Involvement of antioxidant activity of *Lactobacillus plantarum* on functional properties of olive phenolic compounds

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Abstract Eight lactic acid bacteria strains isolated from traditional fermented foods were investigated for their antioxidant activity against DPPH free radicals, β -carotene bleaching assay and linoleic acid test. *L. plantarum* LAB 1 at a dose of 8.2×10^9 CFU/ml showed the highest DPPH scavenging activity, with inhibition rate of 57.07 ± 0.57 % and an antioxidant activity (TAA = 43.47 ± 0.663 % and AAC = 172.65 ± 5.57), which increase with cell concentrations. When *L. plantarum* LAB 1 was administered to oxidative enzymes, residual activities decreased significantly with cell concentrations. The use of *L. plantarum* LAB 1 on olives process, favours the increase of the antioxidant activity (24 %). HPLC results showed a significant increase of orthodiphenols (74 %). Viable cells of strain were implicated directly on minimum media growth with 500 mg/l of olive phenolic compounds. Results showed an increase in their antioxidant activity. CG-SM analysis, identify the presence of compounds with higher antioxidant activity as vinyl phenol and hydroxytyrosol.

Keywords *L. plantarum* · Olive · Antioxidant activity · Phenolic compounds · Competition

Introduction

Nutritional and medical studies point out the benefits of intensified consumption of fruit and vegetables on account of their low caloric value and beneficial effects of their constituents on human organisms. One of the most important merits of fruit and vegetables is their antioxidative properties. According to Prior and Cao (2000) this is above all justified by the presence of ascorbic acid, tocopherol and beta-carotene; however, Rice-Evans et al. (1997) ascribe it to the antioxidative activity of phenolic compounds. In addition to having nutritional and antioxidant proprieties, phenolic compounds influence multiple sensorial food properties, such as flavour, astringency and colour. Phenolic compounds contribute to the aroma and taste of numerous food products of plant origin (Marsilio et al. 2001). The contribution of phenolic compounds to aroma is mainly due to the presence of volatile phenols. Volatile phenols could be produced by hydrolysis of superior alcohols or by the metabolism of microorganisms, yeast and lactic acid bacteria (Rodriguez et al. 2009).

Factors such as non-enzymatic and enzymatic browning, and process conditions like pH, acidity, oxidation, time and temperature, are responsible for the loss of pigment and colour during processing of foods (Ahmed et al. 2002). Growth and metabolism of the indigenous flora of yeasts and bacteria are responsible for changes in organoleptic properties and alteration of food. As other oxidizing enzymes, polyphenol oxidases are responsible of enzymic browning in several fruits and vegetables, which leads to deterioration of their colour, flavour and nutritional qualities. Moreover, lipoxygenases present in almost all-living systems are demonstrated to be responsible for biochemical changes in colour and flavour profiles.

Lactic acid bacteria (LAB) are Gram-positive bacteria, widely distributed in nature, and industrially important as they

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are used in a variety of industrial food fermentations. The role of LAB in the biopreservation of foods is associated with their ability to produce a range of antimicrobial compounds. These include organic acids, bacteriocins, fatty acids, hydrogen peroxide and diacetyl. Fermented products have been consumed for thousands of years. As the number who consumes traditional fermented foods is increasing, there is an increasing interest in enhancing food safety, improving organoleptic attributes, enriching nutrients, and increasing health benefits (Liu et al. 2011). These functional properties have partly been ascribed to the higher antioxidant properties of LAB involved in many fermentations. Kaizu et al. (1993) demonstrated that several lactobacilli have antioxidative activity, and they are able to decrease the accumulation risk of reactive oxygen species during the ingestion of food.

L. plantarum is a lactic acid bacteria species that is frequently encountered in the fermentation of plant materials where phenolic compounds are abundant. Lactic acid bacteria play an important role in organoleptic qualities of fermented food but little is known about the metabolism of phenolic acids in these bacteria. Many microorganisms have the ability to decarboxylate substituted cinnamic acids, such as ferulic and p-coumaric acids, forming the volatile phenols 4-vinyl guaiacol and 4-vinyl phenol respectively (Cavin et al. 1997). These volatile phenols participate positively in the final aroma of fermented food. Moreover, ferulic acid is exploited to produce value-added aromatic compounds. Among different lactic acid bacteria, we have found that the ubiquitous bacterium *L. plantarum*, used as a lactic starter for many vegetable fermentations, was able to decarboxylate p-coumaric and ferulic acid into corresponding 4-vinyl derivatives. Previous work carried out in our laboratory showed that the application of *L. plantarum* during olive oil process preserves the phenolic compounds, essentially orthodiphenols (Kachouri and Hamdi 2006).

