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Biosynthesis and Role of 3-methylbutanal in Cheese by Lactic Acid Bacteria: Major Metabolic Pathways, Enzymes Involved and Strategies for Control

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Review

Biosynthesis and role of 3-methylbutanal in cheese by lactic acid bacteria: Major metabolic pathways, enzymes involved and strategies for control

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Abstract

Branched chain aldehyde, 3-methylbutanal is associated as a key flavor compound to many hard and semi-hard cheese varieties. The presence and impact of this flavor compound in bread, meat and certain beverages has been recently documented, however its presence and consequences regarding cheese flavor were not clearly reported. This paper gives an overview of the role of 3-methylbutanal in cheese, along with the major metabolic pathways and key enzymes leading to its formation. Moreover, different strategies are highlighted for the control of this particular flavor compound in specific cheese types.

Keywords: Cheese, flavor formation, 3-methylbutanal, metabolic pathways, control strategies

1 Introduction

The presence of branched-chain volatile aldehydes has been reported to be perceived either as a malty/off-flavor or as nutty/chocolate-like aroma among various cheese types associated to both hard and soft categories. Special emphasis has been given to 3-methylbutanal, as its concentration or relative abundance always seemed to be higher as compared to 2-methylbutanal and 2-methylpropanal due to the efficient breakdown of leucine by the cheese microbiota (Tucker & Morgan, 1967; Bosset & Gauch, 1993; Centeno *et al.*, 2002; Morales *et al.*, 2003; Ayad *et al.*, 2004b; Deetae *et al.*, 2007; Irigoyen *et al.*, 2007). Some decades ago, the identification, chemical nature of malty flavor and microbial defects in milk resulting from the use of malty flavor producing strain, *Streptococcus lactis* var. *maltigenes* were dealt with in various works (Jackson & Morgan, 1954; MacLeod & Morgan, 1955; Morgan *et al.*, 1966; Morgan, 1970; Sheldon *et al.*, 1971). Since then, the researchers gave attention to the possible consequences of the presence of these flavor compounds while characterising cheese flavor profiles and corresponding cheese microflora.

Recently, many studies have highlighted both the desirable and non desirable role of 3-methylbutanal with respect to various cheese types. It is assumed that the flavor perception might be related to numerous factors including cheese moisture, texture, milk type and more particularly, internal balance of many odorous compounds generated from various sources like proteins, fat and carbohydrates. However, to get a desired flavor from a cheese is yet a very complex and difficult task, as little imbalance between various odorous compounds could impair the final flavor quality (Wallace & Fox, 1997; Møller *et al.*, 2013).

Lactic acid bacteria (LAB) belonging to genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Carnobacterium* and *Enterococcus* are generally supposed to be involved in the formation of 3-methylbutanal in milk and cheese (Miller *et al.*, 1974; Ward *et al.*, 1999; Helinck *et al.*, 2004; Smit *et al.*, 2004; Afzal *et al.*, 2010; Afzal *et al.*, 2012; Sinz & Schwab, 2012; Afzal *et al.*, 2013b; Bergamini *et al.*, 2013). Moreover, some yeasts such as *Debaryomyces hansenii*, *Yarrowia lipolytica* and *Geotrichum candidum* when used as adjunct or starter in soft cheese manufacturing were also found to contribute the formation of 3-methylbutanal (Bintsis & Robinson, 2004; Boutrou & Guéguen, 2005; Sørensen *et al.*, 2011; Gkatzionis *et al.*, 2013).

Extensive studies have been carried out to investigate the possible biosynthetic pathways of 3-methylbutanal in bacteria. The intracellular biosynthesis of 3-methylbutanal from leucine catabolism generally takes place by the two possible metabolic pathways in LAB: either by a direct pathway using α -ketoacid decarboxylase enzyme (KADC) such as proven for *L. delbrueckii* subsp. *lactis* CNRZ 207 (Helinck *et al.*, 2004) or by an indirect pathway comprising α -ketoacid dehydrogenase enzyme (KADH) such as proven for *L. helveticus* CNRZ 32 (Helinck *et al.*, 2004) and *E. faecalis* 10C1 (Ward *et al.*, 1999). Recently, it has been demonstrated that in *Carnobacterium maltaromaticum* both metabolic pathways were present and functional (Afzal *et al.*, 2012).

