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Influence of fruit ripening on 'Cornicabra' virgin olive oil quality A study of four successive crop seasons

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Abstract

The aim of this work was to study the influence of the stage of fruit ripening on analytical parameters which determine oil quality during four successive crop seasons, in an attempt to establish an optimum harvesting time for 'Cornicabra' olives. The majority of the analytical parameters, i.e. peroxide value, UV absorption at 270 nm, pigments, sensory scores, oleic acid and total sterols, diminished during ripening, whereas free acidity, linoleic acid and Δ-5-avenasterol increased. Oil extraction yield, oxidative stability, natural antioxidants and campesterol showed more complex behaviour. On the basis of the evolution of the analytical parameters studied the best stage of maturity of 'Cornicabra' olive fruits for processing seems to be a ripeness index higher than 3.0 and lower than 4.0–4.5. Results indicated that probably less than 25% of commercial 'Cornicabra' virgin olive oils were extracted from olives harvested at an optimum ripeness index. It is therefore of crucial recommendation to the industry to bring forward harvesting to further improve the quality of the virgin olive oil produced.

Keywords: Virgin olive oil; 'Cornicabra' variety; Ripening; Quality

1. Introduction

The maturation of olive fruits lasts several months, and development varies according to the growing area, olive variety, temperature and cultural practices. During ripening, important chemical changes occur inside the drupe which are related to the synthesis of organic substances, especially triglycerides, and to other enzymatic activities (Boskow, 1996) that may affect virgin olive oil quality (Montedoro, Garafolo, & Bertolucci, 1986). In order to obtain a characteristically fragrant and delicately flavoured olive oil, it is therefore imperative that it is properly extracted from undamaged fruits at its best degree of ripeness (Boschelle, Mozzon, & Giomo, 1994).

Changes in chemical composition of fruit and extracted oil taking place during ripening have been studied by several authors (Aparicio & Morales, 1998; Garcia, Seller, & Pérez-Camino, 1996; Gutiérrez, Jimenez, Ruíz, & Albi, 1999; Minquez-Mosquera, Gandul, & Garrido, 1990; Monteleone, Caporale, Carlucci, & Pagliarini, 1998; Vázquez, Maestro, & Graciani, 1971). However, they dealt generally with individual components and with one crop season only, whereas it has been widely demonstrated that the composition of virgin olive oil may differ significantly from one year to another (Salvador, Aranda, & Fregapane, 1998, 1999).

In order to determine the optimal stage for harvesting and to evaluate the degree of ripeness, the ratio between malic and citric acid, and spectrophotometric absorption at 665 and 525 nm has been proposed (Solinas, Marsilio, & Angerosa, 1987), as have the changes in total polyphenol content (Chimi & Atouati, 1994).

The aim of this work was to study the influence of the stage of fruit ripening on analytical parameters which determine oil quality during four successive crop seasons, in an attempt to establish an optimum harvesting time for 'Cornicabra' olives.

The 'Cornicabra' variety used in this study covers an area of 300,000 ha mainly located in the centre of Spain and accounts for 14% of national production. It is a fruit with a fat yield of about 23% of fresh weight and its oil is valued for its high stability and good sensory characteristics (Barranco, Rallo, Uceda, & Hermoso, 1994).

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2. Materials and methods

2.1. Olives

Olive fruits (*Olea europaea*) of the 'Cornicabra' variety were harvested in two different olive groves in the Castilla-La Mancha main production areas of Toledo and Ciudad Real, during the crop seasons from 1995/1996 to1998/1999. Olives were randomly picked at three to five ripening stages, according to their skin colour. Harvesting was done by hand, using rakes. The olives were put into 20 kg boxes and taken to the pilot plant. Only healthy fruits, without any kind of infection or physical damage, were processed.

2.2. Oil samples

Three or four virgin olive oils from each stage of ripening, olive grove and season, were extracted separately using an Abencor system (Comercial Abengoa, S.A., Sevilla, Spain). The oil obtained was separated by decanting and the amount measured. The oil content was expressed as percentage of fresh and dry olive paste weight (Martinez, Munoz, Alba, & Lazón, 1975). Samples were filtered with anhydrous Na_2SO_4 and stored at 4°C in darkness using amber glass bottles without head space until analysis. Eighty-four virgin olive oil samples of 'Cornicabra' variety were obtained for this study from four successive crop seasons.

2.3. Ripeness Index

Various methods have been proposed for expressing the stage of maturity of olives. Among them the International Olive Oil Council has suggested a simple technique which is based on the assessment of the colour of the skins of 100 olives which are randomly drawn from 1 kg of the sample (IOOC, 1984). The first stage of ripening is known as the 'green stage', corresponding to green mature fruits that have reached their final size. Afterwards, chlorophyll pigments in the olive skin are progressively replaced by anthocyanines during fruit ripening. This makes it possible to identify a 'spotted stage', a 'purple stage' and a 'black stage' according to the skin colour of the fruits (Uceda & Frías, 1975).

2.4. Analytical methods

Determination of free acidity, peroxide value, UV absorption characteristics, and fatty acid composition were carried out following the analytical methods described in Regulations EEC/2568/91 and EEC/1429/92 of the Commission of the European Union (1992). Free acidity, given as percent of oleic acid, was determined by titration of a solution of oil dissolved in ethanol–ether (1:1) with ethanolic potash. Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq/kg), was determined as follows: a mixture of oil and chloroform–acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution. K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, with a UV spectrophotometer (Hewlett-Packard, HP 8452A), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Oxidative stability was evaluated by the Rancimat method since it is a fast and reliable analytical procedure (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (h) measured with the Rancimat 679 apparatus (Metrohm), using an oil sample of 3.5 g, warmed to 98°C, and an air flow of 10 l/h.

