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Antioxidant phenolic compounds loss during the fermentation of Chétoui olives

Nada Ben Othman [a,1], Dominique Roblain [b,2], Nadia Chammen [a,1], Philippe Thonart [b,2], Moktar Hamdi [a,*]

[a] Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de la Technologie (INSAT) 2 Boulevard de la terre, BP676 1080 Tunis, Tunisia
[b] Centre Wallon de Biologie Industrielle, Unité de Bio-Industries, Faculté Universitaire des Sciences Agronomiques, Passage des Déportés 2, 5030 Gembloux, Belgium

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ABSTRACT

Evolution of phenolic compounds was studied during spontaneous and controlled fermentations of "Chétoui" cultivar olives at three degree of ripeness. Both spontaneous and controlled fermentations led to an important loss of total phenolic compounds with a reduction rate of 32–58%. Consequently, the antioxidant activity decreased by 50–72%. After fermentations, hydroxytyrosol and caffeic acid concentrations increased, whilst protocatechuic acid, ferulic acid and oleuropein concentrations decreased. The hydroxytyrosol concentration in black olives increased from 165 mg/100 g dry weight to 312 and 380 mg/100 g dry weight, respectively, after spontaneous and controlled fermentation. The oleuropein concentration in green olives decreased from 266 mg/100 g dry weight to 30.7 and 16.1 mg/100 g dry weight, respectively, after spontaneous and controlled fermentation. During olive fermentation, phenolic loss is essentially due to the diffusion of these compounds into the brine; the main phenolic compound identified and quantified in the different brines was hydroxytyrosol. To preserve antioxidant quality of table olives it is necessary to use a controlled process to minimise phenolic compound loss.

1. Introduction

The olive tree (*Olea europaea* L.) is one of the most important fruit trees in Mediterranean countries. Its products, olive oil and table olives, are important components of the Mediterranean diet and are consumed across the world (Pereira et al., 2006). The Chétoui cultivar used in this study is the second most cultivated variety in Tunisia. It is widespread in the north of the country, occurring on plains as well in mountain regions, and shows a high capacity of adaptation to various pedoclimatic conditions. It covers an area of 176,000 ha and accounts for more than 20% of the olive oil produced in Tunisia. The fruit is medium to large with a fat yield of about 20–30% of fresh weight and the oil is characterised by a good content of total phenols, *o*-diphenols, tocopherols and a good resistance to oxidation (Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006).

There are three main preparation methods of table olives: Spanish (or Sevillian)-style green olives in brine, Californian-style black olives in brine, and Greek-style naturally black olives in brine. However, there are many other traditional table olive preparation recipes.

Olive fermentation, like other natural vegetable fermentations, is a spontaneous, traditional lactic acid fermentation based on an empirical process, which relies upon microorganisms present in the raw material and the processing environment (Fernández Díez, 1983; Garrido Fernández, García García, & Brenes Balbuena, 1995). In the traditional process, olives are handled, in order to favour the growth of *Lactobacillus plantarum* in the fermentation brines, which is thought to be essential to provide the amount of lactic acid needed for preservation, as well as for their characteristic flavour (Leal-Sánchez et al., 2003). *Lactobacillus* strains isolated from different brine samples have been identified. *L. plantarum* represents the predominant species, at a percentage of 39.9% of total *lactobacillus* strains; *Lactobacillus pentosus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei* and *Lactobacillus* ssp. represent 27.8%, 16.7%, 11.1% and 1%, respectively (Chammem et al., 2005). Generally, the *L. plantarum* population coexists with a yeast population until the end of the fermentation process and during storage (Ruiz-Barba, Cathcart, Warner, & Jímenez-Díaz, 1994). In some cases, lactic acid is not produced in the amounts needed for the adequate preservation of olives, and spoilage occurs through subsequent contamination by other microorganisms (Fernández Díez, 1983; Garrido Fernández et al., 1995). In this regard, the use of suitable *L. plantarum* starter cultures has the potential to improve the microbiological control of the process, to increase the lactic acid yield and, accordingly, to provide the production of Spanish-style fermented green olives of consistently high quality (Fernández Díez, 1983; Garrido Fernández et al., 1995; Roig & Hernández, 1991; Ruiz-Barba & Jiménez-Díaz, 1995).

Phenolic compounds in table olives have a great relevance because of their contribution to the colour, taste and texture of the

* Corresponding author. Tel.: +216 71703627; fax: +216 71704329.
E-mail address: moktar.hamdi@insat.rnu.tn (M. Hamdi).
[1] Tel.: +216 71703627; fax: +216 71704329.
[2] Tel.: +32 81622305; fax: +32 81614222.

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product (Marsilio, Campestre, & Lanza, 2001), as well as their antioxidant capacity. In fact, phenolic compounds have redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans, Miller, & Paganga, 1997). Olives and derivative products are recognised as a valuable source of so-called "functional food" because of their natural phenolic antioxidant content (Marsilio et al., 2001). Table olives from the Tunisian market represent an important source of antioxidants. The consumption of 50 g of table olives provides about 152 mg of phenolic compounds (Ben Othman, Roblain, Thonart, & Hamdi, 2008).

