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Pectins as Possible Source of the Copper Involved in the Green Staining Alteration of Cv. Gordal Table Olives

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The pectic and pigment compositions and Ca and Cu contents of the alcohol-insoluble solid (AIS) residues were determined in cv. Gordal olives treated with NaOH solution and kept at different constant pH values (3.5−6.5). The same controls were made in table olives presenting green staining alteration. The ratio between the various pectin fractions of the more acid pH experiment samples remained similar in fruits not showing green staining. In altered fruits, the protopectin fraction was lower, and the calcium pectate or EDTA soluble pectins were higher. Regarding the presence of Ca and Cu in the AIS, it was observed that, whereas Ca levels fell at the most acid pH values, those of Cu increased. The concentration of Ca was higher in the AIS of altered olives than in nonaltered ones. The same trend was seen for the zone with or without green staining of an altered fruit. In the case of Cu, the relationship was the opposite: a decrease in the levels of AIS Cu in fruits and zones of fruits with green staining. This result was correlated with the highest concentration of Cu−chlorophyll complexes found in such samples and suggested that pectins might act as a reservoir of Cu involved in the alteration.

KEYWORDS: Pectin; copper; calcium; olive; green staining alteration

INTRODUCTION

The processing of Spanish-style green table olives comprises an alkaline treatment of the olives to hydrolyze the bitter glucoside oleuropein, followed by several washings with water, after which the fruits are placed in brine, where a natural lactic fermentation takes place (*1*). As a result of this fermentation, the pH of the medium falls to ∼3.5 at the end of the process, which causes fruit color changes due to transformations in the chlorophyll molecule (*2*). These transformations include the formation of pheophytins, pyropheophytins, and pheophorbides, but in the particular case of cv. Gordal olives other oxidized chlorophyll derivatives, the formation of which in a foodstuff had not been reported previously, are formed (*3*, *4*).

The Gordal olive has long presented the problem of the occasional appearance of green staining on the surface of fruits processed as table olives, which is known as green staining alteration. The compounds identified as being responsible for the alteration of the fruits are a series of Cu complexes with chlorophyll derivatives in different degrees of oxidation, without the participation of exogenous Cu (*4*, *5*). The reaction of complexation between heavy metals (mainly Zn and Cu) and chlorophyll derivatives is well-known and described in the literature (*6*) and has even been used to retain color in processed vegetables, but always with the use of added metal ions (*7*, *8*). The intrinsic Cu of the plant has never been reported to complex with the chlorophyll derivatives. The formation of Cu−metallochlorophyll pigments detected in olives with green staining suggests that substances inaccessible during usual conditions of processing come into contact, allowing Cu incorporation into the chlorophyll molecule. For this to be possible, it is necessary to have cell disorganization in the olive. Thus, pectins are fruit constituents possibly related with green staining alteration, as they have a very important structural role in tissue integrity.

The pectic substances are a major group of polysaccharides forming part of the primary cell wall and the intercellular region in higher plants. The modification and degradation of the pectins could cause structural−textural ruptures leading to variation in firmness, liberation or bonding of water, tissue disintegration, etc. (*9*). The pectins are natural polymers based on polymerized galacturonic acid, partially esterified with methanol. These polymers can also be considered copolymers, due to the existence of regions linked to neutral sugars (*10*). At the same time, much of the Ca in plants is normally found in the cell wall, forming Ca bridges between galacturonic acid units of adjacent chains. This acts as an intracellular cement, giving firmness to the plant tissues (*11*). However, as expected of a polyanionic polymer in the presence of opposite charges, the pectins interact strongly with cations such as Cu, Zn, Cd, and Ni (*12*, *13*).

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The aim of the present work was to study the effects of processing green table olives of the Gordal cultivar on the fruit pectic composition and the formation of Cu−metallochlorophyll complexes. The action of the NaOH treatment and the influence of the different acid pH values of the fermentative process on the fruits were considered individually.

MATERIALS AND METHODS

Raw Material. The study was carried out on olive fruits of the Gordal cultivar, *Olea europaea regalis* (L.), supplied by the firm Agroaceitunera S.A. (Utrera, Sevilla, Spain). Samples included fresh fruit (20 kg), fruit treated industrially for 8 h with 2% NaOH solution (40 kg), and table olives from fermenters in which green staining alteration had developed (20 kg).

