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Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils

Gabriel Beltrán [a,*], Maria Paz Aguilera [a], Carmen Del Rio [b], Sebastián Sanchez [c], Leopoldo Martinez [d]

[a] Estación de Olivicultura y Elaiotecnia, CIFA 'Venta del Llano', Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Junta de Andalucía, P.O. Box 50, E-23620 Mengibar, Jaén, Spain
[b] CIFA 'Alameda del Obispo', IFAPA, P.O. Box 3092, E-14080 Córdoba, Spain
[c] Dpto. de Ingeniería Química, Ambiental y de los Materiales, Universidad de Jaén, Paraje Las Lagunillas s/n, E-23071 Jaén, Spain
[d] Dpto. de Ingeniería Química, Facultad de Ciencias, Universidad de Granada, Avda. Fuentenueva s/n, E-18071 Granada, Spain

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Abstract

The effect of fruit ripeness on the antioxidant content of 'Hojiblanca' virgin olive oils was studied. Seasonal changes were monitored at bi-weekly intervals for three consecutive crop years. Phenolic content, tocopherol composition, bitterness index, carotenoid and chlorophyllic pigments and oxidative stability were analysed. In general, the antioxidants and the related parameters decreased as olive fruit ripened. The phenolics and bitterness, closely related parameters, did not present significant differences among years. Although in general, the tocopherols decreased during olive ripening γ-tocopherol increased. Differences between crop years were found only for total tocopherols and α-tocopherol, which showed higher content in low rainfall year oils. The pigment content decreased during ripening, chlorophyll changing faster. For low rainfall years, the level of pigments was higher, reaching significant differences between yields. Significant differences among years were found for oil oxidative stability; higher values were obtained for drought years. A highly significant prediction model for oxidative stability has been obtained.

Keywords: Virgin olive oil; 'Hojiblanca'; Olive ripeness; Natural antioxidants; Polyphenols; Tocopherols; Pigments; Oxidative stability

1. Introduction

In Andalusia, the second olive cultivar, by production and cultivated area, is 'Hojiblanca'. This cultivar covers 220,000 ha and its growing area is located mainly in the provinces of Cordoba, Malaga and some areas of Seville and Granada. It is a vigorous cultivar, considered late to start to produce and shows alternate and high yield. It is very aminable to mechanical harvesting due to its fruit size; however, the resistance to drop is very high. The fruit ripeness is considered as late and the oil content low. It is resistant to drought and calcium soils (Barranco et al., 2000). The fruit has duel use: table olives and oil extraction. The oil presents an equilibrated composition, with a medium oxidative stability and its sensorial characteristics are very appreciated by consumers (Uceda, Aguilera, Beltrán, & Jiménez, 2000).

Virgin olive oil naturally presents a group of minor components with a high antioxidant activity; important among these, because of their nutritional and sensorial interest, are the phenolic compounds; they have cytostatic activity, indicating their potential activity as antitumoral compounds (Saenz, Garcia, Ahumada, & Ruiz, 1998), they protect the low density proteins (LDL) against oxidation (Martínez de Victoria & Mañas, 2001;

* Corresponding author. Present address: University of Reading, School of Food Biosciences, Hugh Sinclair Unit of Human Nutrition, P.O. Box 226, Whiteknights, Reading RG6 6AP, UK. Tel.: +44-1183786218/+34-953370150; fax: +44-118931008/+34-953374017.
E-mail addresses: g.beltran@reading.ac.uk, gbelt@eresmas.com (G. Beltrán).

