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Introduction

It has been observed that the size, weight and type and content of chloroplastic pigments of the Gordal olive fruits harvested for industrial processing are heterogeneous. Also, it has been observed that during industrial processing for table olive, some fruits of Gordal variety are affected by an alteration visually detectable as a green spot caused by an abnormal copper chlorophyll derivatives accumulation in the external zone of the fruit (Gallardo-Guerrero et al., 1999). Latest studies established the nature and structure of the pigment involved in the alteration (Gandul-Rojas et al., 1999), although still remain unknown the causes inducing their formation. The own variability of the fruit could be the reason of different pigment transformation patterns when all the fruits are simultaneously treated under identical conditions. The aim of this study was to study the incidence of the alkaline treatment previous to fermentation on individual Gordal olive fruits of different size, weight and pigment content.

Materials and Methods

Sampling and Raw material

Fresh fruit of Gordal olive variety and their corresponding processed fruit by alkaline and neutralization treatment were used. Samples of fresh fruits were collected in the industry from the set daily received for processing. Processed samples of this same set of fruit were collected at the end of alkaline industrial treatment. For both fresh and treated fruits, 25 kg of fruits were taken. Fruits were sorted by size and from the resultant groups, 10% of the fruits were taken for their later analysis. For the industrially treated fruits, after sorted, fruits were introduced in phosphate buffer 0.2 M, pH 7 in order to keep the conditions until analysis. Each individual fruit was weight before and after de-stoned. The raw material for pigment analysis in each individual fruit was obtained by cutting the pulp into small pieces. Random sampling guarantees the presence of all sizes of fruits harvested. To consider all possible variability introduced by the fruit during harvest, the study was repeated during three industrial alkaline treatments coinciding with the beginning, middle and end of harvest.

Pigment analysis

Pigment extraction was performed with N,N-dimethylformamide according to the method described by Mínguez-Mosquera and Garrido-Fernández (1989). Following the method described by Mínguez-Mosquera et al. (1991), pigments were separated by reversed-phase high-pressure liquid chromatography, and identified and quantified by detecting the absorbance at selected wavelengths. The allomerized chlorophylls and metallochlorophyll complexes of copper were identified as was previously described in detail by Mínguez-Mosquera et al. (1996) and Gandul-Rojas et al. (1999). Results were centered in transformation products from chlorophyll "a" related or involved in the green staining alteration. To make comparable fruits with different pigment content, results were expressed as percentage of concentration of each pigment with respect the total pigment concentration of its series.

Results and Discussion

Figure 1 shows the percentage of allomerized chlorophyll "a" derivatives in each one of the analyzed fresh fruits with respect their weight. Results were separated according to the different times of harvesting. In all the analyzed fresh fruits, only one of them was absent of allomerized chlorophyll derivatives, in all the other fruits the average content in this type of compounds ranged between 0.5 y 3%. The derivatives found in fresh fruit are 132-OH-chlorophyll, 132-and 151-MeO-lactone-chlorophyll and absence in all cases of 132-glyoxylic acid chlorophyll. It can be observed that in the three harvests, the highest content in allomerized chlorophyll derivatives appeared in fruits with medium-low weight (9-12g). During Gordal olive fruit harvest season, the proportion of the allomerized chlorophyll derivatives increased. In the first harvest, 33% of the fruits had a content in these pigments higher than 2%. In the second and third harvest, the proportion of fruits with level higher than trace is 53% and 61% respectively. In parallel, there was an increase in the maximum content in these pigments, growing up from 4.5% at the first harvest to 5 and 7% in the second and third harvest.

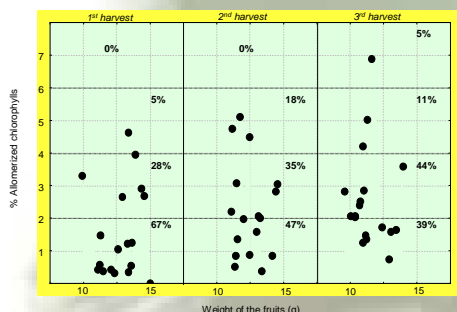


Figure 1. Percentage of allomerized chlorophylls in fresh fruit of Gordal olive variety.

The main difference among the fruits used in the three treatments appeared in the presence of copper glyoxylic acid pheophytin complex. This pigment is one of the usual constituents of the green staining alteration, but this alteration is only visible after the fermentation process. During the alkaline treatment, this pigment was only detected in fruits from the third harvest (figure 3) and its presence was observed in 30% of the fruits analyzed, corresponding to fruits with low-medium weight. The content measured oscillated between 0.1 and 0.5% in these fruits and were also those with highest content in allomerized chlorophylls.

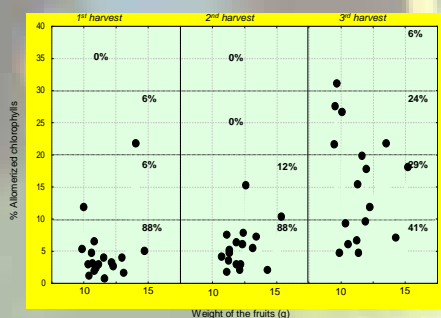


Figure 2. Percentage of allomerized chlorophylls in fruits of Gordal olive variety after the alkaline treatment.

The alkaline treatment provokes in the fruit a strong oxidation of the chlorophyll pigments that in some cases reaches levels of up to 50% of the total of pigments of the series. Upon ordering the analytical results by harvests and weight of the fruits, the percentage of fruits with content in allomerized chlorophylls higher than 10%, passes from 12% in the first and second harvest, to the 59% in the third (figure 2). Equally, the variability interval for these derivatives oscillates 22% - 1% in the first harvest to 32% - 5% in the third.

Alkaline treatment induced the oxidation and allomerization of the chlorophyll pigments, with a different incidence in each fruit due to their different weight and the time of harvest. In fresh fruit, it has been observed a greater degree of oxidation and allomerization especially in the smaller size fruits and fundamentally belonging to latest harvest. During alkaline treatment even copper glyoxylic acid pheophytin complex was formed in these fruits. Our results are similar to those previously found on the green staining alteration. The pigments found in some fruits after alkaline treatment are the same than those found in the green staining alteration and with similar random distribution among fruits but at very low concentration. Analytically, it seems that the alkaline treatment create a group of fruits with predisposition to develop the green staining alteration during the fermentative process.

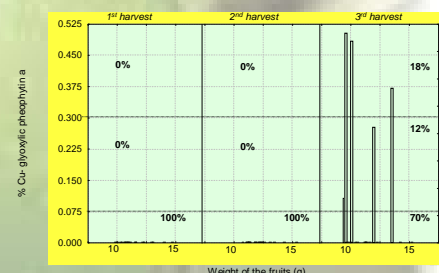


Figure 3. Cu-glyoxylic pheophytin percentage in Gordal olive fruits after the alkaline treatment.

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