The aims of this work were to study the antioxidant activity and oxygen competition of *L. plantarum* strain LAB 1 isolated from traditional fermented olives and to apply its antioxidant potential to produce functional foods with high-added-value compounds, such as antioxidants, from the conversion of olive phenolic compounds.

Material and methods

Bacterial strains Eight lactic acid bacteria strains were used in this study (Table 1), including 2 strains (LAB 1 and LAB 2) isolated from traditional fermented olive (Kachouri and Hamdi 2006), 2 strains (LAB 3 and LAB 4) from traditional fermented dairy Lben (Ziadi et al. 2010), 3 strains (LAB 5, LAB 6 and LAB 7) from dairy product (Ksontini et al. 2011), and a strain (LAB 8) from traditional fermented dairy Raieb (Kraiem et al. 2012). All the strains were identified by API

50CHL kit (biomérieux Inc., France) and 16 s rDNA sequencing analysis. These strains were maintained as frozen (−80 °C) stocks in MRS broth supplemented with 20 % (v/v) glycerol. They were transferred at least three times consecutively using a 1 % (v/v) inoculum in MRS broth at 37 °C for 18 h before use.

Preparation of cells extracts The eight lactic acid bacteria strains were grown in MRS broth at 37 °C for 18 h. The bacterial cells were harvested by centrifugation (6000 g, 10 min, 4 °C), washed twice with deionised water and resuspended in deionised water. The density of cell pellet was adjusted to OD 600 equal to 0.171 (2×10^7 CFU/ml) to 1.1 (8.2×10^9 CFU/ml). **Inoculation of olives** The selected *L. plantarum* strain LAB 1 cultivated on MRS was harvested by centrifugation for 15 min at 6000 g after 18 h of incubation at 37 °C. The cell pellets were then quickly washed with deionized water. Cells were resuspended in sterile saline water (0.9 %). The preparation was used to inoculate a batch of olives.

Olives of the variety “Chetoui” were harvested in the north of Tunisia. The olives were divided in two sets: one set was inoculated with *L. plantarum* LAB 1 (2×10^7 CFU/g) and one uninoculated set was used for control. Both lots were stored for 16 days.

Both lots were ground and were malaxated slowly for 30 min. Extraction of oil was occurred by centrifugation for 15 min at 3000 g. Oil was collected, filtered, filled in dark glass bottles and stored at −20 °C until analyses were performed. Experiments were performed in triplicate.

Inoculation of culture *L. plantarum* LAB 1 was inoculated on minimum media growth (casa-amino acid medium) with 500 mg/l of olive phenolic compounds (p-coumaric acid and tyrosol). The density of suspension was adjusted to OD 600 equal to 1.1 (8.2×10^9 CFU/ml) and incubated at 37 °C for 5 days. Experiments were performed in triplicate.

Extraction for measurement of phenols and total antioxidant activity Five milliliter of olive oil were extracted using 3×5 ml of methanol. The extracts were evaporated under N₂ and taken up with 5 ml of methanol (Boskou et al. 2006). The resulting extract is called ‘phenolic extract’.

The total phenolic contents of olive oil were measured using a modified Folin-Ciocalteu colorimetric method (Boskou et al. 2006). 0.5 ml of phenolic extract were dissolved in 0.5 ml of Folin-Ciocalteu reagent (1:10 dilution) and 1 ml of distilled water. 1.5 ml of 20 % of sodium carbonate were added after 1 min and the final mixture was shaken thoroughly. After 2 h in the dark at 20 °C, the absorbance was measured at 760 nm against blank. The results were expressed in milligram of gallic acid per liter. Values were expressed as means ($N=3$)±standard deviations (S.D.).

