Changes in Texture and Cell Wall Polysaccharides of Olive Fruit during "Spanish Green Olive" Processing

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Olive fruits (*Olea europaea* cv. *Arolensis* Hojiblanca var.) were processed as "Spanish green olives". The treatment included a first step at high pH (lye treatment), a lactic fermentation, and a final period of conservation in a high concentration brine. Four samples were taken in the critical moments of processing, and their cell wall material was isolated and fractionated into several groups of polysaccharides. Lye treatment had its biggest effect on uronic acid-containing fractions such as water (WSF), oxalate (OSF), and chlorite/acetic acid (DEL) soluble fractions. Marked changes were detected in the relative percentage of each of these fractions, suggesting interchange of polysaccharides (mainly homogalacturonans) between them. Fermentation, on the other hand, produced a marked degradation of neutral polysaccharides mostly from hemicellulose A (HA), hemicellulose B (HB), and α-cellulose (CEL), most likely by enzymatic action. Interchange of polysaccharides between these fractions may also occur. Conservation did not produce any change in the solubility characteristics or in the glycoside composition of the different polysaccharides. Texture decreased during the two first steps and did not change during the last one (conservation period).

Keywords: *Olive; cell wall; processing; texture; pectins; hemicelluloses*

INTRODUCTION

To make them suitable for human consumption, olive fruits need to undergo three processing steps. Oleuropeine is a bitter glucoside that makes the fresh fruits unedible, and therefore, it is necessary to deplete the olive flesh of this compound. Treatments with solutions of sodium hydroxide (lye) are known to hydrolyze the oleuropeine. If this treatment is performed three or more times with continuous aeration, "black ripe olives" are obtained. However, if after the lye treatment the olives are placed in a solution of sodium chloride (brine) and in which a lactic fermentation takes place, "Spanish green olives" are obtained (Fernández-Díez, 1985).

Texture is one of the organoleptic characteristics most affected by these treatments. A large number of studies have been undertaken to examine loss of firmness in vegetable products as a result of processing. Thus, Feng et al. (1989) examined ground beets subjected to heat treatment, Chitarra et al. (1989) studied apricots undergoing canning, and Howard and Buescher (1990) looked at cucumbers during lactic fermentation. In all these cases, the influence of factors such as pH, temperature, presence of ions, and enzymatic activity was determined. It is known that calcium improves texture, but sodium has a double effect: it improves texture by reducing the electrostatic repulsion of acidic groups (as happens at low values of pH), and it has the opposite effect on texture by competing with calcium (Van Buren, 1979). Endogenous enzymatic activity produces losses in firmness, as occurs during ripening and in the case of fermentations (Meurer and Gierschner, 1992).

All the polysaccharides of the cell wall must be involved in the changes in texture described, since all of them (pectins, hemicelluloses, and cellulose) form a very complex network in the cell wall structure. However, most of the published studies (Plat et al., 1988; Sajjaanantakul et al., 1989; Van Buren et al., 1988) have only examined the changes occurring in the pectins, the behavior of the hemicelluloses in these technological processes being unknown. In the present study, therefore, a general description is given of the changes that occur in olives during processing to provide preliminary knowledge on the structural changes that take place in each polysaccharide of the olive cell wall during processing. A description of the changes occurring in the hemicellulose fraction is also included.

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MATERIALS AND METHODS

Olive Processing. Olive fruits (*Olea europaea* cv. *arolensis* Hojiblanca var.) harvested in two seasons (years 1988/1989 and 1989/1990) in the province of Seville, Spain, were processed as followed: the fruits were treated with a solution of 2–2.5% sodium hydroxide (lye) in a proportion of 5 kg of olives/3.5 L of lye for approximately 7 h (the lye has to reach two-thirds of the distance to the pit). The olives then underwent one rapid wash followed by another wash which lasted 14–16 h (3.5 L of water each wash). Subsequently, the fruits were placed in a solution of 10–11% sodium chloride (brine) that reached a concentration of ~7% at equilibrium. After the lactic fermentation, which lasts several months and 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 each sample. For texture determination the samples analyzed were as follows: unprocessed fruit (UF), after 4 h of lye treatment (1/2LYE), after 7 h of lye treatment (LYE), after the water wash (WASH), at brine equilibrium (EQ.BRINE), after lactic fermentation (FERM), and after the conservation period (PF). Only the UF, EQ.BRINE, FERM, and PF samples were analyzed to isolate and study the composition of the cell wall.