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Visioli & Galli, 1994) and they protect the oil from autoxidation (Beltrán, Jiménez, Aguilera, & Uceda, 2000; Gutiérrez, Jiménez, Ruiz, & Albi, 1999). These compounds are responsible for the bitterness and pungency in oils (Andrewes, Busch, Joode, Groenewegen, & Alexandre, 2003; Beltrán et al., 2000; Gutiérrez, Albi, Palma, Rios, & Olias, 1989). In olive oils Vitamin E is represented by tocopherols (Boskou, 1996); these antioxidants inhibit LDL oxidation (Jialal, Fuller, & Huet, 1995) and present other nutritional benefits (Gottstein & Grosch, 1990; Meydani & Tengerdy, 1993; Meydani, 1997; Stampfer et al., 1993). Olive oil colour is correlated with its pigment composition, which is considered quality parameter (Mínguez, Rejano, Gandul, Higinio, & Garrido, 1991). The pigments are involved in autoxidation and prooxidation processes in the oil and chlorophyllic pigments show antioxidant activity in the dark but prooxidant in light (Endo, Usuki, & Kaneda, 1984, 1985; Fakourelis, Lee, & Min, 1987). However, other authors did not find prooxidant activity when olive oil was used (Gutiérrez-Rosales, Garrido-Fernández, Gallardo-Guerrero, Gandul-Rojas, & Minguez-Mosquera, 1990). Carotenoids show activity and reduce the cancer risk (Hong & Itri, 1994). During the development of different cancer types, there is a decrease of vitamins; thus an intake of carotenoids should be helpful (Correa, 1988; Moon & Itri, 1983). There is a strong possibility that lutein and zeaxanthin may prevent age-related macular degeneration (AMD) and cataract formation (Basu, Del Vecchio, Flider, & Orthoefer, 2001). Carotenoids are effective inhibitors of photosensitised oxidation by quenching singlet oxygen and their antioxidant effect has been described in virgin olive oils (Fakourelis et al., 1987; Kiritsakis & Osman, 1995).

Therefore, these minor compounds show great importance because of their health benefits and effect on oil shelf-life. However, the oil antioxidant content is not constant; it depends on the cultivar, fruit ripening stage, agroclimatic conditions and olive growing techniques (Beltrán, 2000; Salas, Pastor, Castro, & Vega, 1997; Tovar, Romero, Alegre, Girona, & Motilva, 2003; Uceda & Hermoso, 2001).

Although 'Hojiblanca' is a very important Spanish olive cultivar, few works have described its oil composition (Uceda & Hermoso, 2001; Uceda et al., 2000). Some reports include information about the antioxidant content and changes during ripening (Gutiérrez et al., 1999). However, the effect of the ripening depends on the interactions with the crop year and it is therefore necessary to study the seasonal changes during different crop yields.

Because of the importance of this cultivar for oil production and because of the need to improve knowledge of antioxidants, the aim of this work has been to study the seasonal changes in natural antioxidants of 'Hojiblanca' virgin olive oils during the fruit ripening process and the effect of the crop year.

2. Materials and methods

2.1. Plant material

For this work, 12 sixteen-year-old olive trees of the 'Hojiblanca' cultivar were selected. The trees were spaced 7×7 m and grown in the experimental farm of Estacion de Olivicultura y Elaiotecnia in Mengibar, Jaen, using standard growing techniques. The study was carried out during three crop years: 1996/97, 1997/98 and 1998/99. The rainfalls registered for each crop yield were: 880 (96/97), 596 (97/98) and 348.9 mm (98/99). The monthly values of mean temperatures registered for the three years studied are shown in Table 1.

The fruit samples were harvested from all of the olive trees in duplicate, at bi-weekly intervals, from the middle of September. An aliquot of 100 fruits was taken from each fruit sample in order to determine its ripening index (Uceda & Frías, 1975).

2.2. Oil extraction

The oils were extracted using a laboratory oil mill Abencor (Abengoa, Seville). The mill consists of a hammer mill, a thermobeater and a paste centrifuge. Olive fruits were milled in the hammer crusher, then the olive paste was kneaded for 30 min at 28 °C (Martínez, Muñoz, Alba, & Lanzón, 1975). Micronised microtalc and water were not added. After the vertical centrifugation, the oily must was collected and left to decant. The oil samples were filtered and stored at −24 °C until analyses.

