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Storage of olives (*Olea europaea*) under CO_2 atmosphere: Effect on anthocyanins, phenolics, sensory attributes and in vitro antioxidant properties

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Abstract

Green, unripe olives were subjected to post-harvest treatment under a CO_2 atmosphere for a period of 12 days. The total polyphenol (TP), total flavonoid (TFd) and total anthocyanin (TA) contents, along with the antioxidant and sensory characteristics, were continuously monitored during the treatment on a 24 h-interval basis, in order to identify possible changes in the quality of olives related mainly to changes in the polyphenolic contents. The storage of olives under CO_2 atmosphere resulted in pronounced increases in TP and TF contents, mainly within the first 3-5 days, but TA exhibited a different pattern of evolution. Furthermore, storage under CO_2 contributed to flavour appearance with the development of fruity/floral notes, and reduced bitterness. The in vitro antioxidant properties of the CO_2-treated sample showed notable increases compared with the sample stored under regular atmospheric conditions. It was concluded that storage of olives under a CO_2 atmosphere resulted in the appearance of desired sensory attributes, by decreasing bitterness and developing aroma and colour, and the functional (antioxidant) properties were improved. This approach may be used as an alternative, chemical-free means of table olive debittering.

Keywords: Anthocyanins; Antiradical activity; Carbon dioxide; Debittering; Flavonoids; Modified atmospheres; Olives; Polyphenols; Post-harvest treatments; Reducing power

1. Introduction

There is currently an important interest in the improvement of nutritional value of various plant food commodities, by increasing their contents of biologically active polyphenolic phytochemicals. In this regard, several methods have been proposed, including selection of appropriate varieties and suitable plant breeding (Bliss, 1999), pathway modification (Verhoeyen et al., 2002), implementation of favourable cultural practices, and post-harvest treatments (Cisneros-Zevallos, 2003). The latter concept appears to constitute a promising means of improving the nutritional status of plant foods, since it does not involve gene transfer and manipulation, which raise serious concerns for the consumers, and it can be performed in a relatively simple and cost-effective manner.

Exposure of freshly harvested plant tissues to modified atmospheres (MA) containing CO_2, or exclusively CO_2, has been shown to elicit a spectrum of phenomena related to polyphenolic metabolism, but, from the data that are available to date, it appears that the responses

Abbreviations: AAE, ascorbic acid equivalents; A_{AR}, antiradical activity; CTE, catechin equivalents; CyE, cyanin equivalents; DPPH·, 2,2-diphenyl-picrylhydrazyl radical; GAE, gallic acid equivalents; MA, modified atmospheres; P_R, reducing power; TA, total anthocyanins; TFd, total flavonoids; TP, total poly- phenols; TPTZ, 2,4,6-tripyridyl-*s*-triazine; TRE, Trolox™ equivalents.

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of plant tissues to storage under MA may be affected by several factors, including the nature of the plant tissue itself and the gaseous composition of the atmosphere used for the treatment. Storage of intact clusters of red grapes under CO_2 atmosphere was shown to give rise to increased anthocyanin biosynthesis (Dourtoglou, Yannovits, Tychopoulos, & Vamvakias, 1994), but this finding contrasted with the outcome of studies on strawberries, where storage under CO_2-enriched atmosphere was demonstrated to provoke, either anthocyanin degradation, or decreased synthesis, compared with samples stored under air (Gil, Holcroft, & Kader, 1997; Holcroft & Kader, 1999). In another study on grapes (Artés-Hernández, Artés, & Tomás-Barberán, 2003), anthocyanin and flavonol contents were either preserved or they declined upon storage, irrespective of the presence of CO_2 amounts in the storage atmosphere. Results on post-harvest colour development in asparagus spears (Siomos, Dogras, & Sfakiotakis, 2001) and purple carrots (Alasalvar, Al-Farsi, Quantick, Shahidi, & Wiktorowicz, 2005) were in accordance. Packaging of Swiss chard (*Beta vulgaris*) under CO_2-containing modified atmospheres had no effect on flavonoid contents (Gil, Ferreres, & Tomás-Barberán, 1998), as was also observed for fresh-cut spinach treated (Gil, Ferreres, & Tomás-Barberán, 1999). In apples, ultra low oxygen or CO_2-enriched storage showed that only minimal changes may occur in phenolics and flavonoids (Awad & de Jager, 2000; Rocha & Morais, 2001). Similar results were found for cranberry fruits, which exhibited no large variations in polyphenolic contents in relation to the storage atmosphere, but their antioxidant activity was significantly enhanced upon storage under air than under CO_2 (Gunes, Liu, & Watkins, 2002). Nevertheless, modified atmosphere packaging of artichokes (*Cynara scolymus L.*) positively influenced polyphenolic content, by increasing the levels of 1,5-, 3,5-, 1,4, and 4,5- dicaffeoylquinic acids (Gil-Izquierdo, Conesa, Ferreres, & Gil, 2002).

