



Factors influencing biogenic amines accumulation in dairy products

Daniel M. Linares, Beatriz del Río, Victor Ladero, Noelia Martínez, María Fernández, María Cruz Martín and Miguel A. Álvarez*

Instituto de Productos Lácteos de Asturias, (IPLA – CSIC), Villaviciosa, Spain

Edited by:

Giovanna Suzzi, University of Teramo, Italy

Reviewed by:

Giuseppe Spano, University of Foggia, Italy

Maria Martuscelli, University of Teramo, Italy

*Correspondence:

Miguel A. Álvarez, Instituto de Productos Lácteos de Asturias, Carretera de Infiesto s/n. 33300 Villaviciosa, Spain.
e-mail: maag@ipla.csic.es

Fermented foods are among the food products more often complained of having caused episodes of biogenic amines (BA) poisoning. Concerning milk-based fermented foods, cheese is the main product likely to contain potentially harmful levels of BA, specially tyramine, histamine, and putrescine. Prompted by the increasing awareness of the risks related to dietary uptake of high biogenic amine loads, in this review we report all those elaboration and processing technological aspects affecting BA biosynthesis and accumulation in dairy foods. Improved knowledge of the factors involved in the synthesis and accumulation of BA should lead to a reduction in their incidence in milk products. Synthesis of BA is possible only when three conditions converge: (i) availability of the substrate amino acids; (ii) presence of microorganisms with the appropriate catabolic pathway activated; and (iii) environmental conditions favorable to the decarboxylation activity. These conditions depend on several factors such as milk treatment (pasteurization), use of starter cultures, NaCl concentration, time, and temperature of ripening and preservation, pH, temperature, or post-ripening technological processes, which will be discussed in this chapter.

Keywords: biogenic amines, cheese, producing microorganisms, pasteurization, starters, ripening, chemico-physical factors

INTRODUCTION

Fermented foods, and especially cheese, are within the food products more often related with biogenic amines (BA) poisoning (Sumner et al., 1985; EFSA Panel on Biological Hazards (BIOHAZ), 2011). Under normal conditions, exogenous BA ingested with food are rapidly detoxified by the action of amine oxidases, but whenever the detoxification process is disturbed, or the BA concentration in food is very high, BA become toxic metabolites responsible of serious human health problems (Repka-Ramírez and Baraniuk, 2002; Soufleros et al., 2007; Ladero et al., 2010a; Spano et al., 2010). In the 60s of last century, a close relation between migraine crisis and ingestion of tyramine-rich food (especially cheese) was observed (Blackwell, 1963; Hanington, 1967; Coutts et al., 1986). In fact, these effects of tyramine consumption were coined as *cheese-reaction*. Despite the toxicity of BA, their limit concentrations in fermented foodstuffs have not yet been adequately standardized by regulatory agencies.

The most important BA in dairy foods are histamine, tyramine (produced by enzymatic decarboxylation of histidine and tyrosine, respectively), putrescine (synthesized via ornithine decarboxylation or agmatine deamination), and to a minor extent, cadaverine (originated by lysine decarboxylation; Linares et al., 2011). These amino acid decarboxylating activities are mainly attributable to the microbial groups that participate in the fermentation process.

Many bacteria of different genera and species have the capacity to produce BA. Gram negative bacteria (mainly *Enterobacteriaceae*) that can be present in milk are able to produce histamine, putrescine, and cadaverine (ten Brink et al., 1990; Marino et al., 2000; Pircher et al., 2007). However, the main BA producers in

cheese are mostly lactic acid bacteria (LAB) included in the genera *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Streptococcus* (Fernández et al., 2004; Martín et al., 2005; Bonetta et al., 2008; Calles-Enríquez et al., 2010; Ladero et al., 2011a). These Gram positive bacteria can be present: (i) in the milk, (ii) introduced by contamination throughout the entire process of cheese production, (iii) and may even be part of starter or adjunct cultures. Several authors have reported the presence of tyrosine and histamine decarboxylase activity in strains from various starter cultures (Burdychova and Komprda, 2007; Calles-Enríquez et al., 2010; La Gioia et al., 2011).

Consequently, formation of BA can occur during food processing and storage as a result of bacteria that have amino acid decarboxylase activities. The manufacture of dairy foods is not a sterile process, and BA producers are likely to enter the food chain as non-starter LAB that are indigenous to the raw material. Besides the presence of microorganisms with the appropriate metabolic pathway, BA formation is only possible if there is availability of the free substrate amino acids and the environment conditions are favorable to the decarboxylation activity (Russo et al., 2010). There are different factors related with such conditions: the milk treatments, the use of starter cultures and enzymes, the duration and the temperature of ripening, the level of proteolysis, the pH, the NaCl concentration, the presence of oxygen, the activity of water and the relative humidity, the bacterial density and synergistic effect between microorganisms are the most important (Gardini et al., 2001). In spite of great technological advantages, much of the dairy fermentations are still based on traditional, experienced-based techniques that are strongly rooted in specific geographic

regions. As a result, these factors can not always be modified in order to reduce the accumulation of BA without modifying the organoleptic and sensory properties of the final product.