2.3. Oil analyses

Polyphenols were isolated by extraction with water:methanol (60:40) three times, from an oil-in-hexane solution, according to the method described by Vázquez-Roncero, Janer del Valle, and Janer del Valle (1973), using Folin-Ciocalteau reagent and colorimetric measurement at 726 nm; the results were expressed as

Table 1
Monthly mean air temperatures (°C) registered in Mengibar (Jaén) for the years 1996, 1997 and 1998

Month	1996	1997	1998
January	10.2	9.9	–
February	8.2	–	12.4
March	12.7	16.6	15.6
April	16.8	19	14.8
May	18.8	20.5	19.2
June	26.4	23.8	26.4
July	28.5	28	30.2
August	26.4	27.7	29.7
September	21.1	25.1	24
October	17.4	19.6	16.4
November	13	13	12.2
December	10.4	9.4	7.6

mg of caffeic acid per kilogram of oil. The bitterness index K_{225} has been determined by solid phase extraction (SPE) of the bitter compounds, using SPE C18 cartridges (Baker, J.T). The oil was dissolved in *n*-hexane, the cartridge was conditioned by eluting with methanol and *n*-hexane and the oil solution was applied to the SPE column. The column was washed with hexane that was run through the cartridge and discarded. The bitter compounds were eluted with methanol:water (50:50). The absorbance of the methanolic extract was measured at 225 nm (Gutiérrez-Rosales, Perdiguero, Gutiérrez, & Olías, 1992). The pigments, carotenoids and chlorophyllics, were determined as described by Mínguez et al. (1991). 7.5 g of oil was weighed, dissolved in cyclohexane and taken to a final volume of 25 ml; the carotenoid and chlorophyll pigments were determined by measuring the absorbances at 470 and 670 nm, respectively. The results were expressed as mg/kg. All the absorbance measurements were performed in a HP8042A diodearray spectrometer (Hewlett Packard, Spain).

The tocopherol content and composition were analysed by the IUPAC 2432 method (IUPAC, 1992); 1.5 g of oil was dissolved in the mobile phase (10 ml). The chromatographic separation was performed using a Perkin–Elmer liquid chromatograph equipped with an isocratic pump LC200 and a UV–vis detector Lc295. A normal phase column Lichrosphere Si60 (Merck, Spain) (250 mm length, 4.6 mm i.d. and 5 μm particle size) was used with an injection volume of 20 μl and a flow rate of 1.0 ml/min. The mobile phase was 0.5% isopropanol in *n*-hexane. The absorbance was measured at 295 nm. Tocopherols were quantified by an external standard method; the α-, β- and γ-tocopherol standards were obtained from Sigma Chemical Co. The results were expressed as mg of tocopherol per kg of oil.

The fatty acid methyl esters (FAMEs) were prepared as described by the EU official method (European Union Commission, 1991). The chromatographic separation was carried out by using a Perkin–Elmer Autosystem gas chromatograph (Perkin–Elmer, Spain) equipped with an autosampler, split/splitless injector, flame ionisation detector (FID) and a fused silica capillary column BPX70 of 50 m length × 0.25 mm i.d. and 0.25 μm of film thickness (SGE Scientific PTY Ltd., Australia). Helium was used as carrier gas and the oven temperature was maintained at 198 °C. The injector and detector temperatures were 235 and 245 °C, respectively. The results were expressed as relative area percent of the total.

The oxidative stability was measured by using a Rancimat Model 679 (Metrohmn, Switzerland); 2.5 g of oil was weighed and heated at 98 °C, and air was bubbled through the oil at a flow rate of 10–12 l/h; each oil sample was analysed twice. The results were expressed as induction time in hours (Gutiérrez, 1989).

2.4. Statistical analyses

ANOVA analyses were performed in order to evaluate the effect of ripeness and the crop yield (Statistix for Windows v.1.0). Separation of the means was obtained using Tukey's test. For ANOVA, the common first five harvesting dates were used. Stepwise linear regression analysis was applied to establish the analytical parameters that better explained the oil oxidative stability.

3. Results and discussion

The study was carried out from the middle of September when the fruit colour change began; the ripening index was near to zero. The values of ripening index and harvesting dates for the three crop years are shown in Table 2.

