Olive Fruit Cell Wall: Degradation of Pectic Polysaccharides during Ripening

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Olive fruits at three stages of ripening (green, cherry, and black) have been studied. After cell wall isolation, the compositions of the cell wall and that of the phosphate-soluble polysaccharides were determined. In cell walls, decreases in arabinose, xylose, glucose, and uronic acid levels were observed, together with a slight increase in mannose on ripening. At the beginning of ripening, fragments of pectic polymers were the major constituents of the phosphate-soluble fraction, with the hemicellulosic ones increasing toward the end of the process. The molecular weight of the fragments solubilized was ~6 kDa. After cell wall fractionation, the pectic polysaccharides soluble in imidazole and sodium carbonate were also studied. In both fractions, between the green and cherry stages of ripening, a significant loss of homogalacturonans took place. Between the cherry and black stages of ripening, rhamnogalacturonan side chains were also released in addition to homogalacturonans. In any of the pectic fractions, changes in apparent molecular weight were quantified.

Keywords: *Olive fruit; cell wall; ripening; pectic polysaccharides*

INTRODUCTION

The ripening of most fleshy fruits is usually characterized by softening, with the modification and degradation of cell wall components being the main factors responsible for this process. Changes in the pectic fraction of the cell wall have been the most often studied parameters and can involve increases in solubility, depolymerization, deesterification, and loss of neutral sugar side chains (*1*). Such modifications have been described for persimmon (*2*), apples and kiwifruit (*3*), muskmelon (*4*), bell pepper (*5*), and apricot fruit (*6*).

The olive fruit is a very important crop in Spain, particularly in Andalucia, where olives provide the basis for two important industries: olive oil and pickled olive production. The Hojiblanca variety is one of the most sought after for both industries. In the pickling industry, these olives are processed into “Spanish green olives”, “black oxidized olives”, and “natural olives in brine”. Olive oil from the Hojiblanca is particularly appreciated for its quality, having excellent organoleptic characteristics and a moderate stability to oxidation (*7*).

The quality and yields in both types of production depend on several factors. Among the most important is the ripening stage of fruits and hence the structural characteristics of cell wall polysaccharides. In pickling, each kind of processing is traditionally applied to olives at a determinate ripening stage. In oil production, the yield and quality of oil are directly related to this characteristic. Consequently, the study of cell wall polysaccharides and their changes during ripening is an important key for optimizing output in the pickling and olive oil production industries. The aim of the present study has been to examine the changes that take place in the general composition of cell wall material (CWM) and soluble polysaccharides, changes that would summarize the evolution of pectic and hemicellulosic polysaccharide levels in the olive fruit. Particular attention has been paid to pectic polysaccharides in this paper.

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MATERIALS AND METHODS

Samples. Olive samples of the Hojiblanca variety were provided by Agrosevilla-Aceitunas (La Roda de Andalucía, Sevilla). Olive fruits were sorted by hand according to skin color of the fruit in order to have representative samples of the three stages of ripeness: green (G), cherry (C), and black (B).

Texture and weight per fruit were determined in fresh olives, with samples then frozen at −20 °C until CWM isolation.

Texture Determination. The texture of fruits was measured using an Instron texturometer model 1011 fitted with a shear press cell. The operating speed was set at 200 mm/min, and the force scale was 0−500 N. Results are expressed as the mean of 15 replicates.

Preparation of the CWM. The CWM was prepared according to the method of Selvendran (*8*) adapted for use with olives (*9*): Fifty grams of depitted flesh was homogenized in 200 mL of 20 mM sodium phosphate buffer, pH 7. After 1 h of stirring at 4 °C, the suspension was centrifuged at 17700*g* for 40 min. The supernatant constituted the phosphate-soluble fraction (PSF). This fraction and following were concentrated under vacuum, dialyzed (1200 cutoff for PSF and 12000 for the rest of the fractions), and freeze-dried. The pellet was washed twice in 100 mL of water. The residue was homogenized in 200 mL of 1% (w/v) sodium dodecyl sulfate (for synthesis; Merck, Hohenbrunn, Germany). After stirring overnight at 4 °C, the suspension was centrifuged at 17700*g* for 40 min. The wet residue was homogenized twice in 100 mL of a solution of phenol (BDH, Poole, U.K.)/acetic acid (for analysis; Panreac, Barcelona, Spain)/water (2:1:1, w/v/v),

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washed several times with water, and centrifuged in the same conditions. CWM was washed (suspended, stirred, and filtered) in acetone (extra pure; Scharlau, Barcelona, Spain) until the final supernatant was colorless. CWM was air-dried and stored at −30 °C.

Extraction of Pectic Polysaccharides. The cell wall pectic polysaccharides were fractionated into two groups: the imidazole−HCl soluble fraction (ISF) and the carbonate-soluble fraction (CSF) (*10*), operating in the following way: One gram of the CWM was sequentially extracted with (i) 0.5 M imidazole (puriss.; Fluka, Buchs, Switzerland)/hydrochloric acid (Panreac, puriss.) buffer, pH 7 (two times, 100 mL) at room temperature for 12 h in the case of the first extraction and for 3 h in the second (ISF) and (ii) 0.5 M sodium carbonate (Sigma, Barcelona, Spain), 20 mM in sodium borohydride (Sigma) (two times, 100 mL) for 16 h at 4 °C in the first extraction and for 3 h at room temperature in the second (CSF).