Olives (*Olea europaea*) constitute an integral part of the Mediterranean diet and are considered to contribute to the daily intake of nutritional antioxidants, since they contain an array of polyphenolic phytochemicals, including various hydroxytyrosol derivatives (e.g., oleuropein) and flavone glycosides (Romero, García, Brenes, García, & Garrido, 2002). To the best of our knowledge, post-harvest gaseous treatments, for purposes of manipulating quality characteristics in olives, mainly those related to polyphenolic content, have never been performed, and, therefore, the responses of this fruit upon exposure to CO_2 atmosphere have never been investigated. This study was undertaken to examine the effect of storage of olives under CO_2 atmosphere on certain sensory attributes related to olive quality, and to evaluate the consequences of plausible changes in the polyphenolic contents on the in vitro antioxidant potency of olive extracts. The process described provides new insights into the debittering of olives, and appears as a promising means of table olive processing.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu phenol reagent and ascorbic acid were from Fluka (Steinheim, Germany). Gallic acid, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,2-diphenyl-picrylhydrazyl (DPPH$^{\bullet}$) stable radical, and catechin were from Sigma Chemical Co (St. Louis, MO, USA). Citric acid, sodium nitrite ($NaNO_2$) and aluminium chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) were from Merck (Darmstadt, Germany).

2.2. Plant material and post-harvest treatments

Green, unripe olives were collected on the 20th of September from an olive tree plantation located inside the T. E. I. of Athens. To obtain uniform amounts of fruits, collection was accomplished from three vicinal trees and from different parts of each tree, so as to minimize the effect of watering, sun exposure, and differences related to different maturation stages. After collection, fruits were pooled and randomly divided into two lots, each being approximately 1 kg. One lot was immediately placed under CO_2 atmosphere, in a glass jar, as shown in Fig. 1. The counter lot was spread out on a plastic tray to ascertain full contact of all fruits with air. Sampling was performed on a 24-h interval basis, through a period of 12 days.

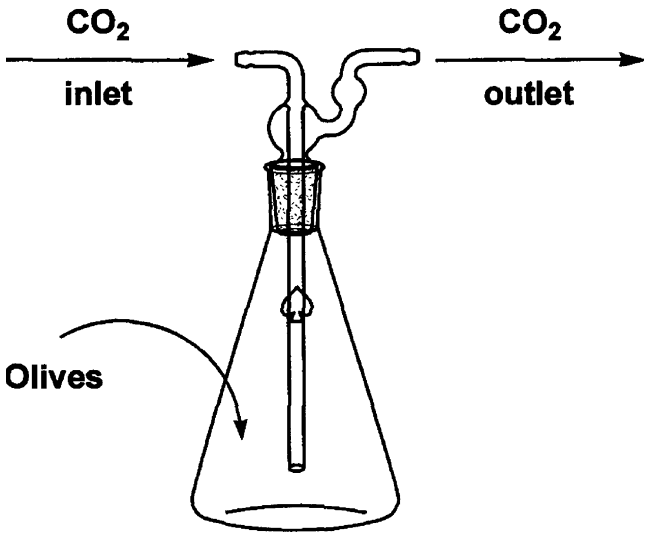

Fig. 1. The device used for the post-harvest treatment of olives. Supply of gas was introduced, at the bottom of the glass jar to ascertain complete enrichment in CO_2.

2.3. Sensory assessment

Sensory evaluation was employed to discriminate the appearance, smell/odour, and bitterness between olive samples treated under CO_2 atmosphere and regular atmospheric conditions, over the period of 12 days. A panel of 6 judges (staff members, post-doctoral researchers and graduate students) with sensory evaluation training and experience were used for assessing the organoleptic characteristics of olives on a daily basis. Bitterness was scored arbitrarily, employing a scale graded from 0 (no bitter) to 5 (the most bitter). Each panellist tasted at least 5 individual fruits, randomly selected, in order to evaluate day-to-day variations.

