Storage influence on the initial content and class of pigments of virgin olive oil

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Introduction

The pigment profile of the virgin olive oil recently extracted is determined by the chlorophyll and carotenoid fractions initially found in the fruits, plus derivatives formed during milling and beating. Therefore, chlorophyll and carotenoid composition of virgin olive oil is mediated for several factors, as ripeness degree and variety of the fruits employed, which will affect to the content, percentage and exclusivity of the pigments (1,2). Usual storage conditions for virgin olive oil in the industry before marketing are at 15ºC in the darkness. It is not known if the chlorophyll molecule undergoes any structure specific change, which modifies the pigment profile associated to the virgin olive oil recently extracted. The aim of the present study was to investigate this fact, considering different content and class of pigment of virgin olive oil.

Materials and Methods

Raw materials. The study was carried out using 5 mono-variety virgin olive oils of 5 different olive cultivars (Arbequina, Blanqueta, Cornicabra, Hojiblanca and Picual). The oils were distributed into amber glass jars of 65 mL capacity, with 3% (v/v) headspace and stored in refrigerated chamber at 15 ºC in darkness. The samples were analysed monthly during one year.

Analysis of pigments. The concentrated pigment extract suitable for chromatographic analysis was obtained by selective separation of components between N,N-dimethylformamide (DMF) and hexane (1). The hexane phase carried over lipids and the carotene fraction while the DMF phase retained chlorophylls and xantophylls. Separation, identification and quantification of pigments were carried out by HPLC-diode array detector (3,4).

Acidity determination. Free acidity, given as % oleic acid, was determined by titration (5).

Results and Discussion

In general, all oils showed similar qualitative composition (Figure 1, time zero). Chlorophylls *a* and *b*, pheophytins *a* and *b*, and in much less quantity the allomerized derivatives 13-OH-pheophytin *a* and 15^1-OH-lactone pheophytin *a* were present. Oil from Arbequina variety differed slightly with absence of pheophytin *b* and presence of dephytilated derivative, pheophorbide *a*. However, quantitatively, the initial composition of oils was different. Oils of the Arbequina and Hojiblanca varieties, whose were of greener apparent colour, had more than 50% of chlorophyll *a* (with respect to total chlorophyll compounds), while those of Cornicabra, Picual and Blanqueta, with a more golden colour, had mainly pheophytin a. Although pheophytinization reaction is mediated by an acid medium, the pheophytin content in the oils was not correlated with the free acidity measured. Therefore, it can be deduced that any acid compound and/or Mg-dequelating substances that are found in the olive fruit and released during milling and beating of the paste in the extraction process of the olive oil, as well as the characteristic of the fresh fruit, will determine the transformation range of chlorophyll into pheophytin.

Subsequently, during oil storage, pigment losses were not produced, but certain transformations of the initial pigments happened. Chlorophylls *a* and *b* were totally or partially transformed into pheophytins *a* and *b*, respectively. Furthermore, the presence of 13-OH- pheophytin *a* and 15^1-OH-lactone pheophytin *a* were increased slightly by allomerized reactions of the isocyclic ring in the pheophytin *a* molecule. In parallel, by descarboxylation of the former pigment, a new compound was formed, pyropheophytin *a*.

The transformation patterns of the chlorophyll pigments were similar in all oils used in the study. The pheophytinization reaction initiated during the oil extraction process, progresses during storage in a different way for each oil but not correlated with the free acidity measured in them. In oils with low initial presence of chlorophyll *a*, this was totally transformed in pheophytin *a* during the first months of storage. In Arbequina and Hojiblanca oils that transformation was slower, so that chlorophyll *a* was still detected after nine and twelve months in both oils, respectively.

With regard to transformation of chlorophyll *b* into pheophytin *b*, was complete only in Cornicabra oil. This behaviour is in agreement with the kinetics studies carried out on the pheophytinization reaction, which conclude that the reaction rate is always higher for chlorophyll *a* than chlorophyll *b* (6,7). In the same way that was noted for chlorophyll *a*, is fulfilled that the smaller initial proportion of chlorophyll *b*, the smaller transformation into pheophytin *b* during storage.

In all oil samples used in the study, similar pattern in the formation of allomerized derivatives was found increasing their initial concentration mainly during the first months. This coincides with the period of greater pheophytin *a* formation. As it has been suggested by Psomiadou and Tsimidou (8), the allomerized derivative formation could be attributed to the oxygen availability in the headspace of the oil bottles. In general, the oil with less initial percentage of allomerized derivatives (Hojiblanca variety) was also the oil with less formation of those compounds during storage.

