Evaluation of volatile compounds produced by Lactobacillus paracasei *I90 in a hard-cooked cheese model using solid-phase microextraction*

Guillermo Hugo Peralta, Irma Veronica Wolf, Carina Viviana Bergamini, María Cristina Perotti & Erica Rut Hynes

Dairy Science & Technology
Official journal of the Institut National de la Recherche Agronomique (INRA)Formerly 'Le Lait'

ISSN 1958-5586

Dairy Sci. & Technol.
DOI 10.1007/s13594-013-0143-4

Springer

Dairy Sci. & Technol.
DOI 10.1007/s13594-013-0143-4

NOTE

Evaluation of volatile compounds produced by *Lactobacillus paracasei* I90 in a hard-cooked cheese model using solid-phase microextraction

Guillermo Hugo Peralta · Irma Veronica Wolf · Carina Viviana Bergamini · María Cristina Perotti · Erica Rut Hynes

Received: 22 February 2013 / Revised: 14 June 2013 / Accepted: 17 June 2013

Abstract Selected strains of mesophilic lactobacilli with key enzymatic activities may be employed to intensify or diversify cheese flavor during ripening. Among enzymes of interest, aminotransferases are most important, as they are involved in the first step of amino acid (AA) catabolism. In previous studies, *Lactobacillus paracasei* I90 has been shown to increase both the secondary proteolysis in soft and semihard cheeses and the production of volatile compounds in minisoft cheeses. In this work, its potential for the formation of volatile compounds derived from AA catabolism was assessed by solid-phase microextraction coupled to GC-FID/MS in a hard-cooked cheese model, consisting of a cheese extract sterilized by filtration. *Lb. paracasei* I90 showed aminotransferase activity towards all the AAs studied (branched-chain AAs, aromatic AAs, aspartic acid, and methionine). The highest levels were observed for aspartic acid, followed by branched-chain AAs and tryptophane. Among the volatile compounds derived from AAs, 2-methylbutanal, 3-methylbutanal, diacetyl, 3-methyl1-butanol, acetic, and 3-methylbutanoic acids were detected at higher level in extracts inoculated with *Lb. paracasei* I90 than in controls. It was concluded that the production of volatile compounds by *Lb. paracasei* I90 in a hard cheese model was consistent with its main aminotransferase activities, making this strain a good candidate for enhancing flavor in hard cheeses.

Keywords Amino acid catabolism · *Lactobacillus paracasei* · Cheese model · Volatile compounds · SPME-GC-FID/MS

G. H. Peralta (✉) · I. V. Wolf · C. V. Bergamini · M. C. Perotti · E. R. Hynes
Instituto de Lactología Industrial (INLAIN-UNL/CONICET), Santiago del Estero 2829, S3000AOM Santa Fe, Argentina
e-mail: gperalta@fiq.unl.edu.ar

Published online: 11 July 2013

1 Introduction

The biochemical events that occur during cheese ripening lead to the production of a large number of volatile compounds, several of them related to flavor (McSweeney and Sousa 2000). Cheese flavor is strongly related to amino acid (AA) catabolism, the lactic acid bacteria (LAB) being the main microorganisms responsible for this transformation. The catabolism of AAs starts with removal of the amino group from the amino acids by aminotransferase or transaminase enzymes in a known transamination reaction, α-ketoglutarate being the main acceptor of amino group. The α-keto acids formed in this reaction are key intermediates in the conversion of AAs into aroma compounds such as carboxylic acids, aldehydes, alcohols, and esters, among others. Aminotransferases (ATs) are widely distributed among microorganisms, and their activities towards different AAs have been detected both in mesophilic and in thermophilic LAB. However, the activity levels are species- and strain-dependent (Yvon 2006).

