PIGMENT CHANGES DURING PROCESSING OF GREEN TABLE OLIVE SPECIALITIES TREATED WITH ALKALI AND WITHOUT FERMENTATION

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ABSTRACT

Green table olive preparations including an alkaline treatment, but not preservation by natural fermentation, such as Castelvetrano-style, are gaining importance in international trade. One of the main highly valued characteristics for this product is a bright green colour, as similar as possible to the fresh fruit. Large proportions of Cu-chlorophyll complexes have been quantified in commercial products of Castelvetrano-style table olives thus in the present work the changes in the chlorophyll and carotenoid composition of olive fruits during this processing style were investigated. The high alkaline pH of the process was the most important feature governing the subsequent transformation of the chlorophyll pigments initially present in the fresh fruit. During processing, the main transformations of pigments were due to allomerization and solvolysis reactions of chlorophylls *a* and *b*, which affected the isocyclic ring of the chlorophyll structure but not the Mg atom that remained, and did not change substantially the pigment colour and, consequently, the fruit colour. No Cu-chlorophyll derivatives were detected. Then, even though the intense alkali treatment of the olives may cause such damage in the fruit cells that enable contact between the chlorophyll derivatives and the endogenous Cu of the fruit, the absence of acidic conditions during the olive processing did not lead to the previous reaction of Mg replacement by 2H, which is necessary for the following insertion of Cu. With respect to the carotenoid fraction, any noticeable change took place.

Keywords: allomerized derivatives; alkaline treatment; carotenoid; Castelvetrano-style; chlorophyll; Cu-chlorophyll derivatives; table olive.

1. Introduction

The olive fruits are mostly destined to obtain olive oil. However, a considerable part of them are processed to different types of table olives for direct human consumption. According to the International Olive Council (IOC, 2013), the provisional world production of table olives was 2,424,500 t in the 2012/2013 season. There are numerous commercial preparations of table olives, being a feature common to all of them the removing of the glucoside oleuropein responsible for the natural bitterness of the olive fruit. The treatment of the fruits with dilute alkaline solution is one of the main procedures used worldwide for this purpose via hydrolysis (Garrido-Fernández, Fernández-Díez, & Adams, 1997).

Table olives are broadly distinguished as green or black, although there are also other trade products with olives turning colour. The main green table olive commercial preparation is the so-called Spanish or Seville-style which comprises an alkaline treatment of the olives, followed by washings with tap water, and subsequent natural lactic fermentation in brine (Garrido-Fernández, et al., 1997; Rejano, Montaño, Casado, Sánchez, & de Castro, 2010). However, there are other green table olive preparations that include an alkaline treatment but not preservation by natural fermentation, which are gaining importance in international trade, being included as "Specialities" in the trade standard applying to table olives (IOC, 2004). Such elaborations have features and specific names in the different producing countries: Campo Real in Spain (De Lorenzo et al., 2000); Castelvetrano in Italy (Rejano et al., 2010); Picholine in France, (Rejano et al., 2010) and green ripe olives in the United States (USDA, 1983). The specific conditions of the alkaline treatment (time and NaOH concentration) and the number and characteristics of the washings are the main differences between the commercial products. In addition to its mild and slightly alkaline taste, one of the main highly valued features for this product is a bright green colour, as similar as possible to the fresh fruit and very different from the characteristic golden-yellow colour of Spanish-style table olives.

Generally, colour is one of the most important quality attributes for table olives and can be considered as a quality index. For preparing green table olives, fruits are picked when they have reached their maxima size and their colour varies between green and yellowish green. At this time, the olive colour is due to the presence of two main families of natural pigments, chlorophylls and carotenoids, which are transformed to some derivatives during fruit processing (Gallardo-Guerrero, Gandul-Rojas, Mínguez-Mosquera, & Roca, 2012). In the case of green table olives treated with alkali but not fermented the bright green colour of the fruits is highly instable, changing to yellowish tones with time and losing their related freshness and consumer acceptability. Consequently, the colour of this type of table olive is sometime adulterated with E-141ii colourant to obtain a permanent colour in the final product (Gandul-Rojas, Roca, & Gallardo-Guerrero, 2012). This colourant is a mixture of numerous Cu chlorin-type compounds derived from natural chlorophyll by alkaline hydrolysis and reaction with copper salts.

Studies carried out by us, at the request of table olive industries (private reports 2005-2010), have revealed a huge presence of metallo-chlorophyll complexes of Cu in commercial green table olives with a striking bright green colour and labelled both, as “Green olives in soda” and Castelvetrano-style. Similar results have been found by Aparicio-Ruiz, Riedl and Schwartz (2011), who estimated percentages of Cu-chlorophyll complexes up to 90% of the total chlorophyll compounds present in commercial samples of bright green table olives. These Cu-chlorophyll complexes are structurally different from those that make up the E-141ii colourant, and they can be generated during the industrial processing of food by adding Cu salts, such as $CuCl_2$ or $CuSO_4$ (Segner, Ragusa, Nank, & Hoyle, 1984; LaBorde & von Elbe, 1996). However, in the case of table olives it would be a fraudulent practice because the use of copper salts is not yet accepted (Codex Alimentarius, 1981).