2.4. Extraction procedure

Olives (≈30 g) were weighed and manually destoned. The tissue was ground with sea sand and a small portion of 1% HCl in MeOH, with a pestle and a mortar, and then placed in a round-bottom flask with 50 ml of the same solvent. The flask was attached to a rotary evaporator, and extraction was performed for 15 min at maximum spin without vacuum, at 40 °C. The extract was filtered through paper filter, and this procedure was repeated twice more. The extracts were pooled and concentrated until all MeOH was removed. The aqueous residue was then extracted three times with petroleum ether (3×30 ml), to remove pigments and fats, and solvent residue was removed under vacuum. The remaining aqueous solution was made up to 25 ml with MeOH, and this solution was used for further analyses. Samples were filtered through 0.45 μm syringe filters prior to determinations.

2.5. Determinations

2.5.1. Moisture content

Olives stored under air showed considerable moisture loss (>8%) from the fifth day of storage. For this reason moisture losses were determined and taken into account for further determinations. Moisture content of olives was estimated after drying in an oven at 105 °C to constant weight.

2.5.2. Total polyphenols

Measurements were carried out according to a previously published protocol (Arnous, Makris, & Kefalas, 2002), employing the Folin-Ciocalteu methodology. Gallic acid was used as the reference standard, and results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh tissue.

2.5.3. Total anthocyanins

An aliquot of extract was combined with ethanolic HCI solution (0.25 M) to give a dilution 1:10. The solution was mixed thoroughly, and the absorbance at 520 nm (A_{520}) was read after 5 min, using the ethanolic HCI solution as blank. Total anthocyanin content was determined as cyanin (cyanidin 3-*O*-glucoside) equivalents (CyE) per 100 g fresh tissue, using $\varepsilon = 26{,}900$ and MW = 449.2.

2.5.4. Total flavonoids

A modified protocol of that described by Kim, Chun, Kim, Moon, and Lee (2003) was employed. A 01 ml aliquot of extract, appropriately diluted, was mixed with 0.4 ml distilled water in a 1.5 ml microcentrifuge tube 0.03 ml of 5% $NaNO_2$ was added and the mixture allowed to react for 5 min. Following this, 0.03 ml of 10% $AlCl_3$ was added and the mixture stood for a further 5 min. Finally, the reaction mixture was treated with 0.2 ml of 1 M Na_2CO_3 and 0.24 ml distilled water, and the absorbance at 510 nm was obtained against a blank prepared similarly, by replacing extract with distilled water. Total flavonoid content was calculated from a calibration curve using catechin as standard, and expressed as mg catechin equivalents (CTE) per 100 g fresh tissue.

2.5.5. Antiradical activity (A_{AR})

Determinations were performed as described previously (Arnous et al., 2002), using the DPPH$^{\cdot}$ assay. All samples were diluted 1:20, immediately before the analysis. An aliquot of 0.025 ml of diluted sample was added to 0.975 ml DPPH$^{\cdot}$ solution (49.6 mg l^{-1} in MeOH), vortexed, and the absorbance was read at $t = 0 (A_{515}^{t=0})$ and $t = 30\,\text{min} (A_{515}^{t=30})$. Results were expressed as trolox® equivalents (mM TRE) per g of fresh tissue using the following equation:

$$A_{\mathrm{AR}} = \left(\frac{0.018 \cdot \%\Delta A + 0.017}{tw} \right) \cdot F_{\mathrm{D}}$$

as determined from linear regression, after plotting $\%\Delta A_{515}$ of known solutions of trolox® against concentration, where $\%\Delta A_{515} = \frac{A_{515}^{t=0} - A_{515}^{t=30}}{A_{515}^{t=0}} \cdot 100$, tw the weight of fresh tissue (g), and F_{D} the dilution factor (20).

2.5.6. Reducing power (P_R)

For the determination of the reducing ability, a protocol based on the ferric reducing power assay was used, as described previously (Arnous et al., 2002). Results were expressed as mM ascorbic acid equivalents (AAE) per g of fresh tissue. All samples were diluted appropriately with distilled water before the analyses.

2.6. Statistics

In all cases, analyses were performed in triplicate, unless otherwise specified, and values averaged. The standard deviation (SD) was also calculated. Correlations

were established using regression analysis at a 95% and 99% significance level. All statistics were performed with Microsoft Excel™ 2000.

