Food phenolics and lactic acid bacteria

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Abstract

Phenolic compounds are important constituents of food products of plant origin. These compounds are directly related to sensory characteristics of foods such as flavour, astringency, and colour. In addition, the presence of phenolic compounds on the diet is beneficial to health due to their chemopreventive activities against carcinogenesis and mutagenesis, mainly due to their antioxidant activities. Lactic acid bacteria (LAB) are autochthonous microbiota of raw vegetables. To get desirable properties on fermented plant-derived food products, LAB has to be adapted to the characteristics of the plant raw materials where phenolic compounds are abundant. *Lactobacillus plantarum* is the commercial starter most frequently used in the fermentation of food products of plant origin. However, scarce information is still available on the influence of phenolic compounds on the growth and viability of *L. plantarum* and other LAB species. Moreover, metabolic pathways of biosynthesis or degradation of phenolic compounds in LAB have not been completely described. 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. Recently, several *L. plantarum* proteins involved in the metabolism of phenolic compounds have been genetically and biochemically characterized. The aim of this review is to give a complete and updated overview of the current knowledge among LAB and food phenolics interaction, which could facilitate the possible application of selected bacteria or their enzymes in the elaboration of food products with improved characteristics.

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1. Introduction

In the last years, researchers and food manufacturers have become increasingly interested in phenolic compounds. The reason for this interest is the recognition of their antioxidant properties, their great abundance in our diet, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer, and cardiovascular and degenerative diseases (Manach et al., 2004).

The term “phenolic compound” described several hundred molecules found in edible plants that possess on their structure a benzenic ring substituted by, at least, one hydroxyl group. These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between phenolic acids (benzoic or hydroxycinnamic acid derivatives), flavonoids, stilbenes, and lignans. The flavonoids may themselves be divided into flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids (Manach et al., 2004).

Traditionally, and from a basic knowledge, phenolic compounds have been considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the utilization of vitamins and minerals, reducing the nutritional values of foods. However, the recent recognition of their antioxidant properties reduced the investigations of their adverse health effects. The presence of phenolic compounds on the diet is beneficial to health due to their chemopreventive activities against carcinogenesis and mutagenesis. The health effects of phenolic compounds depend on

the amount consumed and on their bioavailability (Chung et al., 1998; Shen et al., 2007).

In addition to having nutritional and antioxidant properties, 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. The contribution of phenolic compounds to aroma is mainly due to the presence of volatile phenols. Volatile phenols could be produced by the hydrolysis of superior alcohols or by the metabolism of microorganisms, yeast and LAB. In addition, food phenolics also contribute to food astringency. Some phenolic substances, mostly tannins, present in foods are able to bring about a puckering and drying sensation referred to as astringency which is related to the ability of the substance to precipitate salivary proteins (Lea and Arnold, 1978). Moreover, phenolic compounds are natural food pigments that greatly influence the colour of vegetable food products. Among flavonoids, the anthocyanins are responsible for the pink, scarlet, red, mauve, blue and violet colors of vegetables, fruits, fruit juices and wine (Harborne, 1988). Most flavonoids are present in plant cells in the form of glycosides.

Fruits, vegetables and beverages, such as tea, are the main sources of phenolic compounds in the human diet (Kapur and Kapoor, 2001). The Mediterranean diet includes fermented vegetable food products, such as wine and table olives, for which phenolic compounds are responsible of some of their sensorial and nutritional characteristics.

2. Lactic acid bacteria in fermented food products of plant origin

Vegetables are strongly recommended in the human diet since they are rich in antioxidant, vitamins, dietary fibres and minerals. The major part of the vegetables consumed in the human diet are fresh, minimally processed, pasteurized or cooked by boiling in water or microwaving. Minimally processed and, especially, fresh vegetables have a very short-life since subjected to rapid microbial spoilage and the above cooking processes would bring about a number of not always desirable changes in physical characteristics and chemical composition of vegetables. Among the various technological options, fermentation by lactic acid bacteria (LAB) may be considered as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of vegetables. Lactic acid fermentation of vegetables has nowadays an industrial significance for cucumbers, cabbages and olives. Several other varieties of vegetables (e.g., carrots, French beans, marrows, artichokes, capers and eggplants) also increase their safety, nutritional, sensory and shelf-life properties through lactic acid fermentation under standardized industrial conditions.

Composition of microbiota and its development are important factors influencing fermentation and final product quality. Overall, LAB are a small part of the autochthonous microbiota of raw vegetables. To get desirable properties of fermented vegetable food products, LAB has to be adapted to the intrinsic characteristics of the raw materials. Spontaneous fermentations typically result from the competitive activities of a variety of autochthonous and contaminating microorganisms. Those best adapted to the conditions during the fermentation process will eventually dominate. Initiation of a spontaneous process takes a relatively long time, with a high risk for failure. Failure of fermentation processes can result in spoilage and/or the survival of pathogens, thereby creating unexpected health risks in food products. Thus, from both a hygiene and safety point of view, the use of starter cultures is recommended, as it would

lead to a rapid acidification of the product and thus inhibit the growth of spoilage and pathogenic bacteria, and to a product with consistent quality. Although a large number of LAB starters are routinely used in dairy, meat and baked food fermentations, only a few cultures have been used for vegetable fermentations. *Lactobacillus plantarum* is the commercial starter most frequently used in the fermentation of cucumbers, cabbages and olives (Vega Leal-Sánchez et al., 2003; Ruíz-Barba et al., 1994b).

Table olives are one of the most important fermented vegetables in the world economy. Table olives are produced as Spanish-style green olives in brine, as naturally black olives in brine, and as ripe olives, according to well-established processes. In naturally black olives, yeasts and LAB are responsible for fermentation but in Spanish-style olives this role is mainly played by LAB. In other processing methodologies competition between yeasts and LAB has been reported. The composition of the microbiota of the olives before brine making is one of the factors that could affect the dynamics of the fermentation and the quality of the product. In addition, resident LAB of manufacturing plants LAB are recognized to play an important role in olive fermentation. *L. plantarum* and *Lactobacillus pentosus* are, in fact, regarded as the main species leading this process being often used as starter in guided olive fermentations, although other LAB species such as *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, or *Pediococcus pentosaceus*, among others, have been also isolated (Table 1) (Ruiz-Barba et al., 1994a, 1994b; Nychas et al., 2002; Panagou et al., 2003; Vega Leal-Sánchez et al., 2003; Ercolini et al., 2006; Chamkha et al., 2008; Hurtado et al., 2008).

Cabbage or sauerkraut fermentation involves many physical, chemical, and microbiological changes that influence the quality and safety of the products. This fermentation can be broadly categorized as having successive stages, including an initial

heterofermentative stage followed by a homofermentative stage. Historically, four species of LAB have been identified as organisms that are present in sauerkraut fermentations *L. mesenteroides*, *Lactobacillus brevis*, *P. pentosaceus*, and *L. plantarum*, although recent results indicated that the species of LAB present are more diverse than previously reported and include, among others, *Leuconostoc citreum* and *Lactobacillus paraplantarum* strains (Plengvidhya et al., 2007) (Table 1).