Nevertheless, the formation of the same Cu-chlorophyll complexes with endogenous copper of the fruits has been demonstrated in Spanish-style table olives of the Gordal variety affected by the alteration known as *green staining*, which is seen as bluish-green spots distributed over the skin (Gallardo-Guerrero, Hornero-Méndez, & Mínguez-Mosquera, 2002; Gandul-Rojas, Gallardo-Guerrero, & Mínguez-Mosquera, 1999; Mínguez-Mosquera, Gallardo-Guerrero, Hornero-Méndez, & Garrido-Fernández, 1995). In principle, the olive fruit contains sufficient Cu to complex in vitro all their chlorophyll pigments, but this fact does not usually happen in table olives processed as Spanish-style because the metal ion is not accessible to form the coordination complexes. However, in relation to the *green staining* alteration, the oxidizing capacity of the alkaline treatment used in the olive processing has been connected with a strong oxidative disintegration of the fruit chloroplasts, allowing contact between the chlorophyll pigments and the endogenous copper of the fruit, and enabling the formation, migration and localized accumulation of the Cu-metallo-chlorophyll derivatives responsible for the anomalous regreening (Gallardo-Guerrero, Gandul-Rojas, & Mínguez-Mosquera, 2007; Gallardo-Guerrero, Milicua, Salvador, Jarén-Galán, & Mínguez-Mosquera, 2003).

To date, bright green table olives (such as Castelvetrano and Campo Real-styles) have been scarcely studied for different commercial products (Aparicio-Ruiz et al., 2011; Gandul-Rojas et al., 2012), but no detailed study about the pigment transformation during processing has been carried out enabling us to know the normal pigment composition that could and should be found in these products. The Castelvetrano-style processing is a good model to study the pigment changes in these specialties of green table olives since the olives are kept longer in alkaline conditions than other similar table olive types, such as Campo-Real, Picholine or green ripe olives and, a priori, it could trigger off a further deterioration of the fruit chloroplasts. Moreover, as mentioned above, large proportions of Cu-chlorophyll

complexes have been quantified in commercial Castelvetrano-style table olives (Aparicio-Ruiz et al., 2011).

This work was aimed to clarify whether the intense alkaline treatment, typical of the Castelvetrano-style processing (lasting two weeks in alkaline brine), may cause such damage in the fruit cells that leads to the complexation reaction of the chlorophyll derivatives with endogenous Cu in massive form. With that purpose, the changes on the chlorophyll and carotenoid composition of olive fruits during Castelvetrano-style processing, and their involvement in the colour of the final product, have been studied. Only with a deep knowledge of the proper pigments that should be found in a product, we will be able to evaluate adequately its colour and the factors determining its quality.

2. Materials and methods

2.1. Raw material and sample preparation.

Fruits of the Manzanilla variety (*Olea europaea* L.), harvested in the optimal maturity degree (when colours varied from light green to yellow-green), were processed at laboratory scale as green table olives treated with alkali and without fermentation, according to the Castelvetrano-style as is described (Rejano et al., 2010). The selected olives (around 1.8 kg), were put into four plastic vessels with 3 L total capacity and covered with 1.3 L of NaOH solution at 2% (wt/vol). One hour after this alkaline treatment began 75 g of NaCl was added to each container (equivalent to a final concentration of 5.8% in the NaCl/NaOH solution) and the olives were kept in this alkaline brine for 15 days separated into two batches: one batch was maintained at room temperature (RT) (17±7ºC) and the other was kept in a cold room (CR) at 4ºC. Then, after a brief washing step, the product is ready for marketing. Both experiments were run in duplicate, and samplings were made on fresh fruit (FF), fruits treated

with alkali for 1 hour (FTA), and fruits kept in the alkaline brine for 7 and 15 days, both in a CR or at RT.

2.2. Pigment extraction.

All procedures were performed under dimmed green light to avoid any photooxidation of chlorophylls and carotenoids. The pigment extraction was performed from 10 g of olive sample taken from a homogenized triturate, prepared from 15-20 pitted fruits. Previously to pit the fruits, they were washed several times with distilled water until the washing pH was neutral. To carry out the pigment extraction, the method of Mínguez-Mosquera and Garrido-Fernández (1989), slightly modified as previously described by Gandul-Rojas et al. (2012), was used. The technique is based on the selective separation of components between N,N-dimethylformamide (DMF) and hexane. The hexane phase carried over lipids and carotenes, whereas the DMF phase retained chlorophylls and xanthophylls. The pigments from the DMF phase were later transferred to ethyl ether, concentrated to dryness, and the dry residue was dissolved in 1.5 mL acetone for pigment analysis by HPLC. β-carotene was directly quantified in the hexane phase by absorbance measurement at 450 nm.