Table 1 Lactic acid bacteria strains

Strains	Origin	Code	References
<i>Lactobacillus plantarum</i>	Traditional fermented olive	LAB 1	Kachouri and Hamdi (2006)
<i>Lactobacillus plantarum</i>		LAB 2	
<i>Lactococcus lactis lactis</i>	Traditional fermented dairy Lben	LAB 3	Ziadi et al. (2010)
<i>Lactococcus lactis diacetylactis</i>		LAB 4	
<i>Lactobacillus lactis</i>	Dairy product	LAB 5	Ksontini et al. (2011)
<i>Lactobacillus lactis</i>		LAB 6	
<i>Lactococcus lactis spp. lactis</i>		LAB 7	
<i>Lactobacillus pentosus</i>	Naturally fermented dairy Rayeb	LAB 8	Kraiem et al. (2012)

Quantification of total antioxidant activity

Free radical-scavenging ability The DPPH radical-scavenging capacity of lactic acid bacteria strains was determined according to the method described by Kao and Chen (2006) with some modifications. Briefly, 1.0 ml of lactic acid bacteria cells with 10^7 to 10^9 CFU/ml, was added to 2.0 ml ethanolic DPPH radical solution (0.05 mM). The mixture was mixed vigorously and incubated at room temperature in the dark for 30 min. The controls included only deionised water and DPPH solution. The blanks contained only ethanol and the cells. The absorbance of the resulting solution was measured in triplicate at 517 nm after centrifugation at 8000 g for 10 min. The scavenging ability was defined as:

$$\text{Scavenging activity(\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}} / A_{\text{control}})] \times 100.$$

Total antioxidative activity The total antioxidative activity (TAA) was assessed by using the linolenic acid test (LA-test). This test evaluates the ability of the samples (lactic acid bacteria cells and phenolic extract) to inhibit linolenic acid (Sigma) oxidation (Pähkila et al. 1998). The standard of linolenic acid in 96 % ethanol (1:100) was diluted in isotonic saline (1:125). To 0.4 ml linolenic acid preparation were added 0.01 % sodium dodecyl sulphate (Sigma) and the sample (0.045 ml). Incubation was started by adding 0.1 mM FeSO_4 (Sigma) and was monitored at 37 °C for 60 min. After the interruption of the reaction by adding 0.25 % butylated hydroxytoluene (Sigma), the mixture was treated with 0.5 ml acetate buffer (pH 3.5, Sigma) and heated with freshly prepared 1 % thiobarbituric acid solution (TBA) (Sigma) at 80 °C for 40 min. After cooling, the mixture was acidified by adding 0.5 ml cold 5 M HCl, extracted with 1.7 ml cold 1-butanol (Sigma) and centrifuged 10 min at 3000 g. Absorbance of butanol fraction was then measured. The TAA of the sample corresponds to its inhibition effect on LA-standard peroxidation as follows: $[1 - (A_{534}(\text{sample}) / A_{534}(\text{LA as control}))] \times 100$. The higher numerical value (%) of TAA indicates the higher antioxidant phenomenon in the sample. Peroxidation of LA-standard in the isotonic saline

(without samples) served as control. Values were expressed as means ($N=3$) $\pm$ standard deviations (S.D.).

β -carotene bleaching test The antioxidant activity of samples (lactic acid bacteria cells and phenolic extract) was evaluated by β -carotene-linoleate model system (Jayaprakasha et al. 2001). β -carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of Tween 40 (polyoxy-ethylene sorbitan monopalmitate) were mixed with 0.5 ml of chloroform. Chloroform was removed at 45 °C, under vacuum, using a rotary evaporator. The resulting mixture was diluted with 50 ml oxygenated distilled water. Aliquots (4 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of test samples. A control, containing 0.2 ml of corresponding solvent and 4 ml of the above emulsion, was prepared. The tubes were placed, at 50 °C, in a water bath. Absorbances of all the samples at 470 nm were taken at zero time ($t=0$), measurement of absorbance was continued, until the colour of the β -carotene disappeared in the control reaction ($t=120$ min), at 15 min intervals. A mixture prepared as above, without β -carotene, served as blank. All determinations were performed in triplicate. The antioxidant activity coefficient (AAC) was evaluated in terms of bleaching of the β -carotene using the following formula: $\text{AAC} = [(A_{s(120)} - A_{c(120)}) / (A_{c(0)} - A_{c(120)})] \times 1000$ where $A_{c(0)}$ is the absorbance value measured at zero time of the incubation for test control, $A_{s(120)}$ and $A_{c(120)}$ are the absorbances measured in the test sample and control, respectively, after incubation for 120 min. Values were expressed as means ($N=3$) $\pm$ standard deviations (S.D.).