3. Results

Olives remaining under air did not show apparent colour change, although a slight coloration was observed from the fifth day, whereas morphologically the fruits shrank because of dehydration. The effect of CO_2 atmosphere on olive quality and composition was manifested from the third day of the treatment by significant changes in aroma and appearance. Fruits developed red shades which appeared to darken during the course of the treatment (Fig. 2), while the aroma turned from leafy/neutral at the time of harvesting into the characteristic olive flavour with fruity notes (Table 1). Furthermore, their tastes became more palatable due to a decrease in bitterness. At the same time, no moisture loss or adverse effects on the integrity of the fruits was observed.

For the evaluation of changes that might occur in the polyphenolic content during treatments, three characteristic indices: total polyphenols (TP), total flavonoids (TFd), and total anthocyanins (TA), were considered. Monitoring of TP showed that olives stored under CO_2 atmosphere produced larger quantities, which varied from 1.5- to 1.8-fold compared with the values of the control sample, during the first three days of the treatment (Fig. 3). Thereafter and particularly from the seventh day forward, these differences became less pronounced. TFd content, on the other hand, did not show the same trend, and after a 4.5-fold increase under CO_2 during the first 24 h, there was a declining tendency in both samples toward the end of the treatment (Fig. 4). It is worth mentioning, however, that flavonoid levels in the CO_2-treated sample were always higher. TA evolved differently, in that no detectable amounts were found in olive extracts during the first 3 days of the treatment in both samples (Fig. 5). Nevertheless, olives stored under CO_2 developed reddish shades beginning from the fourth day, and this coloration appeared to be enhanced thereafter, with the highest value being recorded on the tenth day. At the same time olives under air showed a weak coloration between the fourth and eighth day, with faint to undetectable levels afterwards.

In principle, the antiradical activity (A_{AR}) was in the same fashion, and the CO_2-treated sample was more active in scavenging radicals, mainly up to the fifth day (Fig. 6(a)). Similarly, the reducing power (P_R) of CO_2-treated olives varied from 1.3- to almost 2-fold of those stored under air, but differences recorded from the seventh day until the end of the treatment were less important (Fig. 6(b)). Both antioxidant characteristics were significantly correlated with TP and TFd during the first four days (Table 2), suggesting that, during this period, changes in the polyphenolic composition may be pronouncedly reflected in the in vitro antioxidant capacity of olives.

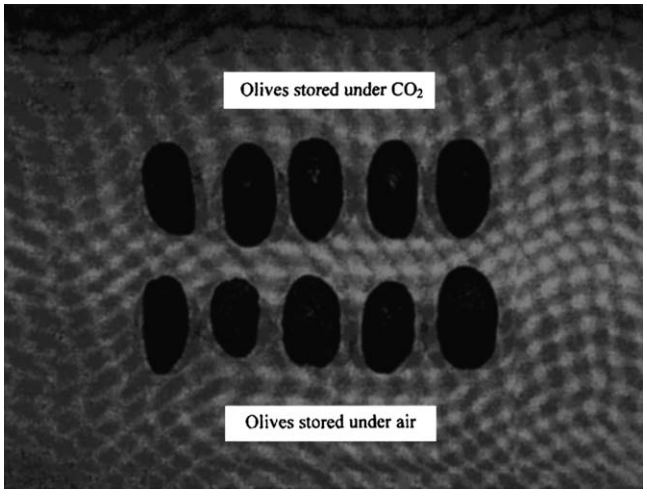

Fig. 2. Olives stored under CO_2 and under air for a period of six days.

4. Discussion

The post-harvest storage of olives and its effects has been documented in relation to the quality of the oil produced (Agar, Hess-Pierce, Sourour, & Kader, 1999; García, Gutiérrez, Barrera, & Albi, 1996a; García et al., 1996b). However, post-harvest treatments for optimization of storability of table olives and improvement of their quality have not been examined. The olive fruit contains a wide spectrum of secondary polyphenolic metabolites (Romero et al., 2002; Vinha et al., 2005), which possess various functional effects (Saija & Uccella, 2001; Soler-Rivas, Espín, & Wichers, 2000). Therefore, their profile and content is of undisputed importance for the nutritional quality of olives.

In this study, an examination of storage of olives was attempted under a CO_2 atmosphere with respect to its impact on quality characteristics, that pertain to both the aesthetic and nutritional properties of the fruit. The storage of olives at ambient temperature was shown to induce increased polyphenol biosynthesis, which, however, was more limited under air (Fig. 3). It is noteworthy that the monitoring of specific classes of polyphenolic constituents, including total flavonoids and total anthocyanin pigments, indicated that not all phenolics were affected in the same manner, as they presented a different evolution pattern throughout the storage period (Figs. 4 and 5).