Similar LAB species, *L. plantarum*, *L. brevis*, *L. pentosus* and *Leuconostoc* spp. have been found to occur mainly in the spontaneous fermentation of cucumbers (Tamminen et al., 2004). It have been also described that *Leuconostoc* and *Lactobacillus* genera predominated during the early hours of fermentation; subsequently, *Lactobacillus* and *Pediococcus* emerged as the dominant genera, and finally, *Pediococcus* appeared as a dominant genera during the late stages of fermentation (Singh and Ramesh, 2008). *Lactobacillus* species were shown to be the only lactic microbiota participating in the process of spontaneous fermentation of “Almagro” eggplant, with *L. plantarum*, *Lactobacillus fermentum* being isolated most frequently and, *L. pentosus* and *L. brevis* being less common (Sánchez et al., 2004; Seseña et al., 2005; Seseña and Palop, 2007). Fermentation of caper and caper berries relies on the spontaneous growth of LAB, being *L. plantarum* the predominant species in the fermentation, although strains of *L. paraplantarum*, *L. pentosus*, *L. brevis*, and *L. fermentum* and pediococci are also isolated (Pérez Pulido et al., 2007). Strains of *L. mesenteroides*, *L. plantarum*, *Enterococcus faecalis*, *P. pentosaceus* and *L. fermentum* were identified from raw carrots, French beans and marrows (Di Cagno et al., 2008). In sourdoughs, microbiological studies have revealed that *L. plantarum*, *L. brevis*, *Weissella cibaria*, and *P. pentosaceus* are the dominant LAB species (Iacumin et al., 2009). *L. plantarum*, *L. brevis*, *L. mesenteroides*, and *L. pseudomesenteroides* have been found to

occur mainly in the spontaneous fermentation of Korean kimchi (Kim and Chun, 2005). Predominant LAB strains associated with fermented bamboo shoot products were identified as *L. plantarum*, *L. brevis*, *P. pentosaceus*, *L. mesenteroides*, among others (Tamang et al., 2008). In cassava, the major staple crop for over 500 million people, *L. plantarum* could be isolated from spontaneous fermentations (Huch et al., 2008). *L. plantarum* and *L. brevis* are the starter cultures most frequently used for fermentation of vegetable juices, e.g., autochthonous *L. plantarum* strains were used to increase health-promoting and sensory properties of tomato juices (Di Cagno et al., 2009). In grape musts and wines, *Oenococcus oeni* is the most important LAB species and is applied as starter culture; however, within species of LAB that may also occur, *L. plantarum*, *L. brevis*, *Lactobacillus hilgardii*, and *L. mesenteroides* strains could be found (Moreno-Arribas et al., 2003; Rodas et al., 2005) (Table 1).

Considering the LAB microbiota found in vegetable fermentations, it seems that only few LAB species are well adapted to growth in raw vegetable material where phenolic compounds are abundant.

3. Influence of phenolics on the growth and viability of lactic acid bacteria

3.1. *Lactobacillus plantarum*

In spite that most vegetable fermentations are spontaneous, *L. plantarum* is the commercial starter most frequently used in the fermentation of vegetable food products. However, only a limited number of studies have been made to study the influence of phenolic compounds on the growth and viability of *L. plantarum* strains.

The role of quinate and shikimate in the metabolism of lactobacilli was studied by Whiting and Coggins (1969). They described that *L. plantarum* reduced quinate and shikimate under anaerobic conditions in the presence of suitable hydrogen donors. Salih et al. (2000) studied the effect of hydroxycinnamic acids, their quinic esters and quinic acid (a non-phenolic acid) on the growth of *L. plantarum*. Results showed that, from the compounds assayed, bacterial growth was only affected by hydroxycinnamic acid at the concentrations used (up to 3 mM). A decreasing inhibitory effect was shown from ferulic acid to *p*-coumaric acid and caffeic acid (Figure 1). Biomass production was not affected, and only the apparent growth rate was affected in *L. plantarum*. Marsilio and Lanza (1998) described that *L. plantarum* growth was significantly reduced in the presence of 1 g/l *p*-coumaric acid and the inhibitory activity increased in the presence of NaCl. Growth was particularly low when *p*-coumaric acid was combined with 40 g/l NaCl and negligible in the presence of 60 g/l NaCl. Lower concentrations (0.5 g/l) of *p*-coumaric acid did not show inhibitory activity against *L. plantarum*. Landete et al (2007) analyzed the antimicrobial activities of ten wine phenolics compounds against *L. plantarum* strains. They reported that inhibition increased in this order: catechin = gallic acid < gallate epicatechin = salicylic acid < methyl gallate = caffeic acid < ferulic acid = tryptophol < *p*-coumaric acid.

In a laboratory medium, the effect of caffeic and ferulic acids on the *L. plantarum* viability was reported to be concentration-dependent (Rozès and Peres, 1998). The same authors reported that low tannin concentrations (0.1 or 0.2 g/l) did not inhibited *L. plantarum* growth, but high amounts of tannin (1 g/l) delayed bacterial growth (Rozès and Peres, 1998).

Ruiz-Barba et al. (1990) studied the bactericidal effect of phenolic compounds from olives on *L. plantarum*. Their results indicated that the phenolic compounds

contained in the non-alkali treated green olive brines exhibit a pronounced bactericidal effect on *L. plantarum*. The bacteriostatic effect of these compounds was avoided by the use of appropriate phenolic inactivating agents. All the phenolic compounds assayed affected the survival of *L. plantarum*, although with differing cellular viabilities. Later, Durán et al. (1993) studied the survival of *L. plantarum* during the first days of ripe olive brining. They reported that the inhibitory effect of diffused phenolic compounds on *L. plantarum* during these days was significant only when it was associated with NaCl (3% NaCl in brines). The combined effect of 6% NaCl and the phenolic concentration caused a marked decrease in survival in 7 days of olive brines. It have been reported that oleuropein, a bitter glucoside present in significant amounts in the pulp of olive fruits, is not the most inhibiting compound of *L. plantarum* growth. Several authors have studied the effects of oleuropein and its hydrolysis products on the survival of bacteria. The results reported are different according to the antibacterial test methods used. Juven and Henis (1970) reported that oleuropein inhibit *L. plantarum* growth. This inhibition was augmented by reducing the concentration of organic nitrogenous compounds, increasing the NaCl concentration in the medium, and decreasing the inoculum size. Marsilio and Lanza (1998) studied the ability of *L. plantarum* to grow in the presence of oleuropein, hydroxytyrosol, and *p*-coumaric acid combined with various concentrations of NaCl. *L. plantarum* grew well in NaCl concentrations up to 60 g/l, levels of 80 g/l delayed the onset of growth rather than retarding the rate and the growth was suppressed in the presence of 100 g/l of NaCl. Under all conditions tested, oleuropein in combination with NaCl increased significantly the bacterial growth and therefore did not appear to have bactericidal effects. Similar results were obtained in the presence of hydroxytyrosol. In the presence

of oleuropein, the cell density was higher than with hydroxytyrosol possibly because glucose released from hydrolysed glucoside was readily metabolised by *L. plantarum*.

