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Determinant Parameters and Components in the Storage of Virgin Olive Oil. Prediction of Storage Time beyond Which the Oil Is No Longer of "Extra" Quality

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This work studies the changes in the quality indices standardized by the European Union, together with the evolution of the oxidative stability and sterols, polyphenols, α-tocopherol, pigments, and fatty acids contents throughout the storage of Picual and Hojiblanca olive cultivar "extra" virgin olive oils at 2 °C + darkness and 30 °C + illumination. Only two quality indices (K_{270} and sensory evaluation) indicate the loss of the extra quality of the oil during storage, and there is an excellent correlation between initial stability and the time to reach the limit of $K_{270} > 0.25$ (after which the oil quality is no longer of "extra" quality). This time can be predicted with an error of <10%, which is of great commercial interest and previously unknown. Also unknown until now is that the changes in polyphenols, pigments, and α-tocopherol with storage time follow first-order kinetics.

KEYWORDS: **Virgin olive oil; storage; quality**

INTRODUCTION

During the shelf life of bottled virgin olive oil, the packaging must adequately protect it against autoxidation processes that cause rancidity (*1*−*3*), one of the best known and most frequent oil alterations, and must also conserve its peculiar sensory characteristics (*4*−*6*). Color, aroma, and taste distinguish virgin olive oil from other edible oils, which are consumed after refining and are therefore odorless, insipid, and almost colorless. In super- and hypermarkets bottled oils are exposed to light and high temperatures (typically 28−30 °C), which are not optimum conditions of preservation for any edible oil, but especially so, for the given reasons, in the case of the virgin olive oil.

A review of the literature on vegetable oils, and in particular on virgin olive oil, reveals that most of the work on commercial packaging and storage conditions was carried out at the Instituto de la Grasa, CSIC (Seville, Spain) (*7*−*15*). These authors studied the changes in the standardized quality indices, oxidative stability, and tocopherol levels. This paper further reports on the evolution in the sterols, polyphenols, pigments, and fatty acids contents.

Manzi et al. (*16*) studied the loss of carotenes, tocopherols, and squalene during olive storage, whereas Mastrobattista (*17*) reported on the decomposition of chlorophyll *a*. On the other hand, Leonardis (*18*) compared the shelf life of glass-bottled virgin olive oil stored in a dark cold room versus ambient temperature with diffuse light. Cinquanta et al. (*19*) studied changes in the phenolic compounds of virgin oilve oil during storage in the dark. All of the olive oils examined in these researches were extracted from several olive cultivars grown in the same Italian region and harvested at different stages of ripeness.

In Spain, 90% of virgin olive oil is packaged in bio-use PVC, PET, and clear glass, with the latter being increasingly used for the packaging and marketing of "extra" quality oils. The claim of this paper is to determine which of the standard quality indices of oil may be used as markers to predict the time when a stored bottled virgin olive oil loses its "extra" quality (acidity ≤ 1%; peroxide value ≤ 20 mequiv/kg; K_{232} ≤ 2.50; K_{270} ≤ 0.25; sensory score ≥ 6.5). Furthermore, we studied the evolution of other important components of virgin olive oil and investigated the relationships among them and with the standard quality indices. The loss of quality of bottled oils is mainly due to oxidative reactions that are catalyzed by light and temperature.

MATERIAL AND METHODS

Materials. Extra virgin olive oils of the Picual and Hojiblanca cultivars, the two most important ones in Spain, were used. Fruits of both varieties were grown in the Experimental Stations Venta del Llano in Menjibar (Jaén) and in Antequera (Malaga). Their oils were extracted in industrial mills by the three-phase continuous system and then packaged in transparent clear glass 1-L bottles. **Table 1** gives the initial characteristics of both oils.

Experimental Design. For each cultivar 46 L of extra virgin olive oils was packaged. Thirty-four bottles of each cultivar were stored in conditions similar to those in consumer sales points: inside a thermostated chamber at 30 °C and placed in shelf units of 3 m long and 3 m high, with illumination (800 lx; 12 h/day). Bottles were sampled weekly during the first 70 days and subsequently every 15 days for complete 6 months of storage. Other 12 bottles of each cultivar were kept in a cool room at 2 °C in darkness and sampled every 15 days.

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Table 1. Initial Values of the Oils

	Picual	Hojiblanca
acidity (%)	0.44	0.26
PV (mequiv/kg)	4.65	5.62
k_{232} (e_x, 1%, 1 cm)	1.44	1.65
k_{270} (e_x, 1%, 1 cm)	0.106	0.117
sensory evaluation	6,8	8.3
stability (h)	69.5	43.3
fatty acids (%)		
palmitic (16:0)	12.09	11.12
stearic (18:0)	2.40	2.70
oleic (18:1)	78.96	75.36
linoleic (18:2)	3.72	8.20
linolenic (18:3)	0.85	0.86
polyphenols (mg/kg, caffeic)	133.8	157.3
o-diphenols (mg/kg, caffeic)	9.00	14.64
α-tocopherol (mg/kg)	287.22	207.08
β-tocopherol (mg/kg)	2.57	1.98
γ-tocopherol (mg/kg)	21.77	13.02
total tocopherols (mg/kg)	311.56	222.08
pigments, chlorophyllics (mg/kg)	11.35	17.91
pigments, carotenoids (mg/kg)	11.26	13.04
sterols (mg/kg)		
cholesterol	1.76	1.31
24-methylenecholesterol	3.76	
campesterol	49.8	61.1
campestanol	6.13	5.67
stigmasterol	10.2	13.7
clerosterol	16.5	15.7
sitosterol	1.511	1.610
sitostanol	9.90	9.8
Δ5-avenasterol	98.3	63.9
Δ5,24-stigmastadienol	7.60	8.61
Δ7-stigmasterol	5.90	6.10
Δ7-avenasterol	5.26	3.18
total	1.724	1.799

Furthermore, 30 L of commercial extra virgin oils of each olive cultivar was bought in a local market, stored in the thermostated room, and sampled in a similar way.

Analytical Determinations. Acidity, peroxide value (PV), specific extinction coefficients K_{232} and K_{270}, sensory analysis, fatty acids and sterols compositions were carried out according to the European Official Method of Analysis (EC1991 Regulation 2568/91) (*20*). Total polyphenols and *o*-diphenols were colorimetrically determined using the Folin−Denis reagent or ammonium molybdate (*21*). Tocopherols were evaluated by HPLC according to IUPAC Method 2432 (*22*). Chlorophyll and carotenoid pigment fractions were analyzed in the absorption spectrum at 670 and 472 nm, respectively (*23*). Stability was determined by the Rancimat method (Metrohm Co., Basel, Switzerland) at 100 °C, with an air flow of 10 L/h, and the results were expressed as hours up to the end of the induction period (*24*).

Sensory analysis was performed by the analytical panel of the Instituto de la Grasa (CSIC), consisting of 12 selected and trained members, working according to the European Official Method of Analysis (Regulation 2568/91). The descriptive analysis used a six-point intensity scale, ranging from 0 (no perception) to 5 (extreme). Overall grading used a nine-point scale from 1 (the lowest quality) to 9 (maximum quality). Oils were classified according to their scores: extra ≥ 6.5; virgin ≥ 5.5; common ≥ 3.5; and lampant < 3.5.