Phenolic compounds were isolated by extraction of a solution of oil in hexane with a water–methanol mixture (60:40), three times. The Folin-Ciocalteau reagent (Merck) was added to a suitable aliquot of the combined extracts, and the absorption of the solution at 725 nm was measured. Values are given as milligrams of gallic acid per kilogram of oil (Gutfinger, 1981; Vázquez, Janer, & Janer, 1973).

Tocopherols were evaluated following the AOCS Method Ce 8-89 (1989). A solution of oil in hexane was analyzed by HPLC (HP 1100) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250 mm×4.6 mm i.d.; Sugerlabor, Madrid, Spain) that was eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 ml/min. A fluorescence detector (Waters 470), with excitation and emission wavelength set a 290 and 330 nm, was used. Chlorophyll and carotenoid compounds (mg/kg) were determined at 472 and 670 nm in cyclohexane, using the specific extinction values, by the method of Minguez-Mosquera, Rejano, Gandul, Sánchez and Garrido (1991).

For the determination of fatty acid composition, the methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2N methanolic potash, and analyzed by GC with a Hewlett-Packard (HP 6890) chromatograph equipped with a FID detector. A fused silica column (50 m length×0.25 mm i.d.) coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor) was used. Helium was used as carrier gas with a flow through the column of 1 ml/min. The temperature of the injector and detector was set at 250°C while the oven temperature was 210°C. An injection volume of 1 μl was used [Regulation EEC 2568/91, corresponding to AOCS method Ch 2-91 (1993)].

Sterols (%) were determined by gas chromatography, Hewlett-Packard (HP 6890), with a capillary column (25 m length×0.25 mm i.d.) coated with SGL-5 (0.25 μm thickness; Sugerlabor). Working conditions were as follows: carrier gas, helium; flow through the column, 1.2

ml/min; injector temperature, 280°C; detector temperature, 290°C; oven temperature, 260°C; injection volume 1 μl [Regulation EEC 2568/91, corresponding to AOCS method Ch 6-91 (1993)]. Analytical determinations were carried out at least in duplicate.

2.5. Sensory analysis

Sensory analysis of the samples was done by 12–15 selected and trained panellists from the analytical panel of the *Instituto de la Grasa* (CSIC, Sevilla), for crop seasons 1995/1996 and 1996/1997, and from that of *D.O. Montes de Toledo* (Toledo; in collaboration with the University of Castilla-La Mancha), for crops 1997/1998 and 1998/1999, according to the method described in Annex XII of the European Union Commission. Oils were scored according to a nine-point scale, 1 being the value for very poor quality and 9 for optimum quality whereas, for positive attributes, like fruitiness and bitterness, a five-point scale was used. The overall grading for each sample was the mean value of 30 scores (15 evaluations in duplicate).

2.6. Statistical Analysis

Statistical analysis was performed using the NCSS 6.0 statistical software (Statistical solutions, 1997). Differences were considered statistically significant when probability was greater than 95% ($P < 0.05$).

3. Results and discussion

3.1. Oil content

The oil content of the olive fruit, expressed as percent of dry matter, generally increased during ripening, as shown in Fig. 1. However, in some cases, at higher ripeness index (> 3.5–4.0) this increase was modest (as in crop seasons 1995/1996a and 1995/1996b) or there was even a decrease in its value (1996/1997 and 1997/1998a). The average oil content, though varying with crop season, ranged from 35–40% at a Ripeness Index (hereafter RI) close to 2 and 40–45% at a RI of about 4. An average of yield increase 5 g of oil per 100 g of dry olive paste yield increase could easily be obtained by choosing an appropriate maturation stage before processing. Chimi and Atouati (1994) and Gutierrez et al. (1999) also observed that olive maturity significantly affects extraction yield, which increases during ripening.

Figs. 2–6 and Tables 1 and 2 list the mean and standard deviation values of the most interesting analytical parameters of the olive oils obtained at different stages of maturity. Table 3 shows the regression coefficient of those parameters exhibiting a linear relationship with RI.

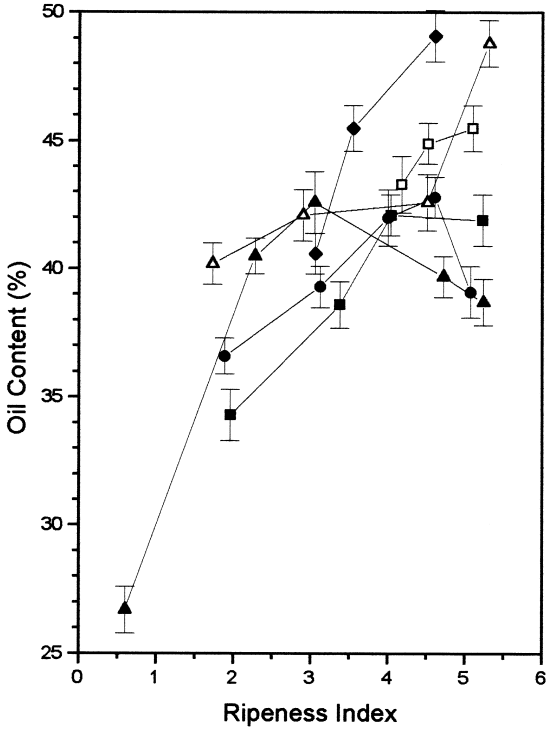

Fig. 1. Changes in oil content of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a;□, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b;◆, 1998/1999.

3.2. Free acidity

An increase was observed in free acidity as ripening progressed (Fig. 2). The same behaviour has been observed for Blanqueta and Arbequina (Garcia et al., 1996) and Picual and Hojiblanca varieties (Gutiérrez et al., 1999).The acidity increased from 0.10–0.15% at a RI of about 2 to 0.2–0.4% at a RI close to 5. In all samples studied, the free acidity was much lower than the upper limit of 1% established for the best commercial quality olive oil, designated 'extra' virgin (Regulations EEC 2568/91). Olives at a later stage of ripening give oils with higher levels of free acidity since they undergo an increase in enzymatic activity, especially by lipolytic enzymes (Martínez Suárez, 1973), and are more sensitive to pathogenic infections and mechanical damage. The relatively lower free acidity observed in the oil samples studied, as compared to commercial Cornicabra 'extra' virgin olive oils (Salvador et al., 1998), is due to the use of only healthy fruits and the typically small scale of the system used for the processing.