Due to the importance of this flavor compound in particular cheese types, numerous strategies have been proposed to control its production. Different approaches have been pointed out using exogenous addition of substrate, microbial adjuncts possessing potential and complementary metabolic pathways, bacteriocin induced lysis, and modifying the environmental conditions by changing oxygen or potential redox. Recently, Smit *et al.* (2009) documented the

origin, presence and impact of branched-chain aldehydes such as 3-methylbutanal, 2-methylbutanal and 2-methylpropanal in bread, meat and certain beverages. The aim of the present review is to clarify and highlight the role and significance of 3-methylbutanal related to particular cheese types and to summarize the current knowledge for its possible control in cheese products.

2 Key enzymes and metabolic pathways involved in the biosynthesis of 3-methylbutanal from leucine catabolism among LAB

The catabolism of leucine during cheese ripening is mainly initiated by the action of microbial aminotransferases, although chemical degradation (Strecker degradation) can also occur (Yvon *et al.*, 1997; Yvon & Rijnen, 2001; Ardo, 2006; Smit *et al.*, 2009; García-Cayuela *et al.*, 2012) (Figure 1). Among LAB, the deamination of glutamate to α -ketoglutarate (α -KG) catalyzed by glutamate dehydrogenase (GDH) is usually linked to a transamination route.

2.1 Aminotransferase and glutamate dehydrogenase

Aminotransferase activity (AT) was found to be present in a large group of cheese related LAB. Activities varied and diversity existed among the strains (Yvon *et al.*, 1997; Smit *et al.*, 2004; Fernández de Palencia *et al.*, 2006; Brandsma *et al.*, 2008; Freiding *et al.*, 2012; García-Cayuela *et al.*, 2012). The major aromatic aminotransferase (AraT) was purified and characterized from *L. lactis* and the role of this enzyme in the initiation of degradation of several

amino acids including leucine was demonstrated by Yvon *et al.* (1997), which was responsible for the synthesis of precursors of aroma compounds usually found active under cheese ripening conditions. Moreover, it was found to exhibit overlapping substrate specificities towards both branched chain and aromatic amino acids (Yvon *et al.*, 1997; Yvon & Rijnen, 2001). The role of both aromatic and branched chain aminotransferases (AraT/BcaT) was studied in a cheese model (Rijnen *et al.*, 2003) and it was demonstrated that both BcaT and AraT were involved in the degradation of leucine. Leucine is converted into α -ketoisocaproate by aminotransferase enzyme and during this conversion the amino group of leucine is transferred to α -ketoglutarate resulting in the formation of glutamic acid.

The importance of GDH activity as major criterion for the selection of flavor-producing LAB strains was pointed to intensify cheese aroma (Tanous *et al.*, 2002). Later on, a beneficial effect on aroma formation was observed by using a combination of GDH positive lactobacilli with *L. lactis* ssp. *cremoris* NCDO763 (Kieronczyk *et al.*, 2004). The biosynthetic pathways for α -KG formation and its impact on cheese aroma development was explored and it has been shown that the citrate-oxaloacetate pathway, that requires citrate permease (CitP), citrate lyase (CitL) and aspartate aminotransferase, was operative for *L. lactis* ssp. *diacetylactis* and hence stimulated the conversion of amino acids (Tanous *et al.*, 2005).

2.2 Major metabolic pathways for the biosynthesis of 3-methylbutanal

The transamination of leucine results in α -ketoisocaproate, which is the central metabolite in leucine catabolism (Smit *et al.*, 2004) and gives rise to 3-methylbutanal either directly as a

result of non-oxidative decarboxylation by α -ketoacid decarboxylase (KADC) or indirectly via an oxidative decarboxylation by the activity of α -ketoacid dehydrogenase (KADH) (Larrouture-Thiveyrat & Montel, 2003; Helinck *et al.*, 2004). In the literature, the direct pathway for the biosynthesis of 3-methylbutanal is very well documented in LAB. On the contrary, the indirect pathway was not studied extensively (Table 1).