Olives stored under CO_2 exhibited phenomena that could be characterised as an acceleration of

Table 1
Results obtained from the sensory assessment of olives stored under a CO_2 atmosphere and regular atmospheric conditions

Days	Atmospheric conditions			CO_2 atmosphere		
	Appearance	Smell/odour	Bitterness	Appearance	Smell/odour	Bitterness
0	Integral	Green/leafy	+++++	Integral	Green/leafy	+++++
3	Integral	Green/leafy	+++++	Integral	Olive like	++++
7	Shrunk	Leafy/neutral	+++++	Integral	Olive like/fruity	+++
11	Dehydrated	Leafy/neutral	+++++	Integral	Olive like/fruity	++

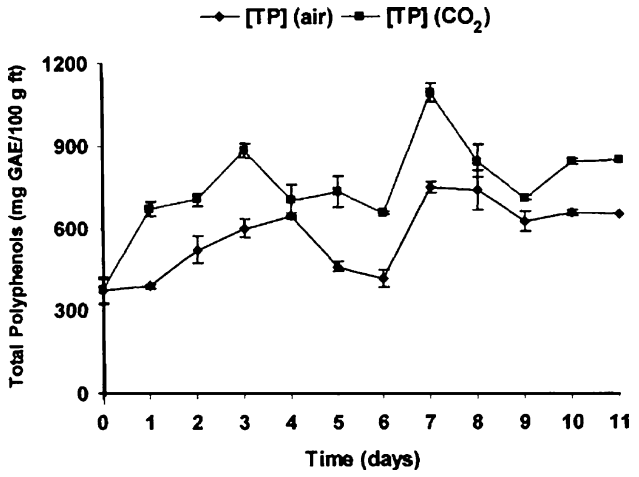

Fig. 3. Changes in the total polyphenol content during post-harvest treatment of olives under CO_2 and regular atmospheric conditions (air).

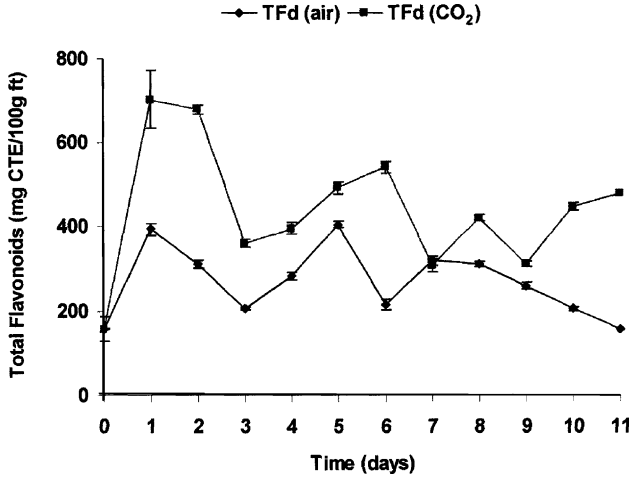

Fig. 4. Changes in the total flavonoid content during post-harvest treatment of olives under CO_2 and regular atmospheric conditions (air).

maturation. In particular, the fruits developed an intense characteristic aroma with floral notes and colour, but the significance of the decrease in the bitter taste should also be stressed (Table 1). Enhancement of the aromatic profile has been observed in various fruits upon exposure to CO_2 atmosphere, including apricots (Bitteur et al., 1990), grapes (Bitteur et al., 1992; Dourtoglou et al., 1994; Etiévant, Issanchou, Marie, Ducruet, & Flanzy, 1989), and strawberries (Guichard, Chambroy, Reich, Fournier, & Souty, 1992). However, the induction of anthocyanin biosynthesis rather contrasts with previous findings on certain plant food commodities (Alasalvar et al., 2005; Gil et al., 1997; Holcroft & Kader, 1999; Ramos, Fleuriet, Rascalou, & Macheix, 1993; Siomos et al., 2001), but is in accordance with others (Dourtoglou et al., 1994).