3.3. Peroxide value and UV absorption

As shown in Table 1, the oils obtained from olives at more advanced stages of maturity showed lower peroxide values (PV), excepting only crop season 1998/1999. This was probably due to an increase in the lipoxygenese activity. In a few cases, as in crop 1995/1996a

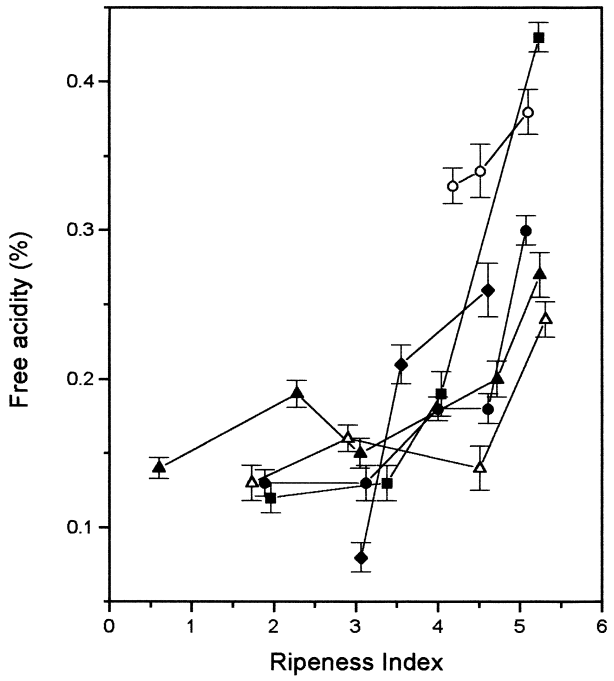

Fig. 2. Changes in free acidity of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a; □, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b; ◆, 1998/1999.

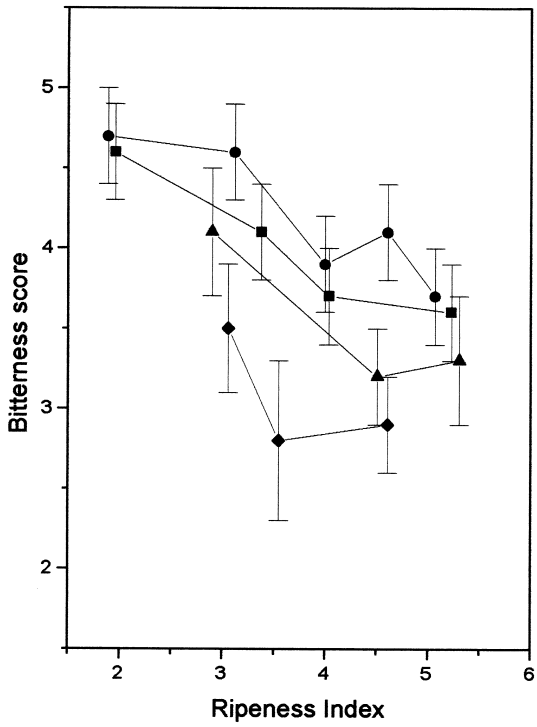

Fig. 4. Changes in bitterness score of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a; □, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b; ◆, 1998/1999.

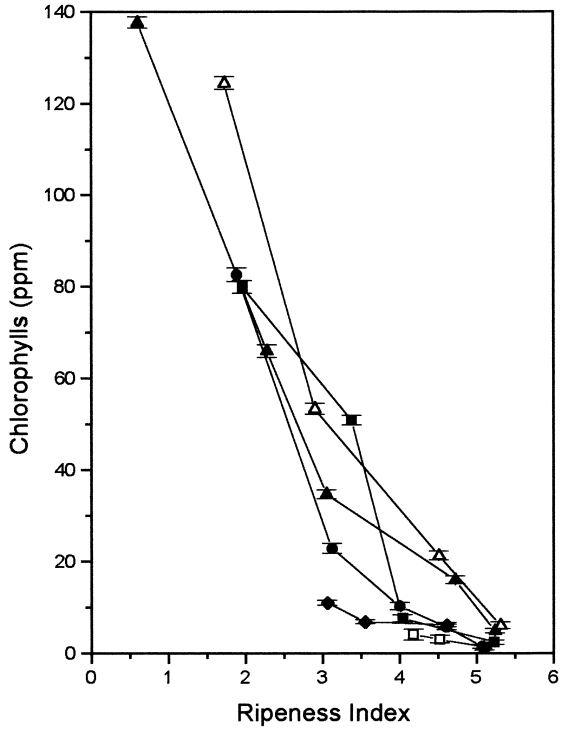

Fig. 3. Changes in chlorophylls of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a; □, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b; ◆, 1998/1999.

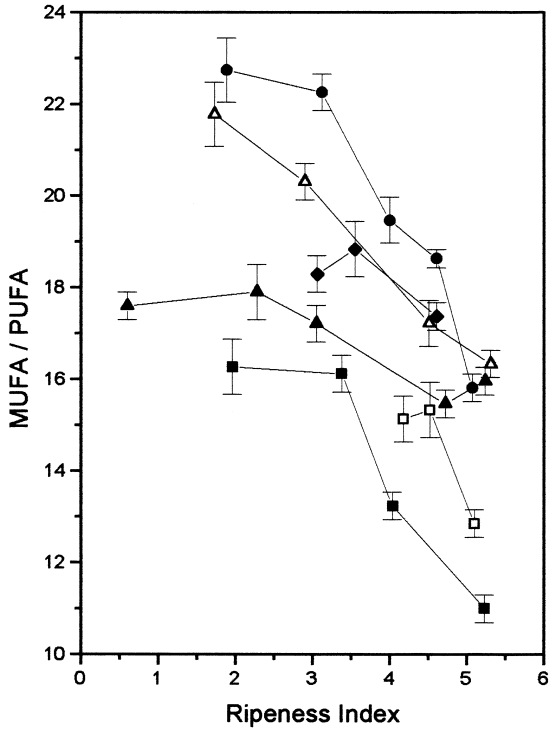

Fig. 5. Changes in the MUFA/PUFA ratio of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a; □, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b; ◆, 1998/1999.