2.3.Pigment analysis by HPLC

Pigment separation was carried out according to a modification of the method of Mínguez-Mosquera, Gandul-Rojas, Montaño-Asquerino and Garrido-Fernández (1991), as is described by Gandul-Rojas et al., (2012). Spectrophotometric detection of pigments was performed at 410, 430, 450 and 666 nm. The on-line UV-Vis spectra were recorded from 350 to 800 nm with the photodiode–array detector. Data were collected and processed with a LC HP ChemStation (Rev.A.05.04). Pigments were identified by co-chromatography with the corresponding standard and from the spectral characteristics as has been described in detail

elsewhere (Mínguez-Mosquera et al., 1991; Mínguez-Mosquera & Gandul-Rojas, 1995; Aparicio-Ruiz et al., 2011). Fig.1 shows the structures and assigned numbers for the chlorophyll pigments relevant to this study. Pigments were quantified using external standard calibration curves prepared with purified standards of each pigment. Analyses were performed in triplicate for the fresh fruit and in quadruplicate for processed olives (each experiment was run in duplicated and each one was analysed by duplicated).

2.4. pH Measurement

The pH was measured in the homogenized triturated resulting from triturating the plant material in a minimum volume of distilled water with a polytron homogenizer at 24000 rpm/min for 1 min. The water was added to facilitate homogenization.

2.5. 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 Corporation, Milford, MA).

2.6. Apparatus

Equipment included a pH meter, model pH 555 (Teknokroma, Barcelona, Spain), an Ultra-Turrax model T-25 polytron homogenizer (Janke Kunker, IKA-Laboratechnik), a Büchi rotavapor, Model R 110 (Laboratoriums-technik AG, Switzerland), a bench-top centrifuge Mikro20 (hettich Zentrifugen, Tuttlingen, Germany), and an HP 1100 Hewlett-Packard (Palo Alto, CA) liquid chromatograph fitted with an HP 1100 automatic injector and diode array detector.

184 *2.7. Statistical analysis*

185 Data were expressed as mean values ± standard deviation (SD). Basic statistic, mean
186 and SD, were calculated for the results with Statistica 6.0 software (Statsoft, 2001).
187 Comparison between mean variables was made by the Duncan's multiple range tests and the
188 differences were considered significant when $p < 0.05$.

189

190 **3. Results and discussion**

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192 At present, the chloroplastic pigment composition in olive fruits is well known, as well
193 as that their quantitative composition depends on the olive variety and the ripeness degree of
194 the fruits (Mínguez-Mosquera & Gallardo-Guerrero, 1995a, Roca & Mínguez-Mosquera,
195 2001, Vergara-Domínguez, Gandul-Rojas, & Roca, 2011). Then, as expected, chlorophyll *a*
196 (1.1*a*) and chlorophyll *b* (1.1*b*) were the main components in the chlorophyll fraction of the
197 fresh fruit, accompanied by minor quantities of their respective epimers, chlorophyll *a´*
198 (1.1*a´*) and chlorophyll *b´* (1.1*b´*). Moreover, 13^2-OH-chlorophylls *a* (1.2*a*) and *b* (1.2*b*),
199 pheophytin *a* (1.3*a*) and other minor catabolic intermediates were also detected. The
200 carotenoid fraction was composed of lutein, β-carotene, neoxanthin, violaxanthin and
201 antheraxanthin. Table 1 shows the initial concentration of the chlorophyll and carotenoid
202 pigments in the fresh olives, as well as the qualitative and quantitative changes in these
203 pigments during the processing of fruits as Castelvetrano-style table olives.

204 Monitoring of the pigments began after the treatment of the fruits with 2% NaOH
205 solution for 1 hour. At this time, the pH of the alkaline solution was 12.74 while in the juice
206 of the fruit was 5.48, i.e., around the internal pH value which may be reached in the case of
207 olives when the cell integrity is broken and the own acids of the fruits are liberated (Garrido-
208 Fernández et al., 1997). This meant that the alkali had scarcely penetrated into the fruit.

Therefore, as the skin of the olive was resistant to the NaOH solution after 1 hour of the alkaline treatment, any qualitative change was observed in the pigment profile of the olive fruits. Nevertheless, a slight increase in the chlorophylls *a´* (1.1*a´*) and *b´* (1.1*b´*) epimers was detected. The epimerization is the mildest transformation possible of the chlorophylls and, in general, is favoured by temperature (Schwartz & Lorenzo, 1990).

It is a common practice in the olive sector industries to maintain a share of production under refrigerated conditions to retard the degradation of the characteristic bright green colour of the product. Because of this, after the addition of NaCl (see *Section 2.1*), replicated containers were stored in a cold room or at room temperature with the aim to study any effect of the temperature on the pigment transformations, and they were monitored for pigment analysis at the middle and at the end time of the process (7 and 15 days, respectively).