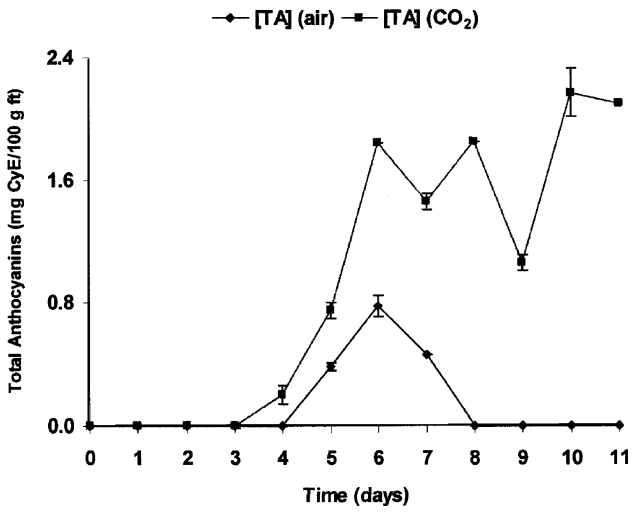

Fig. 5. Changes in the total anthocyanin content during post-harvest treatment of olives under CO_2 and regular atmospheric conditions (air).

The bitter taste of olives is largely ascribed to the content of oleuropein (García, Yousfi, Mateos, Olmo, & Cert (2001); Gutiérrez-Rosales, Ríos, & Gómez-Rey, 2003). In this regard, the gradual loss of bitterness observed during storage under CO_2 may be due to oleuropein decomposition. In the development of olive fruit, three phases are usually distinguished (Soler-Rivas et al., 2000): a growth phase, during which accumulation of oleuropein occurs, a green maturation phase, coinciding with a reduction in the levels of chlorophyll and oleuropein, and a black maturation phase, characterised by the appearance of anthocyanins and during which the oleuropein levels continue to fall. Therefore, oleuropein decomposition with a concomitant decrease in bitterness would appear reasonable,

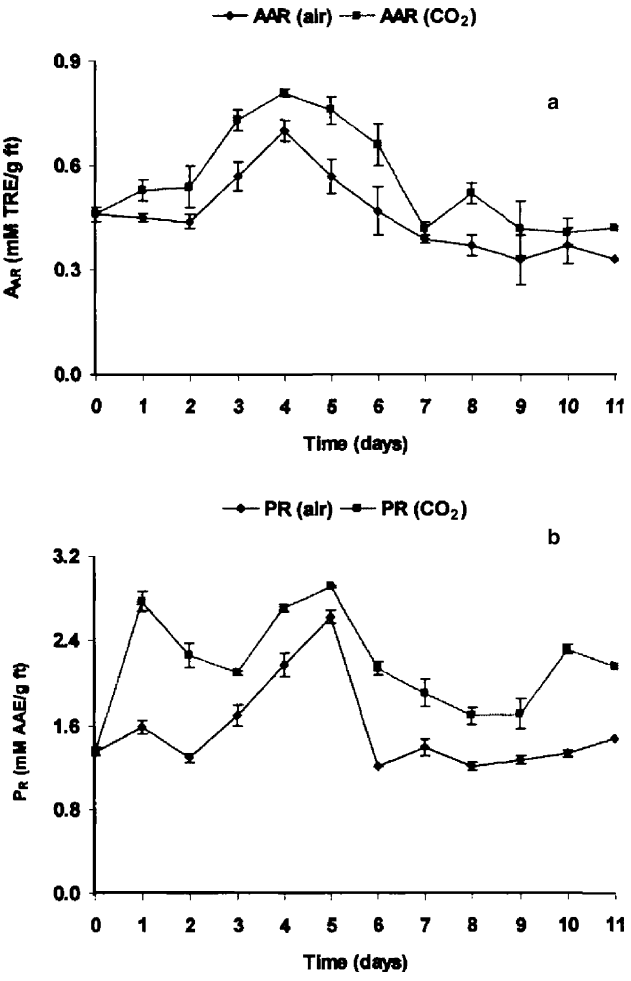

Fig. 6. Evolution of antiradical activity (a) and reducing power (b) of olives during post-harvest treatment under CO_2 and regular atmospheric conditions (air).

Table 2
Correlation coefficients calculated after regression analysis between antiradical activity (A_{AR}) and reducing power (P_R) with total polyphenols (TP) and total flavonoids (TFd)

	A_{AR}		P_R	
	Air	CO_2	Air	CO_2
TP	0.4977	0.7946[a]	0.1275	0.3827
TFd	0.2079	0.0002	0.0098	0.8401[a]

[a] Values statistically significant ($P < 0.05$).

considering that CO_2 actually promoted maturation of unripe olives. At this point it should be noted that a considerable reduction in bitterness did coincide with the appearance of increased anthocyanin levels (Table 1, Fig. 5).