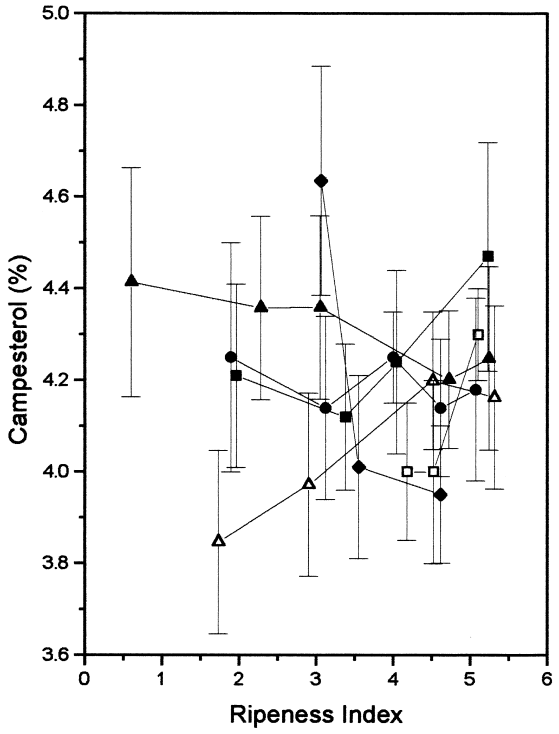

Fig. 6. Changes in campesterol of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a; □, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b; ◆, 1998/1999.

and 1996/1997, an increase in PV was observed at intermediate levels of ripening.

Spectrophotometric absorption at 270 nm (K_{270}) in the UV region also decreased at later RI in all crop seasons studied, whereas, the absorption at 232 nm did not display a clear trend during ripening.

At an intermediate RI, between 3 and 4, the values of these parameters were far below the limits established for 'extra' virgin olive oils (EEC Regulations), with the exception of K_{270} in crop season 1995/1996a and 1995/1996b.

3.4. Pigments: chlorophylls and carotenoids

The stage of olive maturity is very important for pigment, chlorophyll and carotenoid, concentrations in virgin olive oil (Fig. 3 and Table 1). In the early periods of olive picking, pigments were concentrated, i.e. an average chlorophyll content of 80 ppm at RI of about 2 while, by the end of maturity their concentration had diminished to only a few ppm (Fig. 3). This behaviour was similar in the different crop seasons, with a correlation coefficient ranging from −0.93 to −1.0 (Table 3), with the exception of 1998/1999 where the pigment content of the oil was lower than other years. Results agree with the findings of other authors (Gutiérrez et al., 1999; Mínquez-Mosquera et al., 1991).

3.5. Oxidative stability and natural antioxidants

Stability to oxidation, measured by the Rancimat method, of the oils extracted from olives at different stages of ripening are shown in Table 1. In general, the values decreased slightly as fruits ripened, although the trend was not linear and in some cases there was an increase at the highest RI. Values of oxidative stability (OS) for this variety were higher than 100 h. A much lower oxidative stability was observed for oils 1995/1996b, due to the bad climatic conditions of previous years (Salvador et al., 1998).

It is known that the behaviour observed in stability is influenced by some phenolic compounds (Gutiérrez et al., 1999; Gutiérrez et al., 1977; Salvador et al., 1999; Tsimidou, 1998; Tsimidou, Papadopoulos, & Boskow, 1992). During ripening, the concentration of phenolic compounds progressively increases until it reaches a maximum at the 'spotted' and 'purple' pigmentation stage, after which it decreases (Chimi & Atouati, 1994; Montedoro, Bertuccioli, & Anichini, 1978; Monteleone, Caporale, Lencioni, Favati, & Bertuccioli, 1995). The increase in total polyphenols at the last stage of maturity (Table 1) observed in many crops, which is responsible for the corresponding increase in oxidative stability, could be due to the reduction in water content (olive fruit humidity) observed with ripening; this can affect the extraction of partially soluble compounds. In fact, when polyphenols increased significantly, the decrease in humidity was also important (i.e. from 56 to 46% in 1997/1998a) whereas, when a decrease in phenolic compounds was observed, humidity increased (i.e. from 44 to 55% in 1996/1997).

Phenolic components also contribute to oil flavour and aroma (Solinas, Giovacchino, & Mascolo, 1978; Vázquez, 1978), especially to the typical bitter taste of olive oil (Angerosa & Solinas, 1990; Gutiérrez, Perdiguero, Gutiérrez, & Olías, 1992).

The α-tocopherol content varied slightly during ripening and a clear trend with maturity was not clear for this variety (Table 1). Gutierrez et al. (1999) have reported similar behavior for Hojiblanca, whereas for Picual, Verdial and Villalonga varieties a clear decrease as ripening progressed has been observed (Garcia et al., 1996; Gutiérrez et al., 1999).

3.6. Sensory

Sensory evaluation indicated that total scores generally diminished at higher RI (Table 1), although they were always higher than 6.5, the threshold value for 'extra' virgin classification (EEC Regulations). This was probably due to the observed loss in some positive attributes, especially fruitiness (from about 3.0 at RI close to 2, to 1.8 at RI of about 5).