The addition of selected strains of mesophilic lactobacilli with key enzymatic activities to milk cheese has revealed its potential to enhance quality and to intensify or diversify cheese flavor during ripening by increasing secondary proteolysis and amino acids catabolism (Beresford 2003). For this purpose, the cheese-making experiments at pilot-scale are ideal; however, these studies are expensive and time-consuming and require trained panel. Alternatively, model systems consisting of simplified versions of real cheese matrix have been proposed (Hynes et al. 2000; Shakeel-Ur-Rehman et al. 1998). The incubation of these models for short time at high temperature allows accelerating some of the metabolic and biochemical changes that occur during ripening. Recently, a cheese model consisting of the aqueous extract of Reggianito cheese sterilized by filtration has been successfully developed and applied in our institute to describe hard cheese peptidolysis (Milesi et al. 2011).

The production of volatile compounds by microorganisms has been evaluated by different analytical techniques. Although there is no ideal method for extracting volatile compounds in dairy matrices, since each method gives different results in terms of selectivity, sensitivity, and reproducibility, solid-phase microextraction (SPME) has demonstrated to be adequate to compare the volatile profiles due to its high sensitivity and an acceptable reproducibility and suitability for routine analysis (Pinho et al. 2003).

In previous studies, *Lactobacillus paracasei* I90 showed satisfactory technological properties for cheese manufacture, a probiotic capacity, and the ability to increase both the secondary proteolysis in soft and semihard cheeses and the production of volatile compounds in minisoft cheeses (Burns et al. 2012; Milesi et al. 2009, 2010). In the present work, AT activities of *Lb. paracasei* I90 were quantified, and the production of volatile compounds in a hard-cooked cheese model was monitored by SPME coupled to GC-FID/MS.

2 Materials and methods

2.1 Strain and culture conditions

Lb. paracasei I90 belongs to the non-starter lactic acid bacteria (NSLAB) collection of the Instituto de Lactología Industrial (INLAIN, Santa Fe, Argentina). Stock

cultures of this strain were stored frozen at −80 °C in MRS broth (Biokar Diagnostics, Beauvais, France) with 15% (v/v) of glycerol as cryopreservative. Before being used, *Lb. paracasei* I90 was cultivated twice in MRS broth overnight.

2.2 Aminotransferase activity

ATs activities towards Asp, Met, branched-chain (Leu, Val, and Ile), and aromatic (Tyr, Trp, and Phe) amino acids were studied in cell-free extracts (CFE). Duplicate of CFE were obtained from two independent cultures of *Lb. paracasei* I90 in late exponential growth phase on MRS broth, by mechanical disruption with glass beads (106 μm, Sigma) in a Mini-Beadbeater 8TM cell disruptor (Biospec Products, Bartlesville, Ill). The cell lysate was centrifuged at 16,000 g/10 °C during 15 min, and then filtered through membranes 0.45 μm of pore diameter (Millipore, Sao Paulo, Brazil), and constituted the CFE used for the analysis of ATs activities. For that, CFE (15 to 50 μL) was incubated for 20 min at 37 °C in a reaction mixture (250 μL) containing 200 mM Tris buffer (pH 8.00), 1 mM pyridoxal phosphate, 50 mM α-ketoglutaric acid, and 15 mM of each amino acid substrate. Then, the reaction was stopped by heating at 80 °C for 15 min, and the levels of glutamic acid produced in the transamination reaction was quantified using a commercial L-Glu assay kit (Boehringer, Manheim, Germany).

2.3 Cheese model

A hard-cooked cheese sterile extract previously described by Milesi et al. (2011) was prepared. Briefly, a representative portion of mid-ripened 90-days-old Reggianito cheese was disintegrated and homogenized with distilled water (1:1), and the resultant slurry was centrifuged. The soluble fraction was filtered through glass wool, and then pH value and salt content were measured and adjusted to 5.20 and 4%, respectively. Finally, the extract was sterilized by filtration through PVDF membranes of 0.4 μm (Millipore, Sao Paulo, Brazil).

A volume of the extract (70 mL) was inoculated with *Lb. paracasei* I90 at a level of 5×10^4 CFU mL^{-1}, aliquots (15 mL) were distributed in screw cap tubes and incubated at 34 °C for 14 days. In parallel, control extracts without inoculation were incubated. After that, the tubes were maintained at −18 °C until analysis of volatile compounds. All of the extracts were performed in triplicate, by using three independent cultures of *Lb. paracasei* I90 for each inoculation, respectively.