Statistical Analysis. Two bottles of each cultivar were independently analyzed in each sampling, and all of the determinations were carried out in triplicate. The results are expressed as mean ± standard deviation. Duncan's test was used to compare means, and the analysis of variance and correlation studies were performed using CoStat 2.10 software (CoHort Softwares, Berkeley, CA).

RESULTS AND DISCUSSION

Changes in Acidity. Oils of both olive cultivars stored at 2 °C in darkness maintained their initial acidity throughout the 6 months of storage, whereas those stored at 30 °C with illumination showed an increase of acidity with the storage time that became significant ($p < 0.05$) at the end of the storage period (**Figure 1**). This increase of acidity could be explained by the free fatty acids hydrolysis. By this quality index, oils of both cultivars stored in both conditions maintained their extra quality (<1%) at the end of the storage period.

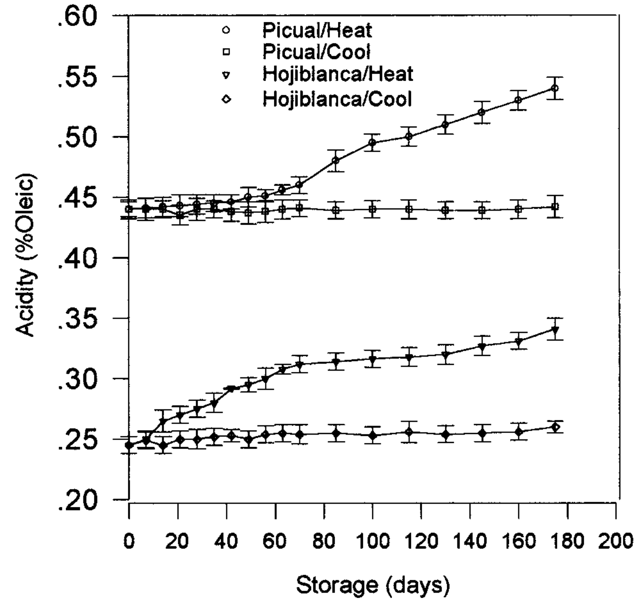

Figure 1. Evolution in free acidity as oleic acid (percent) in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

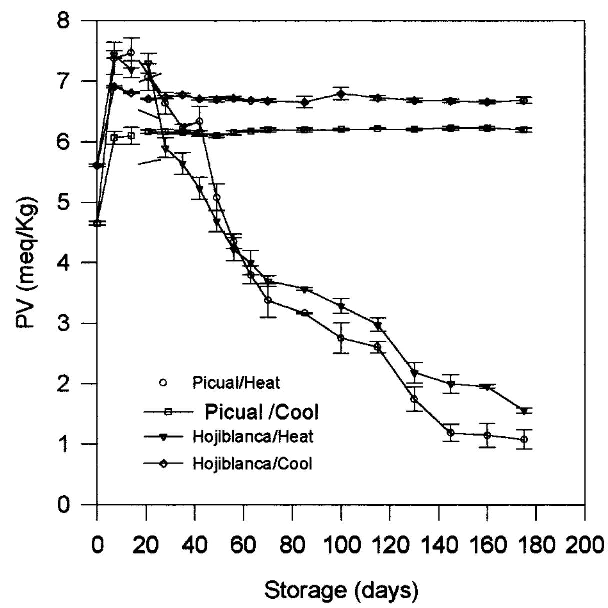

Figure 2. Evolution in peroxide value in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

Changes in Peroxide Value. The changes in PV were similar in oils from the two olive cultivars (**Figure 2**). During the first 21 days a 2-fold increase of the PV was observed. After that, in oils stored at 2 °C in darkness, the PV remained constant, whereas in oils stored at 30 °C with illumination there was a linear decrease with storage time, exhibiting excellent coefficients of correlation ($p < 10^{-5}$). The regression lines obtained for oils stored at 30 °C with illumination were

$$\text{Picual: } PV = -0.04t + 7.2;\ r = 0.9532$$

$$\text{Hojiblanca: } PV = -0.03t + 6.6;\ r = 0.9600$$

These results are in agreement with those of Pérez et al. (*25*). The decrease in the PV is a consequence of the oxygen depletion. The impermeability to oxygen of the container avoided further peroxide production.

Changes in the Specific Extinction Coefficients K_{232} and K_{270}. The K_{232} coefficient notoriously increased in oils stored at 30 °C, although the limit of 2.50 fixed for the extra quality was not exceeded during the storage. In oils stored at 2 °C, K_{232} remained practically constant or very slightly increased (**Figure 3A**). On the other hand, the K_{270} value (**Table 2**) registered a sharp increase in oils stored at 30 °C, which can be partially explained by the peroxide degradation and partially by the effect of light radiation (**Figure 3B**). These results are in agreement with data reported by Olías et al. (*13*) and Gutierrez et al. (*15*). The limiting value of 0.25 for extra oils was exceeded at 85 and 63 days in Picual and Hojiblanca oils, respectively.

Table 2. Initial Values of the Stability and K_{270} of Commercial Oils

	Picual samples					Hojiblanca samples				
	1	2	3	4	5	1	2	3	4	5
K_{270}	0.101	0.106	0.110	0.111	0.115	0.107	0.115	0.116	0.123	0.131
stability (h)	100	73	63	60	54	80	45	40	22	18

The fact that the limit was reached in Picual later than in Hojiblanca could be due to its lower initial level of K_{270} and, as we will see below, higher stability. The coefficient K_{270} in oils stored at 2 °C remained unaltered throughout the storage period.

Because of the significant variation of the K_{270} value during storage, which is easily measured, this parameter may be of capital importance to control the quality of stored virgin olive oils and to determine the time at which they will lose their "extra" category. Commercial samples of Picual oils lost their extra quality at 150, 73, 63, and 54 days, whereas Hojiblanca oils lost it at 80, 45, 40, 22, and 18 days (**Figure 3C,D**), confirming previous data obtained by us.

