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Influence of technology, storage and exposure on components of extra virgin olive oil (Bosana *cv*) from whole and de-stoned fruits

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Abstract

We investigated the influence of technology, storage and exposure on quality parameters, minor components, oxidative stability and antioxidant activity in two extra virgin olive oils of Bosana *cv*, obtained from whole (WO) and de-stoned fruits (DO), processed with a two-phase decanter. DO oils showed great stability and, consequently, had a longer shelf-life than WO oils. During storage, the former maintained lower values of free acidity, higher values of chlorophylls, carotenoids and α-tocopherol, longer oxidative stability and higher antioxidant activity than the latter. Peroxide indices were not significantly different between the two oils, while spectrophotometric indices, during storage, increased more in DO oils than in WO oils. The total phenol content behaviour patterns during storage were very similar in both oil samples, with a higher value in WO oils. Exposure to light significantly decreased the chlorophylls, carotenoid, phenol, α-tocopherol and stability values, as expected, but antioxidant activity was not influenced by exposure conditions.
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Keywords: Extra-virgin olive oil; De-stoned paste; Whole paste; Storage; Exposure

1. Introduction

The consumption of extra virgin olive oil, a typical product of the Mediterranean region, is currently increasing, thanks to its unique sensory and nutritive qualities, since it is the only oil that is consumed unrefined. Recent studies (Boskou, 1996; Hill & Giacosa, 1992; La Vecchia et al., 1998; Owen et al., 2000; Visioli & Galli, 1998) reveal the important role of virgin olive oil in disease prevention, attributing to it healthful effects that are universally recognised. Processing is the most important factor affecting extra virgin olive oil quality. In the past 30 years, olive processing technology has undergone an evolution. Firstly, traditional extraction by pressure was replaced with centrifugation systems of olive paste, able to separate the oil from the other phases (De Stefano, Piacquadio, Servili, Di Giovacchino, & Sciancalepore, 1999; Di Giovacchino, Solinas, & Miccoli, 1994). Centrifugation systems have allowed manufacturing costs to be reduced and, at the same time, because of their high production capacity, the storage time of olives before processing can be dramatically shortened, resulting in the production of better quality oils, principally in those regions where harvesting methods are not very efficient (Cucurachi, 1975).

The centrifugation system shows some disadvantages, such as the reduction in phenol content of the oil due to the addition of warm water to dilute the olive paste (De Stefano et al., 1999; Di Giovacchino et al., 1994; Gimeno, Castellote, Lamuela-Raventos, De la Torre, & Lopez-Sabater, 2002; Salvador, Aranda, Gomez-Alonso,

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& Fregapane, 2003) and waste problems. Some years ago, a new horizontal centrifugal two-phase decanter was manufactured (Gimeno et al., 2002; Ranalli, Costantini, De Mattia, & Ferrante, 2000), able to separate oil from the olive paste without addition of water. This led to both the production of better quality oils, with a higher content of natural phenolic antioxidants (Angerosa & Di Giovacchino, 1996; De Stefano et al., 1999; Di Giovacchino, Sestili, & Di Vincenzo, 2002; Gimeno et al., 2002; Ranalli & Angerosa, 1996) and the generation of negligible quantities of vegetable water. A later technological innovation, now well-established, enabled paste obtained from de-stoned fruits to be used (Frega, Cagliotti, & Mozzon, 1997; Saitta et al., 2003; Siniscalco, Montedoro, Parlati, & Petruccioli, 1989), which in some ways improves the quality of the extra-virgin olive oil. The major advantage in using de-stoned paste is that it improves the sensory qualities and shelf-life of extra virgin olive oil. In the destoning process, in fact, the stones are removed at the beginning of processing, just before the kneading; therefore, the enzymes contained in the seeds do not influence the pulp composition, so it can be supposed that de-stoned oils are more rich in compounds than whole oils because enzymatic reactions, such as the oxidative reactions due to the presence of lipoxygenase, do not occur. Moreover, solid particles, responsible for a rough taste in the olive oil are no longer a problem, and thermal and mechanical activities that cause degradation of minor and major components of olive oil are reduced (Angerosa, Basti, Vito, & Lanza, 1999; Saitta et al., 2003).

