Nitrogen metabolism in lactic acid bacteria from fruits: a review

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Lactic acid bacteria (LAB) are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of the proteins present in raw material. Generally nitrogenous compounds concentration in fruits and their derivate products are poor. Strains of *Oenococcus oeni* from wine show a proteolytic system that provides amino acids that are used for their metabolism and growth. Amino acids requirements and utilization by LAB from fruits show the different behaviors between strains of the same species to growth in a nutritional poor environment. LAB strains produce biogenic amines from decarboxylation of the corresponding amino acid. From arginine degradation via Arginine Dihydrolase System, (ADI) the carbamyl groups formed could react with the alcohol present in a fermented beberage to produce ethylcarbamate (EC) a cancerigen compounds.

Keywords: lactic acid bacteria; nitrogen metabolism; proteolysis; amino acids; biogenic amines; ethylcarbamate

Las bacterias lácticas son microorganismos nutricionalmente muy exigentes. Requieren una fuente exógena de aminoácidos o peptidos que son proporcionados por la proteolysis de las proteínas presentes en la materia prima. La concentración de compuestos nitrogenados en frutas y sus derivados es pobre. Cepas de *Oenococcus oeni*, aisladas de vino presentan un sistema proteolítico que proporciona aminoácidos utilizados para su metabolismo y crecimiento. Los requerimientos y utilización de amino ácidos muestran los diferentes comportamientos entre cepas de la misma especie para crecer en un ambiente de estrés nutricional. Cepas de bacterias lácticas producen aminas biogénicas. La degradación de arginina por el Sistema Arginina Dihidrolasa produce grupos carbamilo que reaccionan con el etanol de bebidas fermentadas produciendo el compuesto cancerígeno, etilcarbamato.

Palabras clave: bacterias lácticas; metabolismo de compuestos nitrogenados; proteólisis; aminoácidos; aminas biogénicas; etilcarbamate

1. Introduction

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The living system is characterized by the ability to direct chemical reaction and organize molecules into specific structures. The ultimate expression of this organization is the self-replication. The chemicals from the environment that are utilized for bacterial growth are called nutrients. During anabolism, nutrients are taken up and are changed into cell constituents in an energy-depending process. Different nutrient groups exist and are broadly classified into those providing energy such as carbohydrates, and those used as components in cellular structures such as peptides. Most of

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microorganisms require an organic compound as their carbon source including carbohydrates, peptides or amino acids, fatty acids, organic acids, nitrogen bases and aromatic compounds [1-3]

Lactic acid bacteria (LAB) have numerous nutritional requirements for growth, especially nitrogen sources. The general assumption is that biomass synthesis in lactic acid bacteria is predominantly from building blocks present in the culture medium. Generally nitrogenous compounds concentration in fruits and their derivate products are poor. The most important application of LAB is their use as starter strains in the manufacture of various fermented products. LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of the proteins present in raw material. After carbon the most abundant element in the cell is nitrogen. A typical bacterial cell is about 12% nitrogen (by dry weight) and it is a main constituent of protein, nucleic acids, and several other constituents in the cell. Bacteria are capable of using ammonia inorganic [4]

2. Proteases

Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation. Microbial proteases account for approximately 40% of the total worldwide enzyme sales [5]. Proteases from microbial sources are preferred to the enzymes from plant and animal sources since they possess almost all the characteristics desired for their biotechnological applications.

In contrast with the well characterized proteolytic system of dairy LAB, the proteolytic system of fruits, fruits juices and fermented fruits LAB remains not extensively studied. Although Davis et al. [6] failed to detect protease activity in several wines LAB examined, including several strains of oenococci, pediococci and lactobacilli, the research group of Manca de Nadra demonstrated by the first time the production, characterization, regulation and purification of an extracellular proteases by strains of *Oenococcus oeni* [7-13], (formerly *Leuconostoc oenos*; Dicks et al. [14]. As the fermented must, produced from grapes, is a nutritionally poor ecological niche the LAB must develop strategies to survive and growth. Guilloux-Benatier et al. [15] reported that wine have few nutrients available for LAB in general and specifically for *O. oeni* strains

O. oeni is the preferred species used to conduct the malolactic fermentation (MLF)) in wines due to its acid tolerance and its contribution to the wine flavor. The MLF is a biological process of wine deacidification in which the dicarboxylic L-malic acid is converted to the monocarboxylic L-lactic acid and carbon dioxide. *O. oeni* strains have complex nutritional requirements [16, 17]. Amino acids produced from proteases hydrolysis are important for the growth of this microorganism, both as nitrogen and carbon source [18, 19].

Two separate extracellular proteolytic activities in four strains of *O.oeni* isolated from Argentinian wine were reported [7]. The first took place in the early growth phase and the second had its maximum at the end of growth phase in all strains. The production of the two proteolytic enzymes had different pH and temperature optima and is affected by divalent cations. They were characterized and maximum production was found with autoclaved grape juice as substrate [8]. The enzymes were thermostables and their activities were unaltered by heating at 70°C for 15 min. Cysteine and ß-merchaptoethanol were strong inhibitors of both enzymes, indicating the involvement of disulphide bridges.