Analysis of phenolic compounds The phenolic extract of olive oil was obtained following the procedure described by Mateos et al. (2001). Ten grams of olive oil were extracted using 3×10 ml of methanol/water (80:20, v/v) containing the internal standards (solution of p-hydroxyphenyl acetic (4.64×10^{-2} mg/ml) and o-coumaric acids (9.6×10^{-3} mg/ml) in methanol). To remove most of the residual oil, the methanolic solution was kept overnight at -20 °C; after filtration, the solution was concentrated in vacuum, keeping the bath temperature under 35 °C, and the syrupy residue was taken up with

10 ml of acetonitrile. To eliminate the residual traces of glyceride, three washing with 13 ml of hexane were performed, and the resulting acetonitrile solution was vacuum evaporated, keeping the temperature under 35 °C. The obtained residue was then dissolved in 2 ml of methanol. Ten microliters of the methanol solution was injected in a high performance liquid chromatography.

The HPLC system was composed of a Spectra-Physics liquid chromatography Model HP 1100 equipped with C₁₈Hypersil column (5 µm; 250×4.6 mm), coupled with a Spectra-Physics UV–Vis detector HP 1100 model. The mobile phase was methanol/acetonitrile 50/50 (A)–acetic acid in water (B) (pH 3.2) 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. Reference compounds were obtained as described elsewhere (Mateos et al. 2001).

Lipoxygenase enzyme assay Lipoxygenase activity was performed at 25 °C by measuring the initial rate of oxidation of linolenic acid (Sigma) at 234 nm (Sekhar-Rao et al. 2002) by using a Jenway 6505 spectrophotometer. The assay mixture contained, in a final volume of 1 ml, 800 µl of different cell concentrations of *L. plantarum* LAB 1 (10⁷ to 10⁹ CFU/ml), 20 µl of enzyme solution (40 nM), 180 µl of linolenic acid emulsion (10 mM) and 0.1 mM glycine buffer, pH 9. Substrate emulsion was: 70 mg linolenic acid and 70 mg Tween 80 in 25 ml water. One unit of the enzyme activity corresponds to an increase of 0.001 absorbance/min. Values were expressed as means ($N=3$)±standard deviations (S.D.).

Polyphenol oxidases assay Polyphenol oxidases activity was performed at 30 °C by measuring the initial rate of oxidation of methylcatechol (Sigma) at 400 nm using a Jenway 6505 spectrophotometer. The assay mixture contained, in a final volume of 2 ml, 1.6 ml of different cell concentrations of *L. plantarum* LAB1 (10⁷ to 10⁹ CFU/ml), 400 µl of enzyme solution and 10 mM 4-methylcatechol in 50 mM sodium citrate buffer, pH 5.5. One unit of the enzyme activity corresponds to an increase of 0.05 absorbance/min (Valgimigli et al. 2001). Values were expressed as means ($N=3$)±standard deviations (S.D.).

GC-MS analysis The cultures were extracted three times with ethyl acetate (v/v). The extract was evaporated in vacuum keeping the temperature under 45 °C, giving a residue. 100 µl of BSTFA were added to 200 µl of the organic extract. The solution was evaporated under N₂ current. The residue was redissolved in ethyl acetate (22 µl) and analyzed by gas

chromatography coupled to mass spectrometry (GC-MS) (Ben Othman et al. 2008). GC-MS was performed with HP model 5872A, equipped with a capillary HP5MS column (30 m length, 0.32 mm internal diameter, 0.32 µm film thickness). The carrier gas was He used at 1.7 ml/min flow rate. The oven temperature program was as follows: 1 min at 100 °C then increased to 260 °C at 4 °C/min and kept 10 min at 260 °C. The identification of the various peaks of the chromatogram was carried out by the use of the mass spectrometry applied to their trimethylsilyls derivatives, analyzed using a CG-SM and compared with composed of reference.

