Evolution of Virgin Olive Oil during Long-term Storage

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Abstract: In this work, the evolution of virgin olive oil from 4 olive varieties when stored at 5°C, 10°C and 20°C for a period of up to 3 years was studied. Free acidity increased progressively for the 4 varieties, staying below the limit for extra virgin olive oil, even at the highest temperature. The peroxide value also increased, reaching its maximum after about 28 months of storage, when some samples stored at 20°C exceeded the limit for extra virgin olive oil. The maximum values for K270 and K232 were reached at the end of the storage period, also exceeding the limit for extra virgin olive oil in the case of some varieties stored at 20°C. Oxidative stability decreased by 38%-50% depending on the storage temperature and the variety. Most of the color indexes increased in value because of the degradation of the oil pigments.

Key words: virgin olive oil, storage, oxidation, peroxide index, oxidative stability, color

1 Introduction

Virgin olive oil (VOO) is one of the most common and valued virgin oils due to its remarkable and unique properties. As a virgin oil, it is not subjected to a refining process aimed at rectifying its chemical properties, as is the case with most other vegetable oils.

However, prolonged or inadequate storage conditions can lead to a degradation of oil quality. Positive attributes of VOO, such as a fruity flavor, can be partly lost during storage, and sensory defects can arise. Furthermore, some of the regulated chemical quality parameters can deteriorate, and, as a result, the commercial category of the oil could be downgraded from extra virgin olive oil (EVOO) to VOO, or from VOO to lampante, not fit for human consumption without further processing.

The olive variety also influences the chemical and sensory degradation that takes place during storage, since each variety has its own profile of minor components that could affect oil stability, such as phenolic compounds, tocopherols and phytosterols, among others.

Stefanoudaki et al. studied the evolution of VOO during 15 months of storage in inside and outside warehouse conditions, but they did not maintain a controlled temperature. Likewise, other works have studied the quality changes of VOO during storage time of up to 6 months at room temperature. Sanmartín et al. compared the evolution of EVOO stored at low (6°C) and room temperature (26°C) conditions, but the storage time was limited to 125 days. In another work, Cinquanta et al. studied the oxidative stability of VOO at 6°C and room temperature during a more extended period of up to 18 months. To our knowledge, there is no other published study on the evolution of the physicochemical quality parameters, stability and color of VOO stored at low or ambient temperatures for long periods of time.

In this work, the evolution of the regulated physicochemical parameters, the stability and the color of four varieties of VOO samples kept at 3 temperatures (5°C, 10°C and 15°C) for up to 3 years was studied.

2 Experimental

2.1 Raw material

The trees corresponding to the 4 studied varieties (Arbequina, Cornicabra, Picual and Hojiblanca) were planted in contiguous plots with similar characteristics belonging to the same farm located in the county of Campos de Hellín (Albacete). The olive samples were taken on the same date, at the beginning of the month of December. For each
variety, 3 samples of 20 kg were collected by the milking system and transported to the ETSIAM laboratory (UCLM) in appropriate mesh bags. Each sample was independently processed and stored to obtain three true replications for the analysis.

2.2 VOO extraction in pilot plant

Olive oil extraction was carried out using the Oliomio TF-30 extractor (Toscana Enológica Mori, Tavarnelle Val di Pesa, FI, Italy), consisting of a hammer mill, a thermobind machine and a horizontal centrifuge with 2 output phases. For each of the olive samples collected, approximately 2 liters of oil were separated. This extraction was carried out under the best processing conditions (i.e., unharmed olives and the use of recommended processing temperatures). The oil was not filtered. Once the oil was decanted, it was introduced into 5 mL amber colored glass bottles for storage.

2.3 Oil storage

The oil was stored in the dark at three different temperatures (5°C, 10°C and 20°C) for a period of 3 years. Every 2 months, a stored oil sample of each treatment was taken in order to analyze its parameters of regulated physicochemical quality. The other parameters were analyzed at the beginning and at the end of the storage period.

2.4 Analytical determinations

The parameters of regulated physicochemical quality (free acidity, peroxide index and absorbance in the ultraviolet spectrophotometer by absorption at 270 and 232 nm respectively, using a UV ˇcyclohexane, with a 1 cm optical path) order to analyze its parameters of regulated physicochemical quality. The other parameters were analyzed at the beginning and at the end of the storage period.

3 Results and Discussion

3.1 Regulated physicochemical parameters

The free acidity of the samples of virgin olive oil stored at the three temperatures increased over time for the four varieties analyzed (Fig. 1). The effect of both temperature and variety on the slope of the trend lines were significant (p<0.05). The highest free acidity values were observed with the Hojiblanca variety at 20°C, but they did not exceed 0.5% and thus remained below the limit of 0.8% for the “extra virgin” olive oil category.