2.4 Microbiological counts

Viable counts of *Lb. paracasei* I90 in experimental extracts were recorded at 0, 3, 7, and 14 days of incubation, by plating on MRS (Biokar Diagnostics, Beauvais, France) agar and incubating at 37 °C for 48 h. Sterility in control extracts was checked by plating samples on skim milk agar and incubating at 37 °C for 48 h.

2.5 Analysis of volatile compounds by SPME-GC-FID/MS

SPME analysis Previously to analysis, cheese extracts were defrosted at 4 °C/12 h, and their contents (15 mL) were transferred in 30-mL glass vials at this low temperature.

The vessels were hermetically sealed with an aluminum seal and Butyl–Teflon septa. During sampling of volatile compounds, the vials were thermostatized at 40±1 °C for 10 min, and then CAR/PDMS 75 μm (Supelco Inc. Bellefonte, PA, USA) fiber was exposed into the headspace for 30 min.

Chromatographic analysis Volatile compounds retained on the fiber-coating phase were thermally desorbed in the injection port (250 °C, splitless mode) equipped with a narrow-bore glass liner (Supelco, Bellefonte, USA) of a gas chromatograph (PerkinElmer model 9000, USA). The compounds were separated on a HP-Innowax column (60 m×0.25 mm×0.25 μm) (Agilent J&W, Agilent Technologies, USA). The oven temperature program was as follows: 45 °C (5 min), increase at 8 °C min^{-1} to 150 °C (3 min) and finally, an increase at 10 °C min^{-1} to 250 °C (5 min). Hydrogen was used as carrier gas at a flow rate of 2.0 $mL.min^{-1}$. The FID detector temperature was setup at 290 °C. Volatile compounds were identified by matching their retention time with those of standard compounds (when available) and by calculating linear retention indexes (LRI) according to the expression proposed by Van den Dool and Kratz (1963).

In order to confirm the presence of the compounds tentatively identified by CG-FID, as well as to detect other volatile compounds, the cheese extracts were also analyzed in a Varian CP-3800 gas chromatograph equipped with a VF-5ht column (30 m×0.25 mm×0.1 μm) and directly interfaced with a Varian Saturn 2000 ion trap mass spectrometer (Varian, Palo Alto, USA). The oven temperature program of gas chromatograph (GC) was the same as that described for GC-FID analysis. In relation to mass spectrometer (MS) operation conditions, the electron impact energy was setup at 70 eV, the data were collected in the range of 40 to 350 amu, and the scan rate was 0.50 scan per second. The gas carrier was helium at a flow rate of 1 $mL.min^{-1}$. Identification was performed by comparing their mass spectra with those of the NIST-98 and Wiley libraries and their retention times with those of authentic standards, when available.

For those compounds identified by FID/LRI and confirmed by MS, quantification was carried out by integrating the peak areas provided by FID, whereas those compounds only identified by MS, quantification was performed by integrating the peak areas of total ion chromatograms. The peak areas were expressed in arbitrary units.

It is important to note that the polarities of the two columns used in the analysis by GC and MS (Innowax and VF-5ht, respectively) are quite different, therefore, volatile compounds eluted in different orders on these stationary phases are expected. This fact is not a trouble in the analysis of the profiles because regardless of retention time, the presence of the compounds identified and quantified by GC–FID was subsequently verified by GC–MS. Thus, only those compounds identified by both techniques as well as those compounds identified only by GC–MS were reported.

2.6 Statistical analysis

The data of ATs activities toward all AAs tested for *Lb. paracasei* I90 and volatile compounds in control and experimental cheese extracts were subjected to one-way analysis of variance in order to detect the differences among them, using the software SPSS 10.0 (SPSS Inc., Chicago, USA).

3 Results and discussion

3.1 Aminotransferase activities

The main AT activity in *Lb. paracasei* I90 was found against aspartic acid (Fig. 1). Indeed, the level of aspartate aminotransferase (AspAT) activity was significantly higher (3–6 times) than the ATs activities for the rest of the amino acids tested ($P<0.05$). In addition, the values of ATs activities toward Met, branched-chain (Leu, Ile, and Val), and aromatic (Phe, Tyr, and Trp) amino acids were similar ($P>0.05$).

