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The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil

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Abstract

We studied the effects of harvesting and two processing systems (two-phase centrifugation and three-phase centrifugation) on olive oil quality. Oils extracted from high quality olives do not differ in free acidity, peroxide value and ultraviolet light absorption. Nor was the fatty acid composition affected. However, the antioxidant content of the oil was higher from green olives than from ripe olives. On the other hand, we determined that neither extraction method affects the presence of α-tocopherol and β-carotene, however, the phenolic content is higher in the two-phase method due to the addition of lukewarm water that is used to dilute the olive paste. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Virgin olive oil; Ripening; Extraction method; Quality; Antioxidant compounds; α-tocopherol; β-carotene; Phenols

1. Introduction

The incidence of atherosclerosis is low in populations using olive oil as their primary source of fat (Assmann et al., 1997; Caruso, Berra, Giovanini, Cortesi, Fedeli, & Galli, 1999; Mancini, Parfitt, & Rubba, 1995). Triacylglycerols comprise about 98% of the oil and oleic acid is its main fatty acid. Virgin olive oil, obtained from the first cold pressing, also contains a range of antioxidants and flavour constituents (Montedoro, Servili, Baldioli, & Miniati, 1992; Visioli & Galli, 1998). The beneficial effects of olive oil are due not only to its high unsaturated/saturated fatty acid ratio, but also to its antioxidants such as vitamin E, carotenoids and phenolic compounds (Caruso et al., 1999; Visioli & Galli, 1998). Some studies have suggested that the antioxidants in virgin olive oil protect against cancer and atherosclerosis by impeding the oxidative modification of LDL and its adherence to the arterial wall (Armstrong et al., 1997; Nicolaiew et al., 1998). These substances also contribute to the stability of the oil (Caruso et al., 1999; Di Giovachino, Solinas, & Miccoli, 1994; Montedoro et al., 1992; Papadopoulos & Boskou, 1991; Ranalli & Martinelli, 1995).

Since olive oil is a natural product, it has a variable chemical composition. The levels of antioxidants depend on several factors such as the variety of olive used, the cultivation environment and the method of oil extraction (Bruni, Cortesi, & Fiorino, 1994; Salas, Pastor, Castro, & Vega, 1997). Virgin olive oil is consumed unrefined and therefore contains several non-saponifiable compounds, especially phenols, which are usually removed when the oil is refined (Caruso et al., 1999; Ragazzi & Veronese, 1973).

Traditional olive oil extraction, based on the use of a pan crusher and a hydraulic press, is not a continuous system. Moreover, the transformation costs are high (Ranalli & Martinelli, 1995). In 1965, olive oil began to be extracted by means of a three-phase centrifuge (with horizontal axle) which separates the oil, water and husk from the olive paste (Ranalli & Martinelli, 1995). Since the centrifuge system reduced the processing time,

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which in turn reduced the excessive storage period of olives, the oils obtained were frequently of higher quality (Alba, 1997; Angerosa & di Giovaccino, 1996; Ranalli & Martinelli, 1995). However, this method of extraction required the addition of lukewarm water to dilute the olive paste. And this addition decreases the levels of phenolic compounds in the oil because of their high solubility in the aqueous phase (Angerosa & di Giovaccino, 1996; Di Giovacchino et al., 1994). Another problem with this extraction method is the considerable increase of vegetable water generated by the processing plant, which increases the waste disposal problems as well as costs. In 1992, several olive oil plant manufactures introduced a new model of decanter that enables the oil phase to be separated from the malaxed olive paste without addition of warm water. Furthermore, the use of this new decanter generates negligible quantities of vegetable water (Angerosa & di Giovaccino, 1996). Oils extracted by a dual-phase decanter have higher concentrations of tocopherols and phenols and show higher stability to oxidation (Angerosa, & di Giovaccino, 1996; Di Giovacchino et al., 1994; Ranalli & Martinelli, 1995) than those obtained by a three-phase decanter. Although the two-phase decanter is widespread, some plant manufacturers still follow the traditional methods. Three-phase decanters can easily be changed to a two-phase system by simply adjusting the crusher (Ranalli & Martinelli, 1995).

The effect of extraction systems on the antioxidant levels of the oil has been evaluated in previous studies (Di Giovacchino et al., 1994; Nergiz & Ünal, 1991; Ranalli & Angerosa, 1996). However, the influence of olive maturation was not considered in these studies. The aim of this paper is to investigate the effect of the extraction system and the maturity grade on several components in the oil: quality parameters, fatty acids, phenolic compounds, vitamin E and β-carotene content. The experiment was performed using two indexes of maturity and two different extraction systems: the two-phase centrifugal decanter and the conventional three-phase equipment.

2. Materials and methods

2.1. Virgin olive oils

Sixty samples made with fruits of the *Arbequina* variety from the two appellations of Catalonia, *Les Garrigues* and *Siurana*, and from two crops (years 1997–1998 and 1998–1999) were provided by the Olive Oil Cooperative Association of Catalonia.