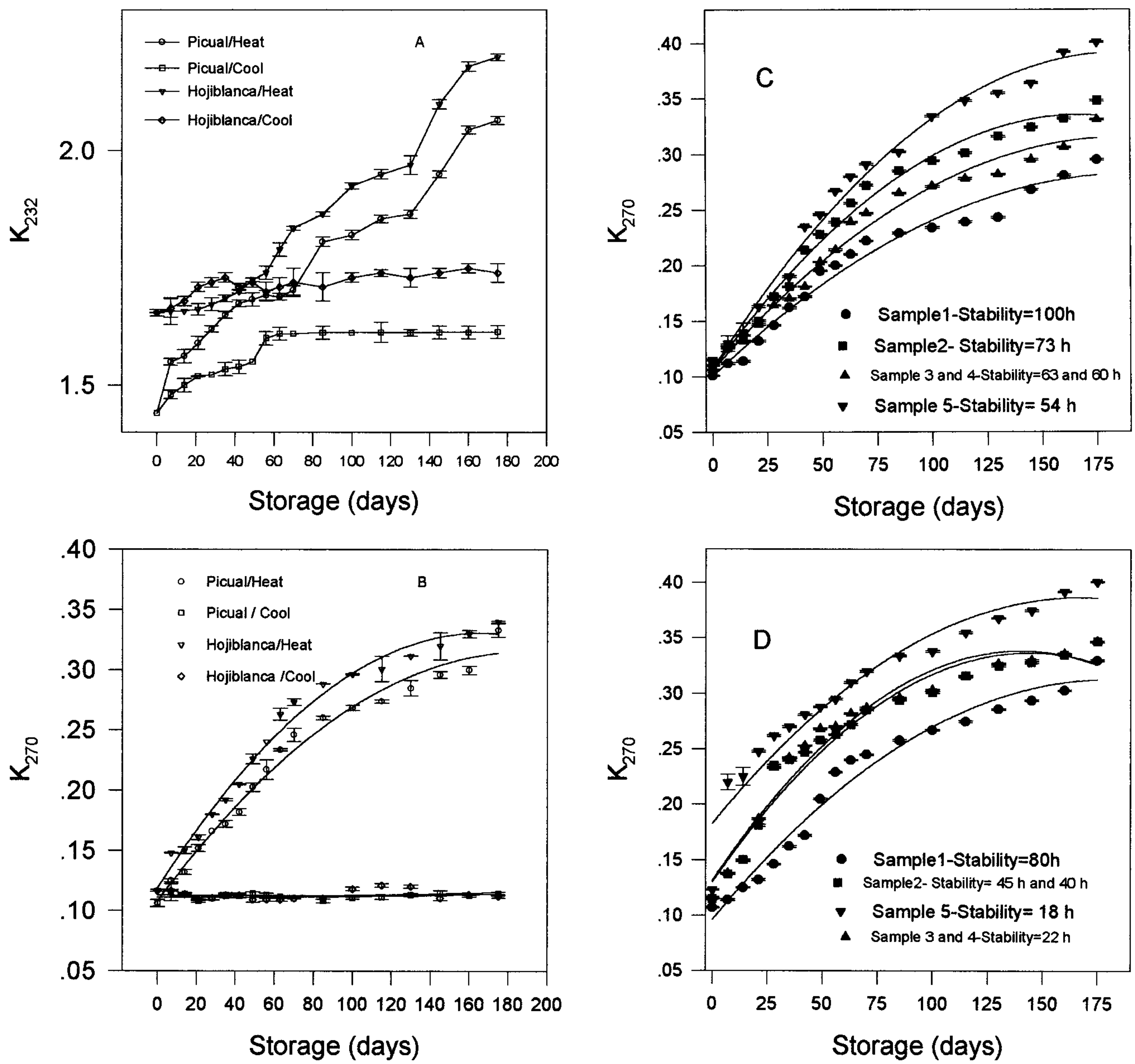

Figure 3. Evolution in the K_{232} coefficient (A) and in the K_{270} (B) in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage and in commercial oils (C and D)

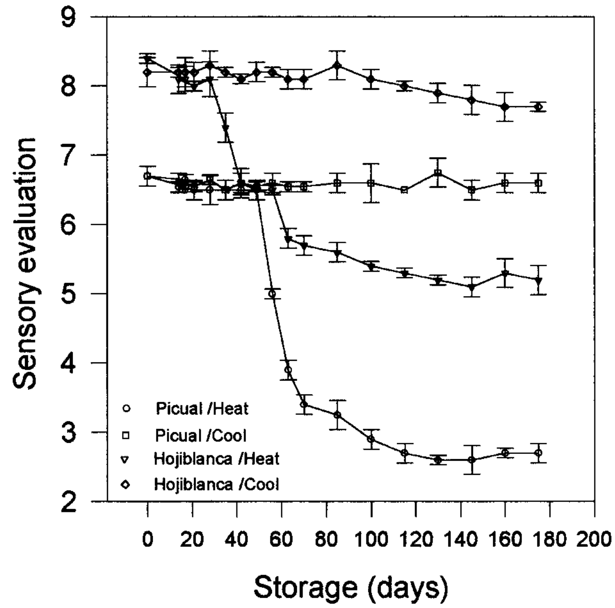

Figure 4. Evolution in organoleptic score in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

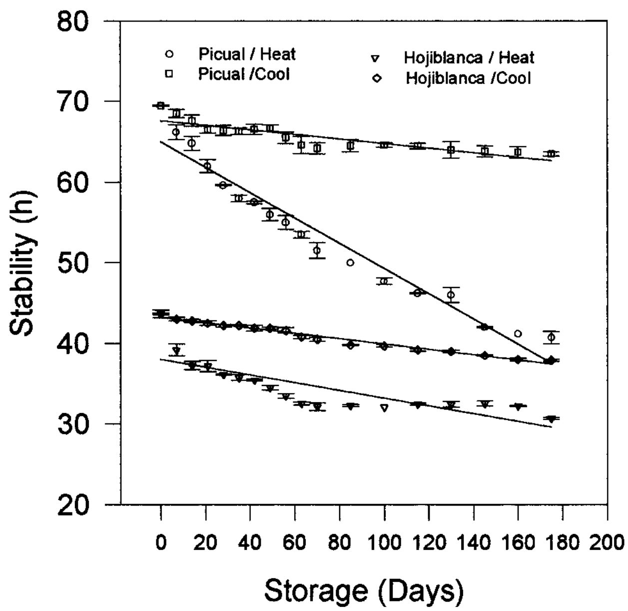

Figure 5. Evolution in the stability in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

Changes in Sensory Score. Storage at 2 °C and in darkness retained the extra quality of oils during 6 months (overall grading > 6.5), whereas storage at 30 °C with illumination originated a sharp decrease of the sensory score (**Figure 4**). Thus, Picual oil lost the extra quality after 49 days, becoming "lampant" (2.7) at the end of the storage period. Hojiblanca oil lost the extra quality after 56 days but retained the "common" quality (5.2) up to 180 days. Quantitative descriptive analysis (QDA) during storage at 30 °C revealed the loss of positive attributes (fruity, apple, green, bitter, piquant, ...) and the appearance of negative ones (winey, muddy, rancid, ...), reaching a score of 3 (on a scale of 0−5) at the end of the period.

Changes in Stability. Oil stability decreased during storage (**Figure 5**), being higher in oils stored at 30 °C with illumination, indicating a higher oxidation by high temperature and light. The oil extracted from Picual fruits showed a higher decrease of its stability, which fell 41%. Hojiblanca oil lost 30% of its initial stability at the end of the storage. The higher stability of the Hojiblanca oil could be explained by its higher polyphenol and *o*-diphenol contents (**Table 1**).