The phenolic compounds present in the virgin olive oils are one of the bases of the nutritional importance of this oil. In 'Hojiblanca' oils, the phenol content varied between 148 and 819 ppm. When oils from each crop year were compared, 1996/97 yield oils showed the higher content although, in the first stages of ripening of the 97/98 crop year, the values were greater. During the olive ripening, the phenolic compounds decreased (Fig. 1). However, this drop is different for each yield. While in 97/98, a linear decrease could be observed, from 819 to 282 ppm, the others showed slower decreases. 'Hojiblanca' oils have reported phenolics within the range 200–450 ppm (Uceda & Hermoso, 2001) with a mean value of around 250 ppm. This value is lower than that obtained in this work (491 ppm), but could be explained by the inclusion of more harvesting dates and crop yields in this work. From the ANOVA analyses, the effect of each factor (ripeness and crop year) as percent of sum of the squares, on the variability of phenolic compounds has been calculated. The crop year had a low incidence 16.97%, while the ripening process showed a greater influence on these compounds. The results are different from those obtained in other olive cultivars in which drought years and water stress conditions caused higher phenol contents (Beltrán, 2000; Salas et al., 1997; Tovar et al., 2003). Therefore, this different behaviour could be explained by the resistance of this cultivar to drought (Barranco et al., 2000).

One parameter related to the polyphenol content is the bitterness index (Beltrán et al., 2000). For the oil analysed, a significant relationship ($p : 0.0001$) was obtained with a r^2 adjusted of 0.89. It is a chemical parameter correlated with the sensorial evaluation by panel test (Gutiérrez-Rosales et al., 1992). From the results, 'Hojiblanca' oils showed a bitterness mean value of 0.42 that should be classified as of highly bitter oil. However, the wide range observed for this parameter shows that oils have large differences, from low bitter to

Table 2
Ripening index and date for the harvesting seasons of 'Hojiblanca' fruits for the three crop years analysed

Harvesting season	Crop year 96/97		Crop year 97/98		Crop year 98/99	
	Date	Ripening index	Date	Ripening index	Date	Ripening index
1	16/09/96	0.10	15/09/97	0.14	16/09/98	0.11
2	30/09/96	0.25	30/09/97	0.38	02/10/98	0.40
3	15/10/96	0.86	15/10/97	0.74	16/10/98	0.71
4	30/10/96	1.42	30/10/97	1.48	03/11/98	1.10
5	15/11/96	2.12	14/11/97	1.89	20/11/98	1.50
6	02/12/96	2.68				
7	20/12/96	3.00				
8	10/01/97	4.03				
9	30/01/97	5.22				

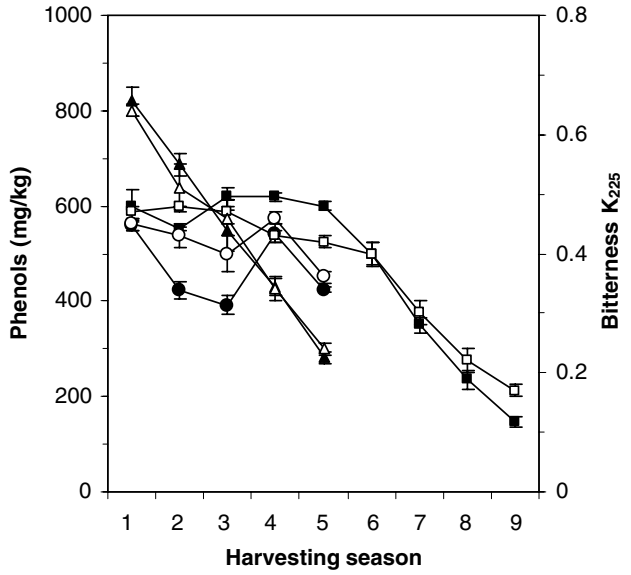

Fig. 1. Seasonal changes in phenol content (black symbols) and bitterness K_{225} (white symbols) during fruit ripening process of 'Hojiblanca' virgin olive oils from three different crop years: 96/97 (■), 97/98 (▲) and 98/99 (●).

very bitter oils. During ripening the bitterness decreased. This trend is the same for the crop years analysed (Fig. 1); significant differences were not found between years.

Other antioxidants, very interesting because of their nutritional activity, are the tocopherols; as found in other cultivars, 'Hojiblanca' oils contain α, β, and γ-tocopherol, and α-tocopherol represents the highest percentage (>97%). Table 4 shows the seasonal changes in tocopherol composition and total content for the three crop years studied. Among agronomic factors, the crop year showed the greatest influence on these compounds; the percentage variations for each tocopherol are shown in Table 3. Differences between years were found for α- and total tocopherol contents, the higher values during the 98/99 yield, being characterised by the low rainfalls registered, while 96/97 had lower values. In general, the tocopherol content decreases during ripening, although the decrease rates were different for each year (Gutiérrez et al., 1999). For γ-tocopherol, there was an increase, as described previously by Rannali, Tombesi, Ferrante, and De Mattia (1998).