Sample Preparation. *pH Experiment.* The olive samples from the industrial alkaline treatment were subjected to successive washings with distilled water for 72 h, until the pH of the pulp was neutral. Ten kilograms of these neutralized fruits was used as control fruit (CF). Eight batches, each of 3.5 kg of fruits, were prepared with the remaining olives and subjected for 30 days to a constant pH. The studied pH values were 3.5, 4.5, 5.5, and 6.5. Each pH value was tested in duplicate. For that purpose, each batch of 3.5 kg of fruits was placed in a glass container in universal buffer containing 0.028 M citric acid, 0.028 M KH_2PO_4, 0.028 M boric acid, and 0.028 M diethylbarbituric acid, at a buffer/fruit ratio of 1:1 (v/w), and the pH was adjusted with 0.2 M NaOH to the experimental pH value. Buffer and fruit pulp pH values were measured every 12 h over 3 days. The buffer was renewed periodically in each batch until the fruit pulp reached the predetermined pH.

Fruits with Green Staining Alteration. Table olives from fermenters in which green staining alteration had developed were also used. Both fruits with green staining (12 kg) and those that —although they had been in the same fermenter—showed no alteration (8 kg) were analyzed. The zone with green staining and the remainder zone of the fruits were also isolated and analyzed. In these cases, the sampling (∼300 g) in the two different parts of the fruit included only the superficial part of the fruits.

Measurement of Fruit pH. The pH was measured in the homogenized sample resulting by triturating the plant material in a minimum volume of distilled water, which was added to facilitate homogenization.

Pectin Fractionation and Analysis. The method previously described by Levi et al. (*9*) was used. The extraction of alcohol-insoluble solids (AIS) was carried out in triplicate for all samples. The pectic fractions were obtained in duplicate, and each pectic fraction was assayed three times for its galacturonic acid (GA) content. The AIS were extracted by homogenizing 250 g of destoned olives in an Ultraturrax homogenizer four times in succession, with 300 mL of 70% ethanol each time, followed by two extractions with 300 mL of acetone. In the case of green-stained and unstained zones of the fruits, 100 g of sample was extracted with 200 mL of ethanol and acetone, respectively, in the same way as for the whole fruit. The residue was dried at room temperature. The different pectic fractions were obtained from 200 mg of AIS. Soluble pectins (SP) were extracted by stirring vigorously for 10 min with 20 mL of distilled water and then centrifuging at 27000*g* for 15 min. This procedure was repeated four times. The supernatants with the SP fraction were pooled, and the pellet was used for the extraction of EDTA-soluble pectins fraction or calcium pectate (CaP), following the same procedure, with a solution containing 0.1 M buffer Tris-HCl and 0.2% EDTA at pH 6.2. The supernatants were collected for CaP determination. The final pellet was extracted once for protopectin (PP) with 50 mL of 0.05 M NaOH. The amount of each pectic fraction was assessed colorimetrically (*14*) for its GA content.

Viscosity of Soluble Pectins. This was determined according to the method of Von Mollendorff and De Villiers (*15*). The viscosity of the SP (η_{sp}) was assayed three times for each AIS, and each one was measured in duplicate. Five grams of the AIS was stirred in 100 mL of 0.1 M NaCl (adjusted to pH 5 with 1 N HCl) for 15 min. The suspension was then centrifuged at 12000*g* for 20 min, and the viscosity of the supernatant solution was determined with a viscosimeter at 30 °C. The η_{sp} was calculated according to Doesburg (*16*) as follows

$$\eta_{sp} = (\eta - \eta_0)/\eta_0$$

where η is the viscosity of the pectin solution and η_0 is the viscosity of the solvent.

Determination of Copper and Calcium. Each sample was analyzed in triplicate. One gram of AIS was dried in a porcelain crucible, and 2 mL of a 5% (wt/v) solution of $Mg(NO_3)_2$ in ethanol was added. The resulting mixture was heated at 450 °C for 8 h, and the resulting ash was bleached (if necessary) with 2 mL of concentrated HNO_3. The ash obtained was dissolved with two portions of 2 mL of hot 6 N HCl, made up to 25 mL with deionized water, and vacuum filtered. Dilution 1:50 and addition of a 5% (w/v) solution of $La(NO_3)_3$ were performed for calcium determinations.