In general, the main transformations of pigments found were due to allomerization reactions of chlorophylls *a* and *b*. The allomerization reactions are those in which the isocyclic ring of the chlorophyll molecule (Fig. 1) is oxidized by triplet molecular oxygen ($^3O_2$), and include a complex series of oxidation on C-13^2 (Hynninen, 1991). As it is shown in Table 1, the content of the allomerized derivative 13^2-OH-chlorophyll *a* (1.2*a*), initially detected in the fresh fruit, increased during the fruit processing, and the formation of some 15^1-OH-lactone-chlorophylls *a* (2.1*a*) and *b* (2.1*b*) was also detected. Some different behaviour was found between chlorophylls *a* and *b*, since for the latter it was not observed any increase of the compound 13^2-OH-chlorophyll *b* (1.2*b*), while the formation of the corresponding 15^1-OH-lactone-derivative (2.1*b*) was proportionally higher. In this respect, differences between the allomerization of chlorophyll *a* and chlorophyll *b* have been described in the literature (Hynninen & Assandri 1973; Hyvärinen & Hynninen, 1999). Moreover it stood out the appearance of new allomerized chlorophyll derivatives characterized by an open isocyclic ring (chlorin- and rhodin-type structures, for the series *a*

and *b*, respectively), resulting from solvolysis reactions of the mentioned ring (Hynninen, 1991). Those compounds were identified as the Mg complex of 15^2-Me-phytol-chlorin e_6 ester (3.3*a*), 15^2-Me-phytol-rhodin g_7 ester (3.3*b*), and 15^2-Me-phytol-isochlorin e_4 (tentative) ester (3.4*a*). No Cu-chlorophyll derivatives were detected.

The Mg-chlorophyll derivatives with chlorin e_6, or rhodin g_7 structure were preliminary identified in Spanish-style table olives of the Gordal variety as Mg-phytol-chlorin e_6 (3.1*a*), and Mg-phytol-rhodin g_7 (3.1*b*) (Mínguez-Mosquera & Gallardo-Guerrero, 1995b), according to their chromatographic characteristics and absorption spectral data (Mínguez-Mosquera & Gandul-Rojas, 1995). Afterwards, the molecular mass data of the corresponding Mg-free-derivative of the series *a*, obtained by positive-ion fast atom bombardment mass spectrometry and positive/negative ion electrospray mass spectrometry led to its identification as purpurin 7 *a* phytyl ester, also called 15-glyoxylic acid pheophytin *a* (3.8*a*) (Mínguez-Mosquera, Gandul-Rojas, & Garrido-Fernández, 1996) and, consequently, the precursor was identified as 15-glyoxylic acid chlorophyll *a* (3.2*a*). This compound has similar chromatographic and spectral characteristics than Mg-phytol-chlorin e_6 (3.1*a*) but differs by 14 units in molecular mass. Nowadays, fragmentation pattern data obtained recently with a Quadrupole/time-of-flight mass spectrometer by Aparicio-Ruiz et al. (2011), suggest new structures such as 15^2-Me-phytol-chlorin e_6 ester (3.6*a*), for the Mg-free derivative of the series *a*, and 15^2-Me-phytol-rhodin g_7 ester (3.6*b*), for the corresponding to the series *b*. Accordingly, their precursor compounds detected in the present work should be identified as the Mg complexes of 15^2-Me-phytol-chlorin e_6 ester (3.3*a*), and 15^2-Me-phytol-rhodin g_7 ester (3.3*b*), respectively. These structures have the same molecular mass than 15-glyoxylic acid chlorophyll *a* (3.2*a*) — or 15-glyoxylic acid chlorophyll *b* (3.2*b*) — but different fragmentation pattern. Similarly, the above authors (Aparicio-Ruiz et al., 2011) propose a structure of 15^2-Me-phytol-isochlorin e_4 ester (3.7*a*) for the Mg-free compound previously

identified as 15-formyl-pheophytin *a* (3.9*a*) by its molecular mass in Spanish-style table olives (Gandul-Rojas, et al., 1999), being their corresponding precursors the Mg complex of 15^2-Me-phytol-isochlorin e_4 ester (3.4*a*) and 15-formyl-chlorophyll *a* (3.5*a*), respectively.

The formation of the chlorin- and rhodin-type compounds fits properly with the mechanism proposed by Hynninen (1973) for the solvolysis of the isocyclic ring of the chlorophyll and their derivatives, as well as with some degradation products of chlorophylls *a* and *b* obtained by alkaline treatment in aqueous media (Mínguez-Mosquera & Gandul-Rojas, 1995), while the 15-glyoxylic acid chlorophyll derivatives previously identified, are mainly found when chlorophylls stand in methanol solution exposed to atmospheric oxygen (Hyvärinen & Hynninen, 1999; Kuronen, Hyvärinen, & Hynninen, 1993; Wooley, Moir, Hester, & Keely, 1998).