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 fractionated into several groups of polysaccharides, according to their solubility in different solutions (hot water, hot oxalate, delignification, and extraction with 10% sodium hydroxide, which provided three more fractions: hemicelluloses A, B, and α-cellulose) (Jiménez et al., 1994a).

Noncellulosic neutral sugars were quantified by trifluoroacetic acid (TFA) hydrolysis (Ruiter and Burns, 1987), reduc-

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Table 1. Relative Percentages of the Main Components of Cell Wall (Cellulose, Uronic Acids, and Noncellulosic Neutral Sugars)[a]

	UF	EQ.BRINE	FERM	PF
season 1988/1989				
yield	5.97 ± 0.14	4.86 ± 0.10	5.91 ± 0.13	5.61 ± 0.12
total carbohydrates	42.48 ± 2.28a	59.09 ± 1.44b	66.68 ± 5.09bc	73.90 ± 5.64c
cellulose	43.35 ± 1.01a	38.87 ± 0.47b	36.07 ± 1.31bc	34.24 ± 1.22c
uronic acids	21.69 ± 0.56a	28.01 ± 0.15b	34.88 ± 2.25c	37.19 ± 0.97c
noncell. neutral sugars	34.94 ± 0.44a	33.11 ± 0.62b	29.04 ± 0.95c	28.56 ± 0.23c
season 1989/1990				
yield	5.58 ± 0.11	4.55 ± 0.06	4.59 ± 0.06	4.94 ± 0.03
total carbohydrates	45.04 ± 2.56a	58.51 ± 2.80b	62.65 ± 3.55b	64.15 ± 2.71b
cellulose	33.92 ± 0.05a	37.90 ± 0.28b	34.14 ± 0.10a	35.68 ± 1.64ab
uronic acids	31.39 ± 0.92a	27.48 ± 0.54b	36.24 ± 0.70c	32.48 ± 0.76a
noncell. neutral sugars	34.68 ± 0.87a	34.61 ± 0.83a	29.61 ± 0.80b	31.83 ± 0.88b

[a] The yields are given as percent on a fresh flesh basis and the total carbohydrates as the sum of the corresponding percentages of the three components on a cell wall basis (statistical analysis: Duncan test, significance level 5%). The numbers from the same row marked with the same character (a–c) are not statistically different.

Table 2. Noncellulosic Neutral Sugar Composition of Cell Wall Material in Both Seasons Studied during Processing[a]

	1988/1989				1989/1990			
season	UF	EQ.BRINE	FERM	PF	UF	EQ.BRINE	FERM	PF
Rha	7.50	8.42	7.95	7.22	8.56	6.85	8.64	7.22
Fuc	0.75	0.67	0.59	0.72	0.68	0.68	0.65	0.72
Ara	42.10	40.40	41.75	45.12	51.37	51.36	51.83	43.32
Xyl	30.08	31.99	31.81	30.68	18.83	22.26	21.60	32.49
Man	3.00	3.37	1.99	1.80	3.42	1.71	2.15	1.80
Gal	9.02	8.41	7.95	9.02	8.56	8.56	8.63	9.02
Glc	7.51	6.73	7.95	5.41	8.56	8.56	6.48	5.41

[a] The data are expressed as a molar fraction. Each result is the average of two replications (CV < 10%).

tion, 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. Cellulose was quantified from the TFA-insoluble residue by the phenol–sulfuric acid method (Dubois et al., 1956).

Texture Determination. The texture of fruits was measured using a texturometer Instron 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. The results given are the mean values of 10 replicates.

Statistical Analysis. The data were statistically analyzed by analysis of variance. Means were compared with Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Processing of olives to obtain the so-called Spanish green olives, includes three main steps: lye treatment, fermentation, and conservation. In order to study the differential effects of these processes in the cell wall composition, four samples were studied: unprocessed fruit (UF), fruits equilibrated in the fermentation brine (EQ.BRINE), fermented fruits (FERM), and fruits after a period of 4 months of conservation in a high concentration brine (PF). Therefore, differences from UP to EQ.BRINE, EQ.BRINE to FERM, and FERM to FP reflect the changes taking place during lye treatment, fermentation, and conservation, respectively.