2.2.1 The direct pathway

The direct pathway appeared to be rare among LAB and KADC activity was found to be highly strain dependent with broad substrate specificity (Smit *et al.*, 2005). KADC activity has been reported in *L. lactis* (Smit *et al.*, 2004), some *L. lactis* wild strains (Ayad *et al.*, 1999; Ayad *et al.*, 2001; De la Plaza *et al.*, 2004; Fernández de Palencia *et al.*, 2006), *L. delbrueckii* (Helinck *et al.*, 2004) and *C. maltaromaticum* (Afzal *et al.*, 2012). The gene encoding KADC enzyme (*kdcA*) was identified by N-terminal sequencing of the partially purified protein in *L. lactis* (De la Plaza *et al.*, 2004) or by screening a mutant library in *L. lactis* (Smit *et al.*, 2005). In *L. lactis*, KADC activity would be of prime importance in order to control desired formation of 3-methylbutanal, as most of the aldehydes could be formed through this pathway (Smit *et al.*, 2004).

2.2.2 The indirect pathway

The indirect pathway constituted the oxidative decarboxylation of α -ketoisocaproate to isovaleryl-CoA by the KADH enzyme complex along with several intermediate enzymes including phosphotransferase (PTA), acyl kinase (ACK), and aldehyde dehydrogenase for the biosynthesis of 3-methylbutanal. This pathway has been reported *in vitro* in *Propionibacterium freudenreichii* (Thierry *et al.*, 2002), *L. helveticus* (Helinck *et al.*, 2004), *C. maltaromaticum* (Afzal *et al.*, 2012) and via the gene product in *Enterococcus faecalis* (Ward *et al.*, 1999). The KADH enzyme activity was determined and found to be dependant of both NAD^+ and NADP^+ in *C. maltaromaticum* (Afzal *et al.*, 2012). The genes encoding KADH complex were found encoded in one operon (*ptb-buk-bkDABC*) in *E. faecalis* (Ward *et al.*, 1999) and in *L. casei* (Liu *et al.*, 2008). In *C. maltaromaticum* LMA 28 strain, these genes encoding KADH enzyme complex (*bkdA*, *bkdB*, *bkdC* and *bkdD*) have been identified by degenerate primer design (Afzal *et al.*, 2012).

2.2.3 Regulation of 3-methylbutanal biosynthesis

The biosynthesis of 3-methylbutanal in bacteria depends on the functionality of intracellular pathways and is mainly regulated by redox environment (NAD^+/NADH , H^+ yield, presence/absence of oxygen). Indeed, high formation of 3-methylbutanal in *L. lactis* (Kieronczyk *et al.*, 2006), *Proteus vulgaris* (Deetae *et al.*, 2011) and *C. maltaromaticum* (Afzal *et al.*, 2013a) has been attributed to the presence of oxygen or stimulation of KADC enzyme activity. It is interesting to note that the resulted aldehyde can be further reduced to alcohol by alcohol dehydrogenase or oxidized to carboxylic acid by an aldehyde dehydrogenase depending upon the

intracellular redox environment (Kieronczyk *et al.*, 2006; Afzal *et al.*, 2012). Oxidative conditions favoured the production of aldehyde while reducing conditions favoured the production of carboxylic acid (Kieronczyk *et al.*, 2006; Afzal *et al.*, 2012; Afzal *et al.*, 2013a).

3 Presence and role of 3-methylbutanal in various varieties of cheeses

An overview of previous studies on flavor characteristics of some cheeses revealed the presence and crucial role of 3-methylbutanal for the unique flavor development in these cheese types (Table 2).

The presence of Strecker aldehyde 3-methylbutanal (73 ó 210 ppb) in hard cheddar cheese made from cow's pasteurized milk was reported to be responsible for a nutty/balanced flavor and was considered as desirable (Avsar *et al.*, 2004; Hannon *et al.*, 2006). However, on the contrary, the presence of 3-methylbutanal in relative peak area (10 ó 143) and relative abundance (1.28 ó 2.17) in Egyptian Ras and Manchego cheese usually made from either cow/buffalo/sheep's raw milk revealed an unclean/burnt flavor (Centeno *et al.*, 2002; Ayad *et al.*, 2004a) which was considered as non desirable. This unclean/burnt flavor was attributed to the high levels of aldehydes and alcohols and was related to the poor quality milk used for cheese manufacture. The flavor perception and desirability of aldehydes in Parmigiano Reggiano, Parmesan and Roncal cheese made from either cow/sheep's raw and pasteurized milk were not clearly reported (Bosset & Gauch, 1993; Barbieri *et al.*, 1994; Irigoyen *et al.*, 2007).