In addition to all of the allomerized derivatives formed in the fruits during processing, a small increase of chlorophyll *a´* (1.1*a´*), as well as the appearance of some pyropheophytin *a* (1.4*a*), was also detected, probably due to the slight raise of temperature that takes place during the alkaline treatment (Tarrado-Castellarnau, Domínguez-Ortega, Tarrado-Castellarnau, & Pleite-Gutierrez, 2013).

Some decrease in the total chlorophylls was detected after the seventh day of the processing, which did not progress later. The pigment decrease should be due to certain degradation to uncoloured compounds, although we should also consider the possibility that the new compounds formed, with chlorin e_6, or rhodin g_7 structure, which are more polar than the native chlorophylls, could diffuse from the fruit inside to the surrounding brine solution due to an osmotic effect. Concerning to this result, it stood out that the higher decrease was found for the chlorophylls of the series *b*, what might be related with the reactivity differences of chlorophyll *a* and chlorophyll *b*, under comparable reaction conditions (Hynninen, Leppäkases, & Mesilaakso, 2006).

With respect to the carotenoid pigments of the fruits, the alkaline treatment did not have any effect on them, since they are stable compounds towards alkali (Schiedt & Liaaen-Jensen, 1995). Therefore, any noticeable change took place throughout the olive processing.

During the whole processing time of green table olives as Castelvetrano-style, fruits were subjected to a high alkaline pH, around 10-11 units. Consequently, reactions replacing the Mg atom of the chlorophyll molecule by two H, as well as isomerization reactions of carotenoids with the 5,6-epoxy group in their structure, to the 5,8-furanoid group, which easily occur under acidic conditions, were not observed. These reactions are however characteristic in the processing of Spanish-style table olives due to the acid pH resulting from the fermentation step (Mínguez-Mosquera & Gandul-Rojas, 1994; Mínguez-Mosquera, Gandul-Rojas, & Mínguez-Mosquera, 1994). During fermentation, sugars pass into the brine and are metabolized by microorganisms with the formation of lactic acid and other organic acids providing a characteristic and distinctive acid flavour to the fermented product. In addition, at the end of the lactic fermentation, the pH values are low enough to allow the preservation of the product in safety. Instead, the taste of the Castelvetrano-style table olives is mild and slightly alkaline, and the product suffers from a short shelf life due to the relatively high pH of the olive pulp at the end of the process. In this case, the use of some sort of thermal treatment in order to preserve olives in the long term is required and, unfortunately, the colour and other organoleptic characteristics are significantly changed during these conservation treatments. In general, similar qualitative changes of chlorophylls were produced both in a cold room and at room temperature, but some quantitative differences were found. To evaluate the overall chlorophyll transformation during the table olive processing, the chlorophyll pigments were grouped into three fractions (in percentage terms), according to the main changes of chlorophylls as follow: native chlorophylls — *a* and *b* (1.1*a* and 1.1*b)*, including their respective epimers *(*1.1*a´* and 1.1*b´)* and the minor Mg-free derivatives present

initially in the fruits or formed during processing (1.3*a* and 1.4*a*) — , allomerized chlorophylls — 13^2-OH-chlorophylls *a* and *b* (1.2*a* and 1.2*b)*, and 15^1-OH-lactone-chlorophylls *a* and *b* (2.1*a* and 2.1*b*) —, and chlorin- and rhodin-type derivatives, resulting from allomerized and solvolysis reactions — the Mg complex of 15^2-Me-phytol-chlorin e_6 (3.3*a*), 15^2-Me-phytol-rhodin g_7 (3.3*b*) and 15^2-Me-phytol-isochlorin e_4 esters (3.4*a*) (Fig. 2). It could be checked that reactions were faster when olives were processed at room temperature, so that the chlorophyll derivatives formed were significantly higher under those conditions than in a cold room for the same sampling date. At the end of the fruit processing in a cold room, the allomerized and the chlorin- and rhodin-type derivatives increased, reaching similar proportions than those formed at the middle of the process (7^{th} day sampling) at room temperature. However, in the last case, only the chlorin- and rhodin-type compounds increased significantly until the end of the process, while a slight but significant decrease in the percentage of the allomerized derivatives was found. Consequently, the main difference found between processing in a cold room or at room temperature, was some higher proportion of the chlorophyll derivatives with structure chlorine or rhodin-type, when fruits were processed at room temperature, reaching levels similar to the native chlorophylls (*a* and *b*), and in no case the Mg was removed from the chlorophyllic structure.

The results of the present study demonstrated that the green colour of olive fruits after processing as Castelvetrano-style was mainly due to the presence of chlorophylls *a* and *b* as well as other allomerized chlorophyll derivatives with Mg in their structures. Since Cu-chlorophyll complexes were not detected at any time, our results did not explain in principle the high presence of these compounds (ranging from 33% to 99% of the total chlorophylls) that has been identified in bright green table olives marketed as Castelvetrano (Aparicio-Ruiz et al., 2011).