Cheese represents an ideal matrix for production of BA because it is not sterile and the casein proteolysis ensures the availability of free substrate amino acids. Therefore, BA can reach concentrations in cheese up to 2000 mg kg⁻¹ (Roig-Sagués et al., 2002; Fernández et al., 2007a). Ingestion of cheese containing such a high concentration of BA is a direct threat to the consumer, especially in places like Europe where cheese is one of the major foods in the diet. The consumption of cheese in Europe in 2009 was 16.6 kg per capita [International Dairy Federation (IDF) Bulletin 446/2010, Statistics Canada]. However, BA concentration differs widely between different cheeses because depends – as indicated previously – on several factors.

Beside cheese, kefir is another fermented dairy product where BA may be present. Recently, Özdeştan and Üren (2010) reported total BA contents in kefir samples between 2.4 and 35.2 mg l⁻¹, being tyramine the prevailing BA. Similarly, Chaves-López et al. (2011) reported a total BA content of 15.31 mg l⁻¹ in kumis, a traditional Colombian fermented cow milk. Nevertheless, these levels are far below the recommended limits. Other milk-based fermented products including buttermilk or yogurt are not likely to contain significant levels of BA (Novella-Rodríguez et al., 2000; Souci et al., 2000). In the milk itself, the polyamines spermidine and spermine are the prevalent accumulated BA (Spano et al., 2010), however it remains unclear whether they are synthesized by microorganisms or they have an endogen origin.

Prompted by the increasing awareness of the risks related to dietary uptake of BA and the general agreement that they should not be allowed to accumulate in food, in this chapter we report about technological aspects affecting BA levels during cheese elaboration and processing. It should be noted that the control of these technological factors can be useful for the reduction of the BA accumulation on the final products.

OCCURRENCE OF BIOGENIC AMINES IN DAIRY PRODUCTS

Milk-based fermented foods, especially cheese, provide an adequate environment for BA production and accumulation (Roig-Sagués et al., 2002; Fernández et al., 2006a). However, big differences have been noted from different types of cheeses, which has been related to bacterial counts in the milk, thermal milk treatment intensity or duration and the length and conditions of the ripening process. The use of starter cultures could also influence the production of BA either directly or indirectly through the interaction with the wild cheese microbiota (Ordóñez et al., 1997). Beside this, a different distribution of BA through the cheese has been observed (Novella-Rodríguez et al., 2003). All these variables contribute to the enormous differences observed between different types of cheeses or even in the same type of cheese depending on the part analyzed or the ripening period. In order to offer an overview of BA content in dairy products, the data available in the literature have been summarized in **Table 1**.

BA-PRODUCING MICROORGANISMS IN DAIRY PRODUCTS

One of the indispensable conditions for the presence and further accumulation of BA in fermented dairy products is the presence of

microorganisms with the capacity to synthesize these toxic compounds. Among those microorganisms that have been identified as usual microbiota of cheeses, some of them have been identified as BA producers including yeast, Gram positive and Gram negative bacteria (**Table 2**), although not all of them have an impact in the presence of high concentrations of BA in the final product.

Several yeasts species have been described as potential producers of aliphatic amines (putrescine and cadaverine; Suzzi et al., 2003). However, only a few strains of *Debaryomyces hansenii* and *Yarrowia lipolytica* isolated from cheese seem to be able to produce histamine and tyramine respectively (Gardini et al., 2006). The presence of *Y. lipolytica*, *Pichia jadinii*, or *Geotrichum candidum* has been related in some cases (Wyder et al., 1999; Roig-Sagués et al., 2002) with an increase in the concentration of histamine and putrescine.

Most of the Gram negative bacteria described as usual contaminants of milk are able to produce histamine, putrescine, or cadaverine, i.e., *Escherichia coli*, *Hafnia alvei*, *Klebsiella pneumoniae*, *Morganella morganii*, *Pseudomonas*, or *Serratia* spp (Marino et al., 2000; Roig-Sagués et al., 2002; Coton et al., 2011). However, the presence of elevated concentrations of Gram negative bacteria – especially *Enterobacteriaceae* – has been only correlated in some cases with an increase in the concentration of putrescine or cadaverine (ten Brink et al., 1990; Pircher et al., 2007; Delbès-Paus et al., 2012). In those cases, BA are result of bad manufacturing practices, poor quality or insufficient hygienic conditions.