Polysaccharide Analysis. Uronic acids (UA) from the different fractions were quantified by using the phenylphenol method (*11*). Absorbance values of samples and standards were measured at 540 nm in a microplate reader (model 550; Bio-Rad, Hercules, CA). Glycosyl compositions of the different fractions were determined by GC and quantified as alditol acetates (*12*). The fractions were hydrolyzed with 2 N trifluoroacetic acid (121 °C, 1 h) (*13*). A Hewlett-Packard 5890 series II chromatograph, fitted with a 30 m fused silica capillary column (SP-2330 from Supelco, Bellefonte, PA) was employed. The oven temperature program used was as follows: initial, 180 °C, 7 min; raised at 3 °C/min up to 220 °C, 15 min. The carrier gas was helium at a flow rate of 1 mL/min. The injector temperature was 250 °C, and the FID temperature was 300 °C. The split ratio was 1/100.

Neutral sugars (NS) from ion exchange fractions were determined colorimetrically according to the anthrone assay of Dische (*14*).

Ion Exchange Chromatography. Five milliliter Hi-Trap columns (anionic exchanger: Q-Sepharose) from Pharmacia (Uppsala, Sweden) were used, equilibrated in 0.01 M imidazole−HCl buffer, pH 7. The flow during injection was 1 mL/min and during elution, 4 mL/min. Neutral polysaccharides were collected in 10 mL fractions and the acidic polysaccharides in 5 mL fractions. The column was washed with 1 bed volume of the equilibrium buffer to collect neutral polysaccharides. Four steps in the fractionation were made with increasing buffer concentrations: 0.5, 1.0, 1.5, and 2.0 M. All fractions were assayed for UA and NS by colorimetry, as described aboved. The chromatographic fractions corresponding with the peaks were collected, dialyzed, and analyzed for sugar composition by GC and molecular weight distribution by HPSEC.

HPSEC Analysis. A Hewlett-Packard series 1100 system, having a filter and precolumn and fitted with two different Tosohaas (Montgomeryville, PA) columns (7.8 mm i.d. × 30 cm) in sequence [TSK gel GMPWXL (dextran MW < 50000 kDa) and TSK gel G3000PWXL (dextran MW < 60 kDa], was used. The eluent buffer was 0.1 M imidazole−HCl, pH 7, at a flow of 0.6 mL/min. Under these conditions, the system was calibrated with dextrans of 250, 110, 40, and 6 kDa (Fluka). The injection volume was 20 μL, and the fractions collected were each of 0.3 mL. NS or UA assay was made on each tube, depending on the major components.

Statistical Analysis. Data were statistically analyzed through their variance. Means were compared by Duncan's multiple-range test ($P < 0.05$).

RESULTS AND DISCUSSION

In this work, three olive samples were analyzed at the green (G), cherry (C), and black (B) stages of ripeness. The textures of the samples were 63.23, 40.58, and 24.45 N/g, respectively, showing a degree of softening between the G and C stages similar to that between the C and B stages.

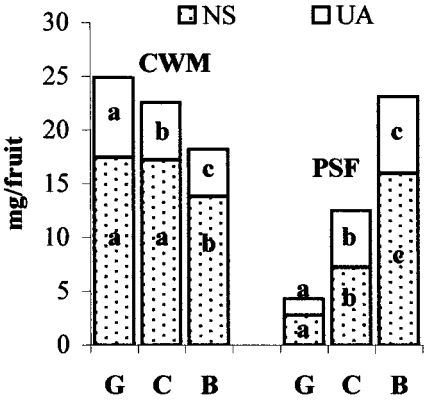

Figure 1. Composition of CWM and PSF of olive fruit at three stages of ripening (G, C, and B). NS and UA are expressed as milligrams per fruit. Bars with different letters indicate significant differences.

CWM was isolated and the fraction of phosphate-soluble polysaccharides obtained (PSF) was studied by GC, ion exchange chromatography, and HPSEC. These same analyses were made on pectic fractions (ISF and CSF). The changes found among these polysaccharides are worthy of comment.

The weight of olive fruits varies over the course of the ripening process due to changes mainly in water and oil contents. Such important compositional changes could produce apparent changes in the analytical results from the CWM, even if there were no actual change at all. Thus, the data have been expressed as milligrams per fruit, such that the results are not affected by changes in other olive fruit components.

CWM and PSF. In Figure 1, the total sugar contents of CWM and PSF are shown, indicating the amount of NS and UA. NS in CWM shows significant differences only between C and B fruits, but UA decreased throughout the ripening process. This fact could indicate that the metabolism of pectic polysaccharides was more active at the beginning of the ripening process, but neutral polysaccharides were metabolized in a higher proportion in subsequent stages. Such a result suggests a sequential degradation of cell wall polysaccharides throughout the ripening process. PSF underwent a very strong increase during the ripening process. In the first stage of ripening, the increases in NS (4.42 mg/fruit) and UA (3.82 mg/fruit) were very similar. In the second stage, the main change was clearly that of NS, which increased by 8.78 mg/fruit between C and B, compared to UA, which increased by only 1.79 mg/fruit between C and B.