The perceived chocolate-like aroma after six weeks of cheese ripening in semi-hard Proosdij-type cheese made from cow's pasteurized milk using a mesophilic strain *L. lactis* ssp.

lactis B851 with acidifying mesophilic and an adjunct thermophilic culture was attributed to the presence of high concentration in relative peak area (15000 - 27000) of 3-methylbutanal (Ayad *et al.*, 2003). A similar chocolate-like flavor was perceived as well due to the presence of 3-methylbutanal (45.5 ng.g⁻¹) in Gouda and in relative peak area (352/1107) in Gouda/Proosdij type cheese and was considered as desirable (Engels *et al.*, 1997; Van Leuven *et al.*, 2008). During the study of aroma development in reduced-fat semi-hard cheese using culture adjunct of *Lactobacillus paracasei* (CHCC 4256), 4 times higher concentration of aldehydes and alcohols (0.9, 8.8 µmol.kg⁻¹ cheese) were determined as compared to controls but flavor perception/desirability was not clearly mentioned (Thage *et al.*, 2005). Some wild strains of *L. lactis* isolated from ewesøraw milk cheeses were reported to produce high levels of aldehydes (0.938 ± 1.179 mg.kg⁻¹ cheese) with respective odour threshold in water (0.06) and alcohols (18.20 ± 20.35 mg.kg⁻¹ cheese) with respective odour threshold in water (4.75), which were considered as responsible for abnormal odours (Morales *et al.*, 2003).

Aldehydes and alcohols were generally present and considered as potent odorants in soft cheeses. At low concentrations, they were perceived as a fruity flavor and considered desirable, while at high concentration, they resulted in rather off-flavor and highly non desirable (Sable & Cotteceau, 1999). The impact of various microorganisms on aromatic profiles of soft cheese has started to be elucidated (Bintsis & Robinson, 2004; Massouras *et al.*, 2006; Deetae *et al.*, 2009; Irlinger *et al.*, 2012). A richer pattern of 3-methylbutanal (37 ± 19 µg.kg⁻¹ cheese) and 3-methylbutanol (1400 ± 1062 µg.kg⁻¹ cheese) was achieved using *Lactobacillus paracasei* subsp. *paracasei* and *Debaryomyces hansenii* as adjuncts in the manufacture of Feta-type cheese (Bintsis & Robinson, 2004). During the study of aromatic profile of Teleme cheese made from

either sheep's or goat's milk or a combination of both, Massouras *et al.* (2006) found the highest level of volatile compounds in arbitrary units including 3-methylbutanal (1766 \pm 2765) and 3-methylbutanol (491 \pm 696) in cheese made from sheep's milk.

4 Strategies for control of 3-methylbutanal concentration in cheese

The significance and impact of 3-methylbutanal in cheese has attracted considerable attention for obtaining desired formation in specific cheese types. A number of strategies have been followed during these recent years to control the formation of 3-methylbutanal in cheese using cheese models at laboratory scale or *in vitro* investigations (Table 3). In this respect, a balanced flavor may be obtained by accelerating cheddar cheese ripening and proteolysis by the addition of free amino acids and enzyme modified cheese powder (EMC), however both strategies seemed to be expensive and not practical at industrial scale use (Wallace & Fox, 1997; Hannon *et al.*, 2006). Most of the volatile compounds are generated through amino acid catabolism, and define final aroma characteristics of particular cheese. Addition of α -kg (transaminase acceptor) in cheese curd has been shown to accelerate amino acid catabolism to enhance volatile compounds (Banks *et al.*, 2001; Williams *et al.*, 2001; Tanous *et al.*, 2002; Kieronczyk *et al.*, 2004). To obtain desired flavor formation, the use of particular isolates with either potential enzyme activities (AT, GDH, KADC) (Ayad *et al.*, 2003; Kieronczyk *et al.*, 2004; Thage *et al.*, 2005; Whetstine *et al.*, 2006; Garde *et al.*, 2007; Pappa *et al.*, 2013; Ruysen *et al.*, 2013; Sgarbi *et al.*, 2013; Salmerón *et al.*, 2014) or enzymes complementing metabolic pathways seemed to be the most promising approach (Ayad *et al.*, 2001; Amárita *et al.*, 2006;