Copper and calcium were determined by atomic absorption spectrophotometry, atomizing the sample in an air/acetylene flame and using a hollow cathode lamp of Cu and Ca. Absorbance was measured at 324.7 nm for Cu and at 422.7 nm for Ca with a slit of 0.5 nm in both cases.

Pigment Extraction. This was performed with *N*,*N*-dimethylformamide according to the method described in a previous work (*17*). The technique is based on the selective separation of components between *N*,*N*-dimethylformamide and hexane. This system yields a solution of pigments free from fatty matter that is characteristic of these fruits and that would interfere with subsequent separation and quantification of pigments. Four replicate extractions of each sample were performed. Sample size was 5 g for whole fruit analysis and 2 g for the analysis of fruit surface zones.

Separation, Identification, and Quantification of Pigments. Pigments were separated, identified, and quantified using reversed-phase high-pressure liquid chromatography (RP-HPLC), following the method described by Mínguez-Mosquera et al. (*18*). The allomerized chlorophylls and metallochlorophyllic complexes of copper were identified as has been described in detail in previous papers (*4*, *19*). Pigments were detected by absorbance at 410, 430, 450, and 666 nm before quantification.

Reagents. For all purposes, analytical grade (American Chemical Society) reagents were used (Panreac, Barcelona, Spain). The solvents used for chromatography were of HPLC grade (Teknokroma, Barcelona, Spain). The deionized water used was obtained from a Milli-Q 50 system (Millipore Corp., Milford, MA).

Apparatus. Equipment included an Ultra-Turrax model T-25 polytron homogenizer (Janke Kunker, IKA-Laboratechnik), an atomic absorption spectrophotometer QBC 932AA, a refrigerated centrifuge Beckman-Coulter Avanty J-25, a Büchi rotavapor, model R 110 (Laboratoriums-technik AG), an HP-1100 Hewlett-Packard (Palo Alto, CA) liquid chromatograph fitted with an HP-1100 automatic injector and an HP-1100 diode array detector, and a Hewlett-Packard 8452A UV−visible spectrophotometer.

RESULTS AND DISCUSSION

Pectic Composition. Figure 1 shows the pectic composition of fresh fruit (FF) and that of olives after treatment with NaOH, followed by washing with water (CF), and after being kept for 30 days at the different constant pH values. In the FF, the main pectin fraction was protopectin (PP), at 88.7%, whereas the fractions of calcium pectate (CaP) and soluble pectins (SP) were only 8.6 and 3.7%, respectively, of the total pectins (TP). Alkaline treatment caused great changes in the pectic composition of the fruits: a drastic reduction in PP (91.2%) and an almost equivalent increase in EDTA-soluble pectin or CaP. The water-soluble pectin fraction increased only slightly.

Under alkaline conditions, the pectins are degraded by two competing reactions: one by β-elimination and the other by demethylation of the methoxylated carboxyl groups. The competing reactions are modulated by the conditions of pH and temperature: β-elimination is more favored as temperature