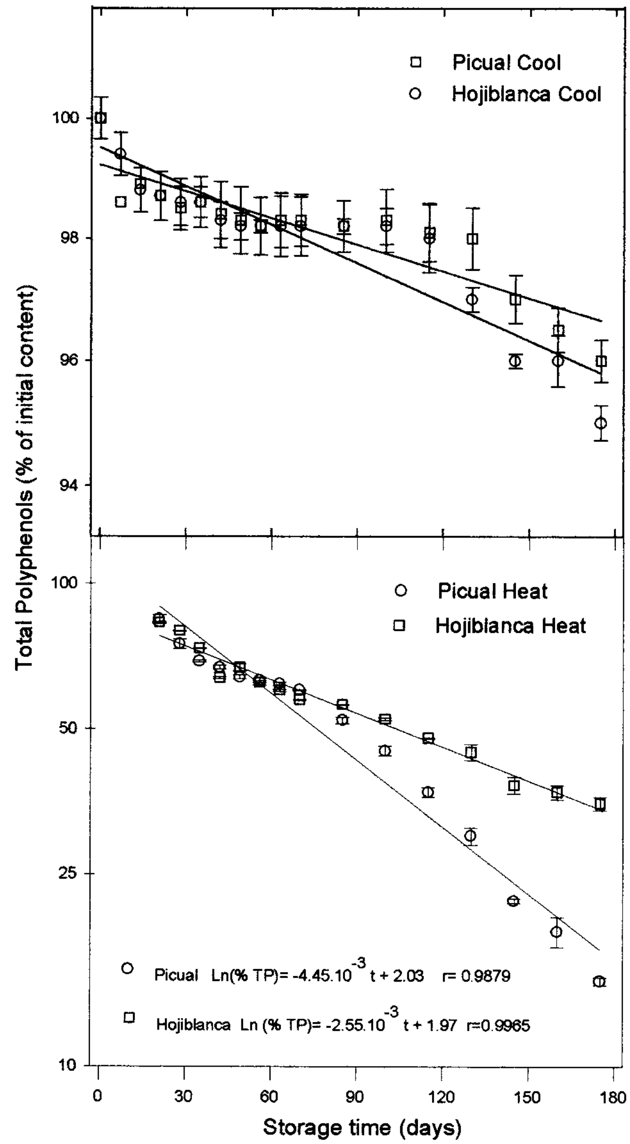

Figure 6. Evolution in the amount of phenols (percent of initial content) in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

Changes in Total Polyphenols. In parallel with stability, the total polyphenol contents (TP) decreased during storage (**Figure 6**). Degradation of these compounds was well fitted to first-order kinetics. These results are in agreement with previous data obtained in our laboratory when oxidation took place under accelerated conditions. The evolution of the *o*-diphenol contents was similar (data not shown).

Changes in α-Tocopherol. α-Tocopherol (vitamin E) is the major tocopherol in virgin olive oil. Changes in the content of this compound are shown in **Figure 7**. During storage at 2 °C and in darkness, oils from both cultivars kept unaltered their α-tocopherol contents. However, in oils stored at 30 °C with illumination the α-tocopherol level fell 97.3% after 180 days. The fact that the content of this compound was higher than zero indicated that the induction period of oxidation was not exceeded. This was observed in previous studies when oils were oxidized under accelerated conditions (data not shown).

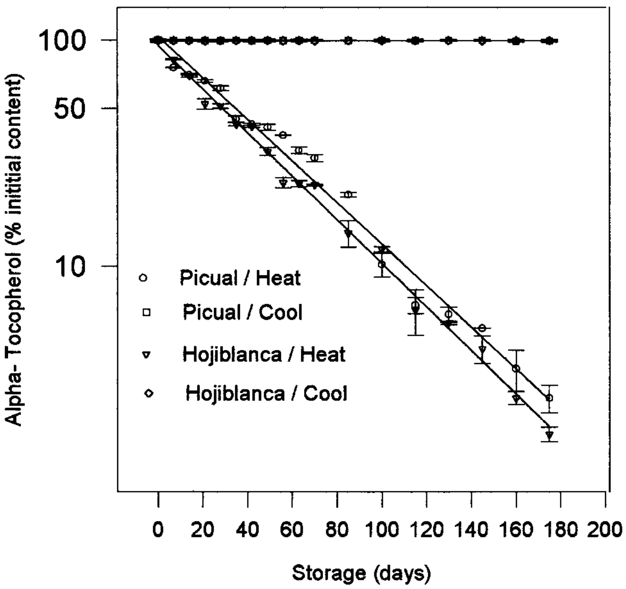

Figure 7. Evolution in the amount of α-tocopherol (percent of initial content) in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

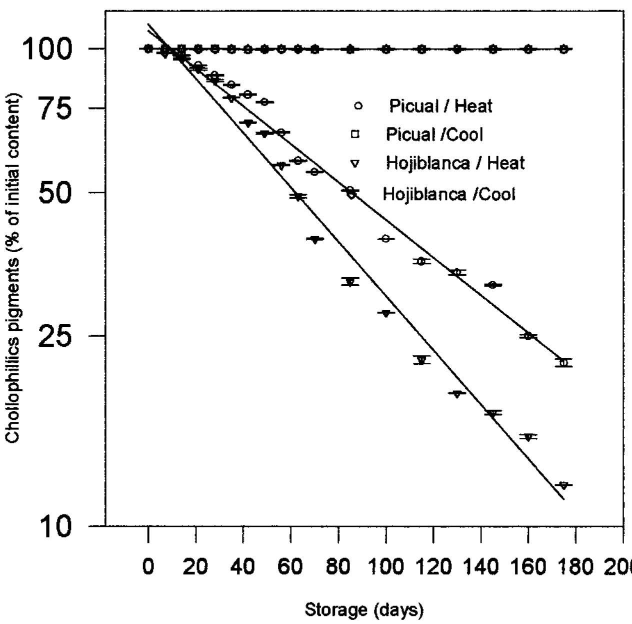

Figure 8. Evolution in the amount of chlorophyllic pigments (percent of initial content) in the virgin olive oils from the olive varieties Picual and Hojiblanca at different times of storage.

During the storage period, the evolution of the α-tocopherol content followed first-order kinetics in oils of both cultivars stored at 30 °C ($r = 0.9922$ and 0.9823 for Hojiblanca and Picual oils, respectively; $p < 10^{-8}$). The marked decrease in the α-tocopherol levels reflects that this natural antioxidant was first destroyed during the oxidative process. Thus, its measurement may be useful to determine the extent of oil oxidation.