The intense alkaline treatment of the olives, typical of the Castelvetrano-style processing, may cause such damage in the fruit cells that enables contact between the chlorophyll derivatives and the endogenous Cu of the fruit (Gallardo-Guerrero et al., 2007). However, it is known that the Cu-chlorophyll complexes are always formed from Mg-free derivatives, never from chlorophylls or Mg-derivatives, and the absence of acidic conditions during the olive processing did not lead to the previous reaction of Mg replacement by 2H, which is necessary for the following insertion of Cu. Nevertheless, we can not rule out the possibility that Cu-chlorophyll complexes can be formed later in the olives if they are subsequently subjected to some thermal treatment (sterilization or pasteurization), which typically produces a massive formation of Mg-free chlorophyll derivatives (Schwartz and Lorenzo, 1990). This point is at present under study since, due to the relatively high pH of the olive pulp after the water washings, and according to the Quality Standards which regulate trade in table olives (Codex Alimentarius, 1981), thermal treatments are frequently used for these types of green table olive specialities in order to preserve the product in the long term.

4. Conclusion

We can conclude that the high alkaline pH of the Castelvetrano table olive processing is the main feature which will govern the subsequent transformation of the chlorophyll pigments initially present in the fresh fruit. During processing, the main transformations of pigments were due to oxidation reactions of chlorophylls *a* and *b*, which affected the isocyclic ring of the chlorophyll structure but not the Mg atom that remained, and did not change substantially the pigment colour and, consequently, the fruit colour. However, the pigment transformation found in the present study for Castelvetrano-style green table olives after processing, was far from the pigment composition identified in marketed olives (Aparicio-Ruiz et al., 2011). At

358 present, the effect of different packing conditions on the final composition of pigments is
359 under study.
360
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476

FIGURE CAPTIONS

Figure 1. Structures and assigned numbers (No) for the chlorophyll pigments relevant to this study.

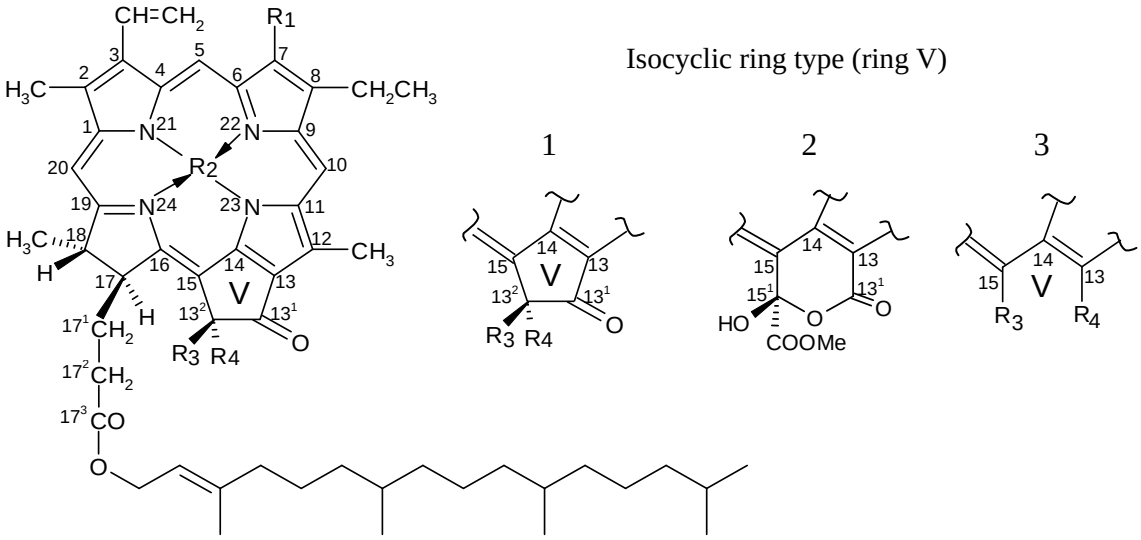
Figure 2. Changes in the percentage composition of the chlorophyll pigments present in olive fruits during processing as Castelvetrano-style table olives. According to the main changes of chlorophylls during processing, pigments are grouped into three fractions as follow: Chls *a* and *b* (sum of chlorophylls *a* and *b* (1.1*a* and 1.1*b*), their respective epimers *(*1.1*a´* and 1.1*b´)* and the minor Mg-free derivatives present in the fruits (1.3*a* and 1.4*a*), allomerized chls *a* and *b* (sum of 13^2-OH-chlorophylls *a* and *b* (1.2*a* and 1.2*b)*, and 15^1-OH-lactone-chlorophylls *a* and *b*) (2.1*a* and 2.1*b*)), and chlorin- and rhodin-type chls (sum of the Mg complex of 15^2-Me-phytol-chlorin e_6 (3.3*a*), 15^2-Me-phytol-rhodin g_7 (3.3*b*) and 15^2-Me-phytol-isochlorin e_4 esters (3.4*a*)).

For each pigment fraction, values with different letters above the standard deviation bars are significantly different ($p < 0.05$). Abbreviations: FF, fresh fruit; FTA, fruits treated with alkali for 1 hour; FCR and FTR, fruits kept in alkaline brine in a cold room and at room temperature, respectively; chls, chlorophylls.