The evolution of the peroxide value of the stored samples can be seen in Fig. 2. The peroxide value increased over time for the four varieties, and the increase was greater at higher storage temperatures (p<0.05). Note that a maximum peroxide value appears after about 28 months. The presence of this maximum is clearer at the highest temperature (20°C). This suggests that the compounds resulting from the primary oxidation of the oils that are generated during storage are in turn transformed into other secondary oxidation compounds that do not contribute to the peroxide index. Aragao et al. developed a mathematical model for the evolution of the peroxide index which predicted the presence of a maximum, but our data did not fit well to that model. The model developed by these authors assumed that the peroxides that appear during lipid oxidation subsequently degrade to other compounds. The lack of fit of our data to this model suggests that a more complex oxidation mechanism is taking place. The peroxide index exceeded the limit of 20 meq / kg for the Arbequina and Hojiblanca varieties stored at 20°C at the peak value that occurred around the 28th storage month. Nevertheless, all the samples were below this threshold at the end of the storage period.

Table 1 shows the initial values of the K232 and K270 indices and their final values after 36 months of storage.
Fig. 1 Evolution of free acidity of virgin olive oil during storage. A: Arbequina, P: Picual, C: Cornicabra, H: Hojiblanca (means of 3 replications).

Fig. 2 Evolution of peroxide value of virgin olive oil during storage. A: Arbequina, P: Picual, C: Cornicabra, H: Hojiblanca (means of 3 replications).
Both indices increased during the storage period \((p < 0.05)\).
A two-way analysis of variance (ANOVA) of the change of these indices during the storage period (Table 3) showed that the increase of \(K_{270}\) depended on the variety, but did not depend on temperature, whereas the increase in \(K_{232}\) depended on the variety and temperature. The interaction \((\text{variety} \times \text{temperature})\) was significant for both indices, revealing a difference in the response of the studied varieties to the storage conditions. All the values of the \(K_{232}\) index were below the regulatory threshold of 2.5 for the Extra Virgin olive oil, while the \(K_{270}\) index slightly exceeded its limit of 0.22 in the case of the oils of the Arbequina and Hojiblanca varieties stored at 20°C towards the end of the storage period.

### 3.2 Oxidative stability

Table 1 also shows the values of oxidative stability measurements at the beginning and at the end of the storage period. Stability decreased with storage time in all cases. The decrease in stability was greater at higher temperatures in Arbequina and Hojiblanca, but not in Picual and Cornicabra, for which no significant differences were observed between the values of stability at the end of the storage period at the three temperatures tested. The oils of the Arbequina and Hojiblanca varieties had the least oxidative stability. This agreed with results published by Gutiérrez et al.\(^{14}\). A two-way ANOVA (Table 3) showed that the effects of variety, temperature, and interaction (variety \( \times \) temperature) on the decrease of stability during the storage period were all significant \((p < 0.05)\). After 36 months, stability fell by about 50% for the less stable varieties (Arbequina and Hojiblanca), while the stability of Cornicabra oil was reduced by 40%. The oil of the Picual variety was the most stable and the one that lost the least stability during storage (38%, approximately).

### 3.3 Color evolution

Table 2 shows the chromatic coordinates of the CIELAB space at the beginning and at the end of the storage period. Luminosity \((L^*)\) increased significantly in all four varieties, regardless of storage temperature. The greatest increases in \(L^*\) occurred for the oil of the Picual variety. The increase in luminosity reflects the degradation of chlorophylls and carotenoids during the storage.\(^{15}\) The \(a^*\) index also increased, eventually switching from negative to positive values. The increase in this index corresponds to the loss of the greenish color of the oil, mainly due to the oxidation of pigments such as chlorophylls.\(^{16}\) The increase of the \(b^*\) index was slight in the case of the Arbequina and Cornicabra varieties, and much more significant in the

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**Table 1** UV absorbance \((K_{232} \text{ and } K_{270})\) and oxidative stability for the virgin olive oil samples at the beginning and at the end of the 36-month storage period at three temperatures.