On the basis of these observations, the aim of this research was to investigate the influence of technology, storage and exposure on quality parameters, minor components, oxidative stability and antioxidant activity on two extra virgin olive oils of Bosana *cv*, obtained from whole and de-stoned fruits, processed with a two-phase decanter. In this work, we decided not to investigate the fatty acid and triacylglycerol contents, since previous works (Frega et al., 1997; Saitta et al., 2003) reported that destoning does not influence the above-mentioned fractions when both de-stoned and whole oil came from the same cultivar (no significant differences between the two oils were found).

2. Materials and methods

2.1. Virgin olive oils

Two homogeneous samples of *cv* Bosana olives, 1000 kg of each, harvested at the same time in December 2002, with no defects and both at an optimal stage of ripening (70% just turned dark-coloured and the rest green), were processed in an industrial oil mill using a two-phase centrifugation system, after defoliation and washing of drupes. One sample was processed after being de-stoned (DO) and the other one was processed whole (WO). Olives were crushed with a fixed-hummer metal crusher and the olive paste was kneaded for 35 min at 30 °C. The paste was then centrifuged with a two-phase centrifugation system (mod. Olimio F2G/D350, TEM, Tavernelle Val di Pesa, Florence, Italy) at a flow rate of 300 kg/h. The oil phases were further clarified in an automated discharge vertical centrifuge.

The oils obtained were stored in hermetically sealed 60 ml colourless transparent glass bottles. Bottles were filled up, leaving a headspace of 3 ml and were left to either normal light exposure or the dark, at room temperature for 16 months. Analyses were carried out as soon as the olive oils arrived at the laboratory and then every two months until the eighth month of storage. The last analyses were performed at the 12th and 16th months of storage.

2.2. Analytical methods

Acidity value, peroxide index and ultra-violet light absorption K232 and K270 were determined by the methods reported in Regulation EEC/2568/91 of the European Union Commission. To determine free acidity, 5 g of oil were added to 60 ml of ethyl alcohol–diethyl ether (1:1 v/v) mixture and neutralized with 0.1 N NaOH. Data obtained were expressed as g of oleic acid per 100 g of oil.

For peroxide index determination, 1 g of oil, weighed precisely, was added to 25 ml of an acetic acid–chloroform (3:2 v/v) mixture. Next, 0.5 ml of a saturated solution of KI was added to this mixture and the sample was put in the dark for 5 min. Afterwards, 75 ml of deionized water and 1 ml of starch paste, as indicator, were added to the mixture and the sample titrated with 0.01 N sodium thiosulphate to complete bleaching. Data obtained were expressed as mEq of O_2 per 1000 g of oil.

To determine spectrophotometric indices, K232 and K270, 0.5 g of olive oil, previously filtered through filter paper and weighed precisely, were put into a 50 ml volumetric flask. The flask was made up to volume with isooctane for spectrophotometry. Samples were analysed in a 10 mm cuvettes, using a Hewlett–Packard 8453 spectrophotometer (Palo Alto, California).

Total phenols were extracted following the method proposed by Montedoro, Servili, Baldioli, and Miniati (1992). Ten grams of olive oil were put into a test tube and 0.2 ml of Tween 20 (Merck) and 10 ml of a methanol:water mixture (80:20 v/v) added. The sample was then homogenized with an Ultra-Turrax T25 (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) macerator for 1 min, then centrifuged at 5000*g* for 10 min at 20 °C. The methanol extract was collected with a pipette and transferred to a 50 ml flask. The same procedure was repeated twice. The extracts were then

assembled, added to anhydrous sodium sulphate and put in a freezer for 6 h. Finally, the sample was filtered through Whatman filter paper and 1 ml taken for analysis, using the Folin-Ciocalteu reagent. The phenol content was determined spectrophotometrically (Spectrophotometer mod. 8453, Hewlett–Packard, Palo Alto, California) at 760 nm and the concentration was expressed as mg of gallic acid per kg of oil.

Carotenoids and chlorophylls were determined according to the method of Mincione, Poiana, Giuffrè, Modafferi, and Giuffrè (1996), as follows: 5 g of olive oil were weighed into a 25 ml flask and isooctane was added up to volume. The spectrophotometric reading was made at 436 and 670 nm for carotenoids and chlorophylls, respectively. Data were expressed as mg of β-carotene per kg of oil and mg of chlorophylls per kg of oil by comparison with a standard response curve.