Glycosyl compositions of CWM and PSF are summarized in Table 1. The neutral sugars that decreased significantly in CWM were arabinose, xylose, and glucose. Xylose and glucose showed significant differences only between C and B, whereas arabinose had a more gradual decrease through the three stages of ripening. Because arabinose is mostly a pectic sugar, its behavior was coincident with that of UA, decreasing during the whole ripening process. Mannose, a minor sugar, was the only one that underwent a small increase between C and B. Similar results have been reported in bell pepper (*Capsicum annum*) (5), where the loss of arabinose and galactose but not of rhamnose suggested a decrease in the proportion of arabinogalactan side chains of pectins, in addition to a loss of homogalactu-

Table 1. Glycosyl Composition of CWM and PSF of Olive Fruit at Three Stages of Ripening (Green, Cherry, and Black)[a]

	Rha	Fuc	Ara	Xyl	Man	Gal	Gle	UA
				Cell Wall Material				
green	0.44 ± 0.11 a	nd	4.33 ± 0.50 a	3.66 ± 0.47 a	0.52 ± 0.02 a	0.83 ± 0.13 a	7.67 ± 0.49 a	7.43 ± 0.63 a
cherry	0.42 ± 0.09 a	nd	3.89 ± 0.50 ab	4.05 ± 0.97 a	0.45 ± 0.05 a	0.76 ± 0.11 a	7.62 ± 0.33 a	5.38 ± 0.33 b
black	0.43 ± 0.01 a	nd	3.26 ± 0.13 b	2.83 ± 0.06 b	0.61 ± 0.02 b	0.82 ± 0.04 a	5.85 ± 0.35b	4.38 ± 0.35 c
				Phosphate-Soluble Fraction				
green	0.16 ± 0.01 a	nd	0.23 ± 0.01 a	0.12 ± 0.01 a	0.20 ± 0.01 a	0.26 ± 0.01 a	1.77 ± 0.04 a	1.52 ± 0.49 a
cherry	0.55 ± 0.05 b	nd	0.86 ± 0.06 b	0.26 ± 0.01 b	0.46 ± 0.02 b	0.64 ± 0.03 b	4.41 ± 0.23 b	5.34 ± 0.95 b
black	0.75 ± 0.02 c	nd	1.73 ± 0.14 c	0.40 ± 0.03 c	1.10 ± 0.08 c	1.26 ± 0.09 c	10.72 ± 0.48 c	7.13 ± 0.34 c

[a] Results are expressed as mg/fruit and are the average value of four replicates. Data with different letters in the same column indicate significant differences. nd, not detected.

Table 2. Glycosol Composition of Both Fractions Eluted by Ion Exchange Chromatography (Neutral and A1) from the PSF[a]

		glycosyl composition (%)								
	%[b]	Rha	Fuc	Ara	Xyl	Man	Gal	Gle	UA	Rha:Ara:Gal:UA
neutral										
green	71.89	3	0	6	10	7	15	52	7	1:2:5:2.3
cherry	64.26	4	2	6	8	6	15	53	6	1:15:3.7:1.5
black	47.49	5	4	8	5	5	12	57	4	1:1.6:3:0.8
A1										
green	20.89	9	0	26	1	2	19	35	8	1.1:3.3:2.4:1
cherry	26.87	11	1	21	0	2	16	39	10	1.1:2.1:1.6:1
black	38.36	12	2	16	1	2	13	44	11	1.1:1.4:1.2:1

[a] Results are expressed as percent. [b] This percentage represents the percentage of the fraction on the total sugar recovered after chromatograph.

ronans. Minor changes in hemicellulosic neutral sugars indicate only small changes in hemicellulose composition.

In PSF, all of the sugars underwent significant increases in both stages of ripening. Between G and C, UA, rhamnose, and arabinose increased by a total of 4.84 mg/fruit, whereas the increase for the rest of the sugars accounted for 3.42 mg/fruit. This finding supports the hypothesis of a higher metabolism of pectic polymers at the first stage studied. However, between C and B the solubilization of sugars related to hemicelluloses was more significant than before, totaling 7.71 mg/fruit (sum of increases of xylose, mannose, galactose, and glucose) compared with 2.86 mg/fruit as the sum of UA, rhamnose, and arabinose. Between C and B the metabolism of pectic polymers decreased and that of neutral polysaccharides showed significant activation. These results are coincident with those described in CWM and support a different metabolism at each stage of ripening. Sakurai and Nevins (*15*) found very similar results working with avocado (*Persea americana* Mill) in which UA increased in the water-soluble fraction by 38% after 1 day of storage at 25 °C. The NS content gradually increased in the first 2 days and then increased markedly. Among the NS, rhamnose and arabinose increased after day 0 and xylose and galactose from day 3. These results have the same pattern as those described in the present study on olive fruits.

If CWM and PSF results are added together, it can be seen that the total amount of sugar increased with ripening. Rhamnose, mannose, galactose, glucose, and UA showed significant differences. Rhamnose increased between G and C, whereas UA displayed a smooth increase through the three stages. These augmentations point to a synthesis of polysaccharides, mainly hemicelluloses, during ripening.

PSF was analyzed by ion exchange chromatography. Three fractions were eluted from each sample, one neutral and two acidic [eluted with 0.5 (A1) and 1.0 M (A2) imidazole buffer]. Because the neutral and A1 fractions were the major fractions eluted, these will be discussed below. Their glycosyl compositions are presented in Table 2.

It is important to point out that the content of UA recovered decreased to about one-fifth of the initial value. As the recovery of NS was good (~80%), it could be concluded that the fragments of homogalacturonans solubilized with the phosphate treatment probably remained linked to the exchanger and could not be released even with 2.0 M imidazole buffer. As ripening progressed, the proportion of the neutral fraction on the total recovered decreased from 72% in G to 47% in B, the A1 increasing from 21 to 38%.