Statistical analysis Statistical analyses were performed using the ANOVA test: DATASET1.ISD by GraphPad in stats demo version 3.0 Software to determine differences between means. Statistic significance was determined at 5 % probability level.

Results and discussion

Antioxidant activity and oxygen competition of *L. plantarum* LAB 1

The results of the in vitro antioxidant activity of the eight lactic acid bacteria strains are shown in Table 2. All the strains demonstrated antioxidant activity in a dose-dependent manner within the test concentration range of 10⁷–10⁹ CFU/ml (result not shown). Among the 8 strains tested, *L. plantarum* LAB 1 had the highest radical-scavenging activity with an inhibition rate of 57.07±0.57 % at 8.2 10⁹ CFU/ml. The total antioxidant activity was 43.47±0.663 % and the antioxidant activity coefficient was 172.65±5.57 at 8.2 10⁹ CFU/ml. The measurement of antioxidant activity of *L. plantarum* LAB 1, showed that the antioxidant activity increased with the cell concentrations. There was correlation between antioxidant activity coefficient ($R^2=0.9911$) and total antioxidant activity ($R^2=0.9018$) and their cellular concentrations (Fig. 1).

Table 2 Inhibition rate (PI), Antioxidant Activity Coefficient (AAC) and Total Antioxidant Activity (TAA) determined respectively by DPPH free radicals, β-carotene bleaching assay and linoleic acid test of lactic acid bacteria strains at a dose of 10⁹ CFU/ml

Strains	PI (%)	AAC	TAA (%)
LAB 1	57.07±0.35	172.65±5.57	43.47±0.66
LAB 2	54.26±0.47	171.45±4.07	42.05±0.04
LAB 3	48.31±0.42	115.1±5.75	42.97±1.02
LAB 4	48.76±0.69	170.05±5.76	42.45±0.04
LAB 5	40.61±0.73	170.05±5.76	36.50±0.95
LAB 6	54.31±0.31	71.93±4.06	42.95±0.37
LAB 7	31.04±0.63	66.54±2.88	29.87±0.83
LAB 8	32.46±0.42	75.53±4.06	41.60±0.02

Fig. 1 Total Antioxidant Activity (a), Antioxidant Activity Coefficient (b) and Inhibition rate (c) determined respectively by DPPH free radicals, β -carotene bleaching assay and linoleic acid test of *L. plantarum* LAB 1 at different cell concentrations

The ability of *L. plantarum* LAB 1 to use the oxygen was estimated by testing its inhibition effect on the activity of both oxidative enzymes (lipoxygenase and polyphenol oxidases), present in olive. Several fruit and vegetables, where phenolic compounds are abundant, have nutritional and antioxidant proprieties. In fact, many factors affect the stability of phenolic compounds, including growth and metabolism of indigenous flora and oxidizing enzymes that are responsible for the deterioration of colour and flavour during processing of food. Up to now, metabolisms of phenolic compounds have been described on LAB. Therefore, there is a potential in further research in this field. The elucidation of these metabolic pathways will lead to obtain biotechnologically useful strains and proteins. These strains or bacterial proteins will be adequate in the elaboration procedures to obtain food with improved sensorial or nutritional characteristics. In addition, it might be possible to use these strains or enzymes to obtain high-added-value compounds, such as antioxidants, from the degradation of phenolic compounds present in food wastes.

In order to study the effect of *L. plantarum* LAB 1 for enzymatic and chemical system on lipoxygenase and polyphenol oxidases activity, different cell concentrations of bacteria were added before activity test (Fig. 2). The initial activity of

Fig. 2 Inhibition of lipoxygenase (□) and polyphenol oxidases (×) at different cell concentrations of *L. plantarum* LAB 1. Initial activity (100 %) of Lip and PPO was respectively 95.6U and 1.74U

lipoxygenase was 95.6 U. When adding $2.2 \cdot 10^7$ CFU/ml of *L. plantarum* LAB 1, lipoxygenase activity inhibition reaches 55 %. The inhibition increased when adding more and more inhibitors. Lipoxygenase activity was totally inhibited when using around $6.8 \cdot 10^8$ CFU/ml of strain.