General Composition of the Cell Wall. Table 1 shows the general composition of the cell wall through the different processes studied. Total carbohydrates increased significantly during lye treatment and remained constant during fermentation and conservation, showing that, during the first process, other components of the cell wall are solubilized in a greater extent than wall carbohydrates.

During lye treatment the relative percentages of cellulose and uronic acid-containing polysaccharides showed small but significant changes, although these were not consistent from one season to the other. During the 1988/89 season there was a decrease in cellulose percentage and an increase in that of uronic acids, suggesting that cellulose was preferentially removed from the cell wall; however, during season 1989/1990 the opposite took place. The relative percentage of noncellulosic neutral sugars, on the other hand, remained stable or changed very little. Also, small but significant ($P < 0.05$) decreases in the relative percentages of both cellulose and other neutral sugars together with an increase in that of uronic acids were detected during fermentation in both seasons, suggesting that this process had its greatest effect on both uronic acid and neutral sugar-containing polysaccharides, with uronic acids affected the most. Finally, during conservation, no changes were detected in any of the components except for a slight decrease in the relative percentage of uronic acids, during the second season.

The relative molar percentages of individual noncellulosic neutral sugars (TFA-hydrolyzable) did not change significantly ($P < 0.05$) during processing (Table 2), meaning that in the steps where neutral sugar solubilization takes place the main neutral polysaccharides must be removed in the same extent or that the sugars which are preferentially solubilized are the minor ones and, as a consequence, the relative percentages do not change significantly.

Solubility Characteristics of Cell Wall Polysaccharides. Cell wall polysaccharides were fractionated into groups by sequential extractions with different solvents (see Materials and Methods). Figure 1 shows the changes taking place in the relative percentages of each of those groups.

Very similar trends of change were found in both seasons during chemical treatment. Among the pectic polysaccharides, the water-soluble fraction (WSF) and the fraction solubilized by delignification (DEL) decreased while the oxalate-soluble fraction (OSF) in-

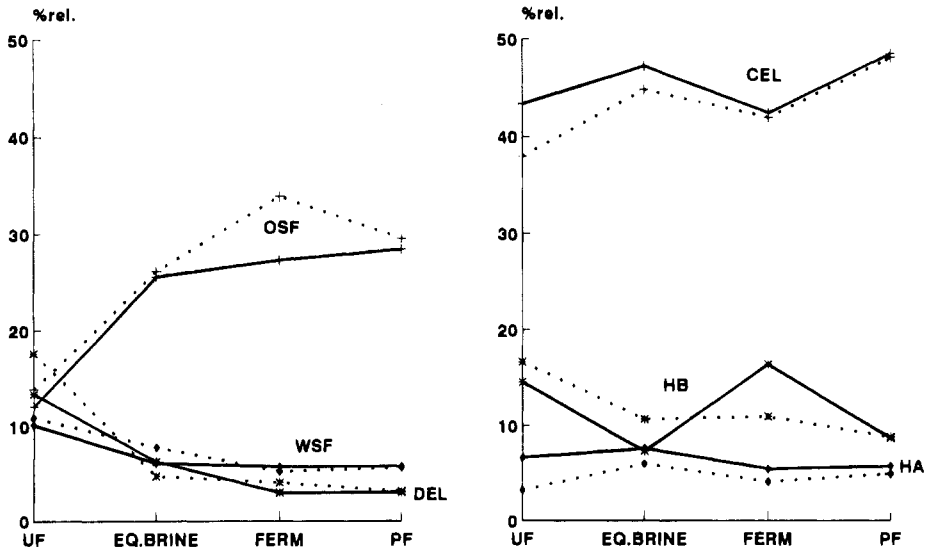

Figure 1. Evolution of the relative yield of each group of polysaccharides (water-soluble fraction, WSF; oxalate-soluble fraction, OSF; fraction solubilized by delignification, DEL; hemicelluloses A, HA; hemicelluloses B, HB; and α-cellulose, CEL) through "Spanish green olives" processing in both seasons studied: —, season 1988/1989; ···, season 1989/1990).