Antioxidant activity was measured in the phenol extract, following the procedure of Brand-Williams, Cuvelier, and Berset (1995), using a decoloration curve of the stable radical, 2,2 diphenyl-1-picrylhydrazyl (DPPH·). Fifty microliters of the sample were made to react with 3 ml of a 6×10^{-5} M solution of DPPH for 1 h at 515 nm and 25 °C, in order to obtain a decrease in absorbance by the radical DPPH·. As the graph of absorbance vs time showed that the decrease followed 4th order kinetics, it was possible to express the antioxidant activity as, $-\mathrm{OD}^{-3}\ \mathrm{min}^{-1}$ mg 10^3, that is, with the following equation $1/A^3 - 1/A_0^3 = -3kt$, where A_0 is the initial absorbance and A is the absorbance at rising time t.

α-Tocopherol was measured by the method reported by Psomiadou, Tsimidou, and Boskou (2000), using a Waters 625 HPLC equipped with a spectrofluorometric detector FL3000 (Thermo Separation, Muskegon, Michigan, USA) at 294 nm for excitation and 330 for emission, a LiChrospher column 60Si, 250×4 mm ID (Agilent Technologies, Palo Alto, California, USA), a mobile phase of *n*-hexane–isopropyl alcohol (99:1 v/v) and a flow rate of 1.2 ml/min. Results were expressed as mg of α-tocopherol per kg of oil by comparison of the chromatographic area with a standard response curve.

Oxidative stability was measured with a Rancimat apparatus (Metrohm Co., Basilea, Switzerland) at 120 °C and 20 l h^{-1} air flow. Stability was expressed as oxidation induction time (hours).

2.3. Statistical analysis

The analyses were carried out on two containers for each sample, making four determinations for every parameter and every sample (DO and WO). Data were evaluated by a three factor completely randomized factorial design, using MSTAT-C software. Storage period, technology and exposure conditions (light or dark) were chosen as variables. Means, where appropriate, were separated by Duncan's multiple range test for $P \leqslant 0.05$ and $P \leqslant 0.01$.

3. Results and discussion

Table 1 shows the influence of storage, technology and exposure on the principal quality parameters. Free acidity values remained below the limits reported by Regulation EEC/1989/2003 (22) of the European Union Commission, which prescribes a value below 0.8 g of oleic acid per 100 g for a virgin olive oil, throughout storage (data not shown). The DO sample value is significantly lower than that of the WO sample (0.33 and 0.41 g of oleic acid per 100 g of oil, respectively), confirming reports in the literature (Saitta et al., 2003). Exposure to light increased the oil's acidity value.

The peroxide index underwent a significant increase up to the fourth month of storage, then slowly decreased up to the 16th month, probably due to the fact that the newly formed oxidation products (inside the bottle headspace) were further converted to secondary ones. This phenomenon was significantly more pronounced in the samples kept in the light, than in those stored in the dark. As regards the influence of technology, the difference between WO and DO samples was not

Table 1
Influence of storage period, technology and exposure on main quality parameters of whole and de-stoned "Bosana" extra-virgin olive oils

Source of variation	Peroxide value (meq O_2/kg oil)	UV spectrophotometric indices	
		K232	K270
Storage period (months)			
0	6.18c[A]	1.825e	0.139e
2	7.26a	1.893c	0.151d
4	7.07ab	1.928a	0.176a
6	6.41bc	1.911b	0.169bc
8	6.29c	1.897c	0.165c
12	4.76d	1.883d	0.172ab
16	4.81d	1.883d	0.176a
Significance	**	**	**
Technology			
De-stoned	6.19a	1.899a	0.171a
Whole	6.03a	1.878b	0.157b
Significance	n.s.	**	**
Exposure			
Light	5.79b	1.888a	0.178a
Dark	6.43a	1.890a	0.150b
Significance	**	n.s.	**

**, significance at $P < 0.01$; n.s., not significant.
[A] Data followed by different letters for each source of variation and column are significantly different by Duncan's multiple range test.

significant, in disagreement with the results of Saitta et al. (2003).

During storage, spectrophotometric indices showed a significant increase. No significance was noted after the 12th month of storage. Values were always under the limits reported by Regulation EEC/1989/2003 of the European Union Commission for an extra virgin olive oil. The difference between WO and DO samples was significant for both K232 and K270, where values were higher for the DO sample, because of a more marked increase during storage, as reported in Saitta et al., 2003 (Fig. 1). Exposure influenced only the K270 index which showed a higher value for the sample stored in the light.