Changes in phenolic profile and content are related to the maturation phase. Oleuropein is the major compound in the flesh of many cultivars; its concentration decreases during maturation whilst hydroxytyrosol concentration increases (Amiot, Fleuriet, & Macheix, 1989). The black maturation phase is characterised by the appearance of anthocyanins (Limiroli et al., 1995). During table olive processing there are important changes in phenolic quantity and quality, which are generally due to several mechanisms caused by the process used. During fermentation of naturally black olives the main reactions that take place are acid hydrolysis of glucosides; therefore, hydroxytyrosol is the most important phenolic compound detected in the final product. Polymerisation of anthocyanin compounds is the cause of the final colour developed in olives (Romero, Brenes, Garcia, Garcia, & Garrido, 2004b). During Spanish-style green olive processing, the hydrolysis of glucosides occurs due to the NaOH treatment, leading to abundant hydroxytyrosol liberation. The formation of caffeic acid has also been observed, caused by verbascoside hydrolysis (Brenes, Rejano, García, Sánchez, & Garrido, 1995).

Changes in simple phenolic compounds during olive processing are widely studied, but there are no data showing antioxidant capacity loss with processing caused by the decrease in total phenolic compounds. The purpose of the current study is to investigate phenolic compounds changes during the fermentation of Tunisian olives of the "Chétoui" cultivar at different degrees of ripeness. Phenolic compounds were monitored in both olive flesh and brine. In particular, the evolution of oleuropein and hydroxytyrosol, the most studied compounds in the literature, was examined. We were also interested by other simple phenolic compounds that have been less focused on.

2. Materials and methods

2.1. Olive samples

Black, varicoloured and green olives of the "Chétoui" cultivar were supplied from the north of Tunisia. After discarding damaged olives and washing the remainder with water, fruits were placed in glass vessels containing freshly prepared brine (8% NaCl). For each olive type one glass vessel underwent spontaneous fermentation and another was inoculated with a selected strain of *L. plantarum*. Experiments were carried out at ambient temperature (22–25 °C).

2.2. Reagents

Methanol, ethanol and acetic acid of analytical grade, and methanol and acetonitrile of HPLC grade were obtained from Riedel de Haën (Sigma–Aldrich, St. Louis, MO). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, Folin–Ciocalteau reagent, sodium carbonate, tyrosol, 4-hydroxyphenylacetic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid and ferulic acid were obtained from Fluka (Buchs, Switzerland). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, vanillin, benzoic acid were obtained from Sigma (St. Louis, MO). Hydroxytyrosol, oleuropein, *m*-coumaric acid, *o*-coumaric acid were obtained from Extrasynthèse, (Genay, France).

2.3. Extraction of phenolic compounds

The procedure for the extraction of polyphenols was the same for kernel and flesh. A quantity (0.5 g) of sample was extracted five times with 5 ml methanol. The extracts were combined, methanol was evaporated under a vacuum, keeping the bath temperature under 45 °C, and the residue was dissolved in 5 ml methanol (Boskou et al., 2006). The extract obtained was used for total phenol, antioxidant activity determination and HPLC analysis.

Phenolics from liquid brine samples (10 ml) were extracted three times with ethyl acetate (v/v) at ambient temperature. The three organic fractions were combined and dried with anhydrous Na_2SO_4 for 30 min. The extract was concentrated to dryness in a rotary evaporator and redissolved in 1 ml methanol (HPLC grade).

2.4. Analysis of total phenolic compounds in extracts

The concentration of total phenolic compounds in olive flesh extracts was determined with the Folin–Ciocalteu assay. To 0.5 ml of diluted extract, 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) were added and, after that (within a time interval from 0.5 to 8 min), 2 ml of Na_2CO_3 (75 g/l) were added. The sample was incubated for 5 min at 50 °C and then cooled. For the control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm (Skerget et al., 2005). The results were expressed in mg of gallic acid per 100 g of dry matter (mg GA/100 g dw) or in mg of gallic acid per 100 g fresh weight (mg GA/100 g fw).

The concentration of total phenolic compounds in brine was determined according to the method used by Caboni et al. (1997). Appropriately diluted samples (3.6 ml) were mixed with 0.2 ml of Folin–Ciocalteu reagent (Merck) and, 3 min later, 0.8 ml of sodium carbonate (20% w/v) were added. The mixture was heated at 100 °C for 1 min. After cooling, the absorbance at 750 nm was measured. Results were expressed as mg gallic acid per litre (mg GA/l).

The level of total phenolic compounds determined according to the Folin–Ciocalteu method are not absolute measurements of the amounts of phenolic compounds but are in fact based on their chemical reducing capacity, relative to an equivalent reducing capacity of gallic acid (Katalinić, Milos, Modun, Musić, & Boban, 2004).

2.5. HPLC/DAD analysis of simple phenolic compounds

The HPLC was performed using a Hewlett–Packard series 1100 liquid chromatographic system, equipped with a diode array UV detector and a ChromSpher C_{18} column (4.6 × 250 mm, particle size 5 μm; Varian, Palo Alto, CA).

The mobile phase was methanol/acetonitrile (50:50 v/v) (solvent **A**) and a mixture of water/acetic acid (97:3, v/v) (solvent **B**) at a flow rate of 1 ml/min. The solvent gradient changed according to the following conditions: from 5% **A** to 30% **A** for 25 min; from 30% **A** to 38% **A** for 10 min; from 38% **A** to 45% **A** for 10 min; from 45% **A** to 52.5% **A** for 5 min; from 52.5% **A** to 100% **A** for 5 min; finally, isocratic at 100% **A** for 5 min. Eluates were detected at 280 nm (Mateos et al., 2001). Phenolic compounds quantification was achieved by measuring the absorbance at 280 nm recorded in the chromatograms relative to external standards.