Finally, the oil storage conditions have favoured the pyropheophytin *a* formation, compound that was absent initially. Although its formation was minimum, the concentration increased with storage time. In general, around 3% of the total chlorophyll pigments was the percentage of pyropheophytin *a* formed after twelve months. On the other hand, the ratio found between pheophytin *a* (precursor pigment) and pyropheophytin *a*, was decreasing until a value included between 20 and 30. Therefore, ratios lower than 20 could be indicative of the fact that the oil has been submitted to other storage conditions less adequate. In general, the pyropheophytin formation in a food is attributed to thermic treatments and the formation extension is mediated by the treatment severity (6). In this sense, in virgin olive oils stored at room temperature (25-35ºC) during 10 months in the darkness, higher proportion of pyropheophytin *a* formation were detected (between 7 and 13 % with respect to the *a* series) (9), which is contrast to the 2.5-3% that was originated in the present experience with lower temperature (15ºC). Therefore, the content and proportion of pyropheophytin *a* that is present in a virgin olive oil can be indicative of its storage conditions.

Conclusions

The content and class of pigments in virgin olive oil are outlined as parameter of varietal origin traceability, process and product quality. Small structural transformations of chl pigments, that are not inherent to the oil extraction process, will be signs of the storage of virgin olive oil, even in soft conditions such as 15ºC in the darkness. Therefore, the qualitative pigment profile, previously established as an authenticity parameter of the virgin olive oil, is also outlined as a traceability index of the quality of the finished product and of its modification until reaches the consumer.

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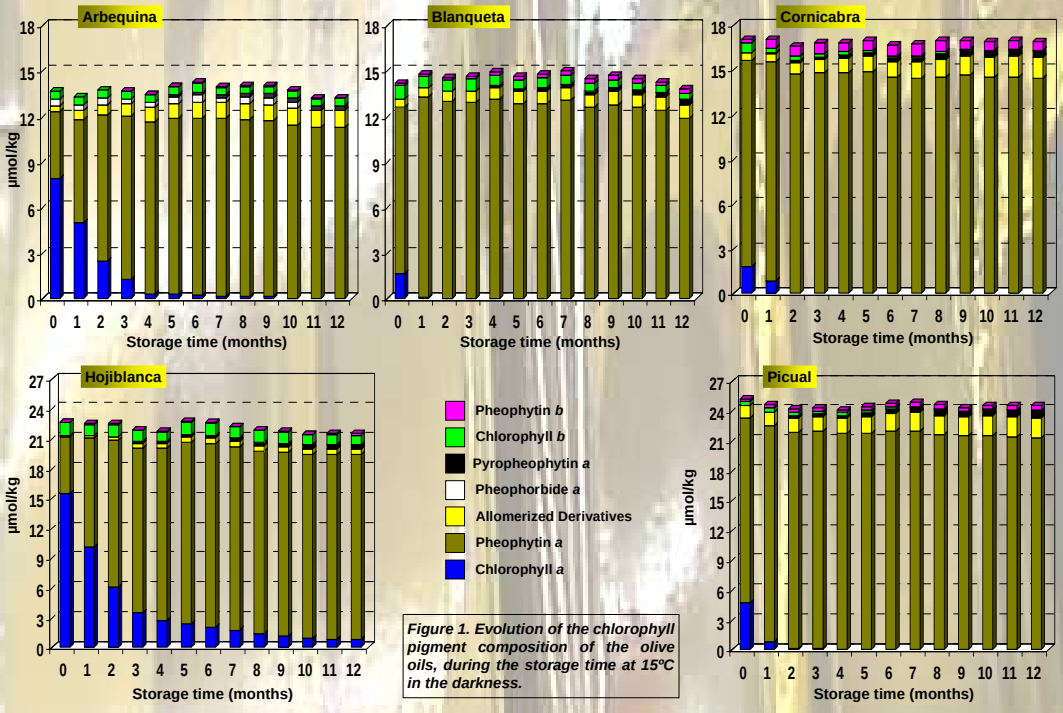

Figure 1. Evolution of the chlorophyll pigment composition of the olive oils, during the storage time at 15ºC in the darkness.

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