Table 2 shows the effects of storage, technology and exposure on chlorophylls, carotenoids, total phenols, α-tocopherol content, oxidative stability and antioxidant activity in WO and DO samples. Chlorophylls decreased significantly up to the twelfth month of storage with losses of nearly 50% of the initial value, confirming

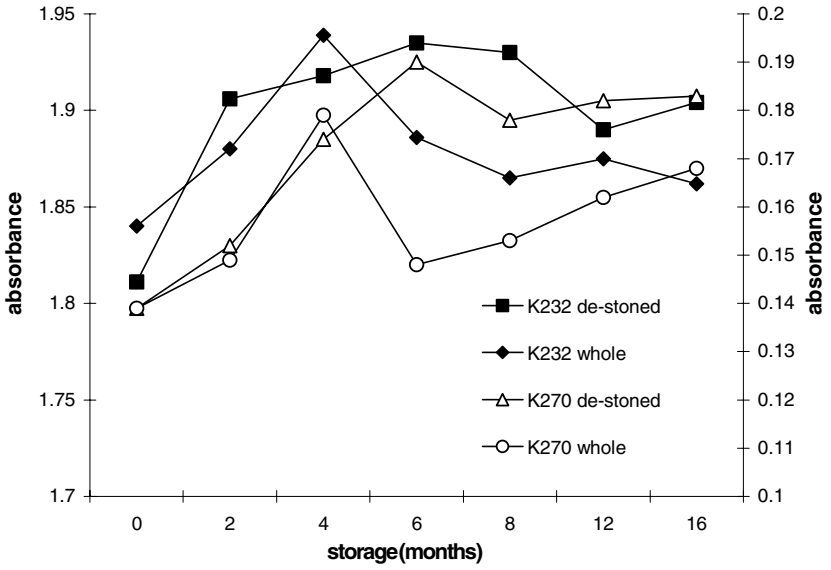

Fig. 1. Changes in spectrophotometric indices during storage of whole and de-stoned "Bosana" extra-virgin olive oil.

Table 2
Influence of storage period and exposure on chlorophylls, carotenoids, total phenols, α-tocopherol, stability and antioxidant activity of whole and de-stoned "Bosana" extra-virgin olive oils

Source of variation	Chlorophylls (mg/kg)	Carotenoids (mg/kg)	Phenols (mg gallic acid/kg oil)	α-tocopherol (mg/kg)	Stability (*h*)	Antioxidant activity ($-OD^{-3}$/min mg 10^3)
0	104b[A]	4.72a	409a	235a	8.79a	0.470a
2	114a	4.27b	355b	216b	8.11b	0.350b
4	80.2c	4.34b	336c	212bc	8.19b	0.341c
6	58.0d	3.75cd	325c	204d	8.34b	0.342c
8	42.4e	3.44d	279d	209c	8.32b	0.287e
12	55.1cd	3.60cd	277d	19e	8.41ab	0.264f
16	62.3d	3.88d	248e	170f	8.18b	0.335d
Significance	**	**	**	**	**	**
Technology						
De-stoned	74.9a	4.38a	313b	230a	8.76a	0.383a
Whole	72.5a	3.62b	324a	181b	7.91b	0.299b
Significance	n.s.	**	**	**	**	**
Exposure						
Light	60.2b	3.81b	314b	195b	8.11b	0.470a
Dark	87.2a	4.19a	323a	216a	8.56a	0.470a
Significance	**	**	**	**	**	n.s.

**, n.s., Significance at $P < 0.01$ and not significant, respectively.
[A] Data followed by different letters for each source of variation and column are significantly different by Duncan's multiple range test.

what is reported in the literature (Morello, Motilva, Tovar, & Romero, 2004), then remained constant until the end of storage. The difference in technology did not affect the chlorophyll content. As expected, light caused a greater decrease in the initial values of extra virgin olive oil samples, compared to dark storage conditions.

The carotenoid contents decreased up to the sixth month of storage, with a loss of 20% of the initial value, smaller than the chlorophylls loss, as reported in the literature (Morello et al., 2004). After that, the carotenoid contents remained constant until the end of storage. Carotenoid content was higher in DO samples than in WO samples, causing better protection of the olive oil against free radical attack, the carotenoids being natural antioxidants (Aparicio, Roda, Albi, & Gutierrez, 1999).