Manca de Nadra and coworkers described that the protease II from *O.oeni* was able to liberate detectable concentrations of amino acids from protein and polypeptide extracts from nitrogenous macromolecular fraction (NMF) of white and red wines [9, 11].

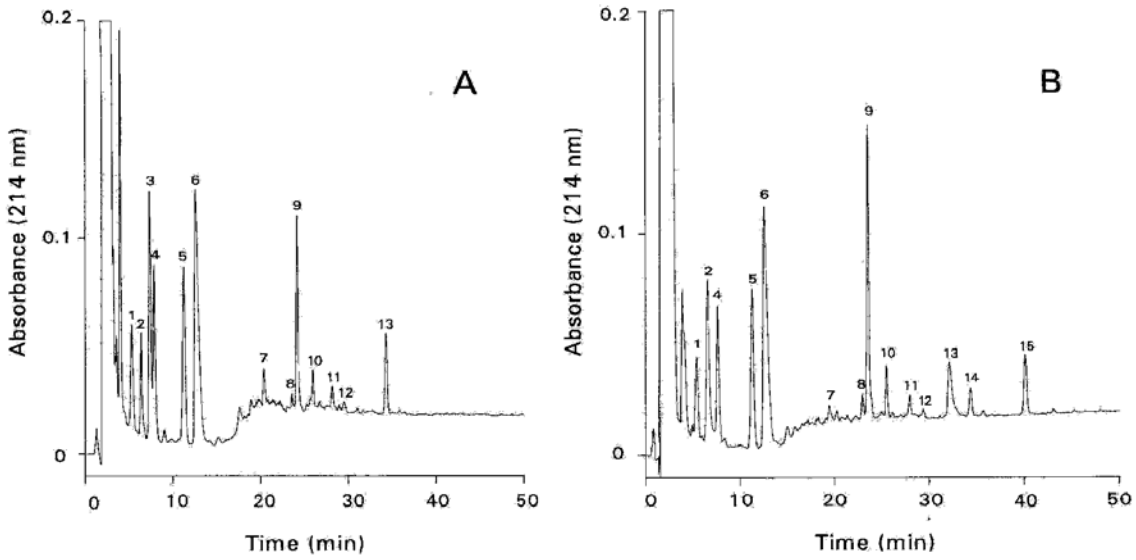


Fig 1. HPLC análisis of peptides before (A) and after (B) of Lc. oenos X2L protease action. With respect to the chromatograph before the protease action, the numbers 14 an 15 indicate peaks without equivalent, numbers 2 and 9 indicate increase peaks, and the number 3 was not detected

Protease action on the NMF from white wine reveals two new peptide peaks and an increase in two other peptide peaks (Fig 1). In total 56.7 mg l^{-1} peptides was liberated by protease action and essential amino acids for *O. oeni* growth were liberated as free amino acids. Arginine, which has a stimulatory effect on *O. oeni* growth, was quantitatively the more important amino acid obtained by protease activity.

The HPLC analysis of peptides before and after the protease activity of *O. oeni* on NMF from red wine shows three new peaks, 6 higher and one lower in the chromatograph obtained after the enzyme action. It produces 148.7 mg l^{-1} of individual amino acids showing a difference of 109 mg l^{-1} with respect to the amino acids produced from white wine. This property is important considering the lower protein concentration in red wine.

The LAB are able to respond to changes in nitrogen availability by regulating the activity of the proteolytic system to ensure proper nitrogen balance in the cell. It was found that the synthesis of many exoproteins is influenced, in part at least, by the levels of individual nutrients in the extracellular environment. Taking account the stress conditions for *O.oeni* in wine, the regulation of a strain exoprotease production and secretion under unfavorable conditions was studied [10]. Starved cells after 2 h incubation at 30°C in citrate buffer 0.05 mmol l^{-1} pH 5 showed greater extracellular proteolytic activity than at the onset of starvation. In the presence of 60 mg l^{-1} SO_2 and 8% or 12% ethanol (both compounds normally present in wine), the proteolytic activity was higher and the role of Ca^{2+} in maintaining the attachment of the proteinase to the cell was determined. In starvation condition the exoprotease production is subject to catabolite repression by glucose and its analogue non-metabolizable 2-DOG. This effect is relieved by the addition of cAMP. The addition of chloramphenicol immediately stopped protease formation, showing that the appearance of extracellular protease in starvation conditions resulted from rapid export of the product of de novo protein synthesis, rather than secretion of protease accumulated intracellulary. The chloramphenicol effect also suggests that the protease activity was not a result of passive cells lysis. This fact was confirmed by the absence of protease activity in the supernatant from the antibiotic treated cells showing that it is a result of deliberate release.

Manca de Nadra et al, [13] evaluated the proteolytic activity of *O. oeni* in living cells in presence of SO_2 and ethanol, using the NMF of red wine as sole nitrogen source. In this stress condition, the rate of amino acids liberation from NMF was two fold higher. HPLC peptide analysis revealed a peak with a retention time of 47 min that diminished markedly during the first 2 h of incubation. The regulatory

mechanism of protease production in stress condition could be related to the survival of *O. oeni* in its natural environment.