Samples were divided into four groups according to the method of extraction and the harvest period. Each producer provided information about the maturity of the olives and the technology used. The maturity index (MI) was calculated after visual colour inspection over a hundred randomly chosen olives, according to the following formula (Hermoso, Uceda, García-Ortiz, Morales, Frías, & Fernández, 1991): $MI = (a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4) + (f \times 5) + (g \times 6) + (h \times 7)/100$. Where a, b, etc. are the number of olives in each of the seven colour classes from dark green to dark black. The two stages of ripening were (1) from November to December and (2) from January to February. Group A includes the oils obtained from green olives (MI = 1.48–2.46) processed by the two-phase method, Group B includes the oils from mature olives (MI = 3.10–4.65) using the same method, Group C contains the oils from green olives (MI = 1.59–2.55) using the three-phase method, and Group D contains the oils from mature olives (MI = 3.00–4.65) by the same method.

All oil samples were stored in dark brown glass bottles at 4 °C until analysed.

Acidity value, peroxide index and ultra-violet light absorption (K_{270}) were determined following the analytical methods described in The Regulation EEC/2568/91 of the European Union Commission.

Fatty acids were transformed into methyl esters and analysed using a Hewlett Packard 6890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionisation detector (FID). Separations of the methyl esters were performed on a fused silica column (30 m×0.20 i.d.) coated with SP-2330 stationary phase [poly (80% biscianopropyl–20% cyanopropylphenylsiloxane), 0.20 μm film thickness] from Supelco (Bellefonte, PA, USA). The split-splitless injector was used in a split ratio of 1:30. We used a HP 7673 automatic liquid sampler injector. The injector volume of the sample was 1 μl. The injector and detector temperatures were kept at 250 and 270 °C, respectively. The oven temperature was programmed at 180 °C, isotherm. Helium was used as the carrier gas with a lineal velocity of 22.5 cm/s and nitrogen was used as the make up gas. The recording of chromatograms was performed with a HP-Chemstation for GC systems (Software G2070AA, version A.0402). The quantification was performed by internal normalisation.

α-Tocopherol and β-carotene were determined by reverse-phase high-performance liquid chromatography (RP-HPLC) in one run following the method proposed by Gimeno, Calero, Castellote, Lamuela-Raventós, de la Torre, and López-Sabater (2000). Briefly, the method involved a rapid saponification and a subsequent extraction with a mixture of hexane–ethyl acetate. The chromatographic system consisted of an ODS-2 column with a mobile phase of methanol–water–butanol and a diode-array detector (DAD).

Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water–methanol (60:40 v/v). Total phenols, expressed as caffeic acid equivalents (ppm), were determined with a UV-visible

(Uv-160A) recording spectrophotometer (Shimadzu, Kyoto, Japan) at 765nm using the Folin-Ciocalteu reagent (Swain & Hillis, 1961)

2.2. Statistical analysis

The assays were carried out in duplicate. The results are shown as tables of mean values and standard deviation. The discussion is based on the one-way analysis of variance (ANOVA; $P<0.05$). All statistical analyses were performed using the SPSS of the windows statistical package (Release 8.0).

3. Results and discussion

3.1. Acidity, peroxide index and UV spectrophotometric index (K_{270})

All oils were extracted from high quality olives and met the standards set by the European Commission for extra virgin quality. All sample parameters analysed were within the levels accepted for each appellation (Spanish Regulations BOE/166/1977 and BOE/298/1979). None of the parameters showed any difference between the two methods of extraction or even between the two harvest periods. Table 1 details the results for each group.

3.2. Fatty acid composition (Table 1)

Fatty acid composition was not affected by the extraction method or the maturity grade, since all the oils were produced from the same variety of olive. The major fatty acids were oleic (69–75%), palmitic (13–15%), linoleic (9–11%), stearic (1,5–2%) and palmitoleic (1–1.5%). These results are in agreement with those obtained by Hidalgo et al. (1993). Nevertheless, Gutierrez, Jimenez, Ruíz, and Albi (1999) described an increase in linoleic acid during ripening due to the oleate desaturase enzyme, which transforms oleic acid into linoleic. They also observed changes in palmitic and linolenic acids during ripening. However, since the maturity range for their study was wider than ours, the results are not comparable.

3.3. α-Tocopherol (Table 2)

No significant difference was found for either methods when we compared the two states of ripening ($P=0.099$). Although the level of antioxidants decreased throughout ripening (Agramont, López, Boatella, & de la Torre, 1986; Gutiérrez et al., 1999), α-tocopherol seems to be the least affected (Gutiérrez et al., 1999). In agreement with Ranalli and Angerosa (1996), neither the technology seems to affect the levels of α-tocopherol ($P=0.283$).

3.4. β-Carotene (Table 2)

The β-carotene difference in maturity is more remarkable. Oils from green olives have more β-carotene than those from ripe olives ($P=0.001$), with no significant difference between the two extraction methods ($P=0.748$). These results are in agreement with those obtained by Gutierrez et al. (1999).