Levels and specificity of AT activities of LAB have demonstrated to be highly strain- and species-dependent (Thage et al. 2005; Yvon 2006). Overall, main AspAT activity have been found in *Lb. paracasei* and *Lb. plantarum* strains, while high levels of ATs with specificity on branched-chain and aromatic amino acids were verified in strains of *Lactococcus lactis* and *Lactobacillus helveticus*, respectively (Kieronczyk et al. 2004; Thage et al. 2004). The ATs profile found for *Lb. paracasei* I90 in the present study is in agreement with these previous results.

3.2 Microbiological counts

Control extracts remained sterile during incubation. In experimental extracts, *Lb. paracasei* I90 reached a maximum of ca. 10^8 CFU mL^{-1} after 3 days of incubation and then decreased by one and two log orders at 7 and 14 days, respectively.

3.3 SPME analysis

The analysis of volatile compounds both in control and experimental extracts by SPME-GC-FID/MS allowed isolating a total of 44 compounds, of which

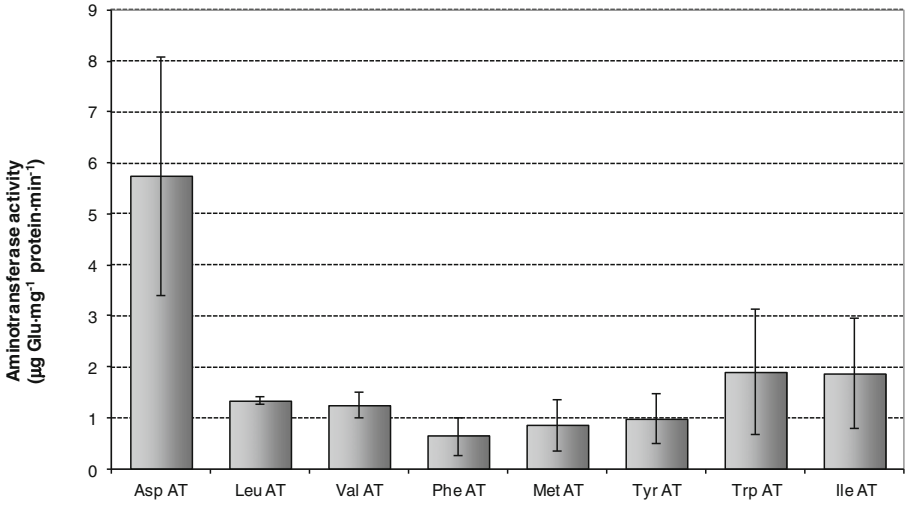

Fig. 1 Aminotransferases activities (*ATs*) of *Lb. paracasei* I90, analyzed on CFEs prepared from the strain grown in MRS broth. Values are the means of duplicate analysis on two CFEs obtained from two independent cultures. *Error bars* represent the standard deviations

approximately 21 compounds have been suggested to be directly or indirectly related to amino acids catabolism. They are listed in Table 1.

Several compounds had higher area values in experimental extracts than in controls. Diacetyl and its reduction product, acetoin, are commonly produced from citrate metabolism by Cit (+) bacteria, although more recently, it has been suggested that they may also derive from aspartic acid by transamination by mesophilic lactobacilli (Kieronczyk et al. 2004; Skeie et al. 2008). A significantly higher level of diacetyl was found in the extracts inoculated with *Lb. paracasei* I90, but no significant differences between control and experimental extracts were detected in the content of acetoin. The production of diacetyl and acetoin by bacteria with AspAT activity, among them *Lb. paracasei* strains, has been demonstrated in a reaction medium containing α-ketoglutarate (Le Bars and Yvon 2008) in model cheeses (Kieronczyk et al. 2004; Milesi et al. 2009) and in semihard reduced-fat cheese (Thage et al. 2005). In the present study, the highest level of AspATs of *Lb. paracasei* I90 was in agreement with the higher level of diacetyl found in the experimental extracts. It is important to remark that *Lb. paracasei* I90 was not able to metabolize citrate in a soft cheese model (Milesi et al. 2010), neither in extracts obtained from soft cheeses (data not published).