TABLE 1. Changes in the chloroplastic pigment composition (µmol/kg pitted fruit) of olive fruits during processing as Castelvetrano-style table olives. [a,b]

	FF	FTA	FCR		FRT	
		Time of processing				
Chlorophyll pigment		1 hour	7 days	15 days	7 days	15 days
Chlorophyll *a*	53.10 ± 2.75[a]	49.86 ± 1.13[a]	21.36 ± 2.50[b]	15.88 ± 0.36[c]	16.64 ± 0.12[c]	13.11 ± 0.75[c]
Chlorophyll *a´*	0.37 ± 0.06[a]	1.45 ± 0.02[b]	3.14 ± 0.06[c]	2.21 ± 0.08[d]	3.91 ± 0.23[c]	2.89 ± 0.08[c]
13^2-OH-chlorophyll *a*	1.48 ± 0.08[a]	1.46 ± 0.47[a]	7.23 ± 0.97[b]	9.81 ± 0.16[c]	9.76 ± 1.04[c]	8.21 ± 0.89[b, c]
Pheophytin *a*	0.65 ± 0.05[a]	0.73 ± 0.06[a]	0.11 ± 0.02[b,c]	1.43 ± 0.06[d]	0.23 ± 0.07[b]	0.07 ± 0.04[c]
Pyropheophytin *a*	-	-	0.09 ± 0.01[a]	0.88 ± 0.22[b]	0.22 ± 0.09[a]	0.15 ± 0.04[a]
15^1-OH-lactone-chlorophyll *a*	-	-	traces	traces	0.82 ± 0.07[a]	0.63 ± 0.22[a]
Mg-15^2-Me-phytol-chlorin e_6 ester	-	-	9.24 ± 0.24[a]	10.76 ± 1.55[a]	10.65 ± 0.03[a]	14.49 ± 1.80[b]
Mg-15^2-Me-phytol-isochlorin e_4 ester	-	-	0.08 ± 0.00[a]	0.33 ± 0.02[b]	0.29 ± 0.02[b]	0.67 ± 0.09[c]
Chlorophyll *b*	12.11 ± 1.41[a]	11.37 ± 0.54[a]	3.95 ± 0.63[b]	3.19 ± 0.19[b,c]	1.54 ± 0.12[c,d]	1.20 ± 0.06[d]
Chlorophyll *b´*	0.48 ± 0.07[a]	1.44 ± 0.03[b]	0.93 ± 0.04[c]	0.67 ± 0.13[d]	0.41 ± 0.01[a,e]	0.27 ± 0.00[e]
13^2-OH- chlorophyll *b*	0.57 ± 0.29[a]	0.24 ± 0.03[a]	0.31 ± 0.03[a]	0.44 ± 0.18[a]	0.35 ± 0.03[a]	0.30 ± 0.04[a]
15^1-OH-lactone-chlorophyll *b*	-	-	0.10 ± 0.00[a]	0.25 ± 0.06[a]	0.18 ± 0.03[a]	0.09 ± 0.09[a]
Mg-15^2-Me-phytol-rhodin g_7 ester	-	-	0.50 ± 0.08[a]	0.80 ± 0.00[a]	0.47 ± 0.02[a]	1.23 ± 0.53[a]
Total series *a*	55.60 ± 2.78[a]	53.50 ± 1.68[a]	41.24 ± 3.77[b]	41.29 ± 1.20[b]	42.51 ± 1.31[b]	40.21 ± 3.93[b]
Total series *b*	13.16 ± 1.19[a]	13.05 ± 0.59[a]	5.80 ± 0.70[b]	5.35 ± 0.58[b]	2.94 ± 0.05[c]	3.10 ± 0.72[c]
Total chlorophylls	68.76 ± 3.97[a]	66.55 ± 2.27[a]	47.03 ± 4.46[b]	46.64 ± 0.63[b]	45.44 ± 1.26[b]	43.31 ± 4.65[b]
Carotenoid pigment [c]						
Lutein	7.65 ± 0.74[a]	7.97 ± 0.52[a]	7.82 ± 1.46[a]	7.30 ± 0.84[a]	8.24 ± 1.23[a]	7.96 ± 1.11[a]
β-carotene	5.74 ± 0.07[a]	5.89 ± 0.08[a]	6.16 ± 0.71[a]	5.96 ± 0.41[a]	5.68 ± 0.06[a]	5.56 ± 0.12[a]
Violaxanthin	3.50 ± 0.44[a]	3.34 ± 0.60[a]	4.08 ± 0.85[a]	3.72 ± 0.14[a]	3.52 ± 0.67[a]	3.15 ± 0.28[a]
Neoxanthin	2.98 ± 0.13[a]	2.88 ± 0.21[a]	3.26 ± 0.47[a]	3.16 ± 0.01[a]	3.12 ± 0.34[a]	2.91 ± 0.26[a]
Antheraxanthin	0.32 ± 0.03[a]	0.54 ± 0.02[b]	0.35 ± 0.07[a]	0.30 ± 0.03[a]	0.36 ± 0.03[a]	0.33 ± 0.02[a]
Total Carotenoids	20.20 ± 1.41[a]	20.66 ± 1.26[a]	21.67 ± 2.86[a]	20.43 ± 1.13[a]	21.62 ± 2.17[a]	20.50 ± 2.00[a]