Rozès and Peres (1996) reported that untreated oleuropein was not inhibitory to *L. plantarum*. However, when the aglycon was formed in the medium, by the action of a β -glucosidase, and with a low sugar concentration, cell viability decreased. Ruiz-Barba et al. (1991) reported that oleuropein extracted from green olives was bactericidal against *L. plantarum* strains isolated from green olive fermentation brines. Heat-treated oleuropein also demonstrated a strong bactericidal effect but not alkali-treated oleuropein, which allowed survival of most of the strains tested. Ruiz-Barba et al. (1993) also tested the viability of *L. plantarum* in the presence of single or combined fractions of the phenolic compounds isolated from NaOH-treated and untreated olive brines. When assayed at the concentrations found in brines, only the single phenolic fraction containing hydroxytyrosol strongly inhibited *L. plantarum*. When tested as single phenolic fractions, vanillic acid, verbascoside and luteolin-7-glucoside, none of them had bactericidal effect against the lactobacilli even at the maximum concentration found in brines. However, inhibition of *L. plantarum* was observed when double phenolic fractions (e.g. the glucosides, oleuropein and verbascoside) were used; this showed a combined effect in the inhibition. Recently Landete et al. (2008a) evaluated inhibitory growth activities of nine olive phenolic compounds against *L. plantarum* strains isolated from different sources. None of the nine compounds assayed (oleuropein, hydroxytyrosol, tyrosol, as well as vanillic, *p*-hydroxybenzoic, sinapic, syringic, protocatechuic and cinnamic acids) inhibited *L. plantarum* growth at the concentrations found in olive products.

The mechanism of growth inhibition is not clear. Some authors proposed that oleuropein and its hydrolysis products induce leakage of glutamate and inorganic

phosphate from the bacterial cell as well as the degradation of the cell wall itself.

Oleuropein had no effect on the rate of glycolysis when added to resting cells of *L. plantarum*, but it caused a decrease in the ATP content of the cells (Juven et al., 1972).

It have been described that the bactericidal effect of phenolic compounds is related to alterations at two different levels of the cellular ultrastructure: cell wall and cytoplasmic membrane (Ruíz-Barba et al., 1990). These alterations possibly led to the disruption of the cell envelope. Scanning electron micrographs of *L. plantarum* whole cells revealed that after 24 h of incubation in phenolic compounds, the bacterial surface become irregular and rough. Transmission electron micrographs of the same cells also revealed that cell wall structures become irregular and several projections appear on the surface of the bacteria (Figure 2). After 11 days of incubation in phenolics, the cell envelope almost disappeared but whole cells contained mesosomes could be seen (Ruíz-Barba et al., 1990). It was also described that the bactericidal effect of oleuropein was accompanied by changes in the typical bacillary structure of *L. plantarum* and also the typical Gram-positive appearance was lost and a Gram-negative profile was observed (Ruiz-Barba et al., 1991). These changes occur between 30-60 min of incubation in oleuropein. The authors suggest that this could indicate that oleuropein promoted disruption of the peptidoglycan, which could lead to cell death by destruction of the cell envelope.

Alterations in the cytoplasmic membrane produced by phenolic compounds have been also described. Rozès and Peres (1998) studied the effects of phenolic compounds on the fatty acid composition of *L. plantarum* membranes. They described that increasing amounts of caffeic and ferulic acids induced a gradual increase in the amounts of myristic, palmitoleic, stearic and 9,10-methylenehexadecanoic acid with a concomitant decrease of lactobacilli acid. On the other hand, the addition of tannins

induced an increase in the lactobacillic acid level at the expense of vaccenic acid content. Their results suggest that, in the presence of acidic phenols, the fatty acid composition is altered in terms of what occurs in response to low temperature or high alcohol concentration. An opposite phenomenon occurs when tannins are added. Their effect seems comparable to the effect of increases in growth temperature.

3.2. Other lactic acid bacteria species

Limited studies have been done on the influence of phenolic compounds on the growth and viability of other LAB species. These studies were mainly focused on wine LAB species such as *O. oeni*, *L. hilgardii* and *L. brevis*.

O. oeni is the main LAB species involved in malolactic fermentation during winemaking. Phenolic compounds are important components of red wine and potentially affect malolactic fermentation. In *O. oeni*, the effect of some phenolic compounds on the physiology, metabolism, and growth inhibition have been studied. The effects of phenolic acids and free anthocyanins was studied by Vivas et al. (1997). Gallic acid and free anthocyanins seems to activated cell growth and the rate of malic acid degradation. Vanillic acid showed a slight inhibiting effect, while protocatechuic acid had no effect. Later, Vivas et al (2000) reported that grape tannins, procyanidins, and oak wood tannins, elagitannins, do no have the same effect on *O. oeni*. Oligomer procyanidins are powerful inhibitors, affecting bacterial viability in non-growing conditions, bacterial growth and malolactic activity. On the contrary, pure ellagitannins appear to be propitious to the viability of *O. oeni*, while total oak extract is also a powerful inhibitor. These authors demonstrated that ellagitannins when oxidized have a

strong inhibiting effect, as opposed to procyanidins which lose all effect when oxidised. They suggest that the adsorption on the bacterial cells may be the mechanisms involved in this observation. Salih et al. (2000) studied the effects of the following hydroxycinnamic acids: ferulic, *p*-coumaric, caffeic, *p*-coumaroyl quinic, 5'-caffeoyl quinic and the non-phenolic acid, quinic acid on *O. oeni* growth. Quinic acid was not active and *O. oeni* growth was only affected by hydroxycinnamic acid concentrations. Apparent growth rate and biomass production decreases in the presence of hydroxycinnamic acids. Similar results were obtained in studies from other authors. Reguant et al. (2000) found that the growth of *O. oeni* is affected by phenolic compounds in different ways, depending on their type and concentration. Generally they have no effects at low concentrations, but hydroxycinnamic acids are inhibitory at high concentrations. Campos et al. (2003) described that hydroxycinnamic acids were more inhibitory to the growth of *O. oeni* than hydroxybenzoic acids, being *p*-coumaric acid which showed the strongest inhibitory effect on growth and survival.

Cultures of *O. oeni* in the presence of phenolic compounds in a synthetic media or under wine conditions were examined to know how these compounds affect *O. oeni* growth. Reguant et al. (2000) in a synthetic medium found that malolactic fermentation was stimulated in the presence of catechin and quercetin, but increasingly delayed with increasing amounts of *p*-coumaric acid. Gallic acid appeared to delay or inhibit the formation of acetic acid from citric acid. Rozès et al. (2003) found that fifty milligrams per litre or more of phenolic compounds stimulated *O. oeni* growth under wine conditions. These authors also described that phenolic compounds reduced the rate of sugar consumption and enhanced citric acid consumption, increasing the yield of acetic acid.

De Revel et al. (2005) described that, in laboratory medium, the heating of wood favoured *O. oeni* viability more than the simple addition of wood shaving. Theobald et al (2007) found that green tea could cause a growth stimulation of *O. oeni* as a result of the phenolic compounds present on green tea, especially epigallocatechin gallate. However, depending on its concentration, this compound could also inhibit *O. oeni* growth. They also described that individual catechins have a minor influence on the growth of oenococci. Figueiredo et al. (2008) studied the effects of phenolic aldehydes and flavonoids on growth and inactivation of *O. oeni*. Of the phenolic aldehydes tested, inapaldehyde, coniferaldehyde, *p*-hydroxybenzaldehyde, 3, 4-dihydrobenzaldehyde, and 3, 4, 5-trihydroxybenzaldehyde significantly inhibited *O. oeni* growth, while vanillin and syrigaldehyde had no effect at the concentration tested. Among the flavonoids, quercetin and kaempferol exerted an inhibitory effect on *O. oeni*. Myricetin, catequin and epicatequin did not affect considerably *O. oeni* growth. Condensed tannins were found to strongly affect *O. oeni* viability.