2.6. The antioxidant activity

The antioxidant activity was assessed as described by Re et al. (1999). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the study of olive flesh extracts, the $ABTS^{\cdot+}$ solution was diluted with ethanol, to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. After addition of 1.0 ml of diluted $ABTS^{\cdot+}$ solution ($A_{734\ nm} = 0.700 \pm 0.020$) to 10 μl of phenolic extract or Trolox standards in ethanol, readings were taken until A_{734} stabilised. Ethanol was used as blank in each assay. All determinations were carried out in triplicate. The percentage of inhibition of absorbance (*OD*) at 734 nm was calculated as follows:

$$\text{inhibition}\% = \frac{OD_{\text{initial}} - OD_{\text{final}}}{OD_{\text{initial}}} \times 100$$

Inhibition% was plotted as a function of concentration of extracts and of Trolox for the standard reference data.

Antioxidant capacity was calculated in terms of the Trolox equivalent antioxidant activity. To calculate the TEAC, the gradient of the plot of the percentage of inhibition of absorbance *vs.* concentration for the antioxidant in question is divided by the gradient of the plot for Trolox. The equation of the response curve for Trolox standard solutions is:

$$y = 38.949x$$

where y is the inhibition percentage and x is the Trolox concentration (mM).

3. Results and discussion

3.1. Effect of maturation of olive fruit on the phenolic compounds composition

Total phenolic contents of fresh olive flesh extracts were determined in green, varicoloured and black olives (Table 1). Green olives have the highest phenolic content of 2558 mg GA/100 g dw, followed by varicoloured and black olives which have phenolic contents of 2233 and 1760 mg GA/100 g dw, respectively. This result confirmed that total phenolic content is dependent on ripening, as it decreased during the olive maturation stage (Amiot, Fleuriet, & Macheix, 1986; Morello, Romero, & Motilva, 2004; Ryan, Robards, & Lavee, 1999).

The simple phenolic compounds analysis showed that the olive flesh of the "Chétoui" cultivar revealed different phenolic profiles at different ripeness degrees, in which several simple phenolic compounds were identified and quantified (Table 1, Fig. 1). The main phenolic compounds detected in fresh olive flesh were protocatechuic acid, hydroxytyrosol, tyrosol, *p*-hydroxyphenylacetic acid, *p*-coumaric acid, ferulic acid, benzoic acid and oleuropein. Gallic, *p*-hydroxybenzoic and syringic acids were detected only in fresh green olives. However, vanillin, *m*-coumaric acid and *o*-coumaric acid were detected only in fresh varicoloured and black olives. Total simple phenolic compounds in green, varicoloured and black olives were 652, 384 and 311 mg/100 g dw, respectively. Phenolic values obtained by the HPLC method are lower than those estimated by the Folin–Ciocalteu method. One reason is that some phenolic compounds may escape determination by chromatogra-

Table 1
Total phenolic content and simple phenolic composition in fresh olives.

	Green olives	Varicoloured olives	Black olives
Total phenolic compounds (mg GA/100 g dw)	2558 ± 139	2233 ± 158	176 ± 40
Simple phenolic compounds (mg GA/100 g dw)			
Gallic acid	6.1 ± 0.7	ND	ND
Protocatechuic acid	7.2 ± 0.3	28.1 ± 1.7	19.4 ± 0.5
Hydroxytyrosol	257 ± 12	165 ± 8.2	135 ± 3.2
Tyrosol	7.2 ± 0.4	5 ± 0.03	2.9 ± 0.02
p-Hydroxybenzoic acid	2.7 ± 0.05	ND	ND
p-Hydroxyphenylacetic acid	35.9 ± 1.3	41.3 ± 1.9	31.7 ± 1.7
Vanillic acid	3.4 ± 0.1	1.1 ± 0.01	ND
Caffeic acid	ND	0.5 ± 0	ND
Syringic acid	1.7 ± 0.02	ND	ND
Vanillin	ND	2.7 ± 0.02	1.6 ± 0.01
p-Coumaric acid	4 ± 0.2	1.9 ± 0.01	2 ± 0.03
Ferulic acid	5.4 ± 0.4	4.7 ± 0.3	3.8 ± 0.05
m-Coumaric acid	ND	0.7 ± 0.01	2 ± 0.02
Benzoic acid	55.3 ± 3.5	19.4 ± 0.2	54.8 ± 1.5
o-Coumaric acid	ND	1.7 ± 0.02	0.5 ± 0.01
Oleuropein	266 ± 11.2	112 ± 4.2	56.9 ± 2.8
Total	652 ± 30.17	384 ± 16.6	311 ± 9.84