creased very significantly. This last change could be partially due to a removal of all the other fractions, including hemicelluloses and α-cellulose, in a greater extent than OSF. However, if this was the predominant process, a greater change in the general composition of the cell wall material should be expected, besides different changes in the relative percentages of each group in both seasons studied. Consequently, the data suggest that together with a limited solubilization of pectic and neutral polysaccharides during lye treatment, the main process that took place was an interchange between groups due to changes in the solubility characteristics of the different polysaccharides. From this point of view the increase of OSF could be explained, at least in part, as an incorporation to this fraction of polysaccharides from WSF and DEL. Water-soluble pectins have, in general, a very high degree of esterification (Stevens and Selvendran, 1980); for olives this was higher than 80% in the unprocessed fruit (Jiménez et al., 1994a). Alkali treatment can induce two main reactions in this kind of polysaccharides: β-elimination and deesterification (Sajjanantakul et al., 1989). The first would lead to a depolymerization that eventually, depending on its degree, could produce some solubilization of these polysaccharides into the processing liquids. On the other hand, deesterification would increase the number of free carboxyl groups and consequently the ability of these polysaccharides to bind ionically in the cell wall, probably by Ca^{2+} ions (Brett and Waldron, 1990), becoming less soluble in water but soluble in oxalate. This process could be favored by an increase in the available calcium that probably occurred due to the disruption of cytoplasmic membranes. Pectins solubilized by chlorite/acetic acid (DEL) have been suggested to be held in the walls by ester, phenolic, and phenolic–ester cross-linkages (Selvendran and O'Neill, 1985; Fry, 1986) at the same time as they are linked to other pectins (OSF) through ionic bonds. Therefore, the data suggest that lye treatment produces a marked saponification of ester linkages yielding these oxalate-soluble polysaccharides. This reaction could have a great effect on cell wall structure and hence on its mechanical and rheological characteristics.

The relative percentages of α-cellulose and HA showed a slight increase due to the chemical treatment, while that of HB decreased proportionally in both seasons.

Table 3. Composition of the Water-Soluble Fraction[a]

	UF	EQ.BRINE	FERM	PF
total carbohydrate	62.32	69.92	63.84	74.12
Rha	3 (7.1)	3 (4.3)	4 (4.9)	3 (3.5)
Fuc	0.2 (0.5)	0.1 (0.1)	0.2 (0.2)	0.1 (0.1)
Ara	34 (80.4)	61 (88.3)	74 (90.5)	79 (93.0)
Xyl	0.6 (1.4)	0.9 (1.3)	1 (1.2)	0.2 (0.2)
Man	0.5 (1.2)	0.1 (0.1)	0.1 (0.1)	0.2 (0.2)
Gal	2 (4.7)	3 (4.3)	2 (2.4)	2 (2.3)
Glc	2 (4.7)	1 (1.4)	0.5 (0.6)	0.6 (0.7)
UA	54	30	18	15
NS/UA	0.68/1	2.31/1	3.60/1	4.55/1
Rha/UA	1/18.01	1/9.32	1/4.87	1/4.71

[a] The total carbohydrate content is expressed as percent of weight in the total weight of the fraction and the sugar composition in a molar fraction (the data in parentheses represent the molar fraction of the neutral sugars).

These changes will be discussed later in relation to the modifications of the glycosyl composition of these fractions.

No significant changes were detected in the relative percentages of the different fractions due to fermentation and conservation, except for a decrease in α-cellulose and a proportional increase of HB in one season and OSF in the other during fermentation.

Glycosyl Composition of the Fractions during Processing. The three pectic fractions (WSF, OSF, and DEL) underwent very different changes during processing. WSF (Table 3) became enriched in neutral sugars as shown by the progressive increase of the ratio NS/UA from 0.68/1 for the unprocessed fruit to 4.55/1 for the processed fruit. Among the neutral sugars, arabinose increased the most (13% in relative percentage), the maximum change being during lye treatment. This fraction in the unprocessed fruit is constituted mainly by polyuronides and arabinans (Jiménez et al., 1994a), the data suggesting that during processing the polyuronides are either solubilized or interchanged with other groups of polysaccharides, the arabinans becoming the main polysaccharide in this fraction for the processed fruit. The increase in the ratio Rha/UA from 1/18 to 1/5 suggests that processing removes homogalacturonans in a greater extent than rhamnogalacturonans from this fraction, a fact that is compatible with the proposed mechanism of interchange, since rhamnoga-

Table 4. Composition of the Oxalate-Soluble Fraction[a]