Lactobacillus hilgardii growth has been identified as a cause of wine spoilage (Rodríguez et al., 2007a). The effects of different concentrations of gallic acid and catequin on *L. hilgardii* growth was studied by Alberto et al. (2001). These phenolic compounds, at concentrations normally present in wine, not only stimulated the growth rate but also resulted in greater cell densities during the stationary phase of growth. During the first hours of growth both phenolic compounds activated the rate of glucose and fructose utilization and only catechin increases the malic acid consumption rate. Later, Alberto et al. (2002) when studied the effect of wine phenolic compounds on *L. hilgardii* viability described that in decolorized wines, which result in tannin losses, the viable cell counts increase. They also found a relationship between *L. hilgardii* tannin binding and its viability loss. The influence of some phenolics acids on growth and

inactivation of *L. hilgardii* was also studied by Campos et al. (2003). *p*-Coumaric acid showed the strongest inhibitory effect on growth and survival, showing caffeic and ferulic acids a beneficial effect on *L. hilgardii* growth. Recently, the effect of phenolic compounds on *L. hilgardii* putrescine production was studied (Alberto et al., 2007). They found that bacterial growth was stimulated by phenolic compounds, except for gallic acid and quercetin. Putrescine formation from agmatine diminished in the presence of protocatechuic, vanillic and caffeic acids, and the flavonoids catechin and rutin. Figueredo et al. (2008) studied the effect of phenolic aldehydes and flavonoids in *L. hilgardii* growth and found that, among the aldehydes tested, it was only inhibited by sinapaldehyde and coniferaldehyde. Similarly to *O.oeni*, myricetin, catechin and epicatechin did not affect considerably *L. hilgardii* growth. However, condensed tannins strongly affect cell viability. The effect of a tannin, tannic acid, on *L. hilgardii* was analyzed by a proteomic assay (Bossi et al., 2007). Although the mechanisms ruling the interaction between tannic acid and *L. hilgardii* cells were not elucidated, the proteomic approach suggests the interference of tannins on cell protein expression. The involvement of important metabolic enzymes, ribosomal and functional membrane proteins could explain the inhibition of cells growing in presence of tannins.

The effects of hydroxycinnamic acids on the growth of two other species of wine-spoilage LAB, *Lactobacillus collinoides* and *L. brevis*, was studied by Stead (1993). At concentrations of 500 and 1000 mg/l, caffeic, coumaric and ferulic acids markedly inhibited growth; coumaric and ferulic acids were more effective than caffeic acid. At concentrations of 100 mg/l, all compounds stimulated growth. In general, *L. collinoides* strains were more susceptible both to inhibition and stimulation by the hydroxycinnamic acids than was *L. brevis*. Later, the effect of chlorogenic, gallic and quinic acids, at concentrations of 100, 500 and 1000 mg/l on *L. collinoides* and *L. brevis*

growth was evaluated by Stead (1994). During early stages of growth, all the complex acids at each concentration stimulated growth of *L. collinoides* but not of *L. brevis*. During stationary phase, chlorogenic and gallic acids produced greater cell densities of both species, whereas quinic acid generally had loss effect.

4. Metabolism of food phenolics by lactic acid bacteria

4.1. Lactobacillus plantarum

Lactobacillus plantarum is a LAB species that is most frequently encountered in the fermentation of plant materials where phenolic compounds are abundant. However, nowadays most of the metabolism of phenolic compounds remains unknown, as well as its induction or repression by the presence of different sugar sources (Muscariello et al., 2001).

As early as 1975, Whiting described that *L. plantarum* in anaerobic conditions reduce quinate to dihydroxycyclohexanecarboxylate and acetic acid (Table 2). This pathway involves eleven steps, catalyzed by inducible enzymes. *L. plantarum* not only reduces quinate but at the same time, even under anaerobic conditions, oxidizes a proportion to catechol, in a pathway involving a NAD-dependent dehydrogenase and a protocatechuic acid decarboxylase that remains genetically uncharacterized (Whiting and Coggins, 1974; Whiting, 1975).

Ciafardini et al (1994) and Marsilio et al. (1996) reported that *L. plantarum* strains degraded oleuropein, the main phenolic glucoside of olive fruit. Strains of *L. plantarum* initially hydrolyze the oleuropein by means of a β -glucosidase action, with formation of an aglycone, and in a second step, this derivative, by means of a esterase

action, gives rise to hydroxytyrosol and elenoic acid (Table 2). β -glucosidase activity was partially inhibited by glucose, however, esterase activity involved in the second step of biodegradation process, was not influenced by glucose (Marsilio and Lanza, 1988). Grimaldi et al. (2005) found that *L. plantarum* Lac26 strain exhibited a detectable capacity for the hydrolysis of the β - and α -D-glucopyranosides. However, contradictory data were obtained in relation to the *L. plantarum* protein possessing this activity. A β -glucosidase, with a molecular mass of 40 kDa, was purified from *L. plantarum* extracts (Sestelo et al., 2004). However, Spano et al. (2005), by sequence similarity, described the coding region of a putative β -glucosidase of 61.2 kDa which expression was analysed by reverse transcriptase (RT-PCR) and Northern-blot analysis. The results indicated that the gene was regulated by abiotic stresses such as temperature, ethanol and pH.

Hydroxycinnamic acids (*p*-coumaric and ferulic acids) derivatives are important in the food aroma. The decarboxylation of these phenolic acids originates the formation of 4-vinyl phenol and 4-vinyl guaiacol that are considered food additives and are approved as flavouring agents (JECFA, 2001). The reduction of these vinyl phenols, originates ethyl phenol and ethyl guaiacol, which are considered the most important flavour components of fermented soy sauce (Yokosuka, 1986) or, on the other hand, are considered as *off flavour* and responsible of sensorial wine alterations (Chatonnet et al., 1992). It have been demonstrated that *L. plantarum* possessed two inducible phenolic acid decarboxylases. The phenolic acid decarboxylase (PDC) decarboxylates *p*-coumaric, ferulic and caffeic acids to their corresponding vinyl derivatives (Figure 1). Knockout of the *pdc* gene from *L. plantarum* reveals the existence of a second phenol acid decarboxylase enzyme, better induced with ferulic acid than with *p*-coumaric acid (Barthelmebs et al., 2000). This second decarboxylase remains uncharacterized. In

addition, *L. plantarum* also displayed an uncharacterized inducible acid phenol reductase activity, able to reduce the vinyl derivatives into ethyl derivatives, and to metabolize *p*-coumaric acid into phloretic acid (Table 2). It have been suggested that the synthesis of these inducible enzymes could be considered a specific chemical stress response to overcome phenolic acid toxicity (Gury et al., 2004).