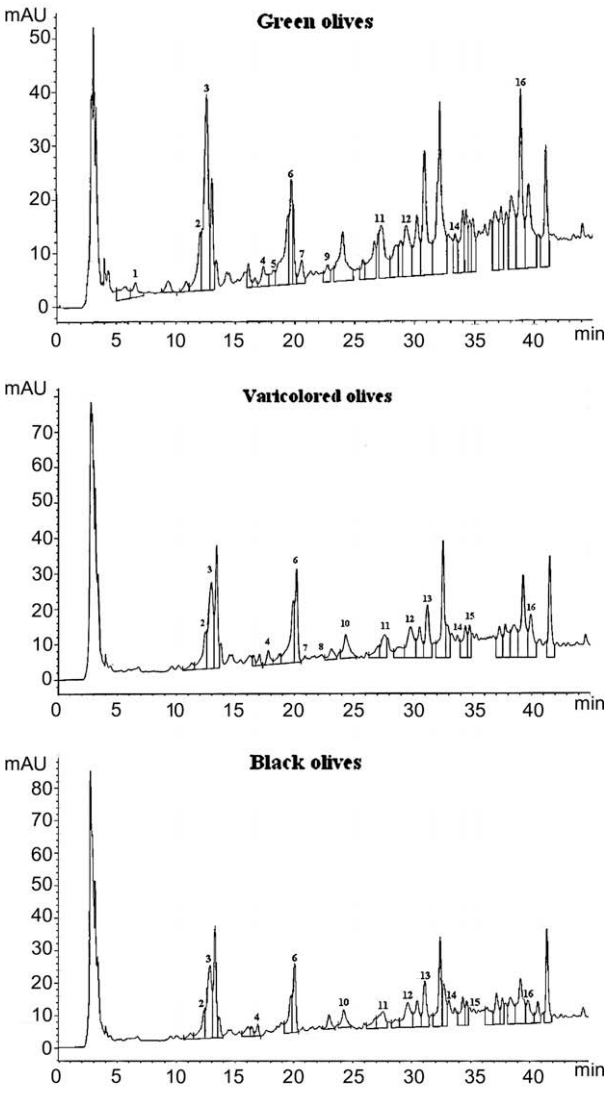

Fig. 1. HPLC chromatograms of flesh phenolic extracts of green, varicoloured and black olives. 1: gallic acid, 2: protocatechuic acid; 3: hydroxytyrosol; 4: tyrosol; 5: *p*-hydroxy-benzoic acid; 6: *p*-hydroxy-phenyl-acetic acid 7: vanillic acid; 8: caffeic acid; 9: syringic acid; 10: vanillin; 11: *p*-coumaric acid, 12: ferulic acid, 13: *m*-coumaric acid, 14: benzoic acid, 15: *o*-coumaric acid, 16: oleuropein.

phy. These can be unknown compounds, compounds present as traces that were not considered in the characterisation of food sources or compounds that are not resolved by chromatography, such as proanthocyanidin polymers and oxidised polyphenols (Santos-Buelga & Scalbert 2000). A second reason is the interference of other reducing substances in phenolic extracts, leading to overestimation of total phenolic contents in the Folin–Ciocalteau colorimetric analysis (Schieber, Keller, & Carle, 2001).

Green olives contain the highest simple phenolics content, of which oleuropein represents about 40.8%. The concentration of some compounds like hydroxytyrosol, tyrosol, *p*-coumaric acid and oleuropein decreased during maturation. Hydroxytyrosol concentrations in green, varicoloured and black olives were 257, 165 and 135 mg/100 g dw, respectively. Oleuropein concentrations in green, varicoloured and black olives were 266, 112 and 56.9 mg/100 g dw, respectively. These observations were proved by Morello et al. (2004) for the Arbequina, Faga and Morrut Spanish cultivars. Furthermore, Amiot et al. (1989), and Esti, Cinquanta, and La Notte Esti (1998) suggested that oleuropein concentration decreased with physiological development of the fruit and continues to decline rapidly during the black maturation phase, which is characterised by the appearance of anthocyanins (Limiroli et al., 1995).

3.2. Total phenolic content evolution in olive flesh during olive fermentation

During olive processing there is an osmotic exchange between fruit and brine (Gandul-Rojas & Mínguez-Mosquera, 2006); major changes in composition are in the soluble sugars, NaCl and phenolic compounds. In the present work we are interested in the phenolic change during olive processing. Green, varicoloured and black olives of the Chétoui cultivar were placed in 8% (w/v) NaCl brine for 67 days. For each olive type there was a sample which underwent spontaneous fermentation and another one was inoculated with a selected strain of *L. plantarum*. Total phenolic content evolutions in olive flesh and brine are reported in Fig. 2. This figure shows that both olives fermented with endogenous microflora and with *L. plantarum* have the same total phenolic evolution profile. The major loss was obtained after 9 days of fermentation. After that total phenolic compounds continue to decrease in the olive fruit, and no more loss of phenolic compounds from the fruit to the brine was observed after 23 days.

Total phenolic compounds in spontaneous fermented green olives dropped from 2558 to 1072 mg GA/100 g dw after 67 days, which is equivalent to a reduction yield of 58.1%. Total phenolics in spontaneous and controlled fermented varicoloured olives were