Changes in Chlorophyllic and Carotenoid Pigments. Chlorophyllic pigments (CP) contents remained practically constant during storage at 2 °C, whereas at 30 °C they were progressively degraded (**Figure 8**). Degradation of these pigments at high temperature and light followed a first-order kinetics. These results are in agreement with previous studies

Table 3. Sterol Composition (Milligrams per Kilogram)[a] of the Virgin Olive Oil from the Variety Picual during Storage in Heat

	0	7	14	21	28	35	42	49
cholesterol	1.31 ± 0.06	1.56 ± 0.44	1.42 ± 0.33	1.54 ± 0.25	1.34 ± 0.16	1.26 ± 0.64	1.48 ± 0.35	1.42 ± 0.06
		1.84 ± 0.01	1.50 ± 0.01	1.60 ± 0.41	1.44 ± 0.09	1.70 ± 0.18	1.47 ± 0.18	1.34 ± 0.06
24-methylenecholesterol								
campesterol	61.1 ± 3.4	61.9 ± 3.5	52.0 ± 8.1	61.5 ± 6.3	57.3 ± 1.3	57.4 ± 4.3	56.4 ± 5.2	61.3 ± 3.6
		67.0 ± 6.2	59.3 ± 3.7	55.9± 1.5	58.1 ± 3.4	61.1 ± 2.3	54.4 ± 2.3	55.6 ± 1.8
campestanol	5.67 ± 0.32	5.76 ± 0.03	5.59 ± 0.45	6.01 ± 0.14	5.90 ± 0.22	5.57 ± 0.03	5.42 ± 0.0	5.77 ± 0.08
		5.92 ± 0.30	5.66 ± 0.56	5.17 ± 0.35	5.77 ± 0.28	5.36 ± 0.37	5.79 ± 0.45	5.50 ± 0.26
stigmasterol	13.7 ± 0.8	13.9 ± 1.8	15.4 ± 0.2	15.2 ± 0.7	13.5 ± 0.3	14.2 ± 0.6	16.3 ± 0.6	14.4 ± 0.8
		15.0 ± 0.3	14.6 ± 3.0	13.9 ± 1.0	13.4 ± 0.3	14.1 ± 0.2	15.0 ± 1.2	12.6 ± 0.3
clerosterol	15.7 ± 1.4	19.7 ± 7.8	13.1 ± 2.6	16.3 ± 1.9	16.2 ± 0.0	17.7 ± 3.8	21.7 ± 0.5	16.6 ± 0.2
		75.5 ± 82.1	15.7 ± 2.3	15.4 ± 2.9	15.8 ± 0.7	15.8 ± 0.7	21.6 ± 5.2	15.2 ± 1.3
sitosterol	1610 ± 87.2	1606 ± 113	1645 ± 77.1	1648 ± 70.2	1598 ± 4.16	1584 ± 24.5	1613 ± 130	1493 ± 74.6
		1594 ± 72.2	1622 ± 57.8	1611 ± 55.9	1578 ± 94.5	1596 ± 45.9	1585 ± 95.9	1593 ± 84.6
sitostanol	9.8 ± 0.9	10.8 ± 0.9	10.4 ± 0.1	10.6 ± 0.1	10.7 ± 0.1	9.8 ± 0.4	10.4 ± 0.2	10.0 ± 0.2
		10.8 ± 0.1	10.2 ± 0.5	10.2 ± 0.1	10.2 ± 0.4	9.7 ± 0.8	10.1 ± 0.3	8.6 ± 0.2
Δ5-avenasterol	63.9 ± 5.3	63.2 ± 1.1	64.2 ± 7.5	68.4 ± 1.4	68.4 ± 1.3	66.2 ± 1.1	65.8 ± 4.7	67.9 ± 2.7
		66.1 ± 3.0	61.7 ± 0.5	66.9 ± 3.4	63.9 ± 2.3	62.2 ± 1.4	62.6 ± 0.3	65.6 ± 0.6
Δ5,24-stigmastadienol	8.61 ± 0.29	8.54 ± 0.29	6.43 ± 0.52	6.70 ± 0.32	6.01 ± 0.08	6.57 ± 0.11	7.72 ± 2.12	5.92 ± 0.03
		7.14 ± 0.02	6.01 ± 1.24	5.97 ± 0.29	6.50 ± 0.43	6.03 ± 0.18	6.19 ± 0.35	6.31 ± 0.05
Δ7-stigmasterol	6.10 ± 0.17	6.01 ± 0.44	5.54 ± 0.69	5.63 ± 0.09	5.42 ± 0.33	5.42 ± 0.33	5.46 ± 0.35	5.39 ± 0.08
		5.99 ± 0.20	6.30 ± 0.46	5.49 ± 0.01	5.14 ± 0.07	5.56 ± 0.07	5.45 ± 0.64	5.33 ± 0.09
Δ7-avenasterol	3.18 ± 0.11	3.17 ± 0.58	3.21 ± 0.43	2.77 ± 0.31	3.22 ± 0.06	2.69 ± 0.39	3.44 ± 0.39	3.04 ± 0.19
		3.16 ± 0.00	2.87 ± 0.22	3.04 ± 0.05	3.69 ± 1.12	3.67 ± 0.35	3.26 ± 0.21	3.56 ± 0.28
total	1799 ± 93.1	1807 ± 134	1822 ± 95.9	1843 ± 62.8	1787 ± 3.2	1772 ± 31.7	1807 ± 128	1685 ± 76.5
		1854 ± 9.2	1806 ± 65.0	1796 ± 46.2	1762 ± 95.1	1781 ± 151	1772 ± 92.9	1772 ± 84.2