The mean value of total tocopherol content found in 'Hojiblanca' oils (301 mg/kg) is similar to that found by Uceda and Hermoso (2001) who described it as a high tocopherol cultivar. The mean tocopherol composition was: α-tocopherol 293 ppm, β-tocopherol 3.3 ppm and γ-tocopherol 5.2 ppm. These results could not be contrasted because there are no data about tocopherol composition, in general, for virgin olive oil and in particular, for the 'Hojiblanca' cultivar. The biological activity of vitamin E is defined as α-tocopherol equivalents (α-TE) (Sheppard, Pennington, & Weihrauch, 1993). Considering the biological activities of β- and γ-tocopherol, the mean vitamin E activity for 'Hojiblanca' oils is 295 mg α-TE kg^{-1}; this value is higher than that described for olive oil (refined olive oil treated with virgin olive oil), but it could not be compared with other results for virgin olive oil because this appears to be the first time that it has been described. The 'Hojiblanca' oils showed a vitamin E activity greater than those described for other vegetable edible oils, such as corn, canola and soybean (Sheppard et al., 1993).

Table 3
Variability percent explained by each variation source for tocopherols of virgin olive oils of 'Hojiblanca' cultivar

Parameter	Crop year (%)	Season (%)	Crop year*Season (%)	Error (%)
α-Tocopherol	53.06	38.94	7.26	0.75
β-Tocopherol	24.84	39.60	24.43	11.14
γ-Tocopherol	6.57	41.26	41.04	11.13
Total tocopherols	53.19	38.73	7.34	0.74

Table 4
Variation of tocopherol content (mg/kg) of 'Hojiblanca' virgin olive oils during olive ripeness from three different crop years

Harvest season	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Total tocopherols
Crop year 96/97				
1	290 ± 5.16[a]	3.94 ± 0.11	4.35 ± 0.62	298 ± 4.64
2	279 ± 6.25	3.40 ± 0.44	5.03 ± 1.13	287 ± 5.56
3	260 ± 6.17	2.39 ± 0.49	2.31 ± 0.98	265 ± 7.64
4	257 ± 3.25	3.05 ± 0.13	2.38 ± 0.79	262 ± 2.59
5	237 ± 10.15	2.41 ± 0.41	2.64 ± 1.13	243 ± 8.61
6	244 ± 7.59	3.93 ± 0.10	8.54 ± 1.26	257 ± 8.75
7	245 ± 7.31	1.65 ± 0.04	9.03 ± 1.61	256 ± 5.74
8	232 ± 4.03	2.89 ± 0.18	12.42 ± 2.08	248 ± 5.93
9	232 ± 4.68	3.04 ± 0.06	11.8 ± 2.73	248 ± 7.47
Crop year 97/98				
1	355 ± 12.46	4.04 ± 0.30	3.52 ± 1.22	362 ± 16.96
2	354 ± 3.48	4.28 ± 0.18	3.54 ± 0.55	362 ± 3.85
3	300 ± 5.37	3.77 ± 0.41	2.21 ± 0.35	306 ± 5.42
4	283 ± 3.66	3.04 ± 0.25	4.13 ± 0.16	290 ± 4.02
5	277 ± 0.83	3.89 ± 0.40	8.38 ± 0.75	290 ± 0.31
Crop year 98/99				
1	374 ± 2.05	3.77 ± 0.06	3.76 ± 0.37	381 ± 1.64
2	370 ± 3.03	3.76 ± 0.23	3.55 ± 0.10	378 ± 3.35
3	370 ± 0.27	3.68 ± 0.13	3.21 ± 0.44	377 ± 0.83
4	322 ± 2.70	2.97 ± 0.20	2.46 ± 0.88	327 ± 1.63
5	283 ± 0.74	2.60 ± 0.30	4.40 ± 0.18	290 ± 1.20

[a] Mean ± SD (standard deviation).