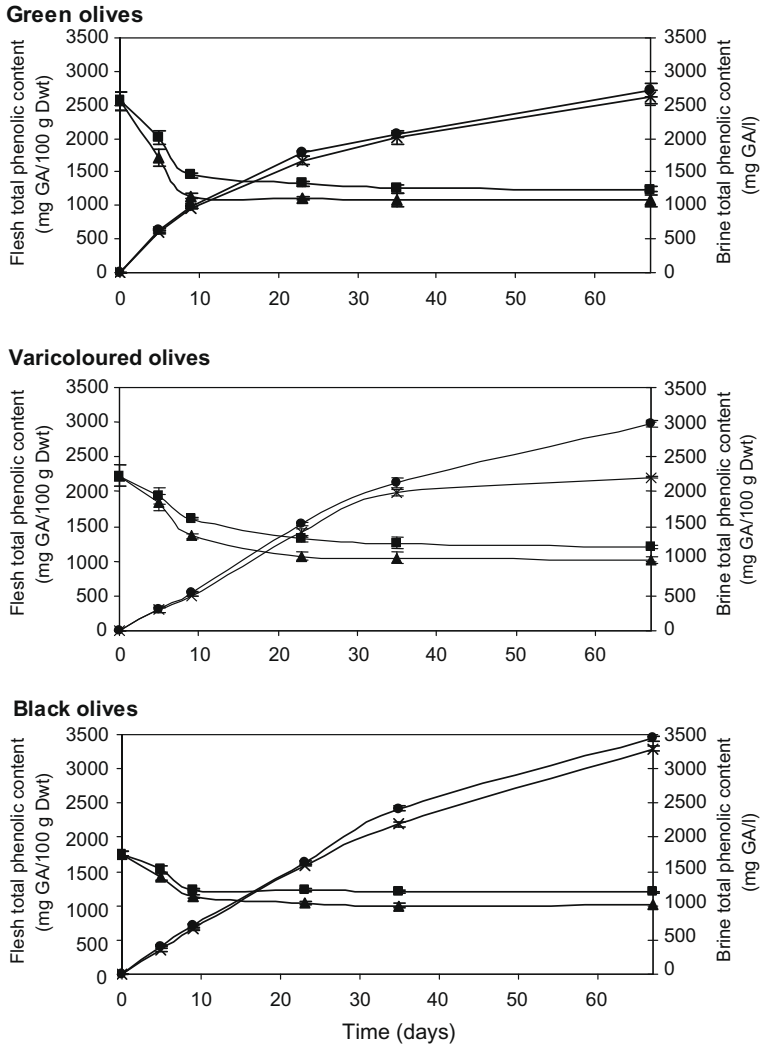

Fig. 2. Evolution of olive flesh total phenolic content during spontaneous (▲) and controlled (■) fermentation and brine total phenolic content of spontaneous (●) and controlled (X) fermentation.

reduced by 55% and 46%, respectively; however in spontaneous and controlled fermented black olives they were reduced by only 43% and 32%, respectively. Total phenolic reductions for the green, varicoloured and black olives are lower in the controlled fermentation, due to the development of a biofilm that can be a barrier for phenolic diffusion. Nychas, Panagou, Parker, Waldron, and Tassou (2002) observed by scanning electron microscopy the presence of yeasts and bacteria on and within the fermented black olives. Yeasts tended to predominate on the skin surface and in the stomal openings, whereas bacteria predominated in the intercellular spaces of the sub-stomal cells.

3.3. pH and total phenolic content evolution in brine during olive fermentation

Profiles of pH in brine were very similar for all fermentations (Fig. 3). pH values decreased rapidly during the first 5 days, especially in controlled fermentations. Then the decrease slowed down but continued until the 10th day. After this moment, pH stabilised. The decrease of pH values in spontaneous fermentations was slower than that of controlled fermentations. Thus, inoculation with *L. plantarum* starter culture leads to a faster pH decrease from 6.18 to 4.6, 4.56 and 4.8 in green, varicoloured and black olives processing, respectively, which help to reduce the risk of spoilage during the first days of fermentation (Leal-Sánchez et al., 2003).

Fig. 2 shows that total phenolic content in brine of spontaneous fermentations increased progressively to rich final concentrations of 2718, 2974 and 3450 mg/l, in green, varicoloured and black olive brines, respectively. During olive fermentation there is an important loss of phenolic compounds, due to the diffusion of these compounds to the brine. Despite, the higher olive flesh phenolic content and phenolic loss that were observed in green olives, the higher brine phenolic content was observed in black olive brines. These results can be explained by the fact that varicoloured and black olives contain high anthocyanin levels and these compounds appear progressively during ripening. Romero et al. (2004a) showed that total anthocyanins increase in brine and in the presence of air they are oxidised and polymerised. In addition, green olives contain higher simple phenolic compounds, which are converted by lactic acid bacteria and other microorganisms. Total phenolic content was lower in controlled fermented olive brines. In fact, the presence of lactic acid bacteria in the controlled fermentation contributes to simple phenolic conversion and the depolymerisation of high molecular weight phenolic compounds. Several authors studied the degradation of phenolic compounds by lactic acid bacteria. Ciafardini, Marsilio, Lanza, and Pozzi (1994) studied oleuropein hydrolysis by β-glucosidase produced by strains of *L. plantarum*. Recently, Rodríguez, Landete, Rivas, and Muñoz (2008) demonstrated that *p*-coumaric, caffeic, ferulic and *m*-coumaric acids are metabolised by *L. planatarum*. Besides simple phenolic compounds conversion, Ayed and Hamdi (2003) showed that *L. plantarum* growth in fresh olive mill wastewaters led to an important phenolic hydrolysis and depolymerisation.

3.4. Simple phenolic compounds evolution in the olive flesh and brine

After fermentation, changes in the profile and the quantity of simple phenolic compounds were observed (Table 2). These changes are mainly due to the diffusion of substances from within the olive fruit to the surrounding medium and vice versa (Bianchi, 2003). Moreover, the presence of endogenous microflora and suitable starters led to the conversion, consumption and production of several substances.