The exoprotease from *O.oeni* produced in stress conditions was purified to homogeneity [12]. The molecular mass was estimated to be 33.1 kDa by gel filtration and 17 kDa by SDS-PAGE and these results suggest that the enzyme consisting of two identical subunits. Optimal conditions of the purified enzyme activity on grape juice were 25°C and pH 4.5. The effect of different inhibitors (EDTA, *o*-Phenanthroline, Iodoacetate, *p*-Hydroxymercuribenzoate, PMSF and 1,4 Pepstatin A) on purified protease activity from *O. oeni*, demonstrated that the enzyme was completely inhibited by pepstatin A. The properties of the enzyme suggest that the protease could be involved in the wine elaboration.

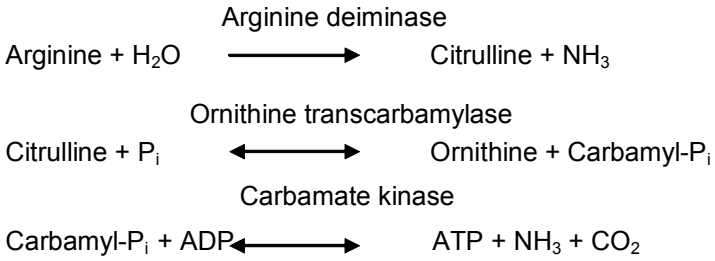
3. Amino acids

Microorganisms with complex nutritional requirements may need small amounts of organic nitrogen compounds for growth because they are substances that the microorganism is unable to synthesize from available nutrients. Such essential nutrients, called growth factors, include purines and pyrimidines (necessary for synthesis of nucleic acids), amino acids (required for synthesis of proteins) and vitamins (needed as coenzymes and functional groups of certain enzymes). The growth factors are not metabolized directly as sources of carbon or energy; rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant bacterial strains that require growth factors not needed by the wild type (parent) strain are referred to as auxotrophs. Amino acid catabolism could play an important role in the ability to obtain energy in nutrient-limited environments; however, the catabolic pathway of many amino acids in LAB is still not clear. Some LAB degrade arginine to citrulline, ornithine and ammonium via the ADI pathway to produce additional energy.

Amoroso et al. [18] demonstrate that the L-malic and citric acids addition individually or in combination to the synthetic media deficient in one amino acid eliminated their amino acid requirements of *O. oeni* strains, suggesting that in terms of nutritional requirements these acids played a beneficial role. In this condition, only one of the four *O. oeni* strains analyzed increased its requirements from four to nine amino acids (DL-alanine, L-asparagine, L-isoleucine, L-leucine, L-Lysine, L-tyrosine, L-threonine, L-valine and L-glycine). Moreover, Saguir and Manca de Nadra [19] demonstrated that both L-malic and citric acids allowed the growth of an *O. oeni* strain, when different combinations of essential amino acids (e.g. L-asparagine and L-isoleucine, L-asparagine and L-cysteine or L-isoleucine and L-cysteine) were successively omitted from synthetic medium. Tracey and Britz [20] found that certain strains of *O. oeni* yielded similar growth in a synthetic medium and in the same medium with L- malic acid where six amino acids were omitted. Thus, the essential amino acids could be synthesized from intermediaries metabolically derived from organic acids in poor nutritional conditions. Saguir and Manca de Nadra [21] demonstrated in an *O. oeni* strain, that the fermentation balance from glucose and citrate catabolism in all media deprived of L-cysteine was always accompanied with a carbon imbalance from glucose or glucose-citrate to D-lactic acid, indicating that glucose metabolism could be involved in the synthesis of L-cysteine. On citric acid metabolism in the media lacking asparagine or isoleucine, the lower recovery of glucose-citrate as D-lactic acid was only observed when citrate was present in the culture medium, indicating that part of citrate metabolism was diverted by *O. oeni* for these amino acids synthesis, via oxalacetate. Several years ago, Manca de Nadra et al. [22, 23] reported that some LAB are able to degrade L-arginine.

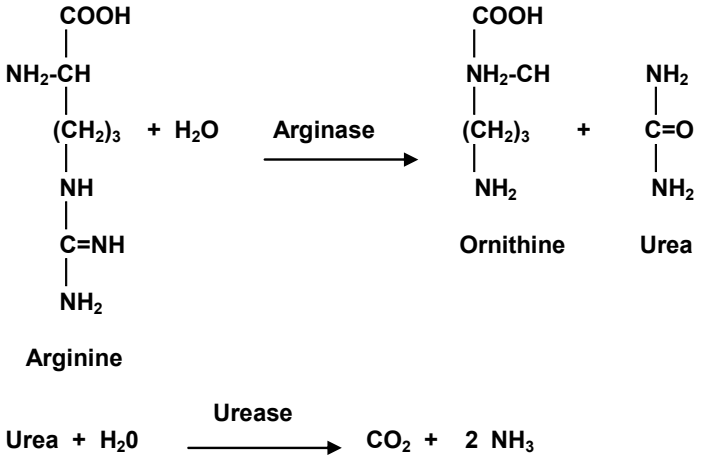
3.1 Arginine

L-arginine is one of the major amino acids found in citrus and grapes juices and wines. Arginine utilization in LAB when is degraded to citrulline, ornithine and ammonium, via ADI pathway, produces additional energy.