	UF	EQ.BRINE	FERM	PF
total carbohydrate	61.51	88.44	105.91	100.19
Rha	3 (11.8)	5 (22.7)	4 (22.1)	4 (18.3)
Fuc	0.3 (1.2)	0.3 (1.4)	0.2 (1.1)	0.2 (0.9)
Ara	19 (74.5)	14 (63.7)	11 (61.0)	14 (63.9)
Xyl	0.4 (1.6)	0.3 (1.4)	0.2 (1.1)	0.3 (1.4)
Man	0.3 (1.2)	0.07 (0.3)	0.4 (2.2)	0.1 (0.5)
Gal	2 (7.8)	2 (9.1)	2 (11.0)	3 (14.0)
Glc	0.5 (2.0)	0.3 (1.4)	0.3 (1.7)	0.3 (1.4)
UA	74	78	82	78
NS/UA	0.28/1	0.23/1	0.18/1	0.23/1
Rha/UA	1/22.79	1/15.67	1/21.26	1/18.07
Rha/Ara	1/5.92	1/2.85	1/2.99	1/3.21

[a] The total carbohydrate content is expressed as percent of weight of the total weight of the fraction and the sugar composition in a molar fraction (the data in parentheses represent the molar fraction of the neutral sugars).

Table 5. Composition of the Delignification Fraction[a]

	UF	EQ.BRINE	FERM	PF
total carbohydrate	22.21	19.82	19.38	21.45
Rha	7 (11.5)	6 (8.6)	6 (9.4)	.6 (9.9)
Fuc	0.7 (1.1)	0.6 (0.9)	0.7 (1.1)	0.7 (1.1)
Ara	44 (72.5)	46 (66.1)	45 (70.6)	41 (67.5)
Xyl	1 (1.6)	6 (9.0)	4 (6.3)	4 (6.6)
Man	1 (1.6)	1 (1.4)	1 (1.6)	1 (1.6)
Gal	5 (8.2)	8 (11.5)	6 (9.4)	7 (11.5)
Glc	2 (3.3)	2 (2.9)	1 (1.6)	1 (1.7)
UA	39	29	35	38
NS/UA	1.56/1	2.4/1	1.82/1	1.60/1
Rha/UA	1/5.73	1/4.53	1/6.13	1/5.93
Rha/Ara	1/6.41	1/7.16	1/7.79	1/6.47

[a] The total carbohydrate content is expressed as percent of weight of the total weight of the fraction and the sugar composition in a molar fraction (the data in parentheses represent the molar fraction of the neutral sugars).

lacturonans cannot bind ionically to other components of the cell wall, due to steric effects of the side chains. Similar results have been found during "black ripe olive" processing (Jiménez et al., 1994b) and during processing of other products such as carrots (Plat et al., 1988).

In contrast with WSF, no changes were detected in either the ratio NS/UA or the Rha/UA one for OSF (Table 4). The only remarkable change taking place on the glycosyl composition of this fraction was a marked decrease of arabinose (11% in relative terms) and a parallel increase of rhamnose during lye treatment that led to an increase in the ratio Rha/Ara from 1/5.92 to 1/2.85, suggesting the degradation of rhamnogalacturonan side chains. Fermentation and conservation did not have any effect on the composition of this fraction.

During lye treatment the ratio NS/UA for DEL (Table 5) increased from 1.56/1 to 2.4/1 and that of Rha/UA from 1/5.7 to 1/4.5 while that of Rha/Ara did not change significantly. The data suggest that, as in the WSF, homogalacturonans are removed from this fraction by the sodium hydroxide solution. Fermentation produced the opposite changes and seemed to have its maximum effect on neutral sugars (among these arabinose increased while xylose, galactose, and glucose decreased). Conservation did not have any effect on the glycosyl composition of this fraction except for a slight decrease of the ratio NS/UA.