The study of the capacity of *L. plantarum* LAB 1 to inhibit another oxidative enzyme (polyphenol oxidases), in order to determine if this inhibition was directly related to oxygen competition or to effect on enzyme, showed that *L. plantarum* LAB 1 is able to inhibit the polyphenol oxydases activity. The initial activity of polyphenol oxydases was 1.74 U. In fact, when $2.2 \cdot 10^7$ CFU/ml of *L. plantarum* LAB 1 were added, a limited inhibition of polyphenol oxydases activity was observed. In presence of $1.1 \cdot 10^8$ CFU/ml of bacteria, the polyphenol oxydases activity decreased by 39.7 %. Total inhibition was obtained with $1.3 \cdot 10^9$ CFU/ml of bacteria.

These results showed that inhibition of the two oxidative enzymes was directly related to oxygen competition and to the antioxidant defense capacity of *L. plantarum* LAB 1. In fact, many micro-organisms possess enzymatic and non-enzymatic antioxidative mechanisms and minimize generation of reactive oxygen species (ROS) to levels that are not harmful to the cells. Lactic acid bacteria lack many of the components of the respiratory chain, which facilitate the utilization of O_2 as a terminal electron acceptor. However, many LAB synthesize the coupled NADH oxidase/NADH peroxidase system which balances the $NAD^+/NADH$ ratio, catalyzes the reduction of O_2 to H_2O_2 and decomposes H_2O_2 to H_2O for the purpose of protection. Jänsch et al. (2011) showed that the defence of *L. sanfranciscensis* to oxygen toxicity is the involvement of oxygen in the metabolism by NADH-oxidase activity. Thus, lactobacilli differ in their response to oxygen, and their ability to use O_2 in central carbon flux.

The current research in antioxidant ability of LAB has shown that some LAB strains are not only able to decrease the risk of reactive oxygen species accumulation through food ingestion but can also degrade the superoxide anion and hydrogen peroxide (Liu and Pam 2010). Furthermore, the intact

cell and cell lysates of some probiotic strains (e.g., *L. fermentum* E3 and *L. fermentum* E18) had some antioxidant properties and could overcome the oxidative stress (Kullisaar et al. 2002).

Effect of the use of *L. plantarum* LAB 1 on olive antioxidant activity The antioxidant activity of olive was evaluated by two methods, β -carotene bleaching assay and linoleic acid test. According to the results presented in Fig. 3, we can note that the phenolic extract of olive showed an antioxidant activity. The total antioxidant activity (TAA) was 70.45 ± 0.03 % and the antioxidant activity coefficient (AAC) was 607.73 ± 0.01 . This may be due to the antioxidant activity of phenolic compounds. Olives and derivative products are recognized as a valuable source of so-called “functional food” because of their natural phenolic antioxidant content (Marsilio et al. 2001). The specificity of the olive fruit from all other drupes is in its chemical composition with a relatively low concentration of sugars and high oil content, and in a characteristic bitter taste. The monounsaturated fat and the minor constituents such as phenolic compounds are the major components

responsible for nutritional benefits of olive products (Simopoulos 2001). In fact, phenolic compounds are one of the main secondary metabolites in olives and they represent 1 to 2 % of fresh fruit. Interest in phenolic compounds is related primarily to their antioxidant activity. Ben Temime et al. (2006) showed that the oil is characterized by good content of total phenols, *o*-diphenols, tocopherols and a good resistance to oxidation. Saura-Calixto and Goñi (2006) showed that the phenolic compounds are quantitatively the main dietary antioxidants. They show an important biological activity in vivo and may contribute to prevent diseases related to oxygen radical formation when this exceeds the antioxidant defence capacity of the human body (Morello et al. 2004). The antioxidant quality of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agent, hydrogen donors, and singlet and triplet oxygen quenchers. They also have metal chelation properties.