Arginine dihidrolase system (ADI)

Arena et al. [24] reported in two strains of *Lactobacillus plantarum* from oranges, that the metabolic products observed during the arginine or citrulline degradation indicates that only the ADI pathway was involved. The arginase urease system was not observed. With different behavior, both *L. plantarum* strains were able to derive energy and ammonia from arginine or citrulline catabolism. This is interesting for microorganisms developing in a stressful environment.



Arginase-Urease System

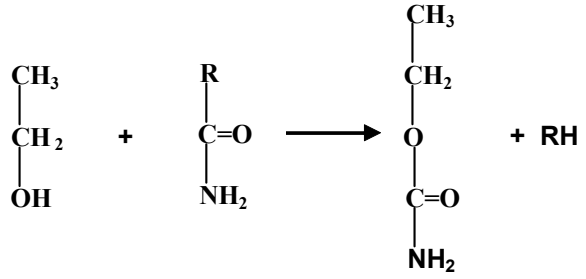
Arena et al. [25] also investigated the catabolism of arginine, citrulline and ornithine in four LAB isolated from wines. They found that only the strain X_1B of *Lactobacillus hilgardii* catabolized arginine and excreted citrulline into the medium. The recovery of arginine as ornithine was lower than the expected theoretical value. As in the LAB from orange, the arginase-urease pathway was not detected indicating that the amino acid degradation was carried out only by the arginine dihydrolase pathway. A strain of *O. oeni* that was not able to utilize arginine, degraded citrulline that was completely recovered as ornithine, ammonia and CO_2. The citrulline utilization by this strain may be important for two reasons: it can gain extra energy for growth from citrulline metabolism, and the amino-acid diminution could avoid the possibility of ethyl carbamate formation from the citrulline naturally present.

Saguir and Manca de Nadra [26] improved a basal synthetic medium for the growth of *L. plantarum* strains and to establish their amino-acid requirements. The basal medium was improved mainly on the basis of annulling limitations with respect to amino acids. In this improved medium cell densities on the order of 10^9 colony-forming units/mL have been achieved, indicating that it could be used for conducting metabolic and genetic studies on *L. plantarum*. The main arginine consumption observed

during growth of *L.plantarum* might be explained as an adaptation response to the conditions of orange juice medium, where arginine concentration is high.

3.2 Ethyl carbamate

One of the major concerns in arginine and citrulline metabolism by wine LAB is their association with the formation of ethyl carbamate (EC). EC precursors can be formed by yeasts [27-29] or



Ethanol **Carbamyl group** **Ethyl carbamate**

Ethyl carbamate formation

bacteria [30]. Several possible sources for the formation of EC have been proposed. This formation is a spontaneous chemical reaction involving ethanol and a compound that contains a carbamoyl group such as urea, citrulline or carbamyl phosphate. A variety of concentrations of EC have been found in wines. Criteria related to consumer concern regarding environmental health issues include the demand for low-alcohol wine and ethyl-carbamate-free beverages. Detection of EC in wines has prompted the selection of strains producing no, or only traces of, ethyl carbamate precursors.

Arena et al. [31] (2005) reported the effects of ethanol and pH on growth and arginine and citrulline metabolism in two heterofermentative LAB from wine and its relation with the formation of EC. Arginine and citrulline are substrates that can be used to keep LAB viable, and the ability of the strains studied to utilize these amino acids in the presence of ethanol is an important property in surviving adverse conditions. Arginine stimulated growth of *L. hilgardii* under all conditions studied, and was partially recovered as citrulline and ornithine. *L. hilgardii* has the potential to contribute to EC formation through the production of citrulline, whereas citrulline utilization by a *O. oeni* strain in the presence of ethanol could possibly avoid the formation of EC.

Tonon and Lonvaud-Funel [32] reported that all *L. hilgardii* strains studied have the ability to degrade arginine via the ADI system. Although LAB effectively degraded arginine, this led to only a moderate pH increase because arginine degradation favored formation of acid from sugar [33]. The total amount of arginine utilized decreased with increasing ethanol concentrations. The higher specific consumption of arginine at pH 3.8 than at 6.5 could be explained by considering the inhibitory effect of the low pH on growth. The arginine used at low pH probably serves more as energy for survival than for growth. The amount of maintenance energy required at low pH is high [34]. When pH decreased, arginine utilization increased. However, specific arginine consumption was not modified by the increase in ethanol, indicating that ethanol only affected bacterial growth.

According to the maximum concentration of EC suggested in wine, 15 ng ml -1 in the USA and 30 ng ml -1in Canada, the citrulline produced by *L. hilgardii* through metabolism of arginine and its subsequent conversion into EC may account for an important amount of this compound. EC was detected in table wine at concentrations between 0 and 102 ng ml -1 [35].

Six percent of Canadian red wines tested were above the permitted level [36]. Azevedo et al. [37] reported that citrulline is apparently the main EC precursor produced by *L. hilgardii* strains in spoiled, fortified wine. The metabolism of arginine by *Pediococcus halophilus* was thought to

be the source of an EC precursor in soy sauce [38]. Uthurry et al. [39] showed that ethanol was not directly correlated with the amount of EC in Spanish wines.