The polysaccharides that constitute hemicelluloses A (Table 6) changed very little during both lye treatment and conservation. Fermentation, however, produced a marked decrease of the ratio NS/UA from 5.7/1 to 4.2/1

Table 6. Composition of the Hemicelluloses A[a]

	UF	EQ.BRINE	FERM	PF
total carbohydrate	39.80	53.51	40.96	41.91
Rha	0.9 (1.0)	1 (1.2)	1 (1.2)	0.8 (1.0)
Fuc	0.3 (0.3)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)
Ara	2 (2.3)	2 (2.3)	2 (2.5)	2 (2.6)
Xyl	81 (94.2)	79 (93.0)	74 (92.4)	73 (93.4)
Man	0.4 (0.5)	0.6 (0.7)	0.5 (0.6)	0.6 (0.8)
Gal	0.4 (0.5)	0.6 (0.7)	0.5 (0.6)	0.6 (0.8)
Glc	1 (1.2)	2 (2.3)	2 (2.5)	1 (1.3)
UA	14	15	19	20
NS/UA	6.1/1	5.7/1	4.2/1	3.9/1
Xyl/UA	5.82/1	5.40/1	3.84/1	3.65/1

[a] The total carbohydrate content is expressed as percent of weight of the total weight of the fraction and the sugar composition in a molar fraction (the data in parentheses represent the molar fraction of the neutral sugars).

Table 7. Composition of the Hemicelluloses B[a]

	UF	EQ.BRINE	FERM	PF
UA	60.69	59.66	81.16	56.08
Rha	2 (2.20)	2 (2.0)	1 (1.2)	1 (1.1)
Fuc	0.7 (0.8)	0.5 (0.5)	0.5 (0.6)	0.5 (0.6)
Ara	10 (11.0)	14 (14.1)	13 (15.0)	13 (15.0)
Xyl	40 (44.1)	22 (22.1)	18 (20.8)	24 (27.8)
Man	9 (9.9)	14 (14.1)	12 (13.9)	12 (13.9)
Gal	8 (8.8)	11 (11.0)	10 (11.6)	10 (11.6)
Glc	21 (23.1)	30 (32.1)	32 (37.0)	26 (30.1)
UA	9	6	14	11
NS/UA	10.1/1	15.6/1	6.2/1	7.9/1
Xyl/Glc	1.87/1	1/1.37	1/1.75	1/1.08
Ara/Xyl	1/3.93	1/1.61	1/1.40	1/1.82

[a] The total carbohydrate content is expressed as percent of weight of the total weight of the fraction and the sugar composition in a molar fraction (the data in parentheses represent the molar fraction of the neutral sugars).

showing, therefore, that the net effect of fermentation on this fraction was a mobilization of xylans either to the processing liquid or to other polysaccharide fractions of the cell wall.

Lye treatment and fermentation had opposite effects on the glycosyl composition of hemicelluloses B (Table 7). During the first process there was a marked increase in the ratio of NS/UA indicating a preferential removal of uronic acids. Among the neutral sugars, there was a very notable decrease of xylose (22% in relative terms) and a parallel increase of several other sugars, implying loss of xylans. In contrast, fermentation produced a dramatic decrease in the ratio NS/UA (from 15.6/1 to 6.2/1) without changes in the relative percentages of individual neutral sugars (Table 7, numbers in parentheses). This fraction in the unprocessed fruit has been shown to be constituted mainly by xylans, xyloglucans, arabinans, and galactoglucomannans (Jiménez et al., 1994a; Coimbra et al., 1994). It can, therefore, be concluded that these polysaccharides undergo extensive degradation during fermentation most likely due to enzymatic activities.

No changes were detected in the ratio NS/UA of the α-cellulose (CEL) residue (Table 8), however significant differences were found in the composition of its neutral sugars during lye treatment and fermentation, characterized by a marked increase in the xylose percentage and a decrease in that of noncellulosic glucose. Small changes were measured during conservation.

On the basis of the changes detected in the glycosyl composition of HA, HB, and CEL, several observations can be made. Lye treatment had two main effects, the

Table 8. Composition of the α-Cellulose[a]

	UF	EQ.BRINE	FERM	PF
NS	12.63	20.68	22.58	17.95
UA	3.48	8.65	13.14	15.97
CELL	76.72	73.26	71.44	72.73
Rha	4 (5.0)	5 (6.8)	5 (7.5)	3 (5.92)
Fuc	0.3 (0.4)	0.2 (0.3)	0.1 (0.2)	0.2 (0.4)
Ara	19 (23.7)	18 (24.6)	17 (25.5)	14 (27.6)
Xyl	9 (11.2)	15 (20.5)	23 (34.4)	16 (31.6)
Man	1 (1.2)	1 (1.4)	0.7 (1.0)	0.5 (1.0)
Gal	6 (7.5)	5 (6.8)	4 (6.0)	5 (9.9)
Glc	41 (51.1)	29 (39.6)	17 (25.4)	12 (23.7)
UA	19	26	33	47
NS/UA	0.24/1	0.35/1	0.49/1	0.93/1

[a] The general composition (neutral sugars, uronic acids, and cellulose) is expressed as percent of weight of the total weight of the fraction and the sugar composition in a molar fraction (the data in parentheses represent the molar fraction of the neutral sugars).