It have been described that *L. plantarum* is a LAB species able to degrade tannins (Nishitani and Osawa, 2003; Nishitani et al., 2004; Vaquero et al., 2004). Gallic acid and the antioxidant pyrogallol were detected as final metabolic intermediates from tannic acid (Rodríguez et al., 2008a) (Table 2). Moreover, the degradation of phenolic compounds present in some plant-derived foods was studied. When the degradation of nine phenolic compounds found in olive products was tested it was found that only oleuropein and protocatechuic acid were metabolized by *L. plantarum* strains (Landete et al., 2008a). Oleuropein was metabolized mainly to hydroxytyrosol, while protocatechuic acid was decarboxylated to catechol (Table 2). The β -glucosidase and decarboxylase activities are involved in these degradations. Similarly, the *L. plantarum* capacity to degrade some phenolic compounds found in wine was studied (Landete et al., 2007). Of the ten compounds analyzed, only some hydroxycinnamic acids, gallic acid, and methyl gallate were metabolized by the *L. plantarum* strains analyzed. Vinyl and ethyl derivatives were originated from hydroxycinnamic acids, and pyrogallol from gallic acid. The enzymes involved on these degradations are a reductase and one (or several) decarboxylase. Since it has been described that *L. plantarum* possess several phenolic acid decarboxylases (Cavin et al., 1997b; Barthelmebs et al. 2000; Rodríguez et al., 2008c), the ability of several *L. plantarum* strains to metabolize nineteen food phenolic acids (hydroxycinnamic and benzoic acids) abundant in food substrates was also studied (Rodríguez et al., 2008d). Only six out 19 phenolic acids were metabolized

by the action of one (or several) decarboxylases and reductases (*p*-coumaric, caffeic, ferulic, *m*-coumaric, gallic, and protocatechuic acids) (Table 2 and Figure 1).

From the above results it could be deduced that *L. plantarum* possess metabolic ability to degrade some phenolic compounds. Nowadays, only tannase, *p*-coumaric acid decarboxylase (PAD), and benzyl alcohol dehydrogenase have been genetically characterized, while other enzymatic activities on phenolic compounds remain biochemical and genetically uncharacterized. The knowledge of the metabolism of phenolic compounds in *L. plantarum* is of great interest in food science and technology, as this bacterium possesses enzymes for the obtention of high-added value compounds, such as powerful antioxidants (hydroxytyrosol and pyrogallol) or food additives approved as flavouring agents (4-vinyl phenol and 4-vinyl guaiacol).

4.1.1. Tannase

Tannase or tannin acyl hydrolase (EC 3.1.1.20) catalyses the hydrolysis reaction of the ester bonds present in the hydrolysable tannins and gallic acid esters. At the moment, the main commercial application of tannase is the elaboration of instantaneous tea or of acorn liquor, and in the production of the gallic acid, which is used in the food industry because is a substrate for the synthesis of propylgallate, a potent antioxidant. Also, tannase is used as clarifying agent in some wines, juices of fruits and in refreshing drinks with coffee flavour, and during manufacture of beer to avoid the undesirable turbidity due to the accomplishing of proteins with tannins (Lehka and Lonsane, 1997; Belmares et al., 2004; Aguilar et al., 2007). Tannase could be used in the treatment of food effluents that contains high amounts of tannins, mainly polyphenols, which are

dangerous pollutants. The use of tannase on these residues represents a cheap treatment and cash for the removal of these compounds

Osawa et al. (2000) reported for the first time tannase activity in *L. plantarum* isolates. Later, this property was confirmed in *L. plantarum* strains isolated from various food substrates (Nishitani and Osawa, 2003; Nishitani et al., 2004; Vaquero et al., 2004). It has been postulated that this enzymatic property has an ecological advantage for this species, as it is often associated with fermentations of plant materials. In all these studies was used methyl gallate, a simple galloylester of methanol, as a substrate to be hydrolyzed by the *L. plantarum* tannase, and then, the gallic acid released from methyl gallate was oxidized to give a green to brown coloration, sufficient to be recognized visually or in a spectrometer (Inoue and Hagerman, 1988). A similar spectrophometric method was used by Lamia and Hamdi (2002) to demonstrate that *L. plantarum* produces an extracellular tannase after 24 h growth on minimal medium of amino acids containing tannic acid. However, Rodriguez et al. (2008b) described that HPLC chromatograms from supernatants of *L. plantarum* grown on a basal medium containing 1mM tannic acid for a week, were similar to the medium incubated in the same conditions. Therefore, if *L. plantarum* is able to produce an extracellular tannase to degrade tannic acid, more differences would be expected. In addition, *L. plantarum* tannase activity on complex hydrolysable tannins, as tannic acid, was firstly demonstrated by Rodriguez et al. (2008b) by using an HPLC analysis.

In order to confirm tannic acid degradation by *L. plantarum*, cell-free extracts obtained from disrupted *L. plantarum* cells were incubated in presence of tannic acid (Rodríguez et al., 2008a). After different incubation times, they were extracted and subjected to HPLC analysis. The results obtained that *L. plantarum* degrades tannic acid, a complex gallotannin, by depolymerization of high molecular weight tannins and

a reduction of low molecular weight tannins (Figure 3). Moreover, *L. plantarum* cell-free extracts, on a 6 h incubation period, degrades as much as 95% of the tannic acid compounds. The authors indicated that the proposed biochemical pathway for the degradation of tannic acid by *L. plantarum* implies that tannic acid is hydrolysed to gallic acid and glucose, and the gallic acid formed is decarboxylated to pyrogallol (Rodríguez et al., 2008a). This metabolic transformation implies the presence of tannase and gallate decarboxylase activities (Table 2). The presence of a gallate decarboxylase in *L. plantarum* has been previously reported (Osawa et al., 2000).

The biochemical properties of *L. plantarum* tannase in cell-free extracts have been described by using a colorimetric assay (Rodríguez et al., 2008b). Tannase activity was optimal at pH 5, whereas at pH 6 the enzyme retained only 40% of maximal activity. The optimum temperature for enzyme activity was found to be 30 °C. With further increase in temperature tannase activity was found to decrease. There was considerably good activity even at 50 °C, this is an additional advantage since some of the processes assisted by tannase are preformed at increased temperature. The effect of metal ions on the *L. plantarum* tannase activity was also studied (Rodríguez et al., 2008b). Tannase was found to be partially inactivated by the presence of Hg^{2+} and Mg^{2+} ions. However, metal ions like K^+ , Ca^{2+} , and Zn^{2+} did not affect *L. plantarum* tannase activity. The addition of some surfactants (Tween 80), chelators (EDTA), inhibitors (DMSO), and denaturing agents (urea) does not affect *L. plantarum* tannase activity at the concentration tested (Rodríguez et al., 2008b).

In order to identify *L. plantarum* tannase, concentrated cell-free extracts were fractionated by non-denaturing SDS-PAGE, and examined for enzyme activity by a zymogram. Tannase activity was localized in a single band (Rodríguez et al., 2008b). Recently, based on the tannase from *Staphylococcus lugdunensis*, Iwamoto et al. (2008)

searched on the complete genome sequence of *L. plantarum* WCFS1 for a similar protein. They found that lp_2956 (TanLp1) showed a 28.8% identity to *S. lugdunensis* tannase (Noguchi et al., 2007). The ORF of TanLp1, spanning 1410 bp, encoded a 469-amino acid protein with several conserved motifs with *S. lugdunensis* tannase, that were absent in other tannases reported for bacteria and fungi. TanLp1 was expressed in *E. coli* and the purified recombinant protein seemed to be a monomer polypeptide of approximately 50 kDa in size. Subsequent enzymatic characterization revealed that TanLp1 was most active in an alkaline pH range at 40 °C (Iwamoto et al., 2008). The results obtained suggest that *L. plantarum* tannase should be classified into a novel family of tannases.

4.1.2. Phenolic acid decarboxylase (PAD or PDC)

There are multiple reasons for improving the understanding of PAD enzymes as these enzymes are involved in the formation of useful volatile phenols derivatives which contribute naturally to aroma in wines and other fermented foods and beverages.