The storage of olive fruits favours the decrease in antioxidant activity accompanying with the decrease in the total phenolic compounds (Fig. 3). Ben Othman et al. (2009) showed that the concentration of phenolic compounds and the antioxidant activity decreased with maturation. The olive processing induced an important loss in phenolic compounds, leading to a reduction in antioxidant value. Unfortunately, phenolic compounds are self-oxidized due first to dispositions taken during the harvest, and more importantly, during the storage of the fruits before trituration. In fact, the phenolic compounds of olive have multiple effects, including the stability to oxidation of extra virgin olive oil during storage. It has been reported that phenolic compounds, particularly ortho-diphenols, show a decrease in their concentration in oil during malaxation (Servili et al. 1999), mainly due to oxidative reactions. Moreover, it was shown that the auto-oxidative of caffeic and gallic acids is directly related to oxygen concentration in the solution (Singleton 1987). This phenomenon was described by certain fundamental work, but without presenting the biochemical mechanisms. Di Gioia et al. (2001) observed an increase in the caffeic acid in a medium, accompanied by a black colouring which could be explained by a spontaneous oxidative reaction and a polymerisation by this phenolic compound.

The use of *L. plantarum* LAB 1 during storage of olive fruits, favours an increase of antioxidant activity with 24 % (Fig. 3b) and an amount of total phenolic compounds (Fig. 3a). The chromatographic profile of phenolic compounds (Table 3) showed that the main phenols identified are alcohol phenols (tyrosol and hydroxytyrosol), benzoic acids (vanillic acid), vanillin, cinnamic acids (*p*-coumaric acid), dialdehydic form of decarboxymethyl of elenolic acid linked to hydroxytyrosol (Hy-EDA), hydroxytyrosyl and tyrosyl acetate and two lignans (pinoresinol and 1-acetoxypinoresinol). The use of *L. plantarum* LAB 1 during olive process favours the increase of the all phenolic

Fig. 3 Effect of the use of *L. plantarum* LAB 1 on phenolic content (a), Total Antioxidant Activity (b) and Antioxidant Activity Coefficient (c) of phenolic extracts ((○) control; (▲) inoculated)

Table 3 Effect of the use of *L. plantarum* LAB 1 on olive phenolic composition (mg/kg)

Compounds	Control	Inoculated
Hy	0.9	4.2
Ty	8.8	13.9
vanillic acid	0.3	0.3
vanillin	0.1	0.1
p-coumaric acid	0.5	0.5
Hy-AC	1.9	2.3
Hy-EDA	1.8	1.5
Ty-AC	13.7	12.2
Pinoresinol	2.6	3.5
1-acetoxypinoresinol	12.8	14.2
Orthodiphenols ^a	4.6	8.0
Non-orthodiphenols ^b	38.8	44.7

^a Sum of hydroxytyrosol (Hy), hydroxytyrosyl acetate (Hy-AC), dialdehydic form of decarboxymethyl of elenolic acid linked to hydroxytyrosol (Hy-EDA)

^b Sum of tyrosol (Ty), vanillic acid, vanillin, p-coumaric, tyrosyl acetate (Ty-AC), 1-acetoxypinoresinol and pinoresinol

compounds (Table 3). Orthodiphenols concentration increased with 74 %, particularly hydroxytyrosol (0.9 mg/kg to 4.2 mg/kg). Among non-orthodiphenols, tyrosol concentration increased with 58 %. The increase may be due to the ability of *L. plantarum* LAB 1 to use the oxygen present in the solution that was responsible for the auto-oxidation of phenolic compounds and/or to the capacity of the strain to convert the phenolic compounds, forming volatile phenols that participate positively in the increase of antioxidant activity. Previous work carried out in our laboratory studied the transformation of phenolic compounds contained in OMW into valuable products using *L. plantarum* in order to increase their transportation from OMW to olive oil. Incubation of olive oil samples with fermented OMW by *L. plantarum* caused polyphenols to decrease in OMW and increase in oil. Fermentation with *L. plantarum* induced reductive depolymerisation of OMW which is more soluble in olive oil. The analysis of the phenolic compounds found in olive oil after storage showed that the application of *L. plantarum* favours the increase of all phenolic compounds in olive oil, especially by depolymerisation and by reductive conversion of phenolic compounds of olive and oxygen fixation (Kachouri and Hamdi 2004). Several authors have focused on oleuropein hydrolysis by β -glucosidase produced by *L. plantarum* (Landete et al. 2008). In the other hand, concentrations of hydroxytyrosol increased after olive fermentations. This compound is present in abundance in fresh olives and its concentration increased after fermentation, due to acid and enzymatic hydrolysis of oleuropein. Recently, Rodríguez et al. (2008) demonstrated that p-coumaric, caffeic, ferulic and m-coumaric acids are metabolized by *L. plantarum*.