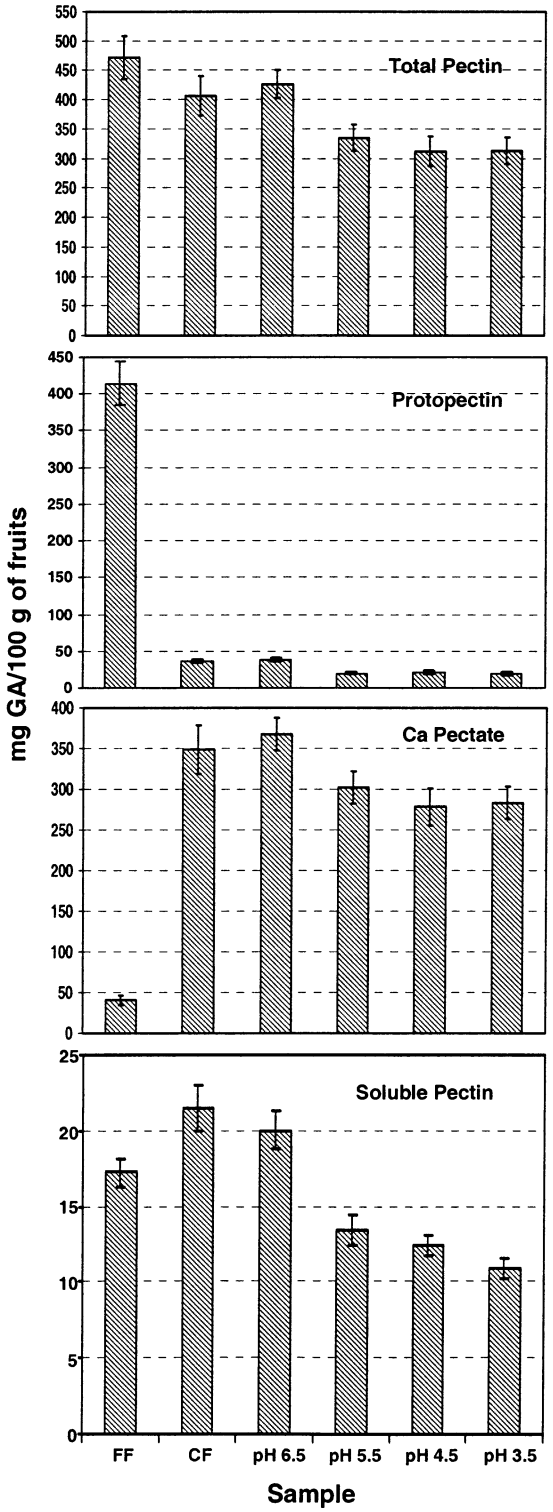

Figure 1. Pectic composition of cv. Gordal olives: fresh fruits (FF), fruits after alkaline treatment (CF), and fruits after being kept at the different pH values. Values represent mean and standard deviation for 18 determinations.

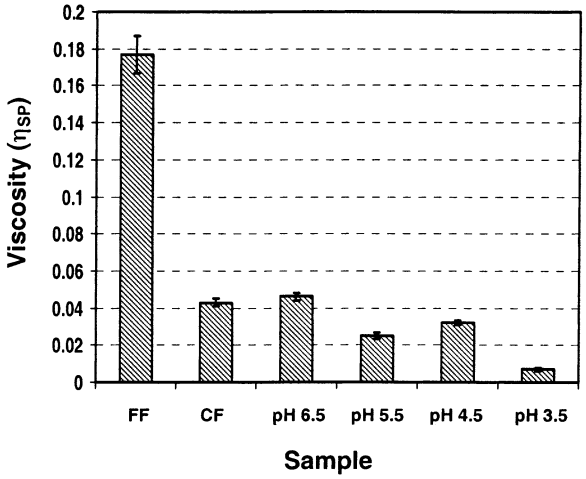

Figure 2. Viscosity of soluble pectins of cv. Gordal olives. (See **Figure 1** for abbreviations.) Values represent mean and standard deviation for 18 determinations.

increases, implying a greater depolymerization of the pectin chain, whereas with increasing pH, the reaction of demethylation by saponification is preferred (*20*).

The high pH of the fruits following alkaline treatment produced de-esterification of the carboxyl groups of the PP. Consequently, the formation of calcium bridges between galacturonic acid units of neighboring pectin chains via exogenous (from the water used in the fruit washing) or endogenous Ca was facilitated. This was to the detriment of the PP that was present in the FF.

With regard to the fruits kept at different pH values, a different effect was shown. In olives kept at pH 6.5, the pectic composition was practically unchanged, and the small difference measured in the TP content could be attributed simply to the intrinsic variability of the raw material. However, when the fruits were kept at a more acid pH, TP decreased (between 17 and 23% compared with CF), presumably as a result of hydrolysis of the pectin to alcohol-soluble short-chain monomers or oligomers. Consequently, there was a greater degradation of pectins in the fruits kept at pH 4.5 and 3.5—the values reached naturally in the fruits and brine at the end of the fermentation process.

The viscosity of the SP varied depending on the sample (**Figure 2**), although low values were found in all cases. The maximum value was measured in the FF, despite the fact that the content in SP was lower than in the CF. The viscosity results showed that the molecular weight and degree of esterification of the SP in the FF were greater than in the rest of the samples, because, according to Doesburg (*16*), the viscosity of the SP depends on the concentration, relative molecular weight, and degree of esterification of the pectins. In the CF, when the highest concentration of SP was found, the viscosity decreased considerably. Moreover, the hydrolysis and saponification of the pectin should yield SP of lower molecular weight and degree of esterification that in the FF.