PAD activity may confer a selective advantage upon microorganisms during growth on plants, where PAD expression could constitute a stress response induced by phenolic acid. The *L. plantarum* phenolic acid decarboxylase (PAD) and its transcriptional repressor (PadR) have been cloned in *E. coli* and characterized at molecular level (Cavin et al., 1997a; Gury et al., 2004). It has been described that, in the absence of phenolic acids, PadR interacts with an operating DNA sequence downstream from the promoter boxes, which blocks the transcription of *padA* (Gury et al., 2004). When *p*-coumaric, ferulic or caffeic acid is added to a growing culture of *L. plantarum*, it inactivates PadR through a mechanism that is not yet entirely elucidated, but which

could need a specific mediator. The resulting production of PAD enzyme rapidly degrades the phenolic acid, and thus eliminates the stress caused by it. In *L. plantarum* the deletion of *padA* gene leads to the inhibition of growth in the presence of *p*-coumaric acid above 3 mM, especially at low pH (Barthelmebs et al., 2000). The deletion of *PadR* leads to a constitutive overexpression of the *padA* gene (Gury et al., 2004). *PadR* is divergently oriented from *padA*; moreover, it forms an operonic structure with *uspI*, a gene whose products display similarity to universal stress proteins. The expression of the three genes is very low in the non-induced condition, while the addition of 1.2 mM *p*-coumaric acid induces an increase in the expression of *padA*, *padR* and *uspI* by factors of 8,000, 37 and 13, respectively (Licantro-Seraut et al., 2008).

The *padA* gene from *L. plantarum* LPCHL2 has been cloned (Cavin et al., 1997a) and the recombinant protein overproduced in *E. coli*. Cavin et al. (1997b) have analyzed the substrate specificity of the purified PAD using 10 hydroxycinnamic acids, concluding that only the acids with a *para* hydroxyl group with respect to the unsaturated side chain and with a substitution of –H or –OH in position *meta* were metabolized. The construction of four chimeric bacterial PAD enzymes, which were functional and which displayed enzymatic activities different from those of the native activity, especially substrate specificity (Barthelmebs et al., 2001).

The complete genome sequence of *L. plantarum* WCFS1 and *L. plantarum* CECT 748, type strain, revealed that their PAD enzymes were identical and differed, mainly in their C-terminal region, from the enzyme previously purified from *L. plantarum* LPCHL2. The PAD from *L. plantarum* CECT 748 was also overproduced in *E. coli* (Rodríguez et al., 2008c) (Figure 4). Contrarily to *L. plantarum* LPCHL2, the recombinant PAD from *L. plantarum* CECT 748 is a heat-labile enzyme, showing

optimal activity at 22 °C. From the nineteen phenolic acids assayed, this PAD is able to decarboxylate exclusively *p*-coumaric, caffeic and ferulic acids (Table 2 and Figure 1). Kinetic parameters indicated that at high substrate concentrations, both *p*-coumaric acid and caffeic acids are much more efficiently decarboxylated than ferulic acid (Rodríguez et al., 2008c). This result indicates that the presence of bulky moieties in the *meta* position of the aromatic ring of the substrate clearly affects the binding step to the enzyme.

The purified PAD from *L. plantarum* behaves as a monomer-dimer associative equilibrium in solution (Rodríguez et al., 2007b). Its crystal structure has been solved and refined at 1.38 Å resolution. *L. plantarum* PAD is a homodimeric enzyme with single-domain subunits which show a novel fold consisting of a central β -sandwich endowed with a pseudo β -barrel topology (manuscript in preparation). Single point mutants have permitted identifying a potential substrate-binding pocket and proposed a catalytic mechanism for decarboxylation of hydroxycinnamic acids by *L. plantarum* PAD (manuscript in preparation).

4.1.3. Benzyl alcohol dehydrogenase

Aroma is an important sensory parameter of food products. LAB have enzymatic activities that could be important in the modification of food aroma. Several studies indicated that *L. plantarum* strains possess some glycosidase activities to hydrolyze odourless non-volatile glycosides to generate odor-active aglycons (Marsilio et al., 1996; Sestelo et al., 2004; Spano et al., 2005). The presence in *L. plantarum* of additional enzymatic activities able to modify the aglycons generated, such as some aromatic alcohols, has been also studied. From the analysis of the *L. plantarum* WCFS1

complete genome sequence, a protein annotated as aryl or benzyl alcohol dehydrogenase (lp_3054) (EC 1.1.1.90) could be identified (Kleerebezem et al., 2003). Benzyl alcohol dehydrogenase catalyzes the reversible oxidation of some aromatic alcohols to aldehydes with the concomitant reduction of NAD⁺. To confirm its enzymatic activity, lp_3054 from *L. plantarum* have been overexpressed and purified (Landete et al., 2008b). Protein alignment indicated that lp_3054 is a member of the family of NAD(P)-dependent long-chain zinc-dependent alcohol dehydrogenases. In lp_3054 all the residues involved in zinc and cofactor binding are conserved. It is also conserved the residue that determines the specificity of the dehydrogenase toward NAD⁺ rather than NADP⁺ and, therefore *L. plantarum* benzyl alcohol dehydrogenase was less active in the presence of NADP⁺ than in the presence of NAD⁺. The purified dehydrogenase exhibited optimal activity at pH 5.0 and 30 °C. Aromatic alcohol dehydrogenases are, in general, specific for aromatic alcohols, with a preference for aromatic alcohols with small substituent groups. Several compounds frequent in food substrates were tested as substrates for *L. plantarum* dehydrogenase (Landete et al., 2008b). Nonalcohol compounds (e.g. *p*-coumaric and caffeic acids) as well as some nonaromatic alcohols (e.g., ethanol) were not reduced by benzyl alcohol dehydrogenase. From the compounds assayed, benzyl alcohol was an effective alcohol substrate for the *L. plantarum* enzyme. The other enzyme substrates were most of them aromatic in nature. It was also described that the electron-withdrawing properties of the substituent groups on the aromatic ring may also be involved in dictating the acceptability of a particular substrate. *L. plantarum* benzyl alcohol dehydrogenase is in general specific for aromatic alcohols or cyclic alcohols such as perillyl, cinnamyl, and coniferyl alcohol (Table 2); however, it was also able to catalyze efficiently the oxidation of some allylic alcohols, such as geraniol and nerol.

Cinnamyl and coniferyl alcohol are both known intermediates of lignin biosynthesis and degradation. Although cinnamyl alcohol or, presumably, coniferyl alcohol cannot serve as sole carbon source for *L. plantarum*, their oxidation could give some energy to the bacteria, and the compounds produced would then be available for metabolism of other microorganisms.

Among the aromatic alcohols oxidized by *L. plantarum* benzyl alcohol dehydrogenase, benzyl alcohol and phenethyl alcohol, as well as the benzaldehyde produced, are volatile compounds important for the aroma of food products, such as grape must or wine (Rosillo et al., 1999).

4.2. Other lactic acid bacteria species

Similarly to *L. plantarum*, the studies on the metabolism of phenolic compounds on other LAB species are scarce. Only a few reports have been described on *L. paracollinoides*, *L. hilgardii* and *O. oeni* metabolism.