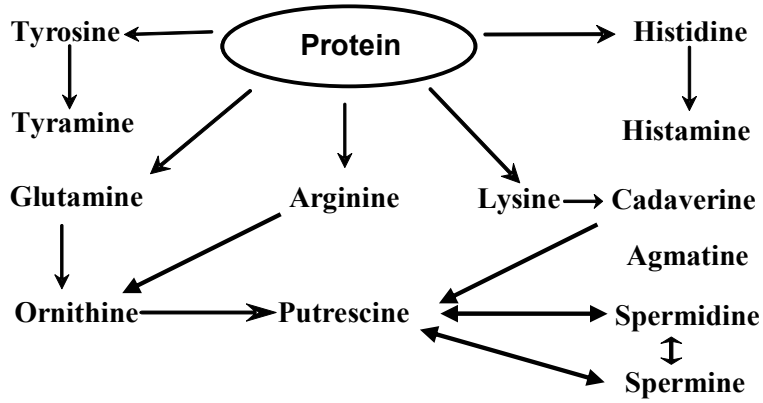
G. Spano et al. [40] determined the activity of the enzymes arginine deiminase and ornithine transcarbamylase in *L. plantarum*.The citrulline and ornithine formed were analysed by HPLC analysis. Although the enzymatic activity was detected in all the strains analysed, a strong variability was observed. One of them was able to accumulate a high concentration of citrulline, precursor of the carcinogenic ethyl-carbamate, as showed by its high arginine deiminasa activity and low ornithine transcarbamylase activity. The ecological flexibility of *L. plantarum* is reflected by the observation that this species has one of the largest genomes known among (LAB) [41]. Although in wine *L. plantarum* is capable of malolactic fermentation, it usually contributes to production of undesirable substances such as biogenic amine and precursors of ethyl carbamate during and after winemaking. [42, 43].

The presence of genes (*arcABC*) coding for enzymes involved in the ADI pathway in wine *L. plantarum* were reported [44, 45]. The high identities among arginine deiminase (ADI), ornithine transcarbamylase (OTCase) and carbamate kinase (CK) protein sequences between *O. oeni* and *L. plantarum* and the induction of *arcABC* genes by arginine suggested that the putative genes cloned controlled arginine catabolism in *L. plantarum*. Arena et al. [46] have sequenced a cluster of *L. hilgardii* encoding the enzymes involved in the ADI pathway and demonstrated that this cluster encodes functional enzymes of this way. The characterization of this way was important in order to achieve a better knowledge of the EC production by *L. hilgardii*.

4. Biogenic amines

Biogenic amines, low molecular weight organic bases, can be formed and degraded as a result of normal metabolic activity in animals, plants and micro-organisms. The amines are usually produced in foods by decarboxylation of the amino acids. Amine production has been associated with protective mechanisms of microorganisms [47].

Toxicological problems may result from the ingestion of food containing relatively high levels of biogenic amines. Putrescine and agmatine has been described as substances that enhance the toxicity of histamine to humans by depressing histamine oxidation [48].



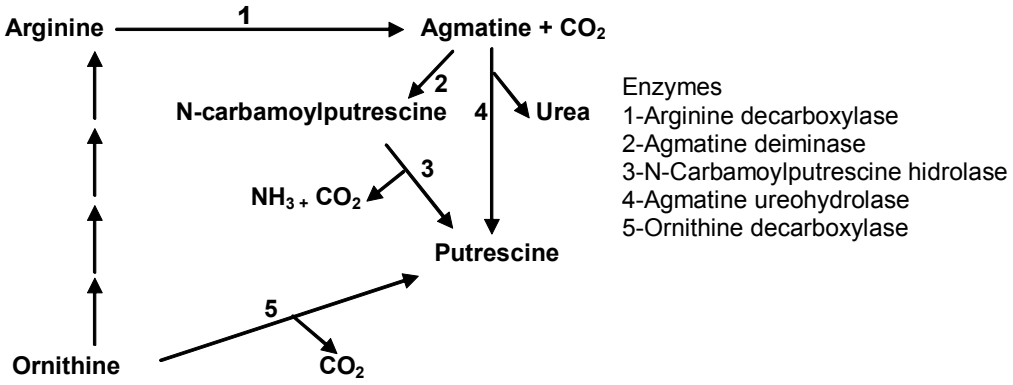
Biosynthesis of biogenic amines

The biogenic amines production was studied in *Lactobacillus* strains from fruits [49]. *Lactobacillus* strains are considered to be generally safe. However the authors demonstrate that a strain of *L. hilgardii* isolated from wine was able to produce agmatine and putrescine from one of the major amino acids found in fruit juices and wine, arginine and its amino acid–derived ornithine.