Glucose was the main sugar in all of the fractions, its percentage ranging between 35 and 57% depending on the fraction. The percentage of glucose increased with ripening in both fractions. In the neutral fraction only galactose exceeded 10%, with rhamnose, arabinose, galactose, and UA being important sugars in A1 in addition to glucose. The ratio between them in the three samples shows that arabinose and galactose decreased over the entire ripening process while rhamnose increased, suggesting that fragments of rhamnogalacturonans arriving in this fraction are different depending on the stage of ripeness. Between G and C, the pectic fragments recovered in this fraction had the same branching degree (the same ratio of Rha to UA) but shorter side chains (lower proportion of arabinose and galactose). However, between C and B, the ratio of Rha to UA increased, thus showing an enrichment in rhamnogalacturonan fragments with shorter side chains (arabinose and galactose continued to decrease).

The molecular size distribution of both fractions, neutral and A1, of the three samples is presented in Figure 2. In green fruits, the neutral fraction showed two peaks, a minor one between 110 and 40 kDa and a major one, lower than 6 kDa. As ripening progressed, the minor peak smoothed and almost disappeared in black fruits, whereas the major peak remained unchanged. More important changes took place in the A1

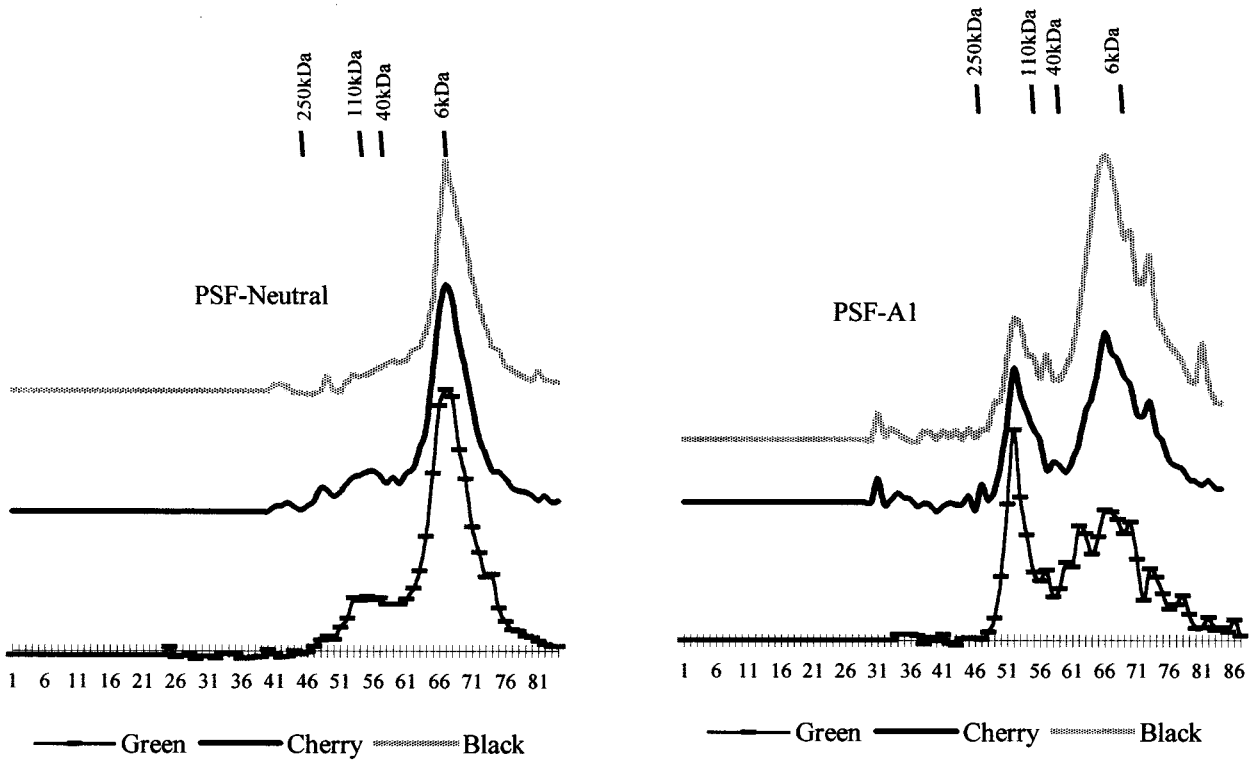

Figure 2. Profiles of elution of neutral and A1 fraction from PSF of olive fruit at three stages of ripening (G, C, and B). Abscissa: absorbance at 630 nm (anthrone assay).

fraction. In G, the first peak eluted near 110 kDa and there was another broad peak centered at ~6 kDa. The evolution of the molecular weight of this fraction during ripening was very clear. While the 110 kDa peak decreased, the 6 kDa peak increased, showing that both glucan and pectic fragments collected with phosphate buffer were of lower molecular weight. The same evolution of molecular weight was found in avocado (*15*), where in the NS elution profile there was a diminution in the minor peak (high molecular weight) but in the elution of UA there was a substantial diminution of molecular weight. This intense depolymerization of pectic polymers seems to be a necessary step for normal ripening, as happens in nectarines (*Prunus persica* var. *nectarina*) (*16*). At the beginning of the normal ripening process of the nectarine, there is a solubilization of pectic polymers of high and intermediate molecular weights, whereas later in the process depolymerization takes place. If this last step does not occur, the fruit becomes mealy, which is signified by an accumulation of high molecular weight pectins with high neutral sugar levels. Therefore, it appears that without the removal of neutral side chains, pectins are not solubilized from the cell wall. Consequently, this process is an essential part of pectic metabolism during ripening.