Pedersen *et al.*, 2013). Moreover, relative AT activities of GDH positive lactobacilli were found to be of major importance for the selection of culture adjuncts in cheese aroma development (Kieronczyk *et al.*, 2004). As most of the flavor compound 3-methylbutanal is formed via KADC/KADH or both pathways from α -ketoisocaproate, which is the central metabolite in leucine catabolism (Smit *et al.*, 2004; Afzal *et al.*, 2012), the inactivation of *panE* gene encoding α -hydroxyacid dehydrogenase activity in *L. lactis* IFPL953 Δ *panE* resulted in enhanced formation of 3-methylbutanal and 3-methylbutanol by making more substrate i.e. α -ketoisocaproate available for decarboxylation and conversion to respective flavor compounds (De Cadinanos *et al.*, 2013). Another interesting strategy for the enhancement of cheese aroma and more particularly, the aldehydes, could be the use of bacteriocin producing strains to induce lysis of the bacteriocin sensitive adjunct cultures and as a consequence, to promote the release of intracellular enzymes and their accessibility towards corresponding substrates hence could contribute in the development of cheese flavor (Martínez-Cuesta *et al.*, 2002; Fernández de Palencia *et al.*, 2004; Martínez-Cuesta *et al.*, 2006). Many studies have started to investigate the influence of various parameters like oxygen or redox potential on the flavor forming pathways, for the control of desired formation of aldehydes/alcohols in cheese (Kieronczyk *et al.*, 2006; Deetae *et al.*, 2011; Afzal *et al.*, 2012; Caldeo & McSweeney, 2012; Afzal *et al.*, 2013a). Indeed, the presence of oxygen/oxidizing agent was found responsible for the increased formation of 3-methylbutanal (Kieronczyk *et al.*, 2006; Deetae *et al.*, 2011). Recently, It has been demonstrated that the curd washing and addition of milk and cream during cheese manufacture could also significantly influence the sensory and volatile profile of cheese (Broadbent *et al.*, 2013; Kaminarides *et al.*, 2013; Hou *et al.*, 2014). García-Cayuela *et al.* (2012) observed high

formation of 3-methylbutanal and 3-methylbutanol in *L. lactis* IFPL730 and they proposed that high levels of volatile compounds can be obtained by increasing level of genes expression of enzymes involved (*bcaT*, *kivD*) under branched chain amino acids starvation. Until now, a chocolate-like flavor due to 3-methylbutanal could not be perceived in soft cheese; instead, main emphasis has been given to obtain a well-balanced flavor.

5 Conclusions

The branched chain aldehyde, 3-methylbutanal is associated as a key flavor compound to many hard/semi-hard cheese varieties, while, it is considered as potent aromatic compound in soft cheese. This flavor compound arises from leucine catabolism either by the direct pathway or indirect pathway or from both depending upon the functionality of these pathways in cheese related microorganisms. In some of the hard/semi-hard cheese varieties, the presence and role of 3-methylbutanal is regarded as chocolate-like and highly desirable. Many strategies have been proposed for the control of this flavor compound. The combination of knowledge of flavor forming pathways, control strategies, final flavor perception could lead to a better control of this specific flavor formation before considering its use in industrial applications.

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Table 1: Demonstration (*in vitro* and via gene product) of direct/indirect pathways for the biosynthesis of 3-methylbutanal in LAB

Bacterial species	Strain	Study model	Pathways KADC/KADH	Literature
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	NIZO B1157	<i>In vitro</i> /via gene product	KADC	(Ayad <i>et al.</i> , 1999; Ayad <i>et al.</i> , 2001; Smit <i>et al.</i> , 2004; Smit <i>et al.</i> , 2005)
<i>Lactococcus lactis</i>	IFPL730	<i>In vitro</i> /via gene product	KADC	(De la Plaza <i>et al.</i> , 2004; Fernández de Palencia <i>et al.</i> , 2006)
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	CNRZ 207	<i>In vitro</i>	KADC	(Helinck <i>et al.</i> , 2004)
<i>Lactobacillus helveticus</i>	CNRZ 32	<i>In vitro</i>	KADH	(Helinck <i>et al.</i> , 2004)
<i>Enterococcus faecalis</i>	10C1	Via gene product	KADH	(Ward <i>et al.</i> , 1999)
<i>Lactobacillus casei</i>	ATCC 334	Via gene product	KADH	(Liu <i>et al.</i> , 2008)
<i>Carnobacterium maltaromaticum</i>	LMA 28	<i>In vitro</i> /via gene product	KADC/KADH	(Afzal <i>et al.</i> , 2012)