Similarly to pectic composition, the viscosity of SP in the fruits kept at pH 6.5 showed no variation compared with that of CF. However, when the fruits were kept at more acid pH values (3.5−5.5), the viscosity of the SP fell, indicating that, as pH decreased, the depolymerization of the pectin to fractions of low molecular weight, soluble in alcohol, was produced, causing the above-mentioned decrease in the TP content of the olives.

A parallel study analyzed the pectic composition of processed olives that presented green staining alteration and of those that, although coming from the same fermenter, did not develop such alteration. With the aim of comparing results, **Table 1** shows the percentage composition for each pectin fraction, with respect to the TP content, of industrially processed fruits with and without green staining and that for fruits kept at pH 4.5 and 3.5 (the values found at the end of fermentation). The data showed that the ratios between the various pectin fractions were

Table 1. Percentage Composition for Pectin Fractions of Olive Fruits Kept at pH 4.5 and 3.5 and for Fruits with and without Green Staining (GS)

	pectin fraction[a] (%)		
sample	SP	CaP	PP
fruit kept at pH 4.5	3.98 ± 0.20	89.30 ± 3.13	6.72 ± 0.26
fruit kept at pH 3.5	3.49 ± 0.15	90.44 ± 2.80	6.07 ± 0.23
fruit without GS	3.47 ± 0.14	90.12 ± 2.22	6.41 ± 0.25
fruit with GS	2.08 ± 0.13	95.68 ± 2.15	2.24 ± 0.09

[a] Data represent mean and standard deviation for 18 determinations. SP, soluble pectin; CaP, calcium pectate; PP, protopectin.

Table 2. Calcium and Copper Concentrations in the AIS Obtained from Different Samples

sample[a]	Ca[b] (μg/g of AIS)	Cu[b] (μg/g of AIS)
fruit without GS	3439.32 ± 189.16	16.57 ± 0.76
fruit with GS	3580.73 ± 193.38	15.81 ± 0.94
RZ	9517.40 ± 570.10	16.58 ± 0.87
GSZ	10733.04 ± 558.12	12.38 ± 0.64

[a] GS, green staining; GSZ, green staining affected zone of the fruit; RZ, remaining zone of altered fruit. [b] Data represent mean and standard deviation for nine determinations.

practically the same in the fruits kept at pH 4.5 and 3.5 and industrially processed ones not showing green staining. However, in the fruits with green staining, it was noted that, although the TP content was equal for the fruits with and without green staining (~400 mg of GA/100 g of fruit), the PP and SP fractions were lower than that in fruits not showing alteration, whereas the CaP was higher. Probably, the CaP fraction increased in the fruits with green staining at the expense of the PP fraction, which must have undergone a measure of de-esterification of the methoxyl groups in such fruits.

Ca and Cu Contents in the AIS. Figure 3 shows the content in Ca and Cu of the AIS obtained from the samples of olives in the pH study. Similarly to what happened with the CaP fraction, the amount of Ca in the FF increased considerably in the olives after alkaline treatment, whereas the Cu concentration decreased. When the fruits were later subjected to fixed pH conditions, a great variation was again observed in the contents of Ca and Cu compared with that of CF. In the samples kept at the different values of pH, there was an appreciable decrease in Ca, much sharper at the most acid values (pH 5.5−3.5), whereas for Cu, the effect was completely the opposite: the concentration

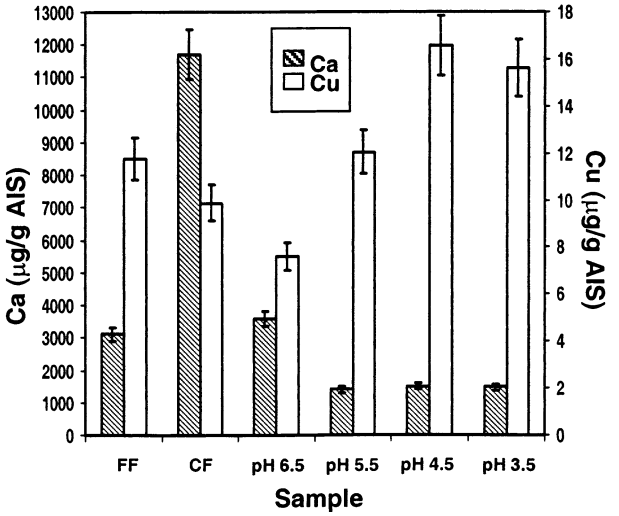

Figure 3. Calcium and copper contents of the AIS obtained from cv. Gordal olives. (See **Figure 1** for abbreviations.) Values represent mean and standard deviation for nine determinations.

increased considerably. This was interpreted as a greater presence of Cu in the pectin chains.