Whiting and Carr (1957) described that chlorogenic acid frequently disappeared during cider fermentation. Studies using cell-extracts from *L. paracollinoides* (formerly named *L. pastorianus* var. *quinicus*) indicated that the first stage into the metabolism of chlorogenic acid was the hydrolysis to caffeic acid and quinic acids. Both products were further metabolised. Later, it was shown that caffeic acid is metabolised with the formation of dihydrocaffeic acid and ethyl catechol (Whiting and Carr, 1959). In addition, *L. paracollinoides* was able to reduce the side-chain of 3, 4-hydroxy and 3-hydroxycinnamic acids. Their products may then be decarboxylated to ethyl catechol and ethyl phenol. Whiting and Coggins (1969) also described that *L. paracollinoides* reduces quinate to shikimate and to dihydroshikimate.

It has been described that *L. hilgardii* degraded gallic acid and catechin (Alberto et al., 2004). *L. hilgardii* was grown in a complex medium containing gallic acid or catechin. In acid gallic-grown cultures, gallic acid, pyrogallol, catechol, protocatechuic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzyl alcohol were detected. In catechin-grown cultures, catechin, gallic acid, pyrogallol, catechol, *p*-hydroxybenzoic acid, acetovanillone, and homovanillic acid were detected.

LoCascio et al. (2006) described that *P. pentosaceus* cultures reduce 21.3% the quercetin present in the culture media, however, the mechanism of quercetin loss (absorption or transport into the cell) remains unknown. It has been also described that *O. oeni* strains, by a glycosidase action, metabolize anthocyanins and other phenolics producing important wine aroma compounds (Vivas et al., 1997; Boido et al., 2002; Ugliano et al., 2002; D’Incecco et al., 2004; de Revel et al., 2005; Bloem et al., 2008). As glycosidases are interesting enzymes for the hydrolysis of glycoconjugated precursors, therefore several surveys of glycosidase activities has been made on *O. oeni* strains, commercial (Grimaldi et al., 2005) as well as selected wild strains (Barbagallo et al., 2004). A remarkable variability was found in the β -glucosidase activity among the analyzed *O. oeni* strains.

Bloem et al. (2007) studied the production of vanillin from simple phenols by wine-associated LAB. They found that LAB were not able to form vanillin from eugenol, isoeugenol or vanillic acid. However, *O. oeni* or *Lactobacillus* sp. could convert ferulic acid to vanillin, but in low yield. Moreover, LAB reduced vanillin to the corresponding vanillin alcohol. Concerning the production of volatile phenols, Cavin et al. (1993) have shown that *p*-coumaric and ferulic acids were decarboxylated by *L. brevis*, *L. plantarum*, and *P. pentosaceus*. Later, Chatonnet et al. (1995) reported that some strains of *L. brevis* and *P. pentosaceus* were able to decarboxylate *p*-coumaric

acid to form 4-vinyl phenol. *Oenococcus oeni* synthesized small quantities of 4-vinyl phenols, and only *L. plantarum* was able to produce ethyl phenols. Recently, Couto et al. (2006) screened the ability to produce volatile phenols in lactic acid bacteria belonging to 20 different species. They found that only species from the *Lactobacillus* genera (*L. brevis*, *L. collinoides*, and *L. plantarum*) were able to reduce vinyl phenols to the corresponding ethyl phenols. *p*-Coumaric acid was not metabolized by the *O. oeni* and the *L. mesenteroides* strains analyzed. *Lactobacillus hilgardii* strains were unable to metabolize *p*-coumaric acid (Couto et al., 2006). Van Beek and Priest (2000) established the wide distribution of the gene *padA* (or *pdc*) encoding a phenolic acid decarboxylase (PAD or PDC) in several strains of *Lactobacillus* isolated from whisky fermentations and described two primers for the PCR detection of the *padA* gene. Recently, based on an alignment of the PAD genes present on the database from the recently sequenced LAB genomes (Makarova et al., 2006; Makarova and Koonin, 2007), De las Rivas et al. (2009) described a PCR assay for the detection of LAB that potentially produce volatile phenols. The *padA* PCR method was applied to strains belonging to the six main wine LAB species. The results showed that *L. plantarum*, *L. brevis*, and *P. pentosaceus* strains produce a positive response in the *padA* PCR assay, whereas *O. oeni*, *L. hilgardii*, and *L. mesenteroides* strains did not produce the expected PCR product. A relationship was found between the presence of the *padA* gene and volatile phenol production; so, the LAB strains that gave a positive *padA* PCR response produce volatile phenols, whereas strains that did not produce a PCR amplicon did not produce volatile phenols.

5. Treatment of food by-products by lactic acid bacteria

Disposal of the waste generated by several food industries constitutes a serious environmental problem due to the presence of phenolic compounds that causes difficulties for their biological treatment (Arvanitoyannis and Kassaveti, 2007). There is a growing interest in the exploitation of these by-products in order to obtain high-added value compounds and to reduce the environmental problem (Lafka et al., 2007; Agalias et al., 2007; Brenes et al., 2004). There are some proposals of the use of LAB for the revaloration of food by-products where phenolics compounds are abundant.

LAB with their capacity to reduce oxygen pressure, redox potential and pH, offers a new promising approach to the bioconversion of phenolic compounds present in olive wastes (Arvanitoyannis and Kassaveti, 2007). Generally the olive industry produces two residues, solids and olive mill wastewater (OMW). OMW is one of the most complex plant effluents. The ecological problem of OMW is due primarily to the presence of phenolic compounds, which make OMW toxic and resistant to biological degradation. OMW is unstable and turns black under aerobic conditions because of the auto-oxidation of phenolic compounds (Arvanitoyannis and Kassaveti, 2007). The effects of *L. plantarum* growth on the reductive decolorization and biodegradation of olive phenolic compounds was evaluated by Lamia and Moktar (2003). *L. plantarum* growth on fresh OMW induced the depolymerization of phenolic compounds of high molecular weight, with a resultant decolorization of fresh OMW. These authors found that approximately 58% of the colour, 55% of the chemical oxygen demand, and 46% of the phenolic compounds were removed when OMW was diluted ten times. The removal of phenolic compounds was associated with the depolymerization, the partial adsorption on the cells and the biodegradation of certain simple phenolic compounds. In addition, it had been described that the application of *L. plantarum* to the olive fruit during crushing could constitute a new microbiological process for olive oil quality

improvement. Kachouri and Hamdi (2004) 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 depolymerization 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. The authors concluded that olive oil mixed with the OMW and fermented by *L. plantarum* had a higher quality and stability because of a higher content of simple phenolic compounds.

Disposal of the waste from wine production has long been a problem from wineries. In total, more than 20% of wine production is waste, comprising thousands of tons, which constitutes a serious environmental problem (Arvanitoyannis et al., 2006). *p*-Coumaric acid is a representative compound of the phenolic fraction of wine distillery wastewater (Arvanitoyannis et al., 2006). *L. plantarum* is able to decarboxylate *p*-coumaric acid to vinyl phenol by the action of PAD; therefore, the growth of *L. plantarum*, or the use of purified *L. plantarum* PAD, in a *p*-coumaric acid solution will convert this phenolic compound into a less toxic derivative, vinyl phenol, which is a valuable intermediate in the biotechnological production of new fragrance chemicals, and also is considered a food flavouring agent (JECFA, 2001). Moreover, leachates, which are solutions of tannins and other compounds that are separated from the grape marc during wine-making elaboration, can cause oxygen depletion in the soil and can infiltrate surface soil and ground water. *L. plantarum*, of its pure tannase enzyme, could be used to obtain high-added-value antioxidants from the degradation of contaminant

phenolic compounds found in wine wastes as *L. plantarum* strains possess tannase activity able to hydrolyze ester bonds present in hydrolyzable tannins, releasing gallic acid which is subsequently decarboxylated to produce pyrogallol. Gallic acid and pyrogallol are powerful antioxidants compounds (Ordoudi and Tsimidou, 2006).