Total phenols underwent a decrease during storage with a loss below to 40% after 16 months. The literature reports that their decrease is due to the decomposition process and the oxidative activity of these compounds (Morello et al., 2004). Contrary to the report by Saitta et al. (2003) of higher phenol content in oils obtained from de-stoned fruits, in our study the initial contents of total phenols were very similar in both DO and WO samples. It was noted that, during storage, DO samples showed a total phenol content that was significantly different from that in the whole fruit samples. The value was lower in the DO samples, confirming data reported in Saitta et al. (2003) after a year of storage. As expected, a significant difference between samples submitted to different exposure conditions was observed, with a lower value for samples stored in the light.

α-Tocopherol content gradually and significantly decreased throughout storage, with losses below 30%, but its total disappearance, as reported by Morello et al. (2004) in their paper, was not observed. Technology affected α-tocopherol content; the value was higher in the DO samples than the WO samples, confirming the results obtained by Saitta et al. (2003). Finally, samples kept in the dark showed significantly higher values than samples stored in the light. Many studies report the very important role of α-tocopherol as an antioxidant in the oxidative stability of an olive oil (Baldioli, Servili, Perretti, & Montedoro, 1996; Morello et al., 2004; Servili, Baldioli, Miniati, & Montedoro, 1996), thus contributing to maintain its shelf-life.

The oxidative stability value, shown in Table 2, decreased in the first two months of storage, then remained constant until the end. Significant differences between DO and WO samples were observed, confirming data reported in the literature (Frega et al., 1997; Saitta et al., 2003) (Fig. 2). In fact, DO samples showed a greater resistance to oxidation than WO samples, lacking the stones, which are rich in enzymes such as lipo-oxygenase, responsible for a decrease in oil stability and consequently reduced shelf-life (Frega et al., 1997). Also, in this case, exposure to the dark extended the oil oxidative stability.

Antioxidant activity decreased significantly during 16 months of storage. The difference between DO and WO samples was considerable: DO samples showed significantly higher values than WO samples, perhaps due to the antioxidant content, principally α-tocopherol and carotenoids. Finally, no differences were found between oil samples exposed to light and those stored in the dark.

Results obtained from analyses of Bosana *cv* DO and WO samples showed that DO samples have great stability, and consequently a longer shelf-life than WO samples: during the entire storage period, DO samples maintained lower values of free acidity, higher values

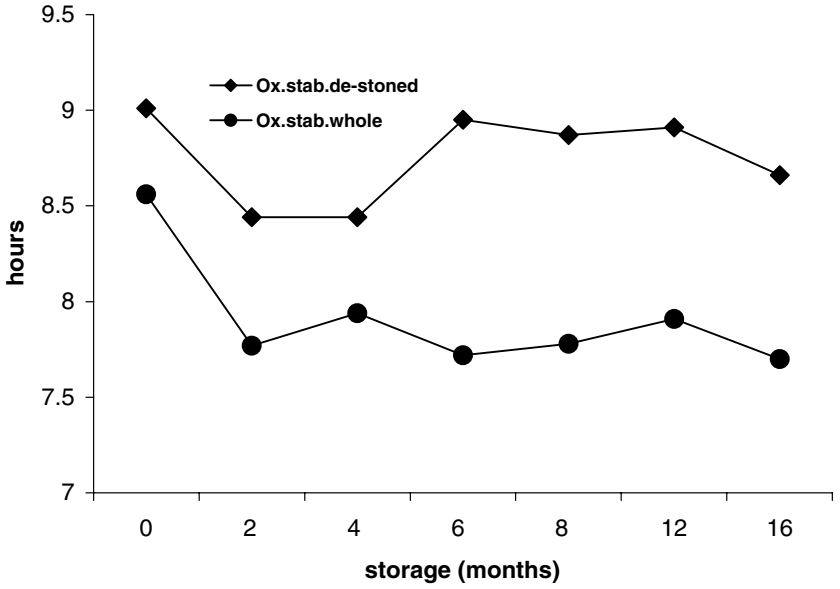

Fig. 2. Changes in oxidative stability during storage of whole and de-stoned Bosana extra-virgin olive oil.

of chlorophylls, carotenoids, α-tocopherol, longer oxidative stability and higher antioxidant activity than WO samples. Peroxide indices, showed no significant differences between the two oil samples, in disagreement with what is reported in the literature (Saitta et al., 2003), while spectrophotometric indices increased more in the DO samples than in the WO samples during storage. Total phenol content behaviour was very similar in both oil samples, with a higher value in WO samples during 16 months of storage.

Our observations, which in part disagree with data reported in the literature, showed the relationships between the de-stoning operation and the olive cultivar and the characteristics of the oils, particularly with regard to the phenol content.

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