The antiradical activity (A_{AR}) was significantly correlated with TP in the CO_2-stored sample during the first four days, where TP values exhibited an increasing tendency. Similarly, TFd showed significant correlation with P_R only in the CO_2-treated sample, where increased TFd levels were observed. It is well known that the antioxidant potency of a plant tissue is in many instances dependent on the totality of the polyphenols and does not usually correlate with specific constituents, a fact also demonstrated for red wines (Arnous, Makris, & Kefalas, 2001; Burns et al., 2000). Thus, the values calculated for both A_{AR} and P_R may be regarded as the integration of the antioxidant potency of all phenolics. Bacause, however, phenolics that possess different antioxidant characteristics (eg simple phenols and flavonoids) did not evolve concomitantly throughout treatments, no sound conclusions could be drawn about the influence of the polyphenolic content on the in vitro antioxidant activity of olive extracts. Nevertheless, it could be stressed that, in general, increased polyphenolic content was accompanied by increased levels of antioxidant potency, and this should be considered as a important evidence for the impact of post-harvest evolution of phenolics on the nutritional value of olives.

5. Conclusions

The most important results of this study may be summarised as follows:

- Post-harvest storage of olives under a CO_2-atmosphere for a period of 12 days resulted in colour and flavour development and reduced bitterness.
- Total polyphenol analysis indicated that biosynthesis was reduced in olives stored under air compared with samples stored under CO_2, and so was seen for total flavonoid content as well.
- Colour onset, that was attributed to anthocyanin biosynthesis, appeared from the third day of the treatment, and the anthocyanin accumulation pattern was different from that of TP and TFd.
- The antioxidant characteristics (antiradical activity and reducing power) were at lower levels in olives stored under air than under CO_2 atmosphere, which is an indication that the functional properties of olives may be enhanced upon CO_2-storage.
- This method appears to provide natural debittering without use of chemicals (e.g alkaline solutions, brine), and merits further investigation for the development of table olive processing that will enable fast olive debittering with minimal environmental impact.

Acknowledgements

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References

Agar, I. T., Hess-Pierce, B., Sourour, M. M., & Kader, A. A. (1999). Identification of optimum preprocessing storage conditions to maintain quality of black ripe 'Manzanillo' olives. *Postharvest Biology and Technology, 15*, 53–64.

Alasalvar, C., Al-Farsi, M., Quantick, P. C., Shahidi, F., & Wiktorowicz, R. (2005). Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. *Food Chemistry, 89*, 69–76.

Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *Journal of Agricultural and Food Chemistry, 49*, 5736–5742.

Arnous, A., Makris, D. P., & Kefalas, P. (2002). Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *Journal of Food Composition and Analysis, 15*, 655–665.

Artés-Hernández, F., Artés, F., & Tomás-Barberán, F. A. (2003). Quality and enhancement of bioactive phenolics in cv. Napoleon table grapes exposed to different postharvest gaseous treatments. *Journal of Agricultural and Food Chemistry, 51*, 5290–5295.

Awad, M. A., & de Jager, A. (2000). Flavonoid and chlorogenic acid concentrations in skin of "Jonagold" and "Elstar" apples during and after regular and ultra low oxygen storage. *Postharvest Biology and Technology, 20*, 15–24.

Bitteur, S., Sarris, J., Chambroy, Y., Bayonove, C., Flanzy, C., & Souty, M. (1990). Influence d'une anaérobiose carbonique sur l'arôme de deux variétés d'abricots. *Sciences des Aliments, 10*, 849–864.

Bitteur, S., Tesniere, C., Sarris, J., Baumes, R., Bayonove, C., & Flanzy, C. (1992). Carbonic anaerobiosis of Muscat grapes. I. Changes in the profiles of free and bound volatiles. *American Journal of Enology and Viticulture, 43*, 41–48.

Bliss, F. A. (1999). Nutritional improvement of horticultural crops through plant breeding. *HortScience, 34*, 1163–1167.

Burns, J., Gardner, P. T., O'Neil, J., Crawford, S., Morecroft, I., McPhail, D. B., et al. (2000). Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *Journal of Agricultural and Food Chemistry, 48*, 220–230.

Cisneros-Zevallos, L. (2003). The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables. *Journal of Food Science, 68*, 1560–1565.

Dourtoglou, V. G., Yannovits, N. G., Tychopoulos, V. G., & Vamvakias, M. M. (1994). Effect of storage under CO_2 atmosphere on the volatile, amino acid, and pigment constituents in red grape (*Vitis vinifera* L. var. Agiorgitiko). *Journal of Agricultural and Food Chemistry, 42*, 338–344.