Biogenic amines like tyramine as well as diamines such as putrescine and cadaverine have been described as precursors of carcinogenic nitrosamines [50]. Alcohol may enhance the effect of amines

present in wine. Lactic acid bacteria are reported as the main producers of biogenic amines in alcoholic beverages. It is assumed that biogenic amines found in foods and wines are produced by specific amino acid decarboxylases from lactic acid bacteria during fermentation [51-53]

Putrescine is the most abundant biogenic amine found in wine [54] and agmatine is the most prevalent one in beer [55]. Arena and Manca de Nadra [56] reported that agmatine was formed as an intermediate in the formation of putrescine from arginine in a strain of *L. hilgardii*, isolated from wine. Putrescine is formed from agmatine through a pathway that does not involve amino acid decarboxylase or formation of urea.



Agmatine and Putrescine formation in bacteria

Production of biogenic amines under laboratory conditions does not imply similar behaviour in fermented products. Wines are complex systems with a wide number of factors influencing microbial growth and metabolism. Grape phenolics are the main compounds responsible for color, taste, oxidation and other chemical reactions in wine and juice. They have received considerable attention because of their potential antioxidant activity. The specific amounts and types of phenolics present in grapes and wines depend on a number of factors, including variety and maturity of the grape, seasonal conditions, storage and the vinification process (phenol carboxylic acids, 100–200 mg/l, catechin, 10–400 mg/l, quercetin, 5–20 mg/l).

Reguant et al. [61] reported that phenolic compounds affected the growth of *O. oeni* in different ways, depending on their type and concentration. In a strain of *L. hilgardii* from wine, [58] it was observed a growth stimulatory effect in the presence of gallic acid and catechin at concentrations normally present in wine. Rodriguez Vaquero et al. [62] reported the effect of non-flavonoid phenolic compounds on the same strain growth. At 100 mg/l gallic acid increased growth, protocatechuic acid decreased growth, whereas vanillic acid did not show any modification. At 10 mg/l caffeic acid did not affect the growth of *L. hilgardii*. The different effect of phenolic compounds on bacterial growth observed among different species and strains of lactic acid bacteria indicate that their effect is strain dependent.

Alberto et al. [57] reports on the influence of phenolic compounds on growth survival and agmatine metabolism of a strain of *L. hilgardii*, a bacterium from wine able to produce important levels of putrescine. The authors demonstrated that agmatine degradation increased growth and survival of the microorganism and the alkalinity of the media. 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. The concentration of phenolic compounds decreased after five days of incubation of the microorganism, except for gallic acid and quercetin. The results indicate that phenolic compounds, besides their already known beneficial properties to human health, seem to be a natural way of diminishing putrescine formation.

As the potential production of biogenic amines is of great toxicological significance, the inhibitory effect on putrescine formation by natural compounds present in wine is important. The phenolic compounds

that have well-known beneficial properties to human health appear to be a natural means of diminishing putrescine formation from agmatine.

Musts and wines are very selective media, which can support growth of only few species of LAB. Four genera are represented: *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus*. During alcoholic fermentation, the LAB population is mainly composed of pediococci along with *O. oeni*. The homofermentative lactobacilli, the major type present on grapes, disappear quickly after the start of alcoholic fermentation in favor of *Leuconostoc mesenteroides* which, at the end of the fermentation, is replaced by *O. oeni* [42].

In wine, several amino acids can be decarboxylated; as a result biogenic amines are usually found. Histamine and tyramine are, besides putrescine, the most abundant amines in wine. Phenylethylamine is also frequently found. Although tryptamine and cadaverine are also found in wine, they are in much lower concentrations than the others mentioned [61]. As the histamine, tyramine and phenylethylamine concentrations found in must are very low or non-existent it is normal that the concentrations of histamine, tyramine and phenylethylamine must be attributed to strains of LAB. Farías et al. [62] did research on the presence of histidine decarboxylase activity in 21 LAB strains isolated from Argentinian wines and found that this activity is not widely distributed among them (Table 1).

Table 1 Histidine decarboxylase activity in lactic acid bacteria from wine

Species	Strain	Histidine decarboxylase activity (nmol CO_2. min^{-1}. mg dry $weight^{-1}$)			
		Basal medium		Basal medium+His[a]	
		-	+Glc[b]	-	+Glc[b]
Pediococcus pentosaceus	9p	0.08	0.08	0	0
	10p	0.34	0	0.05	0.05
	12p	0	0	0	0
	13p	0.41	0.24	0.15	0.12
	Xp	0	0	0	0
	X_2p	0.6	0	0	0
	E_1p	0.36	0	0	0
	E_2p	0.41	0	0	0.32
	E_3p	0.32	0	0	0
	E_5p	0	0	0	0
Leuconostoc oenos	X_2L	0.32	0	0	1.0
	ST	0.08	1.2	2.0	3.7
	L_2	0	0.82	0.21	3.2
	m	0.48	0	0	0.43
Lactobacillus hilgardii	X_1B	0.98	7.1	0.07	12.3
	5w	1.8	20.7	0	19.7
	5s	0.97	5.8	0	32.6
	7k	0	15.7	0	20.7
	6c	0	13.3	0	27.7
	6d	0	28.6	1.4	39.3
	N_5L	0	17.7	2.8	25.3

[a]: 5 g/l histidine added to the culture medium; [b]: 16 mM glucose added to the reaction mixture

This activity occurs significatively in some strains of *L. hilgardii*. In 5w strain of *L. hilgardii*, the activity present constitutive expression and the results with metabolic inhibitors indicated that a proton motive force mediates the histidine transporte. The authors studied the effect of organic acid normally present in wine on histidine decarboxylase of *L.hilgardii* 5 w [51] and observed that the enzyme was inhibited by

L-malic acid. They also observed that citric acid was stimulatory of the enzyme production and the increase was correlated with the citric acid concentration.. When different concentratios of L-malic acid were added to the basal medium supplemented with citric acid, reversion of stimulation was observed. These results suggest that when L-malic acid was present, the additional energy produced by its decarboxylation would become innecessary histidine decarboxylation, and the enzyme production decrease. At the same time, the additional energy necessary for anabolic reactions of the intermediate metabolites derived from citric acid, could be provided by histidine decarboxylation.