[a] Abbreviations: FF, fresh fruit; FTA, fruit treated with alkali for 1 hour, FCR, fruit processed in cold room; FRT, Fruit processed at room temperature. [b] Data represent the mean value ± SD (n = 3 for the fresh fruit, and n = 4 for processed olives). Values with different letters in a row are significantly different ($p < 0.05$). [c] Data include the respective *cis* isomers in those cases that they were present.



Pigment	No	I.ring [a]	R_1	R_2	R_3	R_4
Chlorophyll *a*	1.1*a*	1	CH_3	Mg	H	$COOCH_3$
Chlorophyll *a´*	1.1*a´*	1	CH_3	Mg	$COOCH_3$	H
13^2-OH-chlorophyll *a*	1.2*a*	1	CH_3	Mg	OH	$COOCH_3$
Pheophytin *a*	1.3*a*	1	CH_3	2H	H	$COOCH_3$
Pyropheophytin *a*	1.4*a*	1	CH_3	2H	H	H
15^1-OH-lactone-chlorophyll *a*	2.1*a*	2	CH_3	Mg		
Mg-phytol-chlorin e_6	3.1*a*	3	CH_3	Mg	CH_2COOH	COOH
15-Glyoxylic acid chlorophyll *a*	3.2*a*	3	CH_3	Mg	COCOOH	COOH
Mg-15^2-Me-phytol-chlorin e_6 ester	3.3*a*	3	CH_3	Mg	CH_2COOCH_3	COOH
Mg-15^2-Me-phytol-isochlorin e_4 ester	3.4*a*	3	CH_3	Mg	CH_2COOCH_3	H
15- Formyl -chlorophyll *a*	3.5*a*	3	CH_3	Mg	COH	COOH
15^2-Me-phytol-chlorin e_6 ester	3.6*a*	3	CH_3	2H	CH_2COOCH_3	COOH
15^2-Me-phytol-isochlorin e_4 ester	3.7*a*	3	CH_3	2H	CH_2COOCH_3	H
15- Glyoxylic acid pheophytin *a*	3.8*a*	3	CH_3	2H	COCOOH	COOH
15-Formyl pheophytin *a*	3.9*a*	3	CH_3	2H	COH	COOH
Chlorophyll *b*	1.1*b*	1	COH	Mg	H	$COOCH_3$
Chlorophyll *b´*	1.1*b´*	1	COH	Mg	$COOCH_3$	H
13^2-OH-chlorophyll *b*	1.2*b*	1	COH	Mg	OH	$COOCH_3$
15^1-OH-lactone-chlorophyll *b*	2.1*b*	2	COH	Mg		
Mg-phytol-rhodin g_7	3.1*b*	3	COH	Mg	CH_2COOH	COOH
15- Glyoxylic acid chlorophyll *b*	3.2*b*	3	COH	Mg	COCOOH	COOH
Mg-15^2-Me-phytol-rhodin g_7 ester	3.3*b*	3	COH	Mg	CH_2COOCH_3	COOH
15^2-Me-phytol-rhodin g_7 ester	3.6*b*	3	COH	2H	CH_2COOCH_3	COOH

[a] I.ring: isocyclic ring.

Figure 1

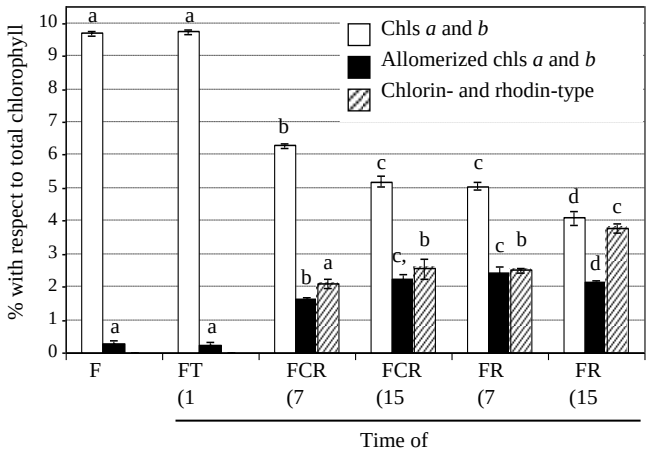

% with respect to total chlorophyll
Chls *a* and *b*
Allomerized chls *a* and *b*
Chlorin- and rhodin-type
F
FT
(1
FCR
(7
FCR
(15
FR
(7
FR
(15
Time of

Figure 2