In addition to their antioxidant activities, the pigments are responsible for the oil colour, which is one of the factors that influence selection by consumers and is considered as an oil quality parameter. In 'Hojiblanca' oils, the chlorophyllic pigments can be found at concentrations between 0.5 and 49.8 mg/kg. Oils with higher content were obtained during the 98/99 yield but lower in 96/97. During the ripening process, there was a decrease in their content (Fig. 2) as described previously (Gutiérrez et al., 1999). From the ANOVA analyses, ripeness was the main source of variability (83.23%). The carotenoid pigments show, for this cultivar, contents within the range 2.26–29.6 mg/kg; as for chlorophyllics, greater concentrations were obtained in the oils from the 98/99 yield, showing significant differences from the others. These differences are difficult to explain because the ripening stages were very similar for all the years, although they could be due to the effect of water stress on the synthesis of the abscisic acid which has the same biosynthesis pathway as the carotenoids (Hartung, Peuke, & Davies, 1999; Rmiki, Schoefs, & Lemoine, 1999). The loss of carotenoids observed during ripening is similar to that described by Garrido, Gandul, Gallardo, and Minguez (1990). The oil pigment losses during fruit ripening were different for each crop year. When the first five common harvesting dates are considered, in 96/97 the decrease of chlorophylls was much greater than that of carotenoids, while in 98/99 there was a greater pigment decrease. There is no explanation for this different behaviour given that the ripening index in 98/99 was lower than 96/97 for the fifth harvesting season.

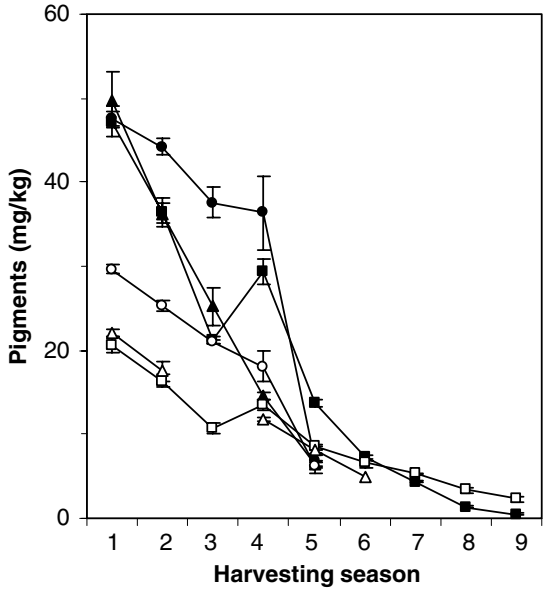

Fig. 2. Changes in chlorophyll (black symbols) and carotenoid (white symbols) pigments in the virgin olive oil extracted from 'Hojiblanca' cultivar during fruit ripening process for three different crop years: 96/97 (■), 97/98 (▲) and 98/99 (●).

Mínguez et al. (1991) found that the ratio between carotenoid and chlorophyllic pigments remained constant during fruit ripening, however, the results obtained for 'Hojiblanca' oils show a clear increasing trend for this value (Fig. 3). These differences are because the chlorophyll pigments decreased faster than the carotenoids. For the crop year 1996/97, lower pigment losses

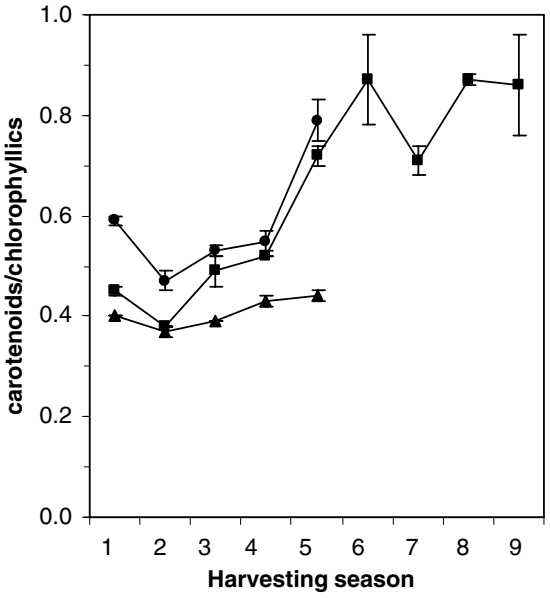

Fig. 3. Seasonal changes of the ratio carotenoid/chlorophyllic pigments through fruit ripeness in 'Hojiblanca' virgin olive oils from three different crop years: 96/97 (■), 97/98 (▲) and 98/99 (●).