In *Oenococcus, Lactobacillus, Leuconostoc* and *Pediococcus* was demonstrated that they can contribute to the histamine synthesis in wine [63, 64]. The authors indicate that histamine-producing strains of *O. oeni* are very frequent in wine. Some of the positive strains might be used as malolactic starter, without prior knowledge of their potential to form biogenic amines. Therefore, the inability to form these compounds needs also to be confirmed for the microorganisms generally regarded as safe. In contrast with these results, formation of histamine was not observed in any species that may be involved in malolactic fermentation [62, 65].

Phenylethylamine production is associated with tyramine production in *L..brevis* and *L.. hilgardii* isolated from wine [66]. This correlation could be explained by the fact that phenylalanine is also a substrate for tyrosine decarboxylase, producing phenylethylamine in a secondary reaction.

References

[1] FM. Saguir and MC. Manca de Nadra, Journal of Applied Bacteriology **81**, 393 (1996)
[2] MG. Gänzle, N. Vermeulen and RF. Vogel, Food Microbiology **24**, 128 (2007)
[3] JB. Brant, EW. Sulzman and DD. Myrold. Soil Biology and Biochemistry **38**, 2219 (2006)
[4] B. Magasanik Journal of Cellular Biochemistry **51**, 34 (1993).
[6] CR. Davis, D. Wibowo, GH. Fleet, and TH.Lee, American Journal of Enology and Viticulture **39,** 137 (1988)
[7] G.C Rollán, ME Farías and MC Manca de Nadra, World Journal of Microbiology and Biotechnology **9**, 587 (1993)
[8] GC Rollán, ME Farías, and MC Manca de Nadra, World Journal of Microbiology and Biotechnology **11,** 153 (1995)
[9] MC. Manca de Nadra, ME. Farías, MV. Moreno-Arribas, E. Pueyo and M C. Polo, FEMS Microbiology Letters **150**, 135 (1997)
[10] GC Rollán, AM. Strasser de Saad, ME Farías, and MC Manca de Nadra, Journal of Applied Microbiology **85,** 219 **(**1998)
[11] MC. Manca de Nadra, ME. Farías, MV. Moreno-Arribas, E. Pueyo and MC. Polo, FEMS Microbiology Letters **174**, 41 (1999)
[12] ME. Farías and MC. Manca de Nadra, FEMS Microbiology Letters **185,** 263 (2000)
[13] MC. Manca de Nadra, ME. Farías, E. Pueyo and MC. Polo, Food Control **16**, 851(2005)
[14] LMT. Dicks, F. Dellaglio and MD Collins. International Journal of Systematic Bacteriology **45**, 395 (1995)
[15] M. Guillox-Benatier, M. Feuillat and B. Colfi, Vitis **24**, 59(1985)
[16] RE Kunkee, Advances in Applied .Microbiology **9,** 235. (1967)
[17] F. Radler, Lactic Acid Bacteria in Beverages and Food, Edited by J. G. Carr, C. V. Cutting and G. C. Whiting, Academic Press, New York and London, **17** (1975)
[18] MJ. Amoroso, FM. Saguir and MC Manca de Nadra, Journal International of Science de la Vigne et du Vin **27,** 135 (1993).
[19] FM. Saguir and MC. Manca de Nadra, Microbiologie-Aliments-Nutrition **15**, 131 (1997).
[20] RP.Tracey and JP. Britz, Journal of Applied Bacteriology **171**, 280 (1989)
[21] FM Saguir and MC. Manca de Nadra, Journal of Applied Microbiology **93**, 295 (2002)
[22] MC. Manca de Nadra, A P. de Ruiz Holgado and G. Oliver, Milchwissenchaft **37**, 669 (1982)
[23] MC. Manca de Nadra, A P. de Ruiz Holgado and G. Oliver, Biochimie **70**, 367 (1988)
[24] ME. Arena, FM. Saguir and MC. Manca de Nadra, International Journal of
Food Microbiol **47,** 203 (1999)
[25] M.E. Arena , F.M. Saguir and M.C. Manca de Nadra, International Journal of
Food Microbiol **52,** 155 (1999)
[26] FM Saguir and MC Manca de Nadra, Current Microbiology **54**, 414 (2007).