Etiévant, P. X., Issanchou, S. N., Marie, S., Ducruet, V., & Flanzy, C. (1989). Sensory impact of volatile phenols on red wine aroma: influence of carbonic maceration and time of storage. *Sciences des Aliments, 9*, 19–33.

García, J. M., Gutiérrez, F., Barrera, M. J., & Albi, M. A. (1996a). Storage of mill olives on an industrial scale. *Journal of Agricultural and Food Chemistry, 44*, 590–593.

García, J. M., Gutiérrez, F., Castellano, J. M., Perdiguero, S., Morilla, A., & Albi, M. A. (1996b). Influence of storage temperature on fruit ripening and olive oil quality. *Journal of Agricultural and Food Chemistry, 44*, 264–267.

García, J. M., Yousfi, K., Mateos, R., Olmo, M., & Cert, A. (2001). Reduction of oil bitterness by heating of olive (*Olea europaea*) fruits. *Journal of Agricultural and Food Chemistry, 49*, 4231–4235.

Gil, M. I., Ferreres, F., & Tomás-Barberán, F. A. (1998). Effect of modified atmosphere packaging on the flavonoids and vitamin C content of minimally processed Swiss chard (*Beta vulgaris* subspecies *cycla*). *Journal of Agricultural and Food Chemistry, 46*, 2007–2012.

Gil, M. I., Ferreres, F., & Tomás-Barberán, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry, 47*, 2213–2217.

Gil, M. I., Holcroft, D. M., & Kader, A. A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry, 45*, 1662–1667.

Gil-Izquierdo, A., Conesa, M. A., Ferreres, F., & Gil, M. I. (2002). Influence of modified atmosphere packaging on quality, vitamin C and phenolic content of artichokes (*Cynara scolymus* L.). *European Food Research and Technology, 215*, 21–27.

Guichard, E., Chambroy, Y., Reich, M., Fournier, N., & Souty, M. (1992). Influence de la concentration cn dioxide de carbone sur la qualité aromatique des fraises aprés entreposage. *Sciences des Aliments, 12*, 83–100.

Gunes, G., Liu, R. H., & Watkins, C. B. (2002). Controlled-atmosphere effects on post-harvest quality and antioxidant activity of cranberry fruits. *Journal of Agricultural and Food Chemistry, 50*, 5932–5938.

Gutiérrez-Rosales, F., Ríos, J. J., & Gómez-Rey, M. L. (2003). Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 51*, 6021–6025.

Holcroft, D. M., & Kader, A. A. (1999). Carbon dioxide-induced changes in color and anthocyanin synthesis of stored strawberry fruit. *HortScience, 34*, 1244–1248.

Kim, D.-O., Chun, O. K., Kim, Y. J., Moon, H.-Y., & Lee, C. Y. (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry, 51*, 6509–6515.

Ramos, T., Fleuriet, A., Rascalou, M., & Macheix, J. J. (1993). The effect of anaerobic metabolism of grape berry skins on phenolic compounds. *American Journal of Enology and Viticulture, 44*, 13–16.

Rocha, A. M. C. N., & Morais, A. M. M. B. (2001). Influence of controlled atmosphere storage on polyphenoloxidase activity in relation to colour changes of minimally processed "Jonagored" apples. *International Journal of Food Science and Technology, 36*, 425–432.

Romero, C., García, P., Brenes, M., García, A., & Garrido, A. (2002). Phenolic compounds in natural black Spanish olive varieties. *European Food Research and Technology, 215*, 489–496.

Saija, A., & Uccella, N. (2001). Olive biophenols: functional effects on human wellbeing. *Trends in Food Science & Technology, 11*, 357–363.

Siomos, A. S., Dogras, C. C., & Sfakiotakis, E. M. (2001). Color development in harvested white asparagus spears in relation to carbon dioxide and oxygen concentration. *Postharvest Biology and Technology, 23*, 209–214.

Soler-Rivas, C., Espín, J. C., & Wichers, H. J. (2000). Oleuropein and related compounds. *Journal of the Science of Food and Agriculture, 80*, 1013–1023.

Verhoeyen, M. E., Bovy, A., Collins, G., Muir, S., Robinson, S., de Vos, C. H. R., et al. (2002). Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway. *Journal of Experimental Botany, 53*, 2099–2106.

Vinha, A. F., Ferreres, F., Silva, B. M., Valentão, P., Gonçalves, A., Pereira, J. A., et al. (2005). Phenolic profiles of Portuguese olives fruits (*Olea europaea* L.): influences of cultivar and geographical origin. *Food Chemistry, 89*, 561–568.