Table 2 shows the concentrations of Cu and Ca found in the AIS of olives presenting green staining and of others not presenting it, although coming from the same fermenter. Table 2 also includes data for the AIS obtained after the green staining affected zone had been distinguished from the remaining zone of the same altered fruits. The Ca content was higher in the altered fruits than in unaltered ones and—within the same fruit—was higher in the zone with green staining than in that without green staining. In these latter samples, corresponding to AIS obtained from surface areas of the fruit, the Ca concentration was very much higher than in AIS from the whole fruit. In the case of Cu, however, the levels found were always of the same order, coinciding with the maximum values found in the pH experiment—those of the AIS obtained from the fruits kept at pH 4.5 and 3.5. The fact that the Cu content of the AIS was always of the same order, independent of the source of the sample (fruit kept at acid pH or fruits and zones of fruits with or without green staining), suggested that the availability of the Cu ion to form part of the pectin chains is limited and that it reaches equilibrium with the rest of the Cu in the fruit. Similar results were found by Axelos et al. (*21*). Furthermore, this constancy indicated that the Cu was linked more strongly than

Table 3. Total Cu–Metallochlorophyll Complexes Concentration and Weight of AIS and Cu Present in the AIS of Each Sample[a]

	Cu–metallochlorophyll complexes				
sample[b]	μmol/kg of fruit	%[c]	g of AIS/100 g of fruits	μmol of Cu in the AIS/kg of fruit	balance of material of Cu[d] (μmol/kg of fruit)
FF	0.00	0.00	5.35 ± 0.32	9.91 ± 0.60	9.91 ± 0.60e
CF	0.00	0.00	3.11 ± 0.17	4.84 ± 0.29	4.84 ± 0.29f
fruit kept at pH 6.5	0.00	0.00	5.24 ± 0.26	6.29 ± 0.30	6.29 ± 0.30f
fruit kept at pH 5.5	1.07 ± 0.06	0.20 ± 0.01	5.52 ± 0.20	10.46 ± 0.53	11.53 ± 0.60e
fruit kept at pH 4.5	0.90 ± 0.05	0.54 ± 0.03	5.59 ± 0.22	14.58 ± 0.75	15.48 ± 0.80g
fruit kept at pH 3.5	1.29 ± 0.08	1.06 ± 0.05	5.56 ± 0.31	13.69 ± 0.61	14.98 ± 0.70g
fruit without GS	0.58 ± 0.02	0.89 ± 0.04	5.58 ± 0.39	14.56 ± 0.71	15.14 ± 0.73g
fruit with GS	2.38 ± 0.14	3.39 ± 0.17	5.03 ± 0.21	12.52 ± 0.58	14.90 ± 0.71g
RZ	1.89 ± 0.09	2.50 ± 0.11	5.01 ± 0.20	13.08 ± 0.66	14.97 ± 0.74g
GSZ	6.09 ± 0.37	9.53 ± 0.57	4.90 ± 0.17	9.55 ± 0.49	15.64 ± 0.84g

[a] Values represent mean and standard deviation for four determinations for the metallochlorophyll complexes analysis, three determinations for the weigth of AIS, and nine determinations for the Cu analysis. [b] FF, fresh fruit; CF, olives after treatment with NaOH, followed by washing with water. For remaing abbreviations see **Table 2**. [c] Percentage of total chlorophyll derivatives. [d] Sum of the Cu forming the metallochlorophyll complexes and that found in the AIS. Mean values in this column wihout a common following letter are significantly different ($p < 0.05$).

the Ca, above all when whole fruit and surface zone of fruit were compared.