Among volatile compounds derived from branched-chain amino acids (Val, Leu, and Ile), 2-methylbutanal, 3-methylbutanal, 3-methyl1-butanol, and 3-methylbutanoic acid were identified in the extracts. In particular, those derived from Leu catabolism such as 3-methylbutanal, 3-methyl1-butanol, and 3-methylbutanoic acid (or isovaleric acid) had higher area values in the extracts inoculated with *Lb. paracasei* I90. Similar results in relation to the higher production of aroma compounds from Leu than those from Ile and Val by *Lb. paracasei* strains have been reported by other authors (Thage et al. 2005). The ability of thermophilic and mesophilic lactobacilli strains to produce aroma compounds derived mainly from branched-chain and aromatic amino acids have been demonstrated in different food matrices or reaction mixture (Klein et al. 2001). Overall, these compounds are considered beneficial to the cheese flavor. Isovaleric acid contributes with sweaty and strong notes; 3-methylbutanal has a green malty aroma, and 3-methyl1-butanol is responsible for the pleasant aroma of fresh cheese, giving an alcoholic floral note (Curioni and Bosset 2002).

A number of volatile compounds derived from aromatic amino acids catabolism were detected: benzaldehyde, 2-hydroxybenzaldehyde, phenylacetaldehyde, phenylethyl alcohol, phenol, and acetophenone. Most of them are identified in fractions with floral rose-like odor (Smit et al. 2005), but they could also cause defects in some varieties of cheeses such as unclean flavors (McSweeney and Sousa 2000). In this work, only phenyl acetaldehyde had higher area value in the experimental extracts than in controls, whereas benzaldehyde and 2-hydroxybenzaldehyde were present in higher amount in the controls. Aminotransferase activity of *Lb. paracasei* I90 towards aromatic amino acids was detectable but the lowest among the amino acids tested. In semihard cheeses added with different strains of *Lb. paracasei*, Thage et al. (2005) did not observed any effect of adjunct cultures on the levels of phenol and benzaldehyde. In the case of benzaldehyde levels, they reported that it was clearly affected by production day, suggesting a contribution to their production by NSLAB or clostridia.

Table 1 Volatile compounds related to amino acids catabolism identified in the extracts with and without *Lb. paracasei* I90

Compounds	AA precursor (1)	Identification method	LRI	Control	Experimental
2-methylbutanal	Isoleucine	FID-LRI/MS	909	50,931±7,411	52,815±1,406
3-methylbutanal	Leucine	FID-LRI/MS	914	83,139±23,184[a]	149,576±12,777[b]
Benzaldehyde	Phenylalanine, Tryptophane	FID-LRI/MS	1,537	1,699,626±338,464[a]	950,990±288,314[b]
Ethanol	Alanine	FID-LRI/MS	934	233,113±126,763	235,379±64,109
Ethyl acetate	Alanine, Glycine, Serine	FID-LRI	882	1,164,152±236,358	1064134±269991
Ethyl butanoate	Alanine	FID-LRI/MS	1,034	30,715±9,475	40637±3497
Isoamyl acetate	Leucine, Glycine, Serine	FID-LRI	1,124	24,008±4,944	22,443±9,064
Diacetyl	Aspartic acid	FID-LRI/MS	974	33,772±9,853[a]	252204±19931[b]
Acetoin	Aspartic acid	FID-LRI/MS	1,291	88,030±15,017	102464±37948
2,3-butanediol	Threonine	FID-LRI/MS	1,546	55,820±28,673[a]	131,659±10,786[b]
Acetic acid	Serine, Glycine, Alanine	FID-LRI	1,465	325,527±84,083[a]	605,608±89,366[b]
Butanoic acid	–	FID-LRI	1,640	468,055±50,867[a]	826,403±62,181[b]
Hexanoic acid	Lysine	FID-LRI	1,861	488,640±49,090[a]	845,090±47,262[b]
3-methylbutanoic acid	Leucine	FID-LRI/MS	1,682	173,369±10,683[a]	311,146±13,063[b]
Phenylethylalcohol	Phenylalanine	FID-LRI/MS	1,925	83,801±21,723	61,442±31,005
Phenol	Tyrosine	FID-LRI/MS	2,021	436,718±41,620	290,255±133,954
3-methyl-butanol	Leucine	FID-LRI/MS	1,210	34,697±4,619[a]	105,812±27,687[b]
Methional	Methionine	MS		16,426±6,906	16,394±7,734
2-hydroxybenzaldehyde	Tyrosine	MS		52,313±3,105[a]	15,956±3,014[b]
Phenylacetaldehyde	Phenylalanine	MS		10,349±1,547[a]	18,921±3,826[b]
Acetophenone	Phenylalanine	MS		10,452±2,533	8,612±3,549