Composition of Pectic Fractions. In Figure 3, the amounts of NS and UA in the pectic fractions are presented. In general, decreases in both pectic fractions (ISF and CSF) were observed. The UA decreased significantly in the three samples studied. The case of NS was different in that no significant differences in amounts were observed between G and C.

The decrease in ISF is not an ever-present event in fruit ripening. In peaches (*Prunus persica*) (*17*), strawberries (*Fragaria ananasa* Duch. cv. Toyonoka) (*18*), and bell peppers (*5*), decreases in chelator-soluble pectins

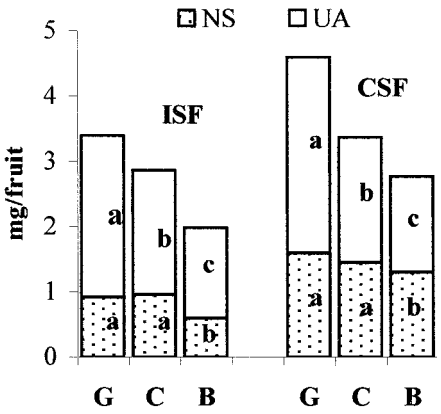

Figure 3. Composition of ISF and CSF of olive fruit at three stages of ripening (G, C, and B). NS and UA are expressed as milligrams per fruit. Bars with different letters indicate significant differences.

have been described. On the other hand, in Spanish pears (*Pyrus communis* cv. Blanquilla) (*19*), apples (*Malus domestica* cv. Golden Delicious) (*20*), nectarines (*16*), and apricots (*Prunus armeniaca* cv. Canino) (*6*), significant increases in this same fraction have been reported. These pectic polymers are of special interest because of their ability to form coordination complexes with calcium. These structures are very important from the point of view of increasing fruit and vegetable texture (*21*−*23*).

ISF. The glycosyl composition of this fraction before and after ion exchange chromatography is presented in Tables 3 and 4. Before ion exchange, in addition to the significant decrease in UA, there was a loss of arabinose. The ratio Rha:Ara:Gal changed from 1.2:11.7:1 to 1:9.9:1 in C and finally to 1.4:7.6:1 in B, suggesting a lowering in the number and/or length of arabinan side chains. There is also reported to be an important loss of

Table 3. Glycosyl Composition of the ISF of Olive Fruit at Three Stages of Ripening (Green, Cherry, and Black), Prior to Ion Exchange Chromatography[a]

	Rha	Fuc	Ara	Xyl	Man	Gal	Gle	UA
green	0.07 ± 0.02 a	nd	0.70 ± 0.09 a	0.02 ± 0.00 a	0.02 ± 0.01 a	0.06 ± 0.01 a	0.04 ± 0.01 a	2.48 ± 0.17 a
cherry	0.07 ± 0.02 a	nd	0.69 ± 0.07 a	0.02 ± 0.00 a	0.02 ± 0.00 a	0.07 ± 0.01 a	0.08 ± 0.05 a	1.91 ± 0.12 b
black	0.07 ± 0.00 a	nd	0.38 ± 0.01 b	0.02 ± 0.01 a	0.01 ± 0.00 a	0.05 ± 0.00 a	0.06 ± 0.01 a	1.39 ± 0.11 c

[a] Results are expressed as mg/fruit and are the average value of four replicates. Data with different letters in the same column indicate significant differences.

Table 4. Glycosyl Compostion of Both Fractions Eluted by Ion Exchange Chromatography (A1 and A2)[a]

	%[b]	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
A1									
green	76.03	3	0	14	0	0	1	1	80
cherry	67.62	4	0	20	0	0	2	3	71
black	61.59	4	1	22	0	0	2	3	67
A2									
green	23.97	2	0	7	0	1	1	2	87
cherry	38.41	2	0	9	0	0	1	3	85
black	32.38	3	0	13	0	1	2	5	76

[a] Results are expressed as percent. [b] This represents the percentage of the fraction on the total sugar recovered after chromatography.

galactose in most fruits because of the degradation on an arabinogalactan, which is very abundant in cell walls as pectic side chains or as a polysaccharide strongly linked to hemicelluloses and/or cellulose (*3*). In the case of olives, this polysaccharide is not present in the CWM in significant amounts, with arabinans being the polymers that seem to be linked to most of the wall polymers (*9*, *10*, *24*−*26*).

Two peaks were isolated using ion exchange chromatography, the first one (A1) eluting with 1.5 M imidazole buffer and the second (A2) with 2.0 M buffer. The behavior of each fraction differed with the stage of ripeness. The A1 peak decreased, whereas A2 increased from G to C but decreased from C to B. The enzymatic activity during ripening could increase the charge density, diminishing the degree of esterification and/or decreasing the molecular weight. These activities could explain the decrease of A1 and the increase of A2 between G and C. At the black stage, both fractions diminished.

In both fractions, there was a more significant loss of UA. The ratio NS:UA increased in A1 from 0.24 to 0.48 and in A2 from 0.16 to 0.31. In A1, the ratio between the main neutral sugars Rha:Ara:Gal remained almost unchanged, but the proportion of UA decreased. These data suggest that there was a major loss of homogalacturonan fragments during ripening. Unlike A1, the changes in A2 were different for the three stages. From G to C, the ratio Rha:Ara:Gal:UA remained almost fixed, showing only small increases in Ara. However, it was only between C and B that a major loss of UA was observed, with NS being in a constant ratio. Again, a more important loss of homogalacturonans could be suggested.