Table 1
Mean values and standard deviations of some analytical parameters of 'Cornicabra' virgin olive oil extracted at different stages of olives maturity[a]

	Crop	RI	PV	K232	K270	Carot	OS	PG	αT	Sens
1	1995/1996a	2.0	7.6±0.4	1.69±0.05	0.21±0.01	31.8±0.6	116±2	326±4	294±2	7.9±0.5
2	1995/1996a	3.4	10.7±0.5	1.76±0.04	0.23±0.01	22.1±0.5	121±2	320±6	311±3	7.8±0.5
3	1995/1996a	4.0	4.6±0.3	1.70±0.06	0.15±0.01	6.6±0.5	94±2	315±4	267±4	7.5±0.5
4	1995/1996a	5.2	3.2±0.4	1.85±0.05	0.16±0.01	2.8±0.3	110±2	430±4	304±5	7.3±0.5
5	1995/1996b	4.2	7.5±0.4	1.81±0.04	0.20±0.01	4.2±0.4	105±2	500±5	192±1	
6	1995/1996b	4.5	6.6±0.4	1.69±0.05	0.15±0.01	3.4±0.5	18±2	318±6	189±2	
7	1995/1996b	5.1	2.3±0.3	1.76±0.05	0.16±0.01	2.1±0.3	21±1	338±3	205±3	
8	1996/1997	1.9	19.1±0.6	1.88±0.04	0.20±0.01	31.5±0.5	178±2	400±3	156±3	8.0±0.5
9	1996/1997	3.1	8.1±0.4	1.84±0.06	0.16±0.01	12.5±0.7	193±3	616±7	194±4	7.9±0.5
10	1996/1997	4.0	13.8±0.5	1.68±0.04	0.14±0.01	8.0±0.2	172±2	351±6	182±2	7.1±0.5
11	1996/1997	4.6	5.9±0.4	1.64±0.07	0.13±0.01	6.0±0.3	128±2	406±5	213±3	7.9±0.5
12	1996/1997	5.1	4.7±0.4	1.52±0.05	0.10±0.01	3.0±0.4	98±2	255±4	218±3	7.6±0.5
13	1997/1998a	0.6	4.5±0.3	1.63±0.06	0.19±0.01	52.5±0.7	146±2	454±5	312±4	
14	1997/1998a	2.3	4.1±0.4	1.70±0.05	0.17±0.01	28.5±0.4	181±2	537±5	253±3	
15	1997/1998a	3.0	3.6±0.2	1.74±0.04	0.16±0.01	17.0±0.5	188±2	561±4	237±2	
16	1997/1998a	4.7	2.5±0.3	1.70±0.05	0.15±0.01	11.1±0.4	138±3	550±2	262±3	
17	1997/1998a	5.2	1.9±0.2	1.72±0.03	0.13±0.01	4.8±0.4	188±2	628±7	199±5	
18	1997/1998b	1.7	4.5±0.3	1.81±0.03	0.22±0.01	46.7±0.6	177±2	496±5	236±4	
19	1997/1998b	2.9	4.2±0.3	1.69±0.05	0.17±0.01	23.8±0.8	176±2	551±6	228±2	7.6±0.5
20	1997/1998b	4.5	4.9±0.4	1.48±0.05	0.11±0.01	12.9±0.3	154±2	378±5	210±3	7.0±0.6
21	1997/1998b	5.3	3.6±0.3	1.54±0.08	0.13±0.01	5.7±0.5	168±2	471±4	178±4	6.8±0.5
22	1998/1999	3.1	4.0±0.3	1.54±0.05	0.12±0.01	18.8±0.5	128±2	317±4	256±5	7.8±0.6
23	1998/1999	3.6	2.3±0.2	1.44±0.04	0.11±0.01	9.9±0.5	119±2	303±6	216±3	7.4±0.6
24	1998/1999	4.6	4.4±0.3	1.47±0.04	0.12±0.01	7.5±0.6	142±3	318±4	213±4	7.3±0.5

[a] RI, Ripeness Index; PV, peroxide value (meq/kg); Carot, carotenoids (mg/kg); OS, oxidative stability (h); PG, polyphenols as gallic acid (mg/kg); αT, α-tocopherol (mg/kg); Sens, sensory score.

Table 2
Mean values and standard deviations of major fatty acids and sterols composition (%) of 'Cornicabra' virgin olive oil extracted at different stages of olives maturity[a]