Total simple phenolic content in flesh increased after the fermentation of varicoloured and black olives, especially in the controlled fermentation. In contrast, phenolic content decreased

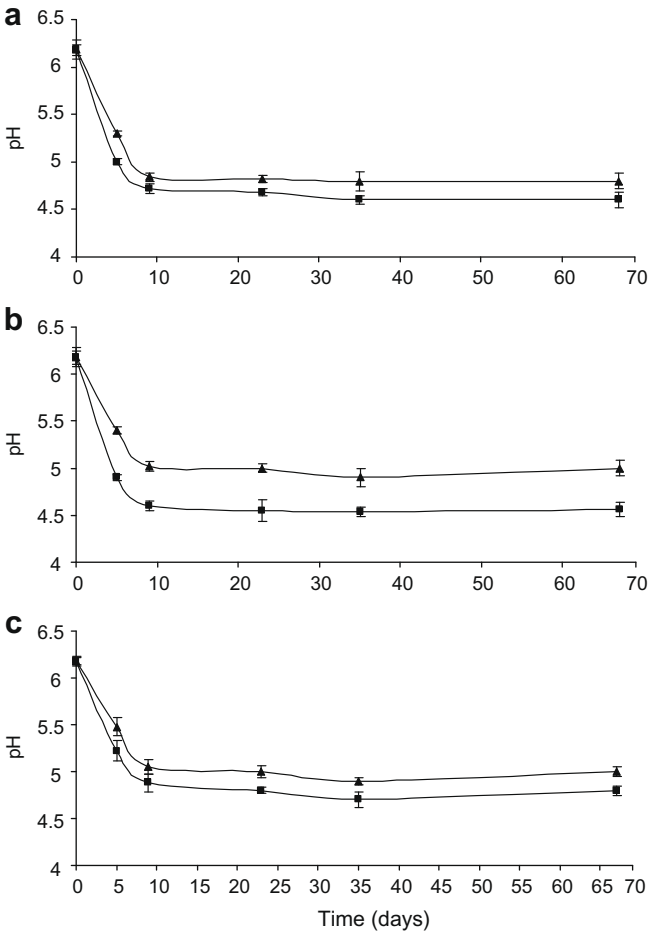

Fig. 3. Evolution of pH during spontaneous (▲) and controlled (■) fermentation of black (a), varicoloured (b) and green (c) olives.

for green olives from 652 to 460 and to 380 in spontaneous and controlled fermentations, respectively. This result could be explained by the liberation of simple phenolic compounds after acid and enzymatic hydrolysis of polymerised phenolic compounds, which are present at high levels in varicoloured and black olives. However, in the case of green olives, the phenolic compounds are present in simple structures and so they were more easily metabolised by microorganisms. (Rodríguez et al., 2008).

The main changes for all types of olives were the decrease in concentrations of protocatechuic acid, ferulic acid and oleuropein. This latter compound is a bitter glucoside, and among the aims of olive processing is the elimination of this compound. After fermentation of all olive types, concentration of this compound decreased. The decrease was more important in the controlled fermentation. In fact, several authors have focused on oleuropein hydrolysis by β-glucosidase produced by *L. plantarum* (Ciafardini et al., 1994; Landete, Curiel, Rodríguez, Rivas, & Muñoz, 2008.). In the other hand, concentrations of hydroxytyrosol and caffeic acid increased after fermentations. A great interest is shown to hydroxytyrosol, essentially because of its important antioxidant activity. This compound is present in abundance in fresh olives and its concentration increased after fermentation, due to acid and enzymatic hydrolysis of oleuropein.

Caffeic acid was not present in fresh olives and appeared after fermentation in all types of olives. Brenes-Balbuena, García-García, and Garrido-Fernandez (1992) reported that this compound is formed from verbascoside degradation. The evolution of other phenolic compounds depends on the olive maturation stage. Gallic,

Table 2
Simple phenolic compounds in olive flesh before and after controlled (CF) and spontaneous (SF) fermentations, expressed in mg/100 g dry weight.

	Green olives (GO)			Varicoloured olives (VO)			Black olives (BO)		
Phenolic compound	Fresh GO	SF GO	CF GO	Fresh VO	SF VO	CF VO	Fresh BO	SF BO	CF BO
Gallic acid	6.1 ± 0.7	ND	ND	ND	4.6 ± 0.1	8.8 ± 0.3	ND	4.4 ± 0.05	10.8 ± 0.09
Protocatechuic acid	7.2 ± 0.3	ND	ND	28.1 ± 1.7	9.4 ± 0.4	10 ± 0.23	19.4 ± 0.5	ND	9.7 ± 0.05
Hydroxytyrosol	257 ± 12	375 ± 8.3	341 ± 7.8	165 ± 8.2	312 ± 6.5	380 ± 10.7	135 ± 3.2	230 ± 9.3	308 ± 10.2
Tyrosol	7.2 ± 0.4	4.9 ± 0.2	4.3 ± 0.3	5 ± 0.03	4.4 ± 0.05	10.6 ± 0.54	2.9 ± 0.02	4.2 ± 0.02	5.6 ± 0.05
p-Hydroxybenzoic acid	2.7 ± 0.05	1.4 ± 0.01	ND	ND	1.6 ± 0.01	4.2 ± 0.02	ND	0.8 ± 0	ND
p-Hydroxyphenyl-acetic acid	35.9 ± 1.3	16.2 ± 0.6	11.2 ± 0.9	41.3 ± 1.9	43.4 ± 1.3	61.8 ± 2.3	31.7 ± 1.7	41.3 ± 3.5	45 ± 2.9
Vanillic acid	3.4 ± 0.1	2.5 ± 0.02	2.2 ± 0.03	1.1 ± 0.01	1.2 ± 0.01	6.4 ± 0.03	ND	3.3 ± 0.02	2 ± 0.02
Caffeic acid	ND	0.6 ± 0	0.7 ± 0.01	0.5 ± 0	1 ± 0.01	2.3 ± 0.01	ND	1.1 ± 0	1 ± 0
Syringic acid	1.7 ± 0.02	ND	ND	ND	ND	3.9 ± 0.02	ND	2.2 ± 0.01	2.7 ± .02
Vanillin	ND	1.7 ± 0.01	ND	2.7 ± 0.02	0.4 ± 0	1.4 ± 0	1.6 ± 0.01	1.1 ± 0.01	1 ± 0.01
p-Coumaric acid	4 ± 0.2	1.6 ± 0.01	1.2 ± 0.05	1.9 ± 0.01	1.9 ± 0.06	4.3 ± 0.04	2 ± 0.03	2.6 ± 0.05	2.7 ± 0.03
Ferulic acid	5.4 ± 0.4	1.9 ± 0.02	1.4 ± 0.03	4.7 ± 0.3	1.4 ± 0.05	2.9 ± 0.03	3.8 ± 0.05	1.2 ± 0	1.2 ± 0.01
m-Coumaric acid	ND	ND	0.5 ± 0	0.7 ± 0.01	2.4 ± 0.1	5.4 ± 0.04	2 ± 0.02	3.5 ± 0.06	3.6 ± 0.04
Benzoic acid	55.3 ± 3.5	21.8 ± 1.3	ND	19.4 ± 0.2	46.4 ± 1.6	96.7 ± 3.1	54.8 ± 1.5	58.3 ± 2.7	67.6 ± 2.9
o-Coumaric acid	ND	1.7 ± 0.005	2.1 ± 0.07	1.7 ± 0.02	0.8 ± 0.01	2.3 ± 0.04	0.5 ± 0.01	1 ± 0.04	1.2 ± 0.01
Oleuropein	266 ± 11.2	30.7 ± 1.2	16.1 ± 0.6	111.8 ± 4.2	30 ± 1.2	20.3 ± 1.5	56.9 ± 2.8	48 ± 2	48.5 ± 1.9
Total	652 ± 30.2	460 ± 11.7	380 ± 9.79	384 ± 16.6	461 ± 11.3	621 ± 18.9	311 ± 9.84	403 ± 17.8	510 ± 18.2