Values are mean peak areas (in arbitrary units) of three extracts and two replicates of analysis±standard deviation

(*1*) Data from: Yvon (2006); Tavaria et al. (2002); Urbach (1995)

LRI linear retention indices as determined on an Innowax column using the homologous series of n-alkanes according to the Van der Dool and Kratz (1963) equation

Different superscripts in the same row indicate significant differences at $P<0.05$. The superscripts have not been included in the cases, where we have not found significant differences ($P>0.05$)

Sulfur-containing compounds are key components in the aroma profile of several cheese varieties. These compounds are described as having strong garlic and very ripe cheese odors (Curioni and Bosset 2002). Methional, a degradation product of methionine, was found at similar levels both in control and in experimental extracts. These results can be explained by the low MetATs of *Lb. paracasei* I90, and by the fact that the methodology employed was not selective to detect sulfur compounds. The existence of specific MetAT in lactobacilli is controversial; it is believed that the activity found in LAB is due to the marginal activity of other ATs on Met (Yvon 2006).

Ethanol is a common compound in cheese and can derive from lactose metabolism by heterofermentative LAB, acetaldehyde reduction, or amino acids catabolism (McSweeney and Sousa 2000; Yvon 2006). Extracts containing the adjunct culture had similar levels of ethanol than the control extracts. Although ethanol is not a relevant aroma component in cheeses, its importance resides in the fact that it participates in the formation of ethyl esters.

Esters are important contributors to cheese flavor. They are formed through a reaction involving an alcohol and a (fatty) acid, in the presence of esterase and lipase activities or also by chemical reactions. Besides carbohydrate and fat metabolism, also amino acid catabolism provides substrates for ester biosynthesis (Smit et al. 2005). No significant differences in the ester levels were found between the extracts with and without *Lb. paracasei* I90. Studies carried out on the ester production by *Lb. paracasei* indicate a great variability in the levels found, which could be associated with the strain as well as physicochemical factors (Liu et al. 1998).

Short-chain (fatty) acids, acetic, butanoic, and hexanoic acids, have different origins in cheese. Acetic acid originates from a number of processes that include the metabolism of serine or alanine via pyruvate by LAB (Skeie et al. 2008). Butanoic acid can be produced from butyric acid fermentation and from lipolysis of milk fat, whereas the most likely origin of hexanoic acid is lipolysis; although a lower proportion of these fatty acids can also originate from the degradation of lactose and amino acids (Urbach 1995). The levels of short-chain fatty acids were higher in the extracts inoculated with *Lb. paracasei* I90. In miniature soft cheeses, an increased content of acetic acid was also observed in cheeses added with *Lb. paracasei* I90 when compared to controls (Milesi et al. 2010).

4 Conclusions

In the present paper, evidence on volatile production by *Lb. paracasei* I90 in a hard-cooked cheese model is provided. This strain mainly produced diacetyl, which could be derived from aspartic acid, and volatile compounds derived from branched chain amino acids, being these results in agreement with the main aminotransferase activities detected. *Lb. paracasei* I90 also increased the level of acetic, butanoic, and hexanoic acids in the extracts.

In parallel, the capability of aroma compound formation of other mesophilic lactobacilli strains of the INLAIN collection, employing the procedure described in this work, is being evaluated. After that, the strains having the best performance to produce desirable volatile compounds will be used as adjunct culture in the manufacture of Reggianito cheese, a typical hard-cooked cheese of Argentina.