[27] WM. Ingledew, CA. Magnus and JR. Patterson, American Journal of Enology and Viticulture **38,** 332 (1987)
[28] CS. Ough, EA. Crowell and BR. and Gutlove, American Journal of Enology and Viticulture **23,** 61 (1988).
[29] FF. Monteiro, EK. Trousdale and LF. Bisson, American Journal of Enology and Viticulture **40,** 1 (1989).
[30] SQ. Liu, G. Pritchard, M. Hardman and G.J. Pilone, , American Journal of Enology and Viticulture **45,** 235 (1994).
[31] ME. Arena and MC. Manca de Nadra, Research in Microbiology **156,** 858 (2005)
[32] T. Tonon, A. Lonvaud-Funel, Food Microbiology **19,** 451 (2002)
[33] R. Mira de Orduña, ML. Patchett, S-Q. Liu and GJ. Pilone, Applied and Environmental Microbiology **67,** 1657 (2001)
[34] T. Henick-Kling, Journal of Applied Bacteriology. Symp. Suppl. **79,** 29 (1995)
[35] R. Battaglia, HBS. Conacher, BD. Page, Food Additives and Contaminants **7,** 477 (1990)
[36] BS. Clegg, R. Frank, Journal of Agricultural and Food Chemistry **36,** 502 (1988)
[37] Z. Azevedo, JA. Couto, T. Hogg, Letters in Applied Microbiology **34,** 32 (2002)
[38] T. Matsudo, T. Aoki and K. Abe, Journal of Agricultural and Food Chemistry **41,** 352 (1993)
[39] CA. Uthurry, F. Varela, B. Colomo, JA. Suarez Lepe, J. Lombardero, JR. García del Hierro, Food Chemistry **88**, 329 (2004)
[40] G. Spano, S. Massa, ME. Arena and MC. Manca de Nadra, Annals of Microbiology **57,** 67 (2007)
[41] D. Molenaar, F.Bringel, FH. Schuren, WM. de Vos, RJ Siezen and M. Kleerebezem, Microbiology **187,** 6119 (2005)
[42] A. Lonvaud-Funel, Antonie van Leeuwenhoek **76,** 317 (1999)
[43] S-Q. Liu, Journal of Applied Microbiology **92,** 589 (2002)
[44] G. Spano, G. Chieppa, L. Beneduce and S.Massa, Journal of Applied Microbiology **96**, 185 (2004).
[45] G. Spano, L. Beneduce, L. De Palma, M. Quinto, A.Vernile and S. Massa, World Journal of Microbiology and Biotechnology **22,** 769 (2006)
[46] ME. Arena, MC. Manca de Nadra and R. Muñoz, Gene **301,** 61 (2002)
[47] A. Halász, A. Barath, I. Simon-Sakardi and W. Holzapel, Trends in food Science and technology **5**, 42 (1994)
[48] SI. Taylor, Critical Review of Toxicology **17**, 91 (1986)
[49] ME. Arena and MC. Manca de Nadra, Journal of Applied Microbiology **90,** 158 (2001)
[51] M. Farías, MC. Manca de Nadra, GC. Rollan and AM. Strasser de Saad, Current Microbiology **31,** 15 (1995)
[52] P. Kalăc, J. Săvel, M. Křížek, T. Pelikánová and M. Prokopová, Food Chemistry **79,** 431 (2002)
[53] P. Lucas, J. Landete, M. Coton, E. Coton and A. Lonvaud-Funel, FEMS Microbiology Letters **229,** 65 (2003)
[54] E. Soufleros, ML. Barrios and A. Bertrand, American Journal of Enology and Viticulture **49,** 266 (1998)
[55] MB. Glória and M. Izquierdo Pulido, Journal of Food Composition and Analysis **12,** 129 (1999)
[56] ME. Arena and MC. Manca de Nadra, Journal of Applied Microbiology **90,** 158 (2001)
[57] MR. Alberto, ME. Arena and MC. Manca de Nadra, Food Control **18,** 898 (2007)
[58] MR. Alberto, ME. Farías and MC. Manca de Nadra, Journal of Agriculture and Food Chemistry **49,** 4359 (2001)
[59] MR. Alberto, ME. Farías and MC. Manca de Nadra, Journal of Food Protection **65,** 211 (2002)
[60] MR. Alberto, C. Gómez-Cordovés and MC. Manca de Nadra, Journal of Agriculture and Food Chemistry **52,** 6465 (2004)
[61] JM. Landete, S. Ferrer, L. Polo, and I. Pardo. Journal of Agriculture and Food Chemistry *53,* 119 (2005)
[62] ME Farías, MC Manca de Nadra, GC Rollán and AM Strasser de Saad, Journal International de la Science de la Vigne et du Vin **27**, 191 (1993)
[63] S. Guerrini, S. Mangani, L. Granchi and M. Vincenzini, Current Microbiology **44**, 374 (2002)
[64] JM. Landete, S. Ferrer and I. Pardo, J. Food Control. Available online 10 January, (2007) doi:10.1016/j.foodcont.2006.12.008.
[65] MV. Moreno-Arribas, MC. Polo, F. Jorganes and R. Muñoz, International Journal of Food Microbiology **84,** 117 (2003)
[66] MV. Moreno-Arribas, S. Torlois, A. Joyeux, A. Bertrand and A. Lonvaud-Funel, Journal of Applied Microbiology **88,** 584 (2000)