Table 3
Simple phenolic compounds in final brine of spontaneous fermented (SF) and controlled fermented (CF) olives, expressed in mg/l.

	Green olive (GO) brine		Varicoloured olive (VO) brine		Black olive (BO) brine	
Phenolic compound	SFGO	CFGO	SFVO	CFVO	SFBO	CFBO
Gallic acid	2.8 ± 0.15	3.7 ± 0.11	ND	ND	2 ± 0.07	ND
Protocatechuic acid	9.6 ± 0.43	6.7 ± 0.24	27.3 ± 1.6	31.9 ± 1.7	71.2 ± 4.8	41.4 ± 2.3
Hydroxytyrosol	980 ± 27	1212 ± 34	873 ± 19	912 ± 37	923 ± 51	1407 ± 45
Tyrosol	30.8 ± 0.6	45.2 ± 0.5	32.8 ± 0.9	33.6 ± 2.1	5.8 ± 0.09	163 ± 7.2
p-Hydroxybenzoic acid	3.3 ± 0.02	5.1 ± 0.17	4.1 ± 0.05	4.4 ± 0.1	15.7 ± 0.7	7.6 ± 0.5
p-Hydroxyphenyl-acetic acid	11.4 ± 0.75	18.9 ± 0.8	ND	16.9 ± 0.8	10.9 ± 0.4	15.8 ± 0.7
Vanillic acid	23 ± 1.2	35.7 ± 1.3	23.3 ± 1.3	6.7 ± 0.2	4.1 ± 0.1	43.6 ± 1.3
Caffeic acid	6.4 ± 0.23	10.6 ± 0.5	10.2 ± 0.6	28.3 ± 2	3.2 ± 0.06	34.9 ± 0.8
Syringic acid	1.9 ± 0.05	2.4 ± 0.03	1.9 ± 0.01	ND	ND	ND
Vanillin	3.9 ± 0.13	3.7 ± 0.02	1.9 ± 0.02	1.8 ± 0.07	1 ± 0.02	3.8 ± 1.2
p-Coumaric acid	9.5 ± 0.4	13.9 ± 0.4	15.7 ± 0.8	5.7 ± 0.15	ND	358 ± 17
Ferulic acid	11 ± 0.9	2.1 ± 0.01	9.9 ± 0.2	ND	18.7 ± 0.4	3.5 ± 0.08
m-Coumaric acid	3.4 ± 0.07	4.2 ± 0.07	1.7 ± 0.07	2.3 ± 0.07	4.4 ± 0.02	3.7 ± 0.05
Benzoic acid	47.5 ± 2.8	79 ± 3.5	ND	ND	ND	ND
o-Coumaric acid	6.8 ± 0.18	10.4 ± 0.9	2.6 ± 0.1	ND	ND	ND
Oleuropein	52.8 ± 1.9	103 ± 4.1	60.8 ± 2.4	71.6 ± 3.4	ND	ND
Total	1204 ± 36.8	1556 ± 46.7	1065 ± 27.1	1115 ± 47.6	1060 ± 57.7	2082 ± 76.1

p-hydroxyphenylacetic, vanillic and benzoic acids concentrations decreased after the fermentation of green olives. However, their concentrations increased for varicoloured and black olives.