The molecular weight distribution of both fractions was determined (Figure 4). A1 polysaccharides from green fruits eluted in a broad peak between 250 and 40 kDa. As ripening progressed, the peak broadened, the maximum being displaced slightly to a higher molecular weight. A2 fractions did not show apparent changes in their profiles, eluting in a sharper peak around 250−110 kDa.

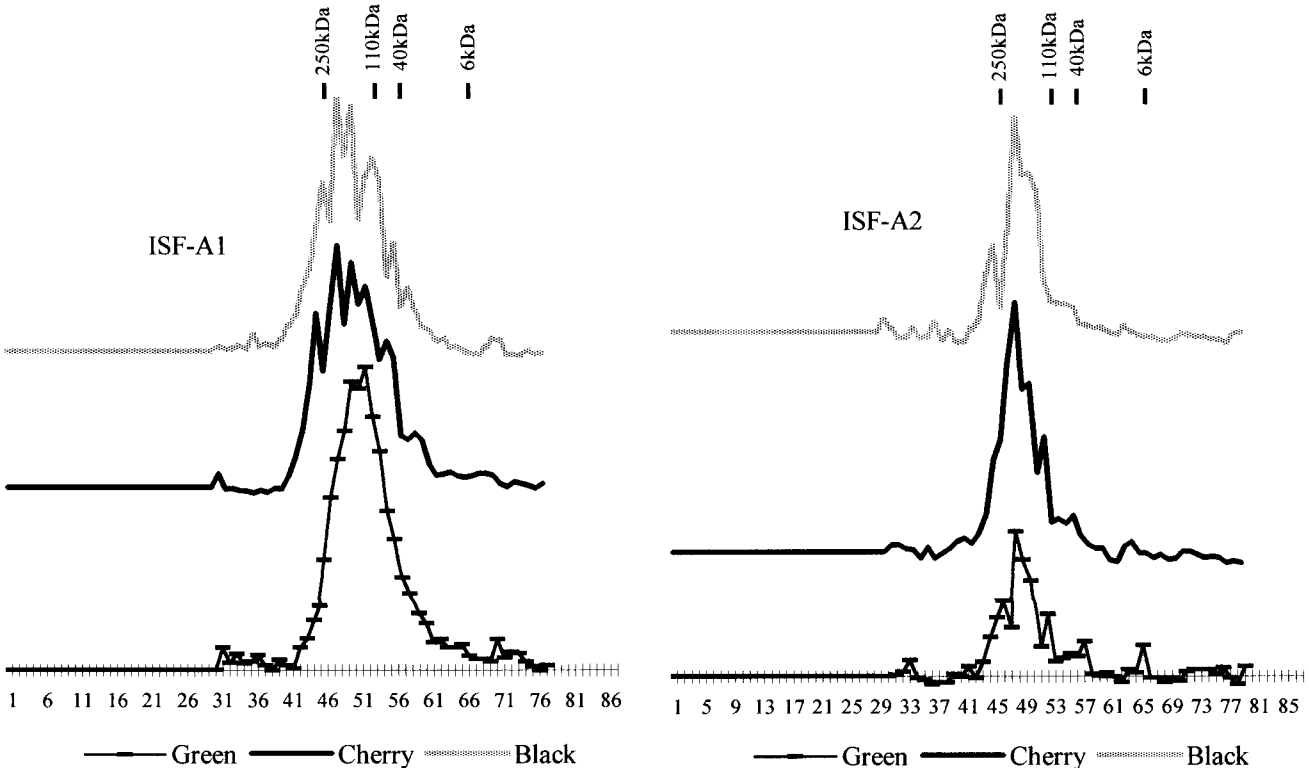

Figure 4. Profiles of elution A1 and A2 fractions from ISF of olive fruit at three stages of ripening (G, C, and B). Abscissa: absorbance at 520 nm (phenylphenol assay).

Table 5. Glycosyl Composition of CSF of Olive Fruit at Three Stages of Ripening (Green, Cherry, and Black), Prior to Ion Exchange Chromatography[a]

	Rha	Fuc	Ara	Xyl	Man	Gal	Gle	UA
green	0.16 ± 0.04 a	nd	1.17 ± 0.02 a	0.04 ± 0.01 a	0.01 ± 0.00 a	0.16 ± 0.02 a	0.05 ± 0.01 a	3.00 ± 0.28 a
cherry	0.16 ± 0.04 a	nd	1.07 ± 0.01 b	0.03 ± 0.00 a	0.01 ± 0.00 a	0.13 ± 0.01 b	0.04 ± 0.00 a	1.92 ± 0.10 b
black	0.13 ± 0.00 a	nd	0.96 ± 0.00 c	0.03 ± 0.01 a	0.01 ± 0.00 a	0.12 ± 0.00 b	0.04 ± 0.01 a	1.47 ± 0.15 c

[a] Results are expressed as mg/fruit and are the average value of four replicates. Data with different letters in the same column indicate significant differences.

Table 6. Glycosyl Compostion of Both Fractions Eluted by Ion Exchange Chromatography (A1 and A2)[a]

	%[b]	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
green	100	3	0	22	1	0	4	2	68
cherry	100	4	0	25	1	0	4	1	66
black	100	4	0	27	0	0	4	3	63

[a] Results are expressed as percent. [b] This represents the percentage of the fraction on the total sugar recovered after chromatography.