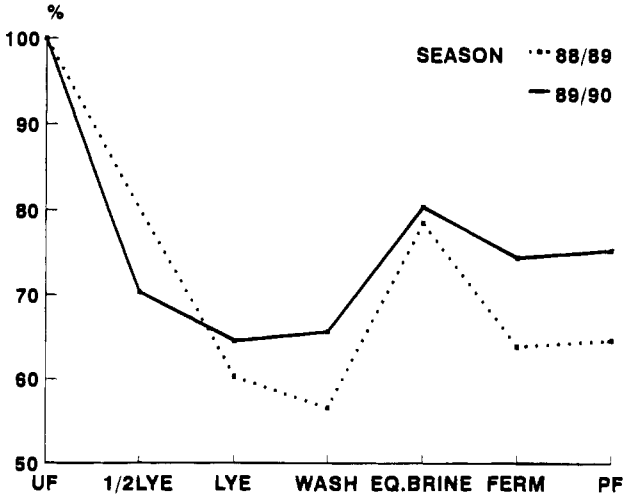

Figure 2. Changes in the olive fruit texture during processing. The results are expressed as relative percentages.

mobilization of arabinoxylans from HB and the solubilization of a glucan from CEL. The mechanism of the first process is unclear, but the decrease of xylose content from HB together with the parallel increase in that of CEL could suggest an interchange between both fractions. Interchange could also happen between HA and HB because of the breakdown of ester linkages, which have been described to hold these polysaccharides in the cell wall (Carpita and Gibeaut, 1993; Selvendran, 1985) or by depolymerization.

Fermentation, on the other hand, produced a marked solubilization and/or interchange of a wide range of polysaccharides including glucans, arabinoxylans, xyloglucans, arabinans, and galactoglucomannans. In olive fruits, α-galactosidase, β-galactosidase, α-mannosidase, α-arabinosidase, α-xylosidase (Heredia et al., 1992, 1993), endoglucanases (Heredia et al., 1991; Fernández-Bolaños et al., 1992) polygalacturonase, and pectinmethylesterase (Mínguez et al., 1978) have been described. A significant proportion of these activities is probably lost during lye treatment; however, if some of them remain, those in the pulp will find a modified cell wall with increased accessibility due to the modifications of the pectic polysaccharides that take place during this treatment. Together with the endogenous enzymes, those coming from some microorganisms that develop during fermentation (Meurer and Gierschner, 1992) should also be considered. The net effect of fermentation on HA was the removal of xylans: no xylanase activity has been detected in olive fruit, implying that if this enzyme is responsible for solubilization it has to be an exogenous microbial enzyme. Alternatively, the action of an arabinosidase could also produce mobilization of xylans since the removal of the arabinose side groups could increase their ability to hydrogen bond to cellulose (this last possibility is supported by the relative increase of xylose in the α-cellulose fraction). The main polysaccharides removed from HB were xyloglucans, arabinans, and galactoglucomannans, endoglucanase being the enzyme which is most probably responsible for the solubilization of the xyloglucans. However, no endogenous arabinase or mannanase has been detected in olive fruits: the solubilization of the other polysaccharides must be due to exogenous enzymes or to the combined action of several glycosidases. A fact supporting this last possiblity is that in dialyzates of the processing liquid only a very limited increased of polymeric material was detected, suggesting that if solubilization takes place the products must have very low molecular weights (data not shown). Finally, the decrease of glucose in the α-cellulose fraction suggests the action of a glucanase, the polysaccharide hydrolyzed probably being a mixed glucan (Jiménez et al., 1994a). The possible presence of the mentioned enzymes during fermentation and the mechanism of polysaccharide solubilization and/or interchange are subjects that are, at present, being investigated.

Unlike the modifications seen in the pectic polysaccharides, which have been thoroughly described in the literature for several vegetable products and processing methods, the changes in hemicelluloses have been studied only during softening related to ripening (Ahmed and Labavitch, 1980; Huber, 1983; Brady, 1987). However, these polysaccharides play an important role in the support of cell wall structure, and very small changes involving them, even changes in their molecular weight, such as takes place in softening during ripening, could produce losses in texture.