	Crop	RI	C16:0	C18:0	C18:1	C18:2	OxSus	β-sito	5-ave	TotSt
1	1995/1996a	2.0	9.68±0.12	3.42±0.01	79.76±0.09	4.16±0.04	350±1	87.0±0.7	5.3±0.3	1910±120
2	1995/1996a	3.4	10.39±0.09	3.35±0.02	79.05±0.11	4.17±0.04	350±1	88.0±0.6	5.1±0.3	1870±100
3	1995/1996a	4.0	10.23±0.06	3.54±0.02	78.00±0.05	5.25±0.05	391±2	86.5±0.7	6.3±0.3	1960±110
4	1995/1996a	5.2	9.49±0.11	3.84±0.03	77.35±0.13	6.37±0.02	444±2	87.2±0.9	5.7±0.2	1840±90
5	1995/1996b	4.2	9.88±0.10	3.29±0.02	79.22±0.06	4.63±0.03	360±1	85.0±0.8	6.9±0.4	1760±80
6	1995/1996b	4.5	10.62±0.08	3.54±0.03	78.36±0.09	4.51±0.03	353±1	86.7±0.5	6.0±0.3	1670±100
7	1995/1996b	5.1	10.29±0.07	3.37±0.02	77.88±0.08	5.43±0.04	399±1	86.1±0.8	6.5±0.3	1650±110
8	1996/1997	1.9	9.45±0.09	2.61±0.02	82.49±0.09	3.13±0.04	279±1	86.0±0.9	6.8±0.2	1010±70
9	1996/1997	3.1	9.64±0.06	2.63±0.01	82.12±0.07	3.19±0.04	282±2	85.3±0.9	7.1±0.3	1450±100
10	1996/1997	4.0	9.39±0.11	2.74±0.02	81.73±0.12	3.68±0.05	307±1	86.0±0.6	6.7±0.2	1550±100
11	1996/1997	4.6	9.45±0.09	2.88±0.02	81.30±0.14	3.84±0.04	314±1	86.5±0.7	6.3±0.3	1580±90
12	1996/1997	5.1	9.30±0.08	3.14±0.01	80.41±0.07	4.56±0.04	347±1	84.4±0.8	8.1±0.4	1640±80
13	1997/1998a	0.6	10.86±0.08	3.07±0.02	79.22±0.09	3.63±0.03	339±1	87.7±0.4	3.3±0.2	1900±120
14	1997/1998a	2.3	10.64±0.09	3.17±0.02	79.44±0.08	3.68±0.04	330±2	87.1±1.0	4.3±0.5	1720±90
15	1997/1998a	3.0	10.13±0.10	3.36±0.02	79.67±0.10	3.95±0.04	334±2	84.6±0.8	5.7±0.4	1620±80
16	1997/1998a	4.7	9.43±0.08	3.25±0.02	79.88±0.06	4.52±0.03	358±1	86.7±0.9	4.7±0.4	1640±100
17	1997/1998a	5.2	8.97±0.12	3.03±0.02	80.85±0.09	4.51±0.04	349±1	84.7±1.1	6.2±0.3	1550±70
18	1997/1998b	1.7	10.01±0.07	2.79±0.02	81.28±0.11	3.07±0.04	293±2	88.2±0.6	3.9±0.2	1620±100
19	1997/1998b	2.9	10.37±0.12	2.78±0.03	80.65±0.07	3.32±0.05	303±1	86.3±0.9	4.6±0.3	1570±90
20	1997/1998b	4.5	9.51±0.09	3.01±0.01	80.65±0.09	4.10±0.03	333±1	85.8±0.5	5.1±0.3	1660±80
21	1997/1998b	5.3	8.97±0.08	3.03±0.02	80.97±0.10	4.45±0.04	341±2	85.9±0.9	4.9±0.4	1570±100
22	1998/1999	3.1	11.00±0.08	3.01±0.02	79.34±0.12	3.74±0.04	317±1	85.6±1.0	5.7±0.4	1620±80
23	1998/1999	3.6	10.07±0.05	3.13±0.02	80.40±0.05	3.73±0.02	311±2	85.8±0.6	5.8±0.2	1620±60
24	1998/1999	4.6	9.70±0.09	3.34±0.01	80.22±0.08	4.10±0.04	325±1	83.0±0.8	6.5±0.3	1510±90

[a] C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; OxSus, oxidative susceptibility; β-sito, β-sitosterol; Δ5-ave, Δ5-avesterol; TotSt, total sterols (mg/kg).

In particular, bitterness scores decreased with RI (Fig. 4 with a correlation coefficient higher than −0.9, with the exception of crop 1998/1999. The oils obtained from green olives were excessively bitter according to the panellists' comments. This does not imply rejection of the oil, but if the level of bitterness is too high it could cause some problems for consumer acceptance (Gutiérrez et al., 1992). A high level of bitterness is a peculiar characteristic of the 'Cornicabra' virgin olive oils; however according to this study, for an optimum sensory quality of these oils it is recommended that the bitterness score should be lower than 4.0–3.5.

3.7. Fatty acids composition

The percentages of major fatty acids are reported in Table 2. Stearic acid (C18:0) generally increased with RI ($r > 0.8$ in many cases). Oleic acid (C18:1) decreased with RI ($r > 0.87$), with the exception of crop season 1997/1998a where an increase was observed. Linoleic acid (C18:2) increased with RI in all cases, showing a good linear correlation, with a regression coefficient ranging between 0.88 and 0.99 (Table 3). A good linear relationship was also obtained with MUFA, PUFA and their ratio (Fig. 5 and Table 3). The change in fatty acid composition with an increase of PUFA increased the oxidative susceptibility (OxSus; Tables 2 and 3), as defined by Cert, Alba, León-Camacho, Moreda, and Pérez-Camino (1996). This should affect the oxidative stability of olive oil, but the latter is also affected by the concentration of natural antioxidants, as discussed above.

3.8. Sterol composition

In the 'Cornicabra' variety the main sterols are β-sito sterol, δ-5-avenasterol and campesterol. Total sterols (Table 2) generally diminished slightly during ripening, with the exception of crops 1997/1998a where the decrease was considerable (from 1900 to 1550 ppm), and 1996/1997 where there was a major increase (from 1010 to 1640 ppm). A decrease in total sterols has been observed by other authors (Gutiérrez et al., 1999; Mariani, Fedeli, Grob, & Arthor, 1991). The content in β-sitosterol generally decreases during ripening, while Δ-5-avenasterol increases, and the presence of desaturase activity has been suggested (Gutierrez et al., 1999).

The high campesterol content of 'Cornicabra' olive oil (Salvador et al., 1998), almost always higher than the limit of 4% established by EEC Regulations, is a peculiar characteristic of this olive oil variety. The change in campesterol content during ripening was unfortunately not clear (Fig. 6). In many cases, the change was minimal, and there was an apparent slight tendency to increase (as in crop 1995/1996a, 1995/1996b and 1997/1998b) or to decrease (1997/1998a and 1998/1999) during ripening.

3.9. Olive quality and ripeness index

On the basis of the behaviour of the analytical parameters studied, in particular free acidity, peroxide value, stability, sensory, oil extraction yield, and their relation to virgin olive oil quality, the best stage of maturity of 'Cornicabra' olive fruits for processing would appear to be a ripeness index higher than 3.0 and lower than 4.0–4.5.

A multiple regression study showed a good correlation between RI and four relevant analytical variables: free acidity, carotenoids, palmitic acid and Δ-5-avena sterol; with a *r*-squared of 0.952 (Fig. 7). Regression coefficients and their *P*-values are reported in Table 4.