Table 1
Quality parameters and fatty acid composition (percent) of the different groups of virgin olive oil ($n=60$)

Groups	Two-phase decanters		Three-phase decanters		Reference values
	A (green)	B (ripe)	C (green)	D (ripe)	
Free acidity (% oleic acid)	**0.14**[a]	**0.14**	**0.14**	**0.14**	**<0.5**
	0.03[b]	*0.06*	*0.03*	*0.03*	
Peroxide value (meq O2/Kg oil)	**10.10**	**10.10**	**9.05**	**9.75**	**<15**
	2.47	*2.33*	*1.89*	*2.44*	
K_{270} (E 1%)	**0.11**	**0.11**	**0.10**	**0.11**	**<0.15**
	0.02	*0.01*	*0.01*	*0.01*	
Fatty acids					
C14:0	**0.01a**	**0.01**	**0.01**	**0.01**	
	0.00b	*0.00*	*0.00*	*0.00*	
C16:0	**13.81**	**13.13**	**14.67**	**13.65**	
	0.65	*0.53*	*0.72*	*0.52*	
C16:1	**1.16**	**1.07**	**1.31**	**1.16**	
	0.09	*0.09*	*0.12*	*0.05*	
C17:0	**0.12**	**0.12**	**0.13**	**0.12**	
	0.01	*0.01*	*0.02*	*0.00*	
C17:1	**0.24**	**0.23**	**0.25**	**0.23**	
	0.02	*0.01*	*0.02*	*0.01*	
C18:0	**1.82**	**1.80**	**1.80**	**1.79**	
	0.05	*0.04*	*0.07*	*0.04*	
C18:1	**71.54**	**72.56**	**69.82**	**71.26**	
	1.41	*1.10*	*1.28*	*1.06*	
C18:2	**9.97**	**9.79**	**10.70**	**10.45**	
	0.65	*0.44*	*0.53*	*0.39*	
C20:0	**0.35**	**0.35**	**0.35**	**0.36**	
	0.01	*0.01*	*0.01*	*0.01*	
C18:3	**0.50**	**0.49**	**0.52**	**0.51**	
	0.04	*0.02*	*0.03*	*0.02*	
C20:1	**0.30**	**0.29**	**0.29**	**0.30**	
	0.03	*0.02*	*0.00*	*0.01*	
C22:0	**0.12**	**0.11**	**0.10**	**0.11**	
	0.03	*0.01*	*0.00*	*0.00*	
C24:0	**0.04**	**0.06**	**0.04**	**0.04**	
	0.01	*0.06*	*0.01*	*0.00*	
SFA	**16.24**	**15.58**	**16.81**	**16.09**	
	0.67	*0.8*	*0.58*	*0.61*	
MUFA	**72.8**	**74.15**	**71.83**	**72.95**	
	1.32	*1.36*	*1.01*	*1.01*	
PUFA	**10.8**	**10.28**	**11.35**	**10.96**	
	0.68	*0.57*	*0.46*	*0.40*	

[a] Mean (in bold).
[b] Standard deviation (in italic).
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2
Content in antioxidants (mg/kg; $n=60$)

	Two-phase decanters		Three-phase decanters	
Groups	A (green)	B (ripe)	C (green)	D (ripe)
Alpha tocopherol	**212.46**[a]	**185.55**	**200.82**	**195.09**
	11.55[b]	*16.04*	*12.77*	*5.07*
Beta-carotene	**2.84**	**1.58**	**2.66**	**1.95**
	0.97	*0.47*	*0.70*	*0.24*
Phenolic compounds	**123.82**	**80.88**	**72.93**	**42.07**
	26.33	*19.53*	*20.13*	*7.30*

[a] Mean (in bold).
[b] Standard deviation (in italic).

3.5. Polyphenolic compounds

Table 2 shows how phenols decrease during ripening ($P=0.047$), in agreement with the results obtained by Gutiérrez et al. (1999). However, the most outstanding difference is found between the two methods of extraction ($P=0.016$). The two-phase decanter preserves more the phenolic content than the three-phase method. Ranalli and Angerosa (1996) found similar results with oils made from others olive varieties. Phenols present in olive paste are soluble in water or oil, according to their partition coefficients and extraction temperature. In the three-phase method, the addition of water to the paste alters the partition equilibrium between the liquid phases and reduces the phenol concentration in the oily phase through dilution in the aqueous phase. Thus, the addition of water to olive oil removes water-soluble phenols. Nevertheless, because of their higher liposolubility, α-tocopherol and β-carotene are not affected.

4. Conclusions

1. There is a higher content of β-carotene and phenolic compounds, and to a lesser extent of α-tocopherol, in oils extracted from green olives as opposed to ripe olives.
2. Regarding the extraction method, the two-phase decanter appears to preserve the phenolic content more than the three-phase decanter. As phenols are more hydrosoluble than α-tocopherol and β-carotene, they are reduced when the warm water is added to the three-phase decanter.
3. These results have proved the two-phase decanter method provides a higher nutritional quality oil.

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