Table 2: Presence and role of 3-methylbutanal in different cheese types

Cheese type	Cheese variety	Milk type	Raw/pasteurized	Bacteria/yeasts	Flavor/perception	Desirability of flavor	Literature
Hard	Cheddar	Cow	Pasteurized	<i>L. lactis</i> ssp. <i>lactis</i> , <i>L. lactis</i> ssp. <i>cremoris</i>	Dark chocolate/malty/nutty	D	(Avsar <i>et al.</i> , 2004)
	Cheddar	Cow	Pasteurized	<i>L. lactis</i> ssp. <i>lactis</i> 303, <i>L. lactis</i> ssp. <i>cremoris</i> 227 ± EMC powder	Balanced	D	(Hannon <i>et al.</i> , 2006)
	Egyptian Ras	Cow/buffalo	Raw	Without addition of starter cultures	Unclean	ND	(Ayad <i>et al.</i> , 2004a)
	Manchego	Sheep/ewes	Raw	<i>Lactococcus lactis</i> (BCV ⁺ , BCV ⁻ , CSC)	Burnt/toasted/unclean/nuts	ND	(Centeno <i>et al.</i> , 2002)
	Parmigiano Reggiano	Cow	Raw	Without addition of starter cultures instead natural whey culture added	NR	NR	(Bosset & Gauch, 1993)
	Parmesan	Cow	Raw	ó	NR	NR	(Barbieri <i>et al.</i> , 1994)
Semi-hard	Roncal	Sheep/ewes	Pasteurized	<i>Lactococcus lactis</i> (CSC), <i>Lactobacillus paracasei</i> (adjunct)	NR	NR	(Irigoyen <i>et al.</i> , 2007)
	Proosdij type/gouda	Cow	Pasteurized	Mixed strain mesophilic starter culture Bos, Mixed strain thermophilic starter culture APS,	Chocolate-like	D	(Ayad <i>et al.</i> , 2003)

Soft	Proosdij type	Cow	Pasteurized	<i>L. lactis</i> ssp. <i>lactis</i> B851 Mixed strain mesophilic starter culture Bos, Mixed strain thermophilic starter culture APS	Chocolate-like/nutty	D	(Engels <i>et al.</i> , 1997)
	Gouda-type	Cow	Raw/pasteurized	<i>L. lactis</i> ssp. <i>lactis</i> biovar <i>diacetylactis</i> , <i>L. lactis</i> ssp. <i>cremoris</i>	Chocolate-like/nutty	D	(Van Leuven <i>et al.</i> , 2008)
	Ewesøraw milk	Sheep/ewes	Pasteurized	<i>Lactococcus lactis</i> (CSC and wild strains)	Roasted hazel/nuts	ND	(Morales <i>et al.</i> , 2003)
	Reduced-fat round-eyed	Cow	Pasteurized	DL-starter (CH-N11, Chr. Hansen A/S), <i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> CHCC 4256	NR	NR	(Thage <i>et al.</i> , 2005)
	Feta-type	Sheep/ewes	Pasteurized	<i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> , <i>Debaryomyces hansenii</i>	NR	NR	(Bintsis & Robinson, 2004)
	Teleme	Sheep/ewes	Pasteurized	<i>L. lactis</i> ssp. <i>lactis</i> , <i>L. lactis</i> ssp. <i>cremoris</i> , <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> , <i>Streptococcus thermophilus</i>	NR	NR	(Massouras <i>et al.</i> , 2006)

Camembert-type cheese model	Cow	Pasteurized	<i>Proteus vulgaris</i> 1M10, <i>Debaryomyces hansenii</i> 304	Fruity	NR	(Deetae <i>et al.</i> , 2009)
Smear soft cheese model	Cow	Pasteurized	<i>L. lactis</i> ssp. <i>lactis</i> , model community (7 bacteria, 4 yeast), \pm <i>Psychrobacter celer</i> 91, \pm <i>Hafnia alvei</i> 2920	Fruity	NR	(Irlinger <i>et al.</i> , 2012)