| Variety      | Time (months) | Temperature (°C) | \(K_{232}\)   | \(K_{270}\)   | Stability (h) |
|--------------|---------------|------------------|---------------|---------------|---------------|
| Arbequina    | 0             | –                | 1.64 ± 0.15   | 0.12 ± 0.01   | 63.80 ± 5.81  |
|              | 36            | 5                | 2.11 ± 0.21\(^{AB}\) | 0.16 ± 0.01\(^{AA}\) | 55.31 ± 4.80\(^{AB}\) |
|              |               | 10               | 2.35 ± 0.18\(^{AB}\) | 0.20 ± 0.02\(^{ABAB}\) | 36.09 ± 4.00\(^{AB}\) |
|              |               | 20               | 2.45 ± 0.18\(^{AB}\) | 0.25 ± 0.02\(^{ABAB}\) | 32.05 ± 3.98\(^{AB}\) |
| Cornicabra   | 0             | –                | 1.50 ± 0.13   | 0.10 ± 0.01   | 132.50 ± 9.12 |
|              | 36            | 5                | 1.42 ± 0.16\(^{AB}\) | 0.11 ± 0.03\(^{AB}\) | 95.52 ± 8.75\(^{ABC}\) |
|              |               | 10               | 1.61 ± 0.15\(^{AB}\) | 0.09 ± 0.02\(^{AB}\) | 82.58 ± 8.52\(^{AB}\) |
|              |               | 20               | 2.09 ± 0.18\(^{AB}\) | 0.22 ± 0.04\(^{ABAB}\) | 79.35 ± 7.25\(^{AB}\) |
| Picual       | 0             | –                | 1.70 ± 0.15   | 0.10 ± 0.02   | 179.90 ± 8.89 |
|              | 36            | 5                | 1.46 ± 0.19\(^{AB}\) | 0.12 ± 0.03\(^{AB}\) | 112.07 ± 9.21\(^{AC}\) |
|              |               | 10               | 1.55 ± 0.21\(^{AB}\) | 0.13 ± 0.02\(^{AB}\) | 118.55 ± 8.46\(^{AC}\) |
|              |               | 20               | 1.65 ± 0.15\(^{AB}\) | 0.16 ± 0.03\(^{AB}\) | 124.99 ± 8.78\(^{AC}\) |
| Hojiblanca   | 0             | –                | 1.77 ± 0.18   | 0.14 ± 0.00   | 85.21 ± 7.91  |
|              | 36            | 5                | 1.60 ± 0.19\(^{AB}\) | 0.19 ± 0.02\(^{ABAB}\) | 59.58 ± 7.20\(^{ABC}\) |
|              |               | 10               | 2.02 ± 0.14\(^{ABAB}\) | 0.21 ± 0.02\(^{ABAB}\) | 51.82 ± 6.58\(^{ABAB}\) |
|              |               | 20               | 2.28 ± 0.20\(^{AB}\) | 0.23 ± 0.01\(^{ABAB}\) | 43.90 ± 5.28\(^{AB}\) |

Mean of 3 samples ± SD. Values in each column with different superscripts lowercase letters present significant differences \((p < 0.05)\) between temperatures for each variety. Values in each column with different capital letters present significant differences \((p < 0.05)\) between varieties for each temperature.
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Table 2  Chromatic coordinates of the CIELAB space for the virgin olive oil samples at the beginning and at the end of the 36-month storage period at three temperatures.