Cu—Metallochlorophyll Complex Content. Chlorophylls *a* and *b* and—in very low proportion—the allomerized derivatives 13^2-OH-chlorophyll *a*, 13^2-MeOH-chlorophylls *a* and *b*, and 15^1-MeOH-lactone chlorophylls *a* and *b* were the chlorophyll pigments present in the FF. In the CF, in addition to all of the aforementioned pigments, 15-glyoxylic acid chlorophylls *a* and *b* were present. In the fruits from the pH study, except at pH 6.5, the Mg-free derivatives pheophytins *a* and *b*, 15-glyoxylic acid pheophytins *a* and *b*, and the Cu−15-glyoxylic acid pheophytin *a* complex were detected. In the samples kept at pH 3.5, the Cu−pheophytin *a* complex was also formed. Therefore, at acid pH, when the presence of Cu detected in the pectic chains was higher, the Mg-free chlorophyll derivatives may be able to form Cu complexes by means of a cation exchange mechanism.

In table olives from fermenters in which green staining alteration had developed, all of the above Mg-free chlorophyll and Cu−chlorophyll derivatives and Cu−15-glyoxylic acid pheophytin *b*, Cu−15-formylpheophytin *a*, and Cu−pyropheophytin *a* were found. Nevertheless, in this study, only the total Cu−metallochlorophyll complexes were taken into account. They were expressed as micromoles per kilogram of fruits and as a percentage of total chlorophyll derivatives.

Table 3 shows the values for total Cu−metallochlorophyll complexes found in each sample, the weight (grams) of AIS per 100 g of fruits, and the Cu (micromoles) present in the AIS per kilogram of fruit. A balance of material with respect to the Cu content localized in the AIS of the fruits was performed. As the pH at which the fruits were kept became more acid, the percentage concentration of the Cu−metallochlorophyll complexes, with respect to the total chlorophyll derivatives, increased. At the same time, the concentration of Cu−metallochlorophyll complexes in the olives with green staining was higher than in the olives without green staining. The same trend was seen when the green-stained and unstained zones of the fruit were compared. When the Cu concentrations in the AIS per kilogram of fruits were compared (**Table 2**), however, the value in the unaltered fruits was higher than that in the altered ones. The same trend was observed in the corresponding values for the two differentiated zones in the same fruit. These results suggested that part of the Cu present in the pectic residue—which might be joining polysaccharide chains—had been released and formed complexes with the chlorophyll derivatives, reducing the Cu content of the AIS as a result of the extraction of pigments during AIS preparation. To confirm this hypothesis, the balance of matter of the involved Cu was calculated as the sum of the Cu forming the metallochlorophyll complexes and that found in the AIS. For all of the samples related with the green staining alteration, the Cu level was constant (**Table 3**). The same result was found in the olive samples with the maximum Cu value in the AIS, that is, those fruits kept at pH 4.5 and 3.5. However, no relationship was observed in the other samples, because there was no equilibrium between the Cu of the pectic chains and that present in the rest of the fruit. Consequently, the results obtained from the balance of matter were consistent with the hypothesis that pectic chains provide the metal of the chlorophyll complexes.

ABBREVIATIONS USED

AIS, alcohol-insoluble solids; GA, galacturonic acid; FF, fresh fruit; CF, olives after treatment with NaOH, followed by washing with water; PP, protopectin; CaP, calcium pectate; SP, soluble pectins; TP, total pectins.

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LITERATURE CITED

(1) Fernández-Díez, M. J.; de Castro y Ramos, R.; Garrido-Fernández, A.; González-Pellisó, F.; Nosti-Vega, M.; Heredia-Moreno, A.; Mínguez-Mosquera, M. I.; Rejano-Navarro, L.; Durán-Quintana, M. C.; Sánchez-Roldán, F.; García-García, P.; de Castro y Gómez-Millán, A. *Biotecnología de la Aceituna de Mesa*; Consejo Superior de Investigaciones Científicas: Madrid, Spain, 1985.

(2) Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Mínguez-Mosquera, J. Mechanism and kinetics of the degradation of chlorophylls during the processing of green table olives. *J. Agric. Food Chem.* **1994**, *42*, 1089−1095.

(3) Mínguez-Mosquera, M. I.; Gallardo-Guerrero, L. Anomalous transformation of chloroplastic pigments in Gordal variety olives during processing for table olives *J. Food Prot.* **1995**, *58*, 1241−1248.