BCV⁺ branched chain volatile compounds producing strains; BCV⁻ branched chain volatile compounds not producing strains;

CSC commercial starter cultures; D desirable; ND non desirable; NR not reported

Table 3: Proposed strategies for control of 3-methylbutanal concentration in cheese

Proposed strategies	Cheese variety/model used	Type	Bacteria used	Resulting flavor	Literature
Addition of free amino acids at intermediate level	Cheddar	Hard	<i>L. lactis</i> ssp. <i>cremoris</i> 223	Clean/mature	(Wallace & Fox, 1997)
Addition of α -kg in cheese curd for accelerating amino acids catabolism	Cheddar	Hard	Mixture of MaO11 and the isolate HRI:C27 (Starter B)	Fruity	(Banks <i>et al.</i> , 2001; Williams <i>et al.</i> , 2001)
Addition of enzyme-modified cheese powder for flavor/ripening acceleration	Cheddar	Hard	<i>L. lactis</i> ssp. <i>lactis</i> 303, <i>L. lactis</i> ssp. <i>cremoris</i> 227 + EMC powder	Balanced	(Hannon <i>et al.</i> , 2006)
Addition of culture adjuncts	Cheddar	Hard	<i>L. lactis</i> 850, <i>L. lactis</i> ATCC 29146 (adjunct)	Nutty/chocolate-like	(Whetstine <i>et al.</i> , 2006)
	Hispanico	Hard	<i>L. lactis</i> ssp. <i>lactis</i> INIA 639, <i>L. lactis</i> ssp. <i>lactis</i> INIA 437, <i>Lactobacillus helveticus</i> LH 92	Intensed	(Garde <i>et al.</i> , 2007)
	Proosdij	Semi-hard	Mixed strain mesophilic starter culture Bos, Mixed strain thermophilic starter culture APS, <i>L. lactis</i> ssp. <i>lactis</i> B851 (adjunct)	Nutty/chocolate-like	(Ayad <i>et al.</i> , 2003)
	Reduced-fat round-eyed	Semi-hard	DL-starter (CH-N11, Chr. Hansen A/S), <i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> CHCC 4256 (adjunct)	Aromatic/sweet	(Thage <i>et al.</i> , 2005)
Strains possessing enzymes complementing metabolic	Gouda/cheddar	Hard	<i>L. lactis</i> ssp. <i>cremoris</i> SK110, <i>L. lactis</i> ssp. <i>cremoris</i> NIZO B1157	Chocolate-like	(Ayad <i>et al.</i> , 2001)

pathways	Milk	-	<i>L. lactis</i> IFPL730, <i>L. lactis</i> IFPL326	Ripened cheese	(Amárita <i>et al.</i> , 2006)
Bacteriocin induced lysis	<i>In vitro</i>	-	<i>L. lactis</i> ssp. <i>lactis</i> IFLP359, <i>L. lactis</i> IFLP105 (lacticin 3147 producer)	Intensed	(Martínez-Cuesta <i>et al.</i> , 2002)
	<i>In vitro</i>	-	<i>L. lactis</i> ssp. <i>lactis</i> IFLP359, <i>L. lactis</i> IFLP730, <i>L. lactis</i> IFLP105 (lacticin 3147 producer)	Intensed	(Martínez-Cuesta <i>et al.</i> , 2006)
	Cheese model	-	<i>L. lactis</i> ssp. <i>lactis</i> IFLP3593, <i>L. lactis</i> IFLP730	Intensed	(De la Plaza <i>et al.</i> , 2004)
Environmental modifications by static and shaking conditions	<i>In vitro</i>	-	<i>Proteus vulgaris</i> 1M10	Off flavor	(Deetae <i>et al.</i> , 2011)
Oxidizing or reducing agents	<i>In vitro</i>	-	<i>L. lactis</i> ssp. <i>cremoris</i> NCDO 763, <i>L. lactis</i> ssp. <i>lactis</i> NCDO 1867	Off flavor	(Kieronczyk <i>et al.</i> , 2006)
Level of genes expressions of enzymes involved in branched chain amino acids catabolism	<i>In vitro</i>	-	<i>L. lactis</i> IFPL730	Potent	(García-Cayuela <i>et al.</i> , 2012)