Simple phenolic compounds in brine were identified and quantified. Results are presented in Table 3. Most simple phenolic compounds were identified in brine. However, there is a difference in the composition between green, varicoloured and black olive brines. In the controlled fermentation a higher simple phenolic content was observed, probably due to the hydrolysis of complex phenolic compounds. In fact, *L. plantarum* depolymerises high molecular weight phenolic compounds to lower molecular weight ones (Ayed & Hamdi, 2003). Hydroxytyrosol was the main simple phenolic compound identified in all brines, its percentage varied between 67.6% and 82% of total simple phenolic compounds. Parinos, Stalikas, Giannopoulos and Pilidis (2007) showed that debittering wastewater, washing wastewater and brine of green olives contain high hydroxytyrosol concentrations, since this compound constitute the main hydrolysis product of oleuropein. Brine of black olives of the Hojiblanca cultivar contains high hydroxytyrosol concentrations. Romero et al. (2004b) suggest that it results from acid hydrolysis of hydroxytyrosol-4-β-glucoside. This compound has been shown to be the main phenolic compound in mature olives (Romero, Brenes, García, & Garrido, 2002).

3.5. Antioxidant activity evolution in the olive flesh

Since olive product antioxidant activity is related to its phenolic content, we focused on the effect of phenolic loss on olive antioxidant activity during fermentation. Curves representing antioxidant activities have the same profiles as those of total phenolic content (Fig. 4), which confirm that antioxidant activity decrease depends on the rate of phenolic loss.

Fresh green, varicoloured and black olives have TEAC values of 133, 102 and 72 μM TEAC per g dw, respectively. After spontaneous fermentation TEAC values decreased to 37, 31 and 29 μM TEAC per g dw in green, varicoloured and black olives, respectively. TEAC values were higher in the controlled fermentation, which is in agreement with the results of the total phenolic analysis.

Saura-Calixto and Goni (2006) showed that phenolic compounds are quantitatively the main dietary antioxidants. However, the biological effects of these substances will depend on their bioavailability (Manach, Scalbert, Morand, Remesy & Jimenez, 2004). Table olives are a basic component of the Mediterranean diet and considered as very good source of phenolic compounds, so their consumption provides a large amount of natural antioxidants (Boskou et al., 2006). Recently, Kountouri, Mylona, Kaliora and Andrikopoulos (2007) focused on the bioavailability of olive

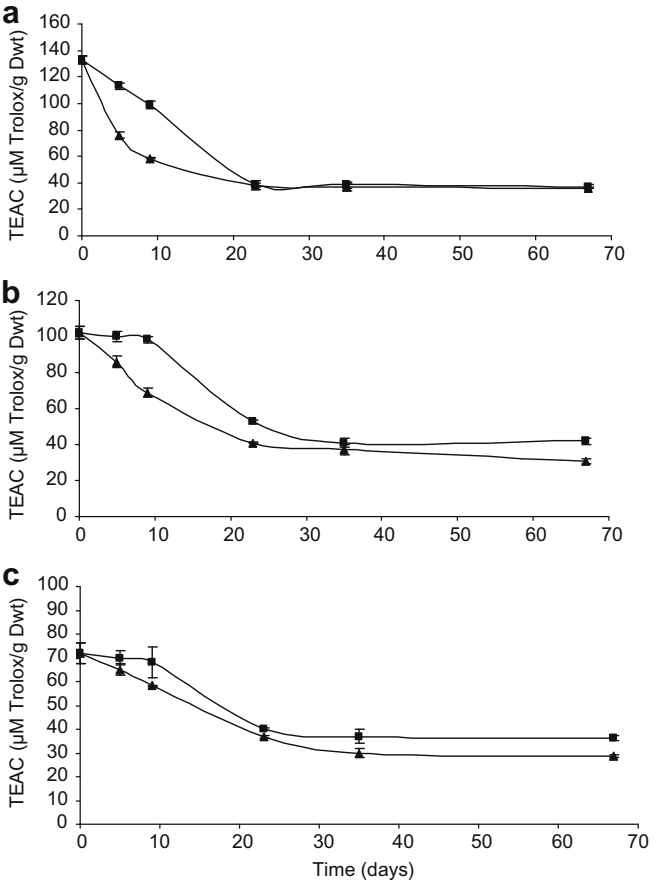

Fig. 4. Evolution of antioxidant activity during spontaneous (▲) and controlled (■) fermentation of green (a), varicoloured (b) and black (c) olives.

phenolic compounds, showing that phenolic content increase in plasma occurred after olives administration to human volunteers. Their results indicated that olive phenolic compounds have good bioavailability, which is in agreement with their antioxidant efficacy.

The results of this work showed that olive processing induced an important phenolic loss leading to the reduction of antioxidant value. The reduction in antioxidant activity after fermentation ranged between 50% and 72%. Fresh olives have greater antioxidant values but there are not edible because of the bitter taste of some phenolic compounds.

4. Conclusion

There is growing scientific evidence that dietary antioxidants may be a critical mediator of the beneficial effects of the Mediterranean diet. Table olives are basic component of Mediterranean diet and considered as very good source of phenolic compounds, so their consumption provide a large amount of natural antioxidants.

The analysis of green, varicoloured and black olives showed a great difference in phenolic compounds at different degree of ripeness. The concentration of phenolic compounds and the antioxidant activity decreased with maturation. Green olives have the highest phenolic content and antioxidant activity of 2558 mg GA/100 g dw and 133 μM Trolox/g dw, respectively. They were followed by varicoloured and black olives. During spontaneous and controlled fermentation there was an important loss of total phenolic content (32–51%) from the olive to the brine. Total phenolic reduction was lower in the controlled fermentation, due to the development of a biofilm that can be a barrier of phenolic compounds diffusion.

The obtained results showed that olive processing induced an important loss in phenolic compounds, leading to a reduction in antioxidant value. Since functional foods are of great importance nowadays, it is necessary to focus on a new olive process with low phenolic loss.

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