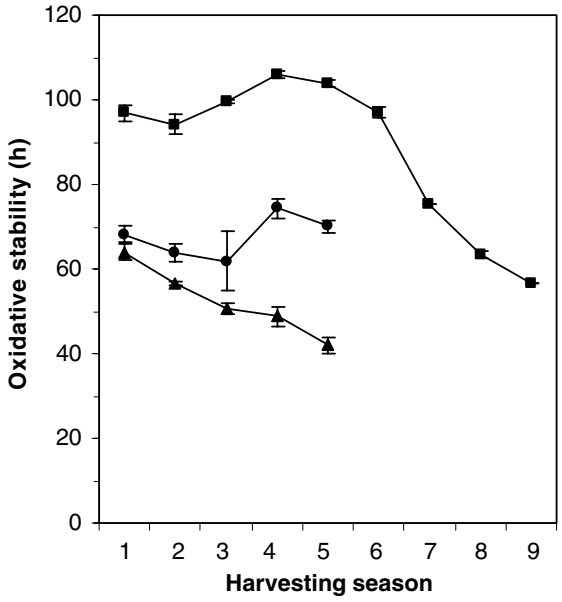

Fig. 4. Seasonal changes of oxidative stability of 'Hojiblanca' virgin olive oils during fruit ripeness for three different crop years: 96/97 (■), 97/98 (▲) and 98/99 (●).

were registered, 70.8% and 58.4% for chlorophylls and carotenoids, respectively, while for 98/99 oils greater losses of both pigments, chlorophyllic 85.7% and carotenoid 79.2% were seen. For the oil samples from fruits harvested later (seasons 6–9) during 96/97, this parameter has values over 4. This means that chlorophyllic pigment had practically disappeared.

The Rancimat method is an accelerated stability test that provides very useful information about the resistance of oil to oxidation; the mean oxidative stability for the oils of this cultivar was 73.4 h, with an interval between 42.1 and 106 h. The mean value obtained in this work is greater than that described by Uceda and Hermoso (2001), probably due to the inclusion of more harvesting dates and the effect of the crop years, since the influence of the year represented 91.43% of the variability found for this parameter, while the ripening stage was 1.38%. The more stable oils were those obtained during the 98/99 crop year, while the oils with lower stability were in 96/97 (Fig. 4). As the ripening occurs, the oil stability decreases according to the results described previously (Gutiérrez et al., 1999). Correlation between phenol content and oil stability has been described in virgin olive oil by several authors (Beltrán et al., 2000; Gutiérrez et al., 1999; Gutiérrez, Janer, Janer, & Gutiérrez, 1977). Fig. 5 shows this relationship for 'Hojiblanca' oils. The correlation obtained for samples overall showed a r value of 0.39. When regression analysis was applied to oils from each crop yield, the significance level was greater for 96/97 and 97/98 yields (Table 5) and trend lines showed better fit, although, for 98/99 oils, the correlation was smaller.

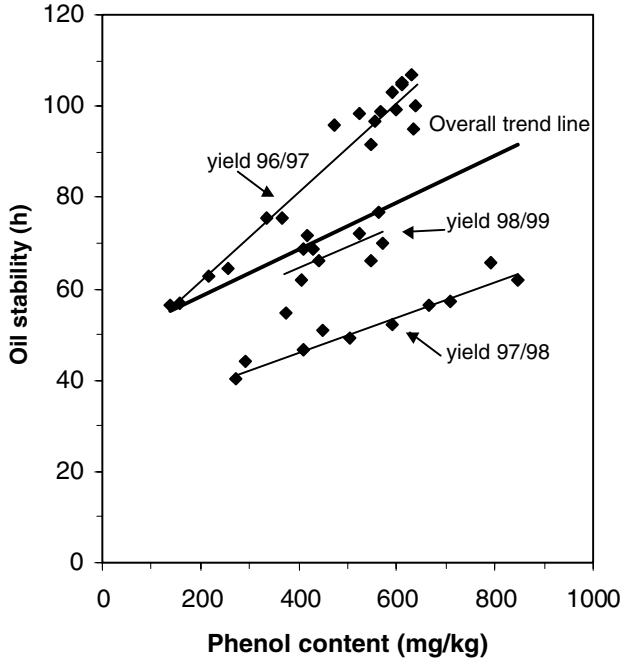

Fig. 5. Relationship between oxidative stability and polyphenol content in 'Hojiblanca' virgin olive oils for each crop year and overall oil samples. Equations of fitted curves, regression coefficients and significance levels are shown in Table 5.