	56	63	70	85	100	115	130	145	160	175
cholesterol	1.21 ± 0.14	1.30 ± 0.03	1.68 ± 0.18	1.37 ± 0.07	3.18 ± 1.76	1.17 ± 0.34	1.41 ± 0.14	2.01 ± 0.19	1.01 ± 0.21	1.54 ± 0.12
	1.18 ± 0.09	1.28 ± 0.11	1.22 ± 0.12	1.19 ± 0.06	2.36 ± 0.34	2.18 ± 0.28	1.99 ± 0.42	1.09 ± 0.47	1.25 ± 0.10	1.72 ± 0.79
24-methylenecholesterol										
campesterol	55.6 ± 0.6	55.7 ± 2.9	62.4 ± 5.0	62.7 ± 1.7	64.1 ± 7.0	57.7 ± 6.3	59.1 ± 0.1	56.9 ± 1.6	67.8 ± 11.8	62.8 ± 10.7
	54.6 ± 2.7	55.0 ± 3.9	61.8 ± 2.0	60.3 ± 1.2	59.5 ± 5.0	58.3 ± 4.6	66.4 ± 4.0	59.4 ± 1.7	62.0 ± 3.1	72.8 ± 3.0
campestanol	5.59 ± 0.15	5.62 ± 0.13	5.75 ± 0.01	5.64 ± 0.06	5.77 ± 0.39	5.41 ± 0.71	5.37 ± 0.23	5.68 ± 0.07	5.79 ± 0.55	5.95 ± 0.31
	5.63 ± 0.02	5.59 ± 0.10	5.65 ± 0.11	5.83 ± 0.24	5.60 ± 0.04	5.62 ± 0.39	5.50 ± 0.14	5.71 ± 0.18	5.59 ± 0.03	5.90 ± 0.22
stigmasterol	12.8 ± 0.1	12.3 ± 0.6	14.2 ± 0.8	14.0 ± 0.3	14.5 ± 1.7	14.8 ± 1.4	13.4 ± 0.1	18.6 ± 0.3	18.3 ± 0.2	24.7 ± 2.3
	12.1 ± 0.5	12.4 ± 0.7	13.5 ± 0.4	13.9 ± 0.1	14.5 ± 1.7	13.8 ± 1.2	15.2 ± 0.9	18.7 ± 0.2	19.8 ± 0.6	26.4 ± 3.3
clerosterol	21.9 ± 9.8	14.8 ± 1.2	16.3 ± 2.3	16.0 ± 0.9	15.4 ± 0.8	14.3 ± 0.7	16.0 ± 0.9	15.4 ± 0.3	15.2 ± 0.6	15.0 ± 1.0
	14.1 ± 0.7	13.9 ± 0.7	15.6 ± 0.3	16.4 ± 0.9	16.4 ± 0.5	15.3 ± 0.6	15.9 ± 0.9	15.3 ± 0.9	16.2 ± 0.7	17.8 ± 1.7
sitosterol	1620 ± 68.8	1620 ± 77.5	1601 ± 97.4	1589 ± 95.2	1635 ± 89.1	1574 ± 4.74	1599 ± 58.4	1576 ± 40.1	1613 ± 34.2	1649 ± 161
	1573 ± 76.7	1609 ± 75.7	1607 ± 16.3	1600 ± 71.9	1609 ± 15.3	1636 ± 7 7.2	1657 ± 120	1587 ± 124	1604 ± 133	1590 ± 28.0
sitostanol	11.4 ± 1.7	10.0 ± 0.0	11.1 ± 0.8	12.0 ± 0.2	10.9 ± 0.1	10.1 ± 0.2	10.2 ± 0.3	9.5 ± 0.2	10.7 ± 0.2	9.9 ± 0.2
	9.7 ± 1.3	10.0 ± 0.1	10.9 ± 0.2	9.8 ± 0.3	10.5 ± 0.5	9.7 ± 0.1	10.5 ± 0.5	9.8 ± 0.5	10.5 ± 0.1	10.7 ± 1.7
Δ5-avenasterol	66.0 ± 0.5	67.3 ± 0.3	69.2 ± 0.5	65.6 ± 4.3	83.2 ± 3.5	84.2 ± 0.8	85.3 ± 6.5	83.1 ± 2.0	84.0 ± 1.6	85.7 ± 1.1
	66.3 ± 4.4	64.6 ± 3.1	65.7 ± 4.2	70.6 ± 2.2	87.9 ± 2.2	85.1 ± 3.9	83.3 ± 2.3	84.8 ± 2.9	83.5 ± 4.8	89.8 ± 7.7
Δ5,24-stigmastadienol	5.99 ± 0.18	6.91 ± 0.08	6.48 ± 0.55	6.74 ± 0.03	6.52 ± 0.51	6.01 ± 0.54	5.78 ± 0.18	6.21 ± 0.08	6.18 ± 0.01	6.28 ± 0.52
	6.49 ± 0.07	6.57 ± 0.51	5.71 ± 0.11	6.00 ± 0.33	6.08 ± 0.52	6.74 ± 0.27	6.07 ± 1.07	6.34 ± 0.31	6.20 ± 0.02	6.33 ± 0.04
Δ7-stigmasterol	5.28 ± 0.37	5.39 ± 0.45	5.57 ± 0.35	5.72 ± 0.15	3.49 ± 0.27	3.82 ± 0.33	3.37 ± 0.41	3.70 ± 0.05	3.50 ± 0.65	3.71 ± 0.01
	5.56 ± 0.28	5.78 ± 0.02	5.71 ± 0.11	5.16 ± 0.95	3.47 ± 0.12	3.35 ± 0.06	3.42 ± 0.25	3.82 ± 0.01	3.08 ± 0.07	3.09 ± 0.09
Δ7-avenasterol	3.49 ± 0.35	3.76 ± 0.10	3.15 ± 0.07	3.49 ± 0.43	2.60 ± 0.39	2.43 ± 0.52	3.29 ± 0.88	2.66 ± 0.27	2.94 ± 0.02	2.49 ± 0.51
	3.31 ± 0.53	3.29 ± 0.03	3.79 ± 0.18	3.81 ± 0.76	2.87 ± 0.54	2.43 ± 0.11	2.74 ± 0.06	2.78 ± 0.93	2.48 ± 0.10	2.57 ± 0.52
total	1809 ± 56.2	1803 ± 81.9	1800 ± 106	1782 ± 97.0	1845 ± 95.9	1826 ± 61.1	1802 ± 79.3	1780 ± 36.8	1827 ± 44.6	1866 ± 174
	1752 ± 75.1	1787 ± 84.6	1797 ± 18.0	1795 ± 72.4	1819 ± 10.6	1838 ± 73.7	1868 ± 122	1794 ± 123	1814 ± 133	1824 ± 45.9

[a] Mean and standard deviation.

Table 4. Sterol Composition (Milligrams per Kilogram)[a] of the Virgin Olive Oil from the Variety Hojiblanca during Storage in Heat