| Variety       | Time (months) | Temperature (°C) | L*         | a*         | b*         | c*         | h*         |
|---------------|---------------|------------------|------------|------------|------------|------------|------------|
| Arbequina     | 0             | –                | 27.98 ± 0.54 | −0.26 ± 0.17 | 14.12 ± 0.16 | 14.1 ± 1.0  | 89.0 ± 10.1 |
|               | 36            | 5                | 30.10 ± 0.75 | 0.38 ± 0.09  | 17.610.14  | 17.6 ± 1.2  | 90.6 ± 9.4  |
|               |               | 10               | 30.38 ± 0.42 | 0.40 ± 0.16  | 18.11 ± 1.2  | 18.1 ± 1.1  | 91.0 ± 9.9  |
|               |               | 20               | 30.10 ± 0.31 | 0.46 ± 0.08  | 17.60 ± 0.10 | 17.6 ± 1.1  | 89.5 ± 10.0 |
| Cornicabra    | 0             | –                | 27.70 ± 0.20 | −0.80 ± 0.10 | 14.40 ± 0.21 | 14.4 ± 0.9  | 93.0 ± 8.8  |
|               | 36            | 5                | 29.95 ± 0.33 | −0.42 ± 0.14 | 17.11 ± 1.9  | 17.2 ± 1.3  | 92.7 ± 8.9  |
|               |               | 10               | 30.30 ± 0.42 | −0.45 ± 0.09 | 18.33 ± 0.22 | 18.4 ± 1.1  | 92.9 ± 9.0  |
|               |               | 20               | 30.20 ± 0.14 | −0.40 ± 0.11 | 18.28 ± 0.20 | 18.2 ± 1.6  | 92.0 ± 9.7  |
| Hojiblanca    | 0             | –                | 23.40 ± 0.68 | −0.70 ± 0.09 | 8.75 ± 0.11  | 8.8 ± 1.5   | 84.3 ± 9.1  |
|               | 36            | 5                | 30.13 ± 0.27 | −0.46 ± 0.05 | 18.26 ± 0.19 | 18.3 ± 1.1  | 91.9 ± 10.4 |
|               |               | 10               | 30.68 ± 0.55 | −0.45 ± 0.05 | 18.89 ± 0.20 | 18.9 ± 1.2  | 92.9 ± 9.5  |
|               |               | 20               | 30.17 ± 0.30 | −0.10 ± 0.10 | 17.60 ± 0.18 | 17.6 ± 1.0  | 91.9 ± 9.6  |
| Picual        | 0             | –                | 22.07 ± 0.56 | 1.10 ± 0.15  | 4.97 ± 0.15  | 5.0 ± 1.1   | 77.6 ± 9.4  |
|               | 36            | 5                | 26.90 ± 0.46 | 1.26 ± 0.14  | 14.20 ± 0.14 | 14.3 ± 1.1  | 87.3 ± 9.5  |
|               |               | 10               | 27.83 ± 0.51 | 1.28 ± 0.16  | 14.80 ± 0.15 | 14.8 ± 1.3  | 88.4 ± 9.9  |
|               |               | 20               | 28.20 ± 0.62 | −0.10 ± 0.10 | 17.60 ± 0.18 | 17.6 ± 1.0  | 91.9 ± 9.6  |
| Cornicabra    | 0             | –                | 26.70 ± 0.56 | 1.10 ± 0.15  | 4.97 ± 0.15  | 5.0 ± 1.1   | 77.6 ± 9.4  |
|               | 36            | 5                | 26.90 ± 0.46 | 1.26 ± 0.14  | 14.20 ± 0.14 | 14.3 ± 1.1  | 87.3 ± 9.5  |
|               |               | 10               | 27.83 ± 0.51 | 1.28 ± 0.16  | 14.80 ± 0.15 | 14.8 ± 1.3  | 88.4 ± 9.9  |
|               |               | 20               | 28.20 ± 0.62 | −0.10 ± 0.10 | 17.60 ± 0.18 | 17.6 ± 1.0  | 91.9 ± 9.6  |

Mean of 3 samples ± SD. Values in each column with different superscripts lowercase letters present significant differences (p < 0.05) between temperatures for each variety. Values in each column with different capital letters present significant differences (p < 0.05) between varieties for each temperature.

Table 3  Percentage sum of squares and R² of the linear model for two-way analysis of variance (ANOVA) of the change in the value of parameters after the 36-month storage period.

| Factor       | K222 | K270 | Stability | L* | a* | b* | c* | h* |
|--------------|------|------|-----------|----|----|----|----|----|
| Variety (V)  | 56.97** | 53.21** | 58.12** | 61.09** | 73.08** | 65.41** | 51.23** | 61.08** |
| Temperature (T) | 6.54NS | 18.32* | 17.90* | 4.25NS | 5.51NS | 4.40NS | 5.92NS | 3.27NS |
| Interaction (V * T) | 25.93** | 16.03* | 13.85* | 5.83NS | 3.28NS | 2.18NS | 2.45NS | 2.19NS |
| R²           | 0.894 | 0.876 | 0.899 | 0.712 | 0.819 | 0.720 | 0.596 | 0.665 |

**. Significant p < 0.01, *. Significant p < 0.05, NS: Not significant.

The *Picual* variety, and especially in *Hojiblanca*. The index c* followed an evolution like b*, while h* only increased in *Picual* and *Hojiblanca*. A two-way ANOVA (Table 3) showed that the variety was the only significant factor on the change of the color parameters during the storage period.

4 Conclusion

Extra virgin olive oil stored at 5-10°C kept its regulated physicochemical parameters within the threshold for its category for up to 3 years. Storage at 20°C allowed for about 24 months before the peroxide index and ultraviolet absorption parameters exceeded their threshold values for *Arbequina* and *Hojiblanca* varieties, while *Picual* and *Cornicabra* could be stored up to 36 months without reaching the limit for these parameters. The chromatic coordinates L*, a*, b* and c* increased during the storage period. *Picual* was the most stable of the four varieties but was also the variety with the greatest change in color.

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