(4) Gandul-Rojas, B.; Gallardo-Guerrero, L.; Mínguez-Mosquera; M. I. Identification of oxidized chlorophylls and metallochlorophyllic complexes of copper in table olives (cv. Gordal) with green staining alteration. *J. Food Prot.* **1999**, *62*, 1172−1177.

(5) Mínguez-Mosquera, M. I.; Gallardo-Guerrero, L.; Hornero-Méndez, D.; Garrido-Fernández, J. Involvement of copper and zinc ions in "green staining" of table olives of the variety Gordal. *J. Food Prot.* **1995**, *58*, 564−569.

(6) Jones, I. D.; White, R. C.; Gibbs, E.; Butler, L. S.; Nelson, L. A. Experimental formation of zinc and copper complexes of chlorophyll derivatives in vegetable tissue by thermal processing. *J. Agric. Food Chem.* **1977**, *25*, 149−153.

(7) Laborde, L. F.; von Elbe, J. H. Method for improving the color of containerized green vegetables. U.S. Patent 5,482,727, 1996.

(8) Segner, W. P.; Ragusa, T. J.; Nank, W. K.; Hoyle, W. C. Process for the preservation of green color in canned vegetables. U.S. Patent 4,473,591, 1984.

(9) Levi, A.; Ben-Shalom, N.; Plat, D.; Reid.; D. S. Effect of blanching and drying on pectin constitutens and related characteristics of dehydrated peaches. *J. Food Sci.* **1988**, *53*, 1187−1203.

(10) Rinaudo, M. Physicochemical properties of pectins in solution and gel states. In *Pectins and Pectinases. Progress in Biotechnolgy*; Visser, J., Voragen, A. G. J., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1996; Vol. 14, pp 21−33.

(11) Alonso, J.; Rodríguez, T.; Canet, W. Effect of calcium pretreatment on the texture of frozen cherries. Role of pectinesterase in the changes in the pectic materials. *J. Agric. Food Chem.* **1995**, *43*, 1011−1016.

(12) Axelos, M. A. V.; Garnier, C.; Renard, C. M. G. C.; Thibault, J. F. Interactions of pectins with multivalent cations; Phase diagrams and structural aspects. In *Pectins and Pectinases. Progress in Biotechnolgy*; Visser, J., Voragen, A. G. J., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1996; Vol. 14, pp 35−45.

(13) Kartel, M. T.; Kupchik, L. A.; Levchenko, I. G. Selectivity of various pectins to heavy metal ions. *Spec. Publ. R. Soc. Chem.* **2000**, *No. 251*, 452−456.

(14) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.

(15) Von Mollendorff, L. J.; De Villiers, O. T. Role of pectolytic enzymes in the development of woolliness in peaches. *J. Hortic. Sci.* **1988**, *63*, 53−58.

(16) Doesburg, J. J. *Pectic Substances in Fresh and Preserved Fruits and Vegetables*; IBVT Communication 25; Veenman, H., Zonen, N. V., Eds.; IBVT: Wageninen, The Netherlands, 1965.

(17) Mínguez-Mosquera, M. I.; Garrido-Fernández, J. Chlorophyll and carotenoid presence in olive fruit (*Olea europaea* L.). *J. Agric. Food Chem.* **1989**, *37*, 1−7.
(18) Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Montaño-Asquerino, A.; Garrido-Fernández, J. Determination of chlorophylls and carotenoids by high-performance liquid chromatography during olive lactic fermentation. *J. Chromatogr.* **1991**, *585*, 259−266.
(19) Mínguez-Mosquera, M. I.; Gandul-Rojas, B.; Garrido-Fernández, J. Preparation of Cu(II) complexes of oxidized chlorophylls and their determination by thin-layer and high-performance liquid chromatography. *J. Chromatogr.* **1996**, *731*, 261−271.
(20) Renard, C. M. G. C.; Thibault, J. F. Pectins in mild alkaline conditions: β-elimination and kinetics of demethylation. In *Pectins and Pectinases. Progress in Biotechnolgy*; Visser, J., Voragen, A. G. J., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1996; Vol. 14, pp 603−608.
(21) Axelos, M. A. V.; Mestdagh, M. M.; Francois, J. Phase diagrams of aqueous solutions of polycarboxylates in the presence of divalent cations. *Macromolecules* **1994**, *27*, 6594−6602.

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