6. Conclusions

Some LAB species are adapted to growth in plant-derived food substrates where phenolic compounds are abundant. Most of the phenolic compounds studied exert an inhibitory effect on LAB growth. This inhibition activity seems to be related to alterations in cytoplasmic membranes and in the cell wall. Up to now, metabolisms of a limited number 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 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.

Acknowledgments

This work was supported by grants AGL2005-00470, AGL2008-01052, Consolider INGENIO 2010 CSD2007-00063 FUN-C-FOOD (CICYT), RM2008-00002 (INIA), and S-0505/AGR/000153 (CAM). We are grateful to M. V. Santamaría and J. M. Barcenilla. We thank C. Ascaso, F. Pinto, and S. Paniagua for the transmission electron micrographs. H. Rodríguez and J. A. Curiel were recipients of predoctoral

fellowships from the I3P-CSIC Program and FPI-MEC, respectively. J. M. Landete was a recipient of a postdoctoral fellowship from the MEC.

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1199

1200 **Figure captions**

1201

1202 Figure 1. Structure of some phenolic acids reported to be metabolized by *L. plantarum*
1203 strains whose growth effects have been also studied.

1204

1205 Figure 2. Transmission electron micrographs of a septum among two *L. plantarum*
1206 CECT 748^T cells grown in the presence of a phenolic compound (0.5 mM tannic acid)
1207 (100000 X). Black bar represents 200 nm.

1208

1209 Figure 3. HPLC chromatograms showing disappearance of tannic acid by cell-free
1210 extracts of *L. plantarum* CECT 748^T after 20 h incubation at 37 °C. Chromatograms
1211 were recoded at 280 nm. AU, absorbance units.

1212

1213 Figure 4. Expression and purification of the PAD protein from *L. plantarum* CECT
1214 748^T. SDS-PAGE analysis of soluble cell extracts of *E.coli* cultures from control cells
1215 (lane 1), and from cells carrying pURI-PAD plasmid (lane 2). Fractions eluted after
1216 affinity column (lane 3 to 8). The positions of molecular mass markers (Bio-Rad) are
1217 indicated on the left.

Table 1. Main LAB species isolated from some vegetable fermentations

Vegetable source	LAB species	References
Olives	<i>Lactobacillus plantarum</i>	Ruiz-Barba et al., 1994a, 1994b
	<i>Lactobacillus pentosus</i>	Nychas et al., 2002
	<i>Leuconostoc mesenteroides</i>	Panagou et al., 2003
	<i>Leuconostoc pseudomesenteroides</i>	Vega Leal-Sánchez et al., 2003
	<i>Pediococcus pentosaceus</i>	Ercolini et al., 2006 Chamkha et al., 2008 Hurtado et al., 2008
Cabbage	<i>Leuconostoc mesenteroides</i>	Plengvidhya et al., 2007
	<i>Lactobacillus brevis</i>	
	<i>Pediococcus pentosaceus</i>	
	<i>Lactobacillus plantarum</i>	
	<i>Leuconostoc citreum</i>	
Cucumbers	<i>Lactobacillus paraplantarum</i>	
	<i>Lactobacillus plantarum</i>	Tamminen et al., 2004
	<i>Lactobacillus brevis</i>	
	<i>Lactobacillus pentosus</i>	
	<i>Leuconostoc</i> sp.	
Eggplants	<i>Pediococcus</i> sp.	
	<i>Lactobacillus plantarum</i>	Sánchez et al., 2004
	<i>Lactobacillus fermentum</i>	Seseña et al., 2005
	<i>Lactobacillus pentosus</i>	Seseña and Palop, 2007
	<i>Lactobacillus brevis</i>	
Caper berries	<i>Lactobacillus plantarum</i>	Pérez-Pulido et al., 2007
	<i>Lactobacillus paraplantarum</i>	
	<i>Lactobacillus pentosus</i>	
	<i>Lactobacillus brevis</i>	
	<i>Lactobacillus fermentum</i>	
Grape must	<i>Oenococcus oeni</i>	Moreno-Arribas et al., 2003
	<i>Lactobacillus plantarum</i>	Rodas et al., 2005
	<i>Lactobacillus brevis</i>	
	<i>Lactobacillus hilgardii</i>	
	<i>Leuconostoc mesenteroides</i>	

Table 2. Metabolism of aromatic compounds by *L. plantarum* strains

Compound assayed	Compound produced	Enzymes involved	References
Benzyl alcohol	Benzaldehyde	Benzyl alcohol dehydrogenase	Landete et al., 2008b
Caffeic acid	Vinyl catechol Ethyl catechol	PAD Reductase	Cavin et al., 1997a Cavin et al., 1997b Barthelmebs et al., 2000 Rodríguez et al., 2008c Rodríguez et al., 2008d
Cinnamyl alcohol	Cinnamyl aldehyde	Benzyl alcohol dehydrogenase	Landete et al., 2008b
Coniferyl alcohol	Coniferyl aldehyde	Benzyl alcohol dehydrogenase	Landete et al., 2008b
<i>m</i> -Coumaric acid	3-(3-hydroxyphenyl) propionic acid	Reductase	Rodríguez et al., 2008d
<i>p</i> -Coumaric acid	Vinyl phenol Ethyl phenol	PAD Reductase	Cavin et al., 1997a Cavin et al., 1997b Rodríguez et al., 2008c Rodríguez et al., 2008d
Ferulic acid	Phloretic acid Vinyl guaiacol Ethyl guaiacol	Reductase PAD Reductase	Barthelmebs et al., 2000 Cavin et al., 1997a Cavin et al., 1997b Rodríguez et al., 2008c Rodríguez et al., 2008d
Gallic acid	Hydroferulic acid	Reductase	De las Rivas et al., 2009
Methyl gallate	Pyrogallol Gallic acid	Decarboxylase Tanase	Rodríguez et al., 2008d Rodríguez et al., 2008d
Oleuropein	Pyrogallol Hydroxytyrosol	Decarboxylase β -Glucosidase Esterase	Rodríguez et al., 2008d Ciafardini et al., 1994 Marsilio et al., 1996 Marsilio and Lanza, 1998 Landete et al., 2008a
Phenethylalcohol	Phenethylaldehyde	Benzyl alcohol dehydrogenase	Landete et al., 2008b
Protocatechuic acid	Catechol	Decarboxylase	Rodríguez et al., 2008d
Quinic acid	Catechol	Several enzymes	Whiting and Coggins, 1971 Whiting and Coggins, 1974 Whiting, 1975
Shikimic acid	Catechol	Several enzymes	Whiting and Coggins, 1971 Whiting and Coggins, 1974 Whiting, 1975
Tannic acid	Gallic acid Pyrogallol	Tannase Decarboxylase	Rodríguez et al., 2008a Rodríguez et al., 2008b

Figure 1

Figure 1

Figure 2

Figure 3

Figure 3

Figure 4

Figure 4