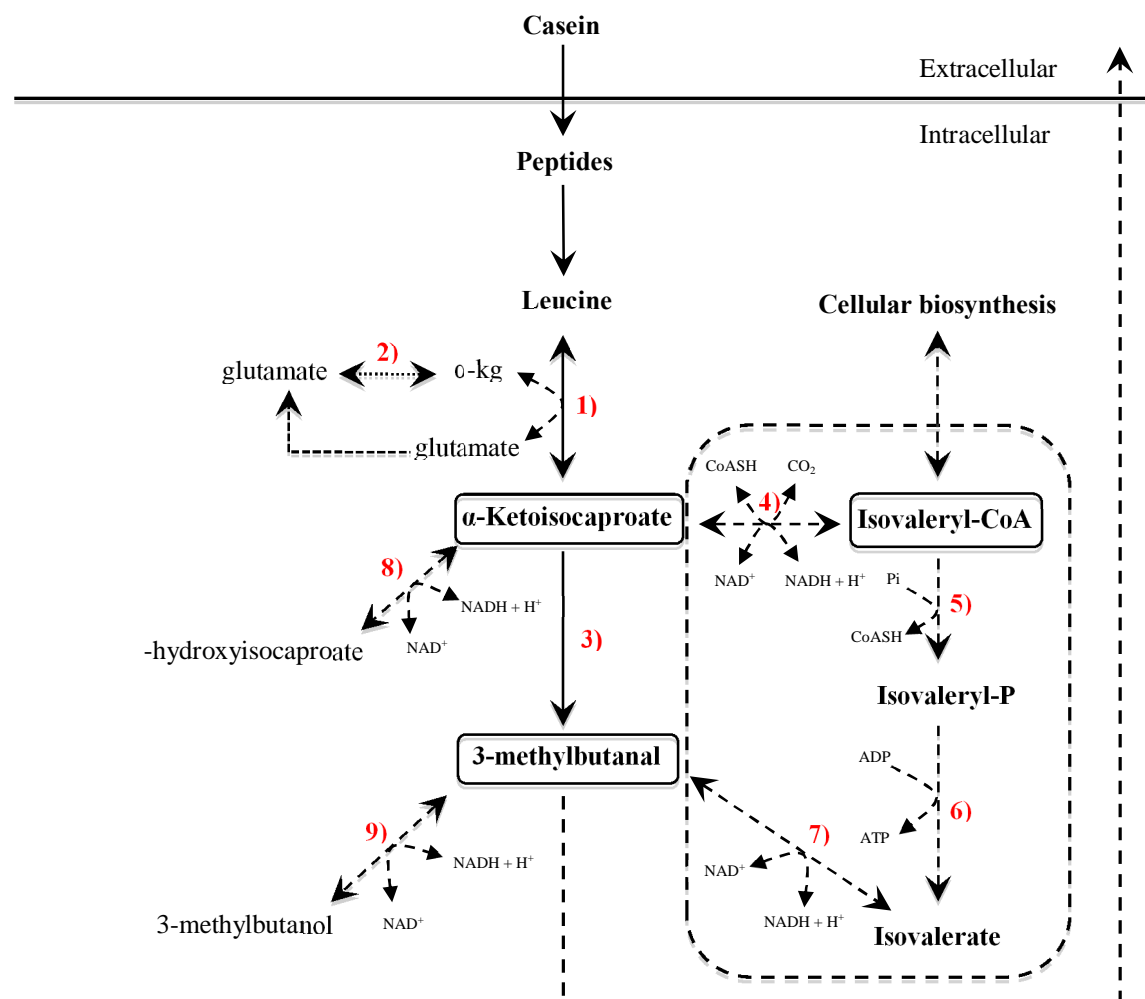


Figure 1: Intracellular possible metabolic pathways for the biosynthesis of 3-methylbutanal from leucine catabolism by LAB (Afzal *et al.*, 2012). Enzymes of the direct pathway (in the solid line): 1) AT, aminotransferase, 2) GDH, glutamate dehydrogenase, 3) KADC, α -ketoacid decarboxylase. Enzymes of indirect pathway (in the dashed lines): 4) KADH, α -ketoacid dehydrogenase, 5) PTA, phosphotransferase, 6) ACK, acylkinase, 7) AldDH, aldehyde

dehydrogenase. Other enzymes include 8) HADH, α -hydroxyacid dehydrogenase, 9) AlcDH, alcohol dehydrogenase