Although it is compulsory to test the mathematical model by using industrial olive oils with a known ripening index of the fruit used, before any routine application to commercial samples, the application of this model to 114 commercial 'Cornicabra' virgin olive oils, obtained from the same crop seasons studied, indicated that probably less than 25% of the oils were extracted from olives harvested with an optimum RI (Table 5). Practical experience in this field confirms this finding: the olive oil industry of Castilla-La Mancha mainly harvests 'Cornicabra' olives with RI greater than 5, due to popular tradition and because it is believed that the oil extraction yield always increases with maturity. It is therefore crucial that the industrial sector that it brings forward the harvesting time of this olive variety to further improve the quality of the virgin olive oil they produce.

Table 3
Regression coefficients between several analytical parameters and the ripeness index of 'Cornicabra' virgin olive oil[a]

	1995/1996a	1995/1996b	1996/1997	1997/1998a	1997/1998b	1998/1999
Chlorophylls	−0.944	−1.000	−0.930	−0.960	−0.958	−0.338
Carotenoids	−0.955	−1.000	−0.953	−0.966	−0.969	−0.401
Bitterness score	−0.963		−0.908		−0.908	−0.648
C18:2	0.906	0.880	0.897	0.950	0.990	0.940
MUFA/PUFA	−0.915	−0.903	−0.933	−0.864	−0.996	−0.771
OxSus	0.899	0.870	0.907	0.677	0.991	0.730

[a] OxSus, oxidative susceptibility.

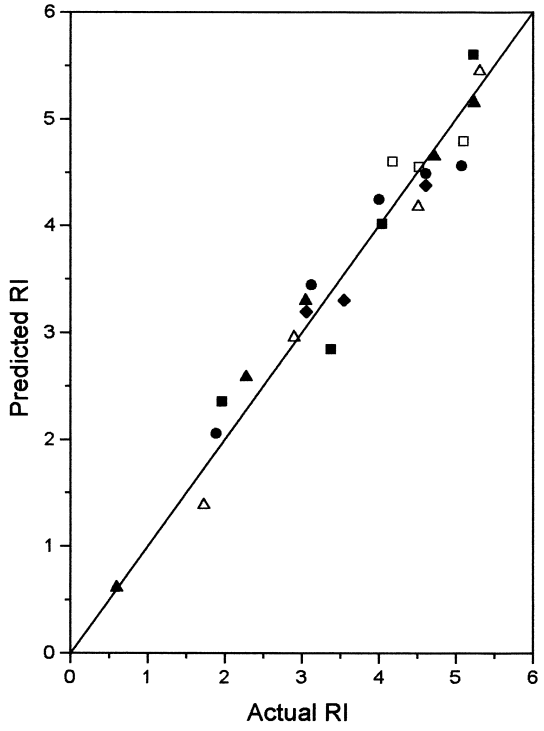

Fig. 7. Predicted vs actual ripeness index of 'Cornicabra' virgin olive oil extracted at different stages of olive maturity. Crops: ■, 1995/1996a; □, 1995/1996b; ●, 1996/1997; ▲, 1997/1998a; △, 1997/1998b; ◆, 1998/1999.

Table 4
Multiple regression equation between four relevant analytical variables and the ripeness index of 'Cornicabra' virgin olive oil[a]

Independent variable	Regression coefficient	Probability level (*P*-value)	Power (5.0%)
Free acidity	2.638	0.008	0.797
Carotenoids	−0.0842	< 0.001	1.000
C16:0[a]	−0.5975	< 0.001	0.994
Δ5-avenasterol	−0.3268	0.001	0.965
Intercept	12.24	< 0.001	1.000

[a] C16:0, palmitic acid.

Table 5
Predicted ripeness index of commercial 'Cornicabra' virgin olive oils ($n = 114$)[a]

Crop	Samples	P10	P25	P50	P75	P90
1995/1996	16	3.6	4.1	5.5	6.1	8.0
1996/1997	19	4.7	5.0	5.1	5.8	7.1
1997/1998	40	4.7	5.1	5.8	6.1	6.9
1998/1999	39	4.3	4.7	5.0	5.5	5.8
Total	114	4.3	4.8	5.4	5.9	6.8

[a] 10th, 25th, 50th, 75th and 90th percentiles.

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References

AOCS. (1989). *Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC*. Official Method Ce 8-89. Champaign, IL: AOCS Press.

AOCS. (1989). *Determination of fatty acids in olive oils by capillary GLC*. Official Method Ch 2-91. *Determination of the composition of the sterol fraction of animal and vegetable oils and fats by TLC and capillary GLC*. Official Method Ch 6-91. Champaign, IL: AOCS Press.

Angerosa, F., & Solinas, M. (1990). *Crushing influence on the olive oil quality*. Presented at the International Seminar 'Olive Oil and Table Olives: Technology and Quality', Cittá S. Angelo, 25–28 April

Aparicio, R., & Morales, T. M. (1998). Characterization of olive ripeness by green aroma compounds of virgin olive oil. *Journal of Agriculture and Food Chemistry, 46*, 1116–1122.

Barranco, D., Rallo, L., Uceda, M., & Hermoso, M. (1994). Fichas varietales del olivo, Cornicabra. *Unaproliva, 4*, 25–28.

Boschelle, O., Mozzon, M., & Giomo, A. (1994). Aceite virgen extra típico: 'Cuáles son sus perspectivas en friuli-venezia giulia'. *Olivae, 50*, 31–35.

Boskow, D. (1996). *Olive oil: Chemistry and technology*. Champaign, IL: AOCS Press.

Cert, A., Alba, J., León-Camacho, M., Moreda, W., & Pérez-Camino, M. C. (1996). Effects of talc addition and operating mode on the quality and oxidative stability of virgin oils obtained by centrifugation. *Journal of Agriculture and Food Chemistry, 44*, 3930–3934.