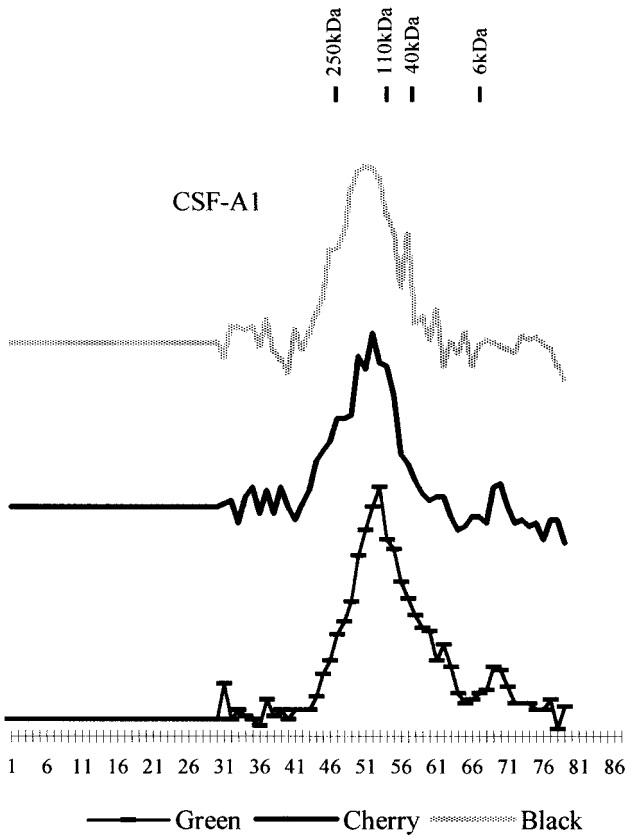

Figure 5. Profiles of elution of the only fraction from CSF of olive fruit at three stages of ripening (G, C, and B). Abscissa: absorbance at 520 nm (phenylphenol assay).

CSF. This fraction lost UA and NS during ripening, with the loss of UA being more considerable. The glycosyl composition is presented in Tables 5 and 6. There were significant losses of arabinose and galactose. Besides homogalacturonans, a small proportion of the side chains of rhamnogalacturonans was lost.

In ion exchange experiments, this fraction eluted in only one peak (A1) with 1.5 M imidazole buffer. The significant decrease in UA resulted in an increased proportion of arabinose, although its net amount decreased with ripening.

The molecular weight distribution of A1 was determined (Figure 5). There were no changes in the profiles, the polysaccharides from the three stages of ripeness eluting in a single peak with a maximum near 110 kDa.

Very few reports have been published of changes in molecular weights of pectic fractions, chelator-soluble fractions, and CSF in fruits. Recently, only Hegde and Maness (*27*), working with peaches, showed increases in the apparent molecular weights of both pectic fractions. However, in most cases, as with olives, no changes have been described (*16*, *18*, *28*). Some authors (*29*, *30*) came to the conclusion that pectin aggregation potentially makes gel filtration a poor technique for molecular weight determination of pectins, particularly at ionic strengths <100 mM.

Final Remarks. Pectic fractions are widely affected by the ripening process. In general, a release of homogalacturonans and a loss and/or shortening of side chains in rhamnogalacturonans take place, although there is a gradation in both events. Between green and cherry olives, there is a major loss of homogalacturonans. Between cherry and black olives, in addition to the loss of homogalacturonans, rhamnogalacturonan side chains are also released. This difference in metabolic activity could explain the changes in composition of the PSF, although in the composition of this fraction the newly synthesized oligo- and/or polysaccharides during ripening could also be influential. Both NS and UA increased to almost the same extent in the first stage of ripening, whereas NS clearly increases in the second stage studied. Pectin-related sugars (UA, rhamnose, and arabinose) increased most in the first stage, whereas the hemicellulose-related sugars (xylose, mannose, galactose, and glucose) increase later in the ripening process.

ABBREVIATIONS USED

B, black stage; C, cherry stage; CSF, carbonate-soluble fraction; CWM, cell wall material; G, green stage; ISF, imidazole-soluble fraction; NS, neutral sugars; PSF, phosphate-soluble fraction; UA, uronic acids.

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LITERATURE CITED

(1) Seymour, G. B.; Gross, K. C. Cell wall disassembly and fruit softening. *Postharvest News Inf.* **1996**, *7*, 45−52.
(2) Cutillas-Iturralde, A.; Zarra, I.; Lorences, E. P. Metabolism of cell wall polysaccharides from persimmon fruit. Pectin solubilization during fruit ripening occurs in apparent absence of polygalacturonase activity. *Physiol. Plant.* **1993**, *89*, 369−375.
(3) Redgwell, R. J.; Fisher, M.; Kendal, E.; MacRae, E. A. Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. *Planta* **1997**, *203*, 174−181.
(4) Ranwala, A. P.; Suematsu, C.; Masuda, H. The role of β-galactosidases in the modification of cell wall components during muskmelon fruit ripening. *Plant Physiol.* **1992**, *100*, 1318−1325.