Effect of *L. plantarum* LAB 1 on phenolic compounds antioxidant activity In order to study the ability of *L. plantarum* LAB 1 to preserve and/or to convert phenolic compounds, which participate positively in the increase of antioxidant activity, viable cells of bacteria were implicated directly in presence of 500 mg/l of phenolic compounds present in olive fruit (tyrosol and p-coumaric acid). The evaluation of the antioxidant activity of both phenolic compounds showed that the application of *L. plantarum* LAB 1 favours the increase in their antioxidant activity (Fig. 4). This increase may be due to the antioxidant defence capacity of this strain to oxygen radical formation by the involvement of oxygen in the enzymatic of reductive conversion of phenolic compounds. This will give the capacity of this strain or enzymes to produce high-added-value compounds, such as antioxidants, from the degradation of phenolic compounds present in the olive. Results obtained in *L. plantarum* showed that *L. plantarum* was able to degrade some food phenolic compounds giving compounds influencing food aroma as well as compounds presenting increased antioxidant activity (Rodríguez et al. 2009). The antioxidant activity of phenolic compounds depends on their chemical structures, number, and arrangement of the hydroxyl groups (Rice-Evans et al. 1997).

The chromatographic analysis in gas phase coupled with the mass spectrometry (CG-SM) was carried out on the inoculated ethyl acetate extracts of the mediums containing different phenolic compounds after 5 days of incubation. The identification of the various peaks of the chromatogram was carried out by the use of the mass spectrometry applied to their trimethylsilyls derivatives, analysed using a CG-SM and compared with composed of reference.

Fig. 4 Effect of *L. plantarum* LAB 1 on Total Antioxidant Activity (a) and Antioxidant Activity Coefficient (b) of tyrosol (Ty) and p-coumaric acid (p-Cou) (□) control; (■) inoculated

In the inoculated medium containing p-coumaric acid, the identification of the various peaks (Fig. 5a) showed the presence of lactic acid and 4-vinyl phenol. Traces of the p-coumaric acid were also identified. Cavin et al. (1997) showed that the phenolic acids and in particular the cinnamic acids (mainly p-coumaric and ferulic acids) are metabolized by various microorganisms like *L. plantarum* into derived 4-vinyl then reduced into derived 4-ethyl. The vinyl derivatives are required in the industry of fine chemistry and the industry of the biopolymers because of their faculty of regeneration of the free radicals. These derivatives are also required in cosmetic

and in food industry. Indeed, the derivatives vinyls and ethyls are volatile phenols which contribute potentially to the flavours of the fermented food.

The tyrosol is transformed into hydroxytyrosol and 3, 4-dihydroxyphenylacetic acid (Fig. 5b) in the presence of *L. plantarum* LAB 1. Allouche et al. (2004) showed that *Pseudomonas aeruginosa* transforms the tyrosol into hydroxytyrosol, during the phase of adaptation of growth, without consumption of these aromatic compounds. After this period, the concentration of the hydroxytyrosol decreases in the medium but the degradation of the aromatic compounds

Fig. 5 Behavior of p-coumaric acid (a) and tyrosol (b) with *L. plantarum* determined by GC-SM analysis

continuous and the traces of 3, 4- dihydroxyphenyl acetic and p-hydroxyphenyl acetic acids appear immediately in the medium. The hydroxytyrosol is a phenolic compound present in the virgin olive oil and which ensures its stability. Moreover, it was proven that the hydroxytyrosol and its derivatives present several aspects interesting for human health and consequently, they are very required in various industries like cosmetic industry and industry pharmaceutical. Thus the transformation of the tyrosol into hydroxytyrosol and acid 3, 4-dihydroxyphenyl acetic by the viable cells of *L. plantarum* will increase its antioxidant effect which plays a very important part in the stability of the olive oil during its conservation because of improvement of its antioxidant activity.

Conclusion

The use of *L. plantarum* LAB 1 on olives favours the increase of the antioxidant activity correlated with an increase of orthodiphenols. Results showed that the strain has a capacity to produce high-added-value compounds, such as vinyl compounds with higher antioxidant activity, from the conversion of phenolic compounds present in the olive. *L. plantarum* LAB 1 could be constituted a new microbiological process for olive oil quality improvement.

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