60 000	30 000	20 000
Rha	ND	3 [3]	ND	ND	ND
Fuc	ND	ND	ND	ND	ND
Ara	8 [9]	23 [26]	19 [22]	10 [12]	7 [8]
Xyl	55 [64]	29 [33]	40 [47]	55 [64]	60 [72]
Man	2 [2]	6 [7]	8 [9]	7 [8]	5 [6]
Gal	5 [6]	12 [13]	8 [9]	6 [7]	5 [6]
Glc	16 [19]	16 [18]	11 [13]	9 [11]	7 [8]
UA	14	11	14	14	17

a The data in brackets represent the molar fraction of the neutral sugars. ND, nondetected.

ticularly noteworthy. In UF their average MW was 260 000 but after processing, a range of 170 000 to 10 000 was recorded. Furthermore, the two kinds of polysaccharides that composed this fraction, were isolated: the first two peaks consisted mainly of xyloglucans, whereas the lower MW peaks consisted mainly of galactoglucomannans. The composition of the first acidic fraction of PF (Table 5) confirmed the existence of very pure arabinoxylans: their MW also decreased (ranging from >400 000 to 20 000). However, all of the peaks isolated had a very similar compositions. As discussed previously (Jiménez et al., 1995), these polysaccharides are probably mainly affected by the activities of endogenous and/or exogenous enzymes, such as cellulases, xylanases, or even xyloglucan endotransglycosylases (XET), as well as microbial enzymes. The extreme pH values (high during lye treatment and low during fermentation) are another factor to take into account, because both of them could alter these polysaccharides. High pH values may affect them due to alkaline degradation, and a low pH could lead to hydrolysis to some extent, as was mentioned above with respect to arabinans.

Final Remarks. During olive processing two main steps are required (treatment at high pH values and a lactic fermentation). These steps lead to marked changes in the composition and structure of cell wall polysaccharides. In a previous paper (Jiménez et al., 1995), these changes were described for the first time and each of them was correlated with the steps during processing. A hypothesis was put forward on the textural changes and their relationship with polysaccharides. Because of the lye treatment and the subsequent wash, texture decreased by about 40% of the initial value. There was a decrease in the WSF and in the pectins solubilized by delignification, and an increase in the OSF, this probably being caused by the high pH values. After reaching equilibrium in brine, the fruits recovered about 20% of the original texture. Fermentation leads to a further decrease in firmness. In this last step, only changes in hemicelluloses were quantified, which could be related to enzymatic activity and/or low pH values.

The findings presented in the current paper lend support to the hypothesis put forward previously to explain the very complex changes in firmness during processing. The increase of OSF and the decrease in its DE could be caused by the high pH values during lye treatment: ester and phenolic linkages may be broken, leading to an increase in OSF and probably also affecting the hemicelluloses to some extent. Owing to the electrostatic repulsion caused by the low DE, quantified during the present work, and to the breakdown of inter- and/or intrapolymer covalent bonds, the firmness of the fruit decreases greatly. However, the texture increases when the fruits are soaked in brine: again the low DE and the high proportion of these deesterified polysaccharides could be the main factors responsible, as cations, such as Na^+, present in brine may stabilize the negatively charged polymers. The rapid decrease in the pH that takes place at the beginning of the fermentation could also contribute in the same way, facilitating the recovery of firmness. The great decrease in molecular weight experienced by the hemicelluloses, observed in the present study, together with the changes in their solubility during fermentation, described previously (Jiménez et al., 1995), could explain the loss of texture after fermentation. This effect could be due to the low pH values (<4) experienced or enzymatic activities. The possible effects of enzymatic activities on the complex modifications to cell wall polymers and the implications that these have on texture remain to be clarified and will be the subject of future research.

ABBREVIATIONS USED

UF, unprocessed fruit; PF, processed fruit; WSF, water-soluble fraction; OSF, oxalate-soluble fraction; DE, degree of esterification; NS, neutral sugars; UA, uronic acids; MW, molecular weight.

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