	0	7	14	21	28	35	42	49	56	63	70	85	100	115	130	145	160	175
cholesterol	1.76 ± 0.24	1.48 ± 0.33	1.39 ± 0.01	1.81 ± 0.23	1.09 ± 0.06	1.13 ± 0.03	1.59 ± 0.04	1.42 ± 0.04	1.24 ± 0.08	1.56 ± 0.08	1.23 ± 0.12	1.50 ± 0.03	1.20 ± 0.02	1.13 ± 0.04	1.15 ± 0.01	1.12 ± 0.02	1.13 ± 0.03	1.13 ± 0.02
		1.82 ± 0.14	1.82 ± 0.19	1.24 ± 0.16	0.99 ± 0.16	1.30 ± 0.02	1.31 ± 0.08	1.23 ± 0.09	1.37 ± 0.08	1.60 ± 0.32	1.08 ± 0.11	1.50 ± 0.10	1.13 ± 0.01	1.06 ± 0.02	1.13 ± 0.03	1.17 ± 0.03	1.15 ± 0.04	1.11 ± 0.14
24-methylenecholesterol	3.76 ± 0.08	3.80 ± 0.18	3.67 ± 0.03	3.17 ± 0.17	3.74 ± 0.11	3.70 ± 0.06	3.85 ± 0.16	3.60 ± 0.07	3.57 ± 0.06	3.69 ± 0.01	3.79 ± 0.18	3.47 ± 0.36	3.42 ± 0.16	3.98 ± 0.03	3.25 ± 0.21	3.52 ± 0.25	2.83 ± 0.32	3.19 ± 0.37
		3.69 ± 0.03	3.61 ± 0.04	3.78 ± 0.20	3.70 ± 0.04	3.77 ± 0.11	3.60 ± 0.02	3.490.02	3.670.05	3.760.11	3.62 ± 0.08	3.46 ± 0.03	4.04 ± 0.80	3.24 ± 0.34	3.54 ± 0.62	4.04 ± 0.23	3.39 ± 0.16	3.47 ± 0.20
campesterol	49.8 ± 0.5	56.6 ± 2.1	56.8 ± 1.1	56.1 ± 0.0	54.7 ± 2.5	56.5 ± 3.4	53.7 ± 0.4	55.1 ± 4.3	55.1 ± 4.2	56.1 ± 3.9	49.7 ± 0.6	51.9 ± 0.1	57.9 ± 1.2	54.0 ± 0.4	51.7 ± 1.0	54.8 ± 0.9	56.9 ± 2.8	55.8 ± 1.2
		55.8 ± 0.2	57.3 ± 1.1	55.6 ± 1.4	51.0 ± 0.8	58.8 ± 1.0	53.5 ± 2.0	54.6 ± 2.7	51.5 ± 0.9	53.9 ± 0.1	52.5 ± 1.5	51.8 ± 1.4	55.9 ± 1.3	53.7 ± 1.4	54.9 ± 2.2	57.9 ± 2.8	54.9 ± 1.0	53.7 ± 0.5
campestanol	6.13 ± 0.08	6.3 ± 0.0	5.69 ± 0.12	6.10 ± 0.03	6.49 ± 0.21	6.09 ± 0.06	6.18 ± 0.01	6.02 ± 0.08	5.99 ± 0.11	6.24 ± 0.06	5.86 ± 0.06	5.89 ± 0.08	5.93 ± 0.04	5.94 ± 0.05	6.02 ± 0.09	5.76 ± 0.09	5.92 ± 0.11	6.09 ± 0.34
		5.9 ± 0.0	5.90 ± 0.30	6.01 ± 0.10	6.01 ± 0.10	5.94 ± 0.03	5.91 ± 0.07	6.14 ± 0.31	5.85 ± 0.07	6.16 ± 0.13	5.92 ± 0.04	6.13 ± 0.22	6.08 ± 0.10	5.66 ± 0.11	5.78 ± 0.06	5.60 ± 0.10	5.74 ± 0.78	5.75 ± 0.22
stigmasterol	10.2 ± 1.0	10.2 ± 0.2	9.94 ± 1.1	10.6 ± 0.7	11.1 ± 0.8	11.9 ± 1.5	12.6 ± 0.3	11.8 ± 0.4	12.6 ± 0.0	13.9 ± 0.7	13.2 ± 0.0	13.9 ± 0.3	12.1 ± 0.4	12.8 ± 0.51	12.1 ± 0.5	12.1 ± 1.2	12.1 ± 1.1	12.3 ± 0.9
		10.7 ± 0.2	11.1 ± 0.1	11.1 ± 1.2	12.1 ± 0.2	13.2 ± 0.3	11.1 ± 0.4	12.1 ± 0.0	12.4 ± 0.0	13.8 ± 0.2	13.8 ± 0.3	14.8 ± 0.2	13.4 ± 1.7	1.82 ± 0.23	11.6 ± 0.8	11.7 ± 0.8	11.2 ± 1.2	12.0 ± 1.1
clerosterol	16.5 ± 2.0	18.8 ± 0.4	18.5 ± 1.2	17.5 ± 1.5	17.0 ± 1.6	17.2 ± 0.6	16.8 ± 1.3	18.0 ± 2.1	18.0 ± 1.1	17.8 ± 1.0	18.0 ± 1.5	17.6 ± 1.3	18.6 ± 0.8	20.9 ± 1.1	19.5 ± 0.7	19.7 ± 1.5	19.3 ± 0.2	19.3 ± 0.9
		17.5 ± 1.0	17.7 ± 1.1	18.1 ± 2.1	19.2 ± 0.5	18.2 ± 1.0	17.7 ± 0.1	16.9 ± 2.2	18.8 ± 1.0	18.1 ± 0.5	18.9 ± 1.9	16.8 ± 1.3	20.1 ± 0.5	19.9 ± 2.1	19.6 ± 1.1	20.8 ± 0.5	19.7 ± 1.2	18.1 ± 0.4
β-sitosterol	1511 ± 71.4	1544 ± 9.4	1620 ± 31.7	1544 ± 39.6	1606 ± 30.2	1518 ± 80.0	1587 ± 47.7	1622 ± 5.8	1685 ± 81.4	1628 ± 25.2	1637 ± 75.4	1606 ± 13.1	1807 ± 3.9	1797 ± 41.6	1806 ± 132	1674 ± 46.4	1681 ± 46.7	1736 ± 10.8
		1564 ± 53.1	1607 ± 11.0	1563 ± 75.0	1600 ± 25.5	1476 ± 72.4	1579 ± 78.1	1656 ± 4.9	1727 ± 65.3	1692 ± 8.5	1670 ± 78.5	1574 ± 82.5	1892 ± 37.5	1704 ± 53.0	1805 ± 62.4	1709 ± 122	1790 ± 202	1709 ± 4.0
sitostanol	9.90 ± 0.01	10.9 ± 1.1	9.6 ± 0.1	9.9 ± 0.8	10.9 ± 1.0	10.8 ± 0.3	10.5 ± 0.7	10.0 ± 1.0	9.4 ± 0.2	9.4 ± 0.3	10.2 ± 0.2	10.6 ± 0.8	10.6 ± 0.2	11.4 ± 0.3	10.7 ± 0.3	11.7 ± 1.2	11.1 ± 0.4	10.5 ± 0.3
		10.0 ± 0.2	9.4 ± 0.1	10.3 ± 0.4	10.9 ± 0.5	10.4 ± 0.1	10.0 ± 0.1	9.8 ± 0.2	9.6 ± 0.4	10.3 ± 0.2	11.5 ± 0.7	9.8 ± 0.4	11.5 ± 0.3	10.3 ± 0.0	11.4 ± 0.6	11.6 ± 0.7	11.3 ± 0.8	10.8 ± 0.0
Δ5-avenasterol	98.3 ± 2.4	100.8 ± 2.1	105.8 ± 1.7	94.3 ± 5.8	109.8 ± 8.5	100.5 ± 1.5	100 ± 12.4	100.2 ± 0.9	108.0 ± 8.0	113.9 ± 3.3	105.4 ± 9.3	107.4 ± 6.6	114.1 ± 0.5	116.3 ± 4.7	117.6 ± 2.5	107.8 ± 6.9	109.7 ± 1.7	108.3 ± 2.2
		108.6 ± 2.9	107.1 ± 1.9	103.6 ± 5.7	106.6 ± 8.3	99.3 ± 1.4	99.6 ± 2.3	111 ± 10.1	110 ± 11.3	117.0 ± 0.1	113.4 ± 4.7	101.1 ± 1.8	123.9 ± 3.3	108.5 ± 2.4	119.4 ± 1.7	113.8 ± 1.9	109.7 ± 4.3	106.6 ± 1.1
Δ5,24-stigmastadienol	7.60 ± 0.05	7.62 ± 0.47	7.43 ± 0.66	7.88 ± 0.11	7.52 ± 0.37	7.76 ± 0.13	8.32 ± 0.67	7.72 ± 0.35	8.56 ± 0.30	11.0 ± 1.00	10.9 ± 1.13	12.1 ± 1.10	10.2 ± 0.36	9.91 ± 0.20	9.59 ± 0.56	9.70 ± 0.08	9.61 ± 0.09	8.80 ± 0.08
		8.07 ± 0.14	6.98 ± 0.81	7.87 ± 0.09	7.86 ± 0.13	7.96 ± 0.02	7.38 ± 0.44	9.05 ± 0.86	8.71 ± 0.01	10.3 ± 0.11	10.7 ± 0.84	11.9 ± 0.18	9.99 ± 0.45	9.30 ± 0.26	9.64 ± 0.00	10.38 ± 0.4	10.31 ± 0.6	8.72 ± 0.26
Δ7-stigmasterol	5.90 ± 0.04	5.07 ± 1.25	4.29 ± 0.05	5.63 ± 0.40	5.83 ± 0.03	4.97 ± 0.44	4.27 ± 0.60	4.67 ± 0.38	4.71 ± 0.01	4.47 ± 0.54	4.53 ± 0.57	5.22 ± 0.40	3.76 ± 0.62	4.28 ± 0.57	4.13 ± 0.40	4.17 ± 0.10	3.92 ± 0.26	4.03 ± 0.39
		4.93 ± 0.30	5.08 ± 0.98	5.17 ± 0.12	5.05 ± 0.12	4.73 ± 0.60	5.52 ± 0.23	4.60 ± 0.25	4.39 ± 0.44	4.62 ± 0.39	4.62 ± 0.06	4.41 ± 0.24	4.01 ± 0.06	3.82 ± 0.45	3.92 ± 0.18	4.10 ± 0.19	4.09 ± 0.17	4.06 ± 0.38
Δ7-avenasterol	5.26 ± 0.53	4.00 ± 0.30	4.83 ± 0.54	4.72 ± 0.52	4.28 ± 0.86	5.45 ± 0.13	4.53 ± 0.37	4.49 ± 0.69	4.40 ± 0.40	4.67 ± 0.14	5.86 ± 0.41	7.95 ± 0.16	4.71 ± 0.33	4.07 ± 0.07	4.53 ± 0.28	4.95 ± 0.03	4.58 ± 0.39	4.61 ± 0.20
		4.04 ± 0.20	4.01 ± 0.01	5.32 ± 0.53	5.73 ± 0.79	4.86 ± 0.16	4.46 ± 0.41	4.58 ± 0.16	4.38 ± 0.21	4.66 ± 0.35	6.71 ± 0.10	8.09 ± 0.71	4.36 ± 0.82	4.39 ± 0.14	4.73 ± 0.17	4.83 ± 0.46	4.48 ± 0.13	4.52 ± 0.20
total	1724 ± 73.4	1770 ± 7.6	1856 ± 44.1	1762 ± 41.2	1838 ± 37.2	1744 ± 77.4	1820 ± 59.4	1855 ± 0.2	1911 ± 101	1870 ± 30.6	1858 ± 90.0	1798 ± 80.5	2049 ± 5.9	2041 ± 36.6	2046 ± 133	1910 ± 36.2	1918 ± 65.7	1970 ± 10.2
		1795 ± 48.3	1837 ± 14.7	1791 ± 81.9	1829 ± 34.2	1704 ± 73.9	1799 ± 78.5	1890 ± 14.9	1958 ± 77.1	1936 ± 9.4	1942 ± 82.8	1805 ± 86.8	2146 ± 32.7	1930 ± 65.7	2050 ± 69.6	1950 ± 133	1975 ± 279	1894 ± 60.7