Because of the effect of fatty acid composition on oxidative stability of virgin olive oils (Aparicio, Roda, Albi, & Gutiérrez, 1999; Uceda & Hermoso, 2001), FAMEs were analysed. Mean data for the three crop years studied are shown in Table 6. A stepwise regression analysis was applied in order to establish the

Table 5
Regression equations between oxidative stability (dependent variable) and phenol content (independent variable) in 'Hojiblanca' virgin olive oil for each crop yield and oil samples overall

Oil samples	Equation	Regression coefficient (adjusted)	p value
Yield 96/97	$y = 0.0985x + 41.927$	r : 0.97	0.000
Yield 97/98	$y = 0.0385x + 31.115$	r : 0.96	0.000
Yield 98/99	$y = 0.0513x + 43.615$	r : 0.57	0.048
Overall samples	$y = 0.0513x + 48.171$	r : 0.39	0.009

Table 6
Mean fatty acid composition of 'Hojiblanca' virgin olive oils for the crop years 1996/97, 1997/98 and 1998/99

Fatty acid	Crop year		
	1996/97	1997/98	1998/99
C16:0[b]	9.97 ± 1.85[a]	13.0 ± 0.76	13.0 ± 1.09
C16:1	0.59 ± 0.10	0.73 ± 0.07	0.68 ± 0.07
C18:0	2.24 ± 0.32	1.81 ± 0.20	2.05 ± 0.23
C18:1	78.9 ± 1.12	70.5 ± 1.46	75.5 ± 0.60
C18:2	5.58 ± 0.92	10.7 ± 2.31	6.57 ± 0.54
C18:3	0.70 ± 0.07	1.03 ± 0.08	0.92 ± 0.09
MUFAs/PUFAs	12.9 ± 1.63	6.37 ± 1.40	10.3 ± 0.56
Oleic/linoleic	14.5 ± 2.22	6.94 ± 1.72	11.6 ± 0.85

[a] Mean value ± SD.
[b] C16:0 palmitic, C16:1 palmitoleic, C18:0 stearic, C18:1 oleic, C18:2 linoleic, C18:3 linolenic, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids.

Table 7
Prediction model for oxidative stability of 'Hojiblanca' virgin olive oils obtained by applying a stepwise regression analysis

Variable	Coefficient	Standard error	T-Student	p-level
Intercept	41.424	9.030	4.59	0.00006
MUFAs/PUFAs	4.549	0.382	11.90	0.00000
Total phenols	0.053	0.007	7.28	0.00000
Total tocopherols	−0.139	0.025	−5.44	0.00005

Adjusted $R^2 = 0.8958$; SE: 6.47; $F(3, 34) = 107.06$; $p < 0.00000$.

compounds that better explained the oxidative stability. The values of p-to-enter and p-to-remove statistical variables were p : 0.01. The regression model obtained is shown in Table 7; the value of R^2 was 0.8958, which is highly significant $p < 0.0000$. From the variance analyses of the stepwise regression model, the fatty acid composition, MUFAs/PUFAs (monounsaturated/polyunsaturated), was the major contributor to the oxidative stability of 'Hojiblanca' oils (73.57%), while total phenols and total tocopherols showed lower contributions of 8.52% and 8.34%, respectively.

This work describes the mean antioxidant composition of the 'Hojiblanca' virgin olive oils, establishing changes due to both, ripening process and crop year, the main agronomical factors. In general, there was a decrease of the natural antioxidants content, the most important factor being the conditions of the crop year, mainly water stress. For the driest year, the oils showed a higher concentration, except for polyphenols that, in this cultivar, show different behaviour against drought. A highly significant prediction model was obtained for oxidative stability. These results improve knowledge of the effect of ripening and crop year on the antioxidant content that could help to establish the optimum fruit harvesting date according to the nutritional, sensorial and commercial advantages.

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