(5) Sethu, K. M. P.; Prabha, T. N.; Tharanathan, R. N. Postharvest biochemical changes associated with the softening phenomenon in Capsicum annun fruits. *Phytochemistry* **1996**, *42*, 961−966.
(6) Femenia, A.; Sánchez, E. S.; Simal, S.; Roselló, C. Developmental and ripening-related effects on the cell wall of apricots (*Prunus armeniaca*) fruit. *J. Sci. Food Agric.* **1998**, *77*, 487−493.
(7) Uceda, M.; Hermoso, M. La calidad del aceite de oliva. In *El Cultivo del Olivo*; Barranco, D., Fernández-Escobar, R., Rallo, L., Eds.; Junta de Andalucia-Ediciones MundiPrensa: Madrid, Spain, 1998; pp 547−572.
(8) Selvendran, R. R. Analysis of cell wall material from plant tissues: extraction and purification. *Phytochemistry* **1975**, *14*, 1011−1017.
(9) Jiménez, A.; Guillén, R.; Fernández-Bolaños, J.; Heredia, A. Cell wall composition of olive fruit. *J. Food Sci.* **1994**, *59*, 1192−1196, 1201.
(10) Sánchez-Romero, C.; Guillén, R.; Heredia, A.; Jiménez, A.; Fernández-Bolaños, J. Degradation of pectic polysaccharides in pickled green olives. *J. Food Prot.* **1998**, *61*, 78−86.
(11) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.
(12) Englyst, H. N.; Cumming, J. H. Simplified method for the measurement of total non-starch polysaccharides by gas-chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937−942.
(13) Ruiter, J. M.; Burns, J. C. Characterization of trifluoroacetic acid hydrolyzed of subtropical forage grass cell walls. *J. Agric. Food Chem.* **1987**, *35*, 308−316.
(14) Dische, Z. Color reactions of carbohydrates. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfram, M. L., Eds.; Academic Press: New York, 1962; pp 477−512.
(15) Sakurai, N.; Nevins, D. J. Relationship between fruit softening and wall polysaccharides in avocado (*Persea americana* Mill) mesocarp tissues. *Plant Cell Physiol.* **1997**, *38*, 603−610.
(16) Dawson, D. M.; Melton, L. D.; Watkins, C. B. Cell wall changes in nectarines (*Prunus persica*). Solubilization and depolymerization of pectic and neutral polymers during ripening and in mealy fruit. *Plant Physiol.* **1992**, *100*, 1203−1210.
(17) Hegde, S.; Maness, N. O. Sugar composition of pectin and hemicellulose extracts of peach fruit during softening over two harvest seasons. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 1162−1167.
(18) Nogata, Y.; Yoza, K.-i.; Kusumoto, K.-i.; Ohta, H. Changes in molecular weight and carbohydrate composition of cell wall polyuronide and hemicelluloses during ripening in strawberry fruit. In *Pectins and Pectinases*; Visser, J., Voragen, A. G. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; pp 591−596.
(19) Martín-Cabrejas, M.; Waldron, K. W.; Selvendran, R. R. Cell wall changes in Spanish pear during ripening. *J. Plant Physiol.* **1994**, *144*, 541−548.
(20) Ben-Shalom, N.; Hanzon, J.; Klein, J. D.; Lurie, S. A postharvest heat treatment inhibits cell wall degradation in apples during storage. *Phytochemistry* **1993**, *34*, 955−958.
(21) Izumi, H.; Watada, A. E. Calcium treatment affect storage quality of shredded carrots. *J. Food Sci.* **1994**, *59*, 106−109.
(22) Nabais, R. M.; Malcata, F. X. Firmness of carrot slices submerged in brines: Experimental data and mathematical model. *J. Food Process. Preserv.* **1996**, *20*, 295−314.
(23) Jiménez, A.; Heredia, A.; Guillén, R.; Fernández-Bolaños, J. Correlation between soaking conditions, cation content of cell wall, and olive firmness during "Spanish Green Olive" processing. *J. Agric. Food Chem.* **1997**, *45*, 1653−1658.
(24) Coimbra, M. A.; Waldron, K. W.; Selvendran, R. R. Isolation and characterisation of cell wall polymers from olive pulp (*Olea europaea* L.). *Carbohydr. Res.* **1994**, *252*, 245−262.
(25) Jiménez, A.; Guillén, R.; Sánchez, C.; Fernández-Bolaños, J.; Heredia, A. Molecular weight and ionic characteristics of olive cell wall polysaccharides during processing. *J. Agric. Food Chem.* **1996**, *44*, 913−918.
(26) Sánchez-Romero, C.; Guillén, R.; Heredia, A.; Jiménez, A.; Fernández-Bolaños, J. Degradation of hemicellulosic and cellulosic polysaccharides in pickled green olives. *J. Food Prot.* **1998**, *61*, 87−93.
(27) Hegde, S.; Maness, N. O. Changes in apparent molecular mass of pectin and hemicellulose extracts during peach softening. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 445−456.
(28) Mitcham, E. J.; McDonald, R. E. Cell wall modification during ripening of "Keitt" and "Tommy Atkins" mango fruits. *J. Am. Soc. Hortic. Sci.* **1992**, *117*, 919−924.
(29) Fishman, M. L.; Gross, K. C.; Gillespie, D. T.; Sondey, S. M. Macromolecular components of tomato fruit pectin. *Arch. Biochem. Biophys.* **1990**, *274*, 179−191.
(30) Mort, A. J.; Moerschbacher, B. M.; Pierce, M. L.; Maness, N. O. Problems encountered during extraction, purification, and chromatography of pectic fragments, and some solutions to them. *Carbohydr. Res.* **1991**, *215*, 219−227.

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