Textural Changes. The same trends in texture were observed in both seasons studied, as shown in Figure 2. The lowest texture value was found after the water wash, this being around 65% of the initial ones. When brine equilibrium was reached, the texture values increased to almost 80% of initial values and then decreased again after fermentation. No changes were detected during conservation.

The treatment with sodium hydroxide was the step that produced the greatest changes. Together with the loss of cell turgidity due to the disorganization of the cytoplasmic membrane and the breakdown of linkages between pectic and hemicellulosic polysaccharides already discussed, another factor that could be important in order to explain the losses in texture during this process could be the deesterification of pectins. This process would dramatically increase the charge density in the pectic chains due to the free carboxyl groups. The presence of these negative charges could lead to a destabilization of the wall structure because of both inter- and intrachain electrostatic repulsion that would change their conformation and, therefore, their ability to form tridimensional gels. The effect of the electrostatic repulsions could be reinforced by the high pH of the processing liquid since this would assure a total ionization of the carboxyl groups. A fact that suggests a role for this factor is the increase in texture observed after equilibrium is reached in brine since sodium ions

could partially neutralize the negative charges of the carboxyl groups.

During this first step, the behavior of seasons 1988/1989 and 1989/1990 was not similar with regard to cell wall compositiom changes (Tables 1 and 2), showing the same trend in changes of polysaccharides solubility (Figure 1). This suggests that the loss of texture during lye treatment, washes, and soaks in brine would be due to the variations in polysaccharide solubility, disorganization of the cell wall because of the breakdown of the different kinds of bonds (H-bonding, ionic, covalent, ···), and of course, to the decrease in cellular turgidity caused by the disorganization of the plasma membrane.

After fermentation the firmness decreased, showing that the activities of endogenous and/or exogenous enzymes may play an important role in fruit softening (Fernández-Bolaños et al., 1992; Meurer and Gierschner, 1992; Heredia et al., 1993). In this case, the variations in cell wall composition were the same in both seasons studied (Table 1), neutral sugars and cellulose decreasing and there being no very marked changes in polysaccharide solubility (Figure 1), except for HB and CEL: firmness seems to be related in this step with the hemicellulosic and α-cellulose fractions. These facts support the idea that enzyme activity is of relevance in this phase of processing. The losses of texture due to enzymatic activities are likely to be greater than those found because the decrease in the pH value during fermentation probably causes protonization of carboxyl groups and, therefore, a decrease in electrostatic repulsions and consequently an increase in texture values.

Final Remarks. Textural changes of fruits and vegetables during processing have often been ascribed to modifications of particular cell wall polysaccharides, mostly pectic ones. The results of the present paper suggest that texture is a multifactor dependent characteristic and that in order to understand the chemistry of its modification during processing the cell wall has to be considered as a dynamic whole where the combined action of several factors is likely to be necessary in order to change its mechanical and rheological characteristics.

Olive processing involved three main steps: lye treatment, fermentation, and conservation. Texture decreased during the two first processes and did not change during the last one. Electrostatic interactions and the consequent changes in gel structure of pectic polysaccharides, together with the breakdown of (most likely ester and/or phenolic) linkages between pectic and hemicellulosic polysaccharides, could be the most important factors in determining the losses of texture during lye treatment. A different enzymatic mechanism of hemicellulose degradation could be, on the other hand, suggested in order to explain changes during fermentation. Experiments to determine the relative importance of the mentioned factors, as well as other possible ones like changes in the molecular weights of the different polysaccharides, are currently being carried out.

ABBREVIATIONS USED

CWM, cell wall material; WSF, water-soluble fraction; OSF, oxalate-soluble fraction; DEL, fraction solubilized by delignification; HA, hemicelluloses A; HB, hemicelluloses B; CEL, α-cellulose residue; NS, neutral sugars; UA, uronic acids; CELL, cellulose quantified colorimetrically; UF, unprocessed fruit; 1/2LYE, sample after 4 h of lye treatment; LYE, sample after 7 h of lye treatment; WASH, sample after water wash; EQ.BRINE, sample at brine equilibrium; FERM, sample after lactic fermentation; PF, processed fruit; TFA, trifluoroacetic acid.

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