References

Beresford T (2003) In: Smit G (ed) Dairy processing. Improving quality, 1st edn. Woodhead Publishing Ltd, Cambridge

Burns P, Cuffia F, Milesi M, Vinderola G, Meinardi C, Sabbag N, Hynes E (2012) Technological and probiotic role of adjunct cultures of nonstarter lactobacilli in soft cheeses. Food Microbiol 30:45–50

Curioni P, Bosset J (2002) Key odorants in various cheese types as determined by gas chromatography–olfactometry. Int Dairy J 12:959–984

Hynes E, Ogier JC, Delacroix-Buchet A (2000) Protocol for the manufacture of miniature washed-curd cheeses under controlled microbiological conditions. Int Dairy J 10:733–737

Kieronczyk A, Skeie S, Langsrud T, Le Bars D, Yvon M (2004) The nature of aroma compounds produced in a cheese model by glutamate dehydrogenase positive *Lactobacillus* INF15D depends on its relative aminotransferase activities towards the different amino acids. Int Dairy J 14:227–235

Klein N, Maillard M-B, Thierry A, Lortal S (2001) Conversion of amino acids into aroma compounds by cell-free extracts of *Lactobacillus helveticus*. J Appl Microbiol 91:404–411

Le Bars D, Yvon M (2008) Formation of diacetyl and acetoin by *Lactococcus lactis* via aspartate catabolism. J Appl Microbiol 104:171–177

Liu S-Q, Holland R, Crow V (1998) Ethyl butanoate formation by dairy lactic acid bacteria. Int Dairy J 8:651–657

McSweeney P, Sousa M (2000) Biochemical pathways for the production of flavor compounds in cheeses during ripening: a review. Lait 80:293–324

Milesi M, Vinderola G, Sabbag N, Meinardi CA, Hynes E (2009) Influence on cheese proteolysis and sensory characteristics of nonstarter lactobacilli strains with probiotic potential. Food Res Int 42:1186–1196

Milesi M, Wolf I, Bergamini C, Hynes E (2010) Two strains of nonstarter lactobacilli increased the production of flavor compounds in soft cheeses. J Dairy Sci 93:5020–5031

Milesi M, Bergamini C, Hynes E (2011) Production of peptides and free amino acids in a sterile extract describes peptidolysis in hard-cooked cheeses. Food Res Int 44:765–773

Pinho O, Pérès C, Ferreira I (2003) Solid-phase microextraction of volatile compounds in "Terrincho" ewe cheese: comparison of different fibers. J Chromatogr A 1011:1–9

Shakeel-Ur-Rehman, McSweeney P, Fox P (1998) Protocol for the manufacture of miniature cheeses. Lait 78:607–620

Skeie S, Kieronczyk A, Naess R, stlie H (2008) *Lactobacillus* adjuncts in cheese: their influence on the degradation of citrate and serine during ripening of a washed curd cheese. Int Dairy J 18:158–168

Smit G, Smit B, Engels W (2005) Flavor formation by lactic acid bacteria and biochemical flavor profiling of cheese products. FEMS Microbiol Rev 29:591–610

Tavaria F, Dahl S, Carballo F, Malcata F (2002) Amino acid catabolism and generation of volatiles by lactic acid bacteria. J Dairy Sci 85:2462–2470

Thage BV, Rattray FP, Laustsen MW, Ardö Y, Barkholt V, Houlberg U (2004) Purification and characterization of a branched-chain amino acid aminotransferase from *Lactobacillus paracasei* subsp. *paracasei* CHCC 2115. J Appl Microbiol 96:593–602

Thage BV, Broe ML, Petersen MH, Petersen MA, Bennedsen M, Ardö Y (2005) Aroma development in semihard reduced-fat cheese inoculated with *Lactobacillus paracasei* strains with different aminotransferase profiles. Int Dairy J 15:795–805

Urbach G (1995) Contribution of lactic acid bacteria to flavor compound formation in dairy products. Int Dairy J 5:877–903

Van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J Chromatogr 11:463–471

Yvon M (2006) Key enzymes for flavor formation by lactic acid bacteria. Aust J Dairy Technol 61:16–24