[a] Mean and standard deviation.

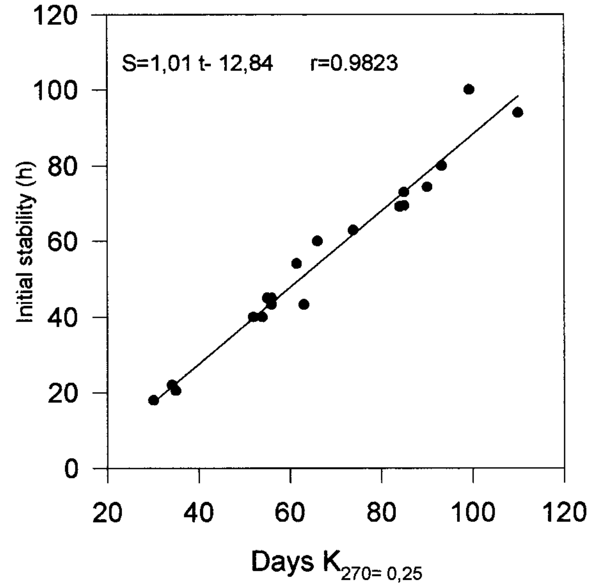

Figure 9. Initial stability/time ratio for the achievement of $K_{270} = 0.25$ during storage.

carried out in our laboratory with oils oxidized under accelerated conditions. The fitting was excellent, with equations

$$\text{Ln(CP)} = -0.11t + 12.34;\ r = 0.9848;\ p < 10^{-7}$$

$$\text{Ln(CP)} = -0.26t + 18.92;\ r = 0.9810;\ p < 10^{-7}$$

for Picual and Hojiblanca oils, respectively. It is interesting that despite the higher initial chlorophyllic pigments contents in Hojiblanca oils (**Table 1**), their degradation was significantly higher ($p < 0.05$). This could contribute to a faster increase of K_{270} (**Figure 3B**). Our results cannot be compared because there are not other studies about this topic. Carotenoid pigments content evolution followed a trend similar to that of chlorophyllic ones but with significantly lower slopes (data not shown), a fact of interest with regard to the provitamin A value of β-carotene. Light is the main cause of pigment degradation in the stored oils.

Changes in Fatty Acid Composition. The studied storage conditions did not appear to have any effect on the fatty acid composition of either oil (data not shown). This could be a consequence of the impermeability of the containers to oxygen. A slight decrease in linoleic and linolenic acids was observed in oils stored at 30 °C with illumination, but they were not statistically significant.

Changes in Sterol Content (Tables 3 and 4). The sterolic fraction remains constant during storage at 2 °C, and a significant ($p < 0.05$) increase in only the stigmasterol level was observed in oils stored at 30 °C (data not shown). These results agree with others previously obtained by us (*26*). The increase in the stigmasterol content could be explained by the thermal hydrolysis of sitosterol, the major sterol in olive oil, and has a clear influence in the loss of the sensory score of oils.

Correlation between Stability and Time To Reach the Limit $K_{270} = 0.25$. Results obtained in this paper indicate that a correlation between stability and the time to reach the limit $K_{270} = 0.25$ exists. The ratio between these two variables shows an excellent correlation, with $p < 10^{-8}$ (**Figure 9**). The coefficient K_{270} is of great importance to the control of oil quality, because it measures the accumulation of oxidation secondary products, and these metabolites could originate offflavors with negative effects on sensory score. Furthermore, it is a totally objective and easily measurable parameter that besides sensory analysis suffers high variations during storage. Thus, from the initial stability of the oil can be predicted the time in which an oil stored under similar conditions will lose its extra quality. We have been able to confirm these data in our laboratory in another study on different olive cultivar oil, demonstrating that this relationship is applicable to oils bottled in glass containers.

Conclusion. In summary, the present paper reports on the study of extra virgin olive oils extracted from two different cultivars, bottled in commercial clear glass and stored under conditions similar to those found in supermarkets. Our results clearly indicate which indices determine the loss of the extra quality during storage. Furthermore, we have demonstrated that the time beyond which the oils lose their extra quality can be predicted as a function of their initial stability. The results of this study could be interesting for virgin olive oil packagers and marketers to estimate the caducity date of an extra quality oil.

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