Chimi, H., & Atouati, Y. (1994). Determination of the optimal stage for harvesting Moroccan picholine olives by monitoring change in total polyphenols. *Olivae, 54*, 56–60.

European Union Commission. (1991). Regulation EEC/2568/91 on the Characteristics of Olive and Olive Pomance Oils and their Analytical Methods. Annexes II, III, IX and XII. *Official Journal of the European Community*. *L248*, 6–36. Regulation EEC/142/92 on the Modifications to the Regulation EEC/2568/91. *ibid.* (1992)

García, J. M., Seller, S., & Pérez-Camino, M. C. (1996). Influence of fruit ripening on olive oil quality. *Journal of Agriculture and Food Chemistry, 44*, 3516–3520.

Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemists' Society, 58*, 966–968.

Gutiérrez, F. (1989). Determination of oxidative stability in virgin olive oils: comparison between active oxygen method and rancimat method. *Grasas y Aceites, 40*, 1–5.

Gutiérrez, F., Jiménez, B., Ruíz, A., & Albi, A. (1999). Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on different components involved. *Journal of Agriculture and Food Chemistry, 47*, 121–127.

Gutiérrez, F., Perdiguero, S., Gutiérrez, R., & Olías, J. M. (1992). Evaluation of bitter taste in virgin olive oil. *Journal of the American Oil Chemists' Society, 69*, 394–395.

Gutiérrez, R., Janer del Valle, C., Janer del Valle, M. L., Gutierrez, F., & Vazquez Roncero, A. (1977). Relación entre los polifenoles y la calidad y estabilidad del aceite de oliva virgen. *Grasas y Aceites, 28*, 101–106.

IOOC. (1984). *Document no 6*. Madrid: International Olive Oil Council.

Mariani, C., Fedeli, C., Grob, K., & Arthor, A. (1991). Indagini sulle variazioni dei componenti minori liberi di esterificati di oli ottenuti di olive in funzione della madurazione e dello stoccaggio. *Rivista Italiana delle Sostanze Grasse, 50*, 179–186.

Martínez Suárez, J. (1973). M, Recientes estudios de la almazara experimental del instituto de la grasa. *Rivista Italiana delle Sostanze Grasse, 50*, 325–330.

Martínez, J. M., Munoz, E., Alba, J., & Lazón, A. (1975). Report about the use of the "Abencor" yields analysis. *Grasas y Aceites, 26*, 379–385.

Mínguez-Mosquera, M. I., Gandul, B., & Garrido, J. (1990). Pigments present in virgin olive oil. *Journal of the American Oil Chemists' Society, 67*, 192–196.

Mínguez-Mosquera, M. I., Rejano, L., Gandul, B., Sánchez, A. H., & Garrido, J. (1991). Colour-pigment correlation in virgin olive oil. *Journal of the American Oil Chemists' Society, 68*, 322–337.

Montedoro, G., Bertuccioli, M., Anichini, F. (1978). Aroma analysis of virgin olive oil by head space volatiles and extraction techniques. In *Flavour of foods and beverages. Chemistry and technology* (pp. 247–281), G. Charalambous, & G. Inglett, Academic Press, NY.

Montedoro, G. F., Garafolo, L., & Bertuccioli, M. (1986). Factors shaping the quality characteristics of an olive oil. *Industrie Alimentari, 25*, 549–555.

Monteleone, E., Caporale, G., Carlucci, A., & Pagliarini, E. (1998). Optimisation of extra virgin olive oil quality. *Journal of the Science of Food and Agriculture, 77*, 31–37.

Monteleone, E., Caporale, G., Lencioni, L., Favati, F. & Bertuccioli, M. (1995). Optimization of virgin olive oil quality in relation to fruit ripening and storage. In *Food flavors: Generation, analysis and process influence* (pp. 397–418), Amstedam: Elsevier Science B.V.

Salvador, M. D., Aranda, F., & Fregapane, G. (1998). Chemical composition of commercial Cornicabra virgin olive oil from 1995/96 and 1996/97 Crops. *Journal of the American Oil Chemists' Society, 75*, 1305–1311.

Salvador, M. D., Aranda, F., & Fregapane, G. (1999). Contribution of chemical components of Cornicabra virgin olive oils to oxidative stability. A study of three successive crop seasons. *Journal of the American Oil Chemists' Society, 76*, 427–432.

Solinas, M., Giovacchino, L., & Mascolo, A. (1978). The polyphenols of olives and olive oils. Note III. Influence of temperature and kneading time on the oil polyphenol content. *Rivista Italiana delle Sostanze Grasse, 55*, 19–23.

Solinas, M., Marsilio, V., & Angerosa, F. (1987). Behaviour of some components of virgin olive oils flavor in connection with the ripening of the olives. *Rivista Italiana delle Sostanze Grass, 64*, 475–480.

Statistical solutions NCSS 6.0. (1997). Cork, Ireland.

Tsimidou, M. (1998). Polyphenols and quality of virgin olive oil in retrospect. *Italian Journal of Food Science, 2*(10), 99–116.

Tsimidou, M., Papadopoulos, G., & Boskow, D. (1992). Phenolic compounds and stability of virgin olive oil. Part I.. *Food Chemistry, 45*(2), 141–144.

Uceda, M. & Frías, P. (1975). *Épocas de recolección. Evolución del contenido graso del fruto y de la composición del aceite*. Córdoba: Caja Provincial de Ahorros de Córdoba

Vázquez, A. (1978). Les polyphénols de l'huile d'olive et leur influence sur les caracteristiques de l'huile. *Revue Francaise de Corps Grasse, 25*, 21–26.

Vázquez, A., Janer, C., & Janer, M. L. (1973). Determination of total polyphenols in olive oils. *Grasas y Aceites, 24*, 350–357.

Vázquez, A., Maestro, R., & Graciani, E. (1971). Changes in olive polyphenols during ripeness. *Grasas y Aceites, 22*, 366–371.