Nevertheless, the main BA producers in cheese are Gram positive bacteria, being LAB the main histamine and tyramine producers (Linares et al., 2011). Moreover, recent discoveries pointed out the importance of this bacterial group as putrescine producers (Ladero et al., 2011a, 2012a). The genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus* include some strains belonging to different species that have been described as BA producers (**Table 2**). Other LAB have been described as BA producers in some reports such as tyramine producing lactococci or lactobacilli (Fernández et al., 2004; Bunková et al., 2009; Nieto-Arribas et al., 2009). However, the identification of the species is not based on the sequencing of the 16S *rRNA* gene, or other gene with taxonomical relevance, which could lead to misclassification (Delgado and Mayo, 2004; Fernández et al., 2007b). All these BA-producing LAB belong to species that are normal microbiota of milk and cheese (Varnam and Sutherland, 1994) and can be potentially present during the elaboration of any fermented dairy product (Novella-Rodríguez et al., 2002; Ladero et al., 2009; Linares et al., 2011).

FACTORS INFLUENCING BIOGENIC AMINE PRODUCTION IN DAIRY PRODUCTS

The biosynthesis and accumulation of BA in dairy foods requires the presence of bacteria with decarboxylase activity, the appropriate environmental conditions for their growth and the activity of the decarboxylases, and the presence of the substrate amino acids. The influence of all these parameters in BA production is addressed in this section.

Table 1 | Biogenic amine content in dairy products (mg/kg).

Source	Type		Cad	His	Phe	Put	Tyr	Spn	Spd	Reference	
Milk	Raw	Cow	nd	nd	nd	nd	nd	0.18	nd	Novella-Rodríguez et al. (2000)	
		Ewe	–	0.3	–	–	–	–	–	Bodmer et al. (1999)	
	Pasteurized	Ewe	–	–	–	–	–	–	nd	Pinho et al. (2004)	
		Cow	–	0.7	–	–	–	–	–	Bodmer et al. (1999)	
Yogurt			–	13	–	–	–	–	–	Bodmer et al. (1999)	
			0.27	nd	nd	nd	nd	0.43	0.34	Novella-Rodríguez et al. (2000)	
Cheese (raw milk)		Sheep	123.07	50	–	107.69	125	115.38	369.23	Mercogliano et al. (2010)	
		Sheep	26.8	0	28.6	394.1	499.6	96.4	–	Schirone et al. (2011)	
		Goat	349.72	15.6	9.39	217.84	216.28	nd	nd	Pinho et al. (2004)	
		Goat	88.7	88.4	11.7	191.8	830.5	–	–	Novella-Rodríguez et al. (2002)	
		Goat	94	117	160	941	258	70.5	23.5	Galgano et al. (2001)	
		Cow	nd	nd	nd	nd	nd	nd	nd	Fernández et al. (2007b)	
	Parmigiano Extra hard grana	Cow	nd	nd	nd	nd	nd	nd	nd	Fernández et al. (2007b)	
		Cow	3.2	10.9	–	1.8	6.4	4.4	–	–	Mayer et al. (2010)
		Cow	15.56	28.55	9.51	75.87	29.89	7.71	4.46	Innocente and D'Agostin (2002)	
		Cow	–	249	–	–	18	–	–	–	Mayer et al. (2010)
Cheese (pasteurized milk)	Feta	Goat	82.8	84.6	4.94	193	246	nd	nd	Valsamaki et al. (2000)	
	Emmental Hard	Cow	98.3	23.5	–	38	52.2	–	16.8	Mayer et al. (2010)	
	Cheddar	Cow	nd	25.4	–	4.8	–	8.5	18.2	Mayer et al. (2010)	
	Semi hard	Cow	nd	–	–	–	24.3	5.0	17.3	Mayer et al. (2010)	
	Gouda	Cow	nd	–	–	–	2.43	0.5	1.73	Mayer et al. (2010)	
	Edam	Cow	nd	3.2	–	–	–	–	–	Mayer et al. (2010)	
	Gorgonzola	Cow	nd	23.7	–	–	–	–	–	Mayer et al. (2010)	
	Gorgonzola	Cow	33.7	255.3	–	3.2	13.2	10.8	17.2	Mayer et al. (2010)	
	Smear	Cow	748.2	168.3	–	31.3	247.6	18.5	16.6	Mayer et al. (2010)	
	Semiripenned	Cow	33.49	24.38	25.75	22.6	32.92	24.35	23.4	Latorre-Moratalla et al. (2009)	
		Cow	nd	65.42	–	175.39	80.9	–	–	Fernández et al. (2007b)	
		Goat	nd	27.68	–	18.12	30.48	–	–	Fernández et al. (2007b)	
	Pecorino Abruzzese	Sheep	nd	nd	–	nd	301.6	–	–	Fernández et al. (2007b)	
Sheep		80	90	300	200	280	nd	20	Martuscelli et al. (2005)		
Cheese (blue)	Roquefort		8.9	9.9	7.7	18.3	–	4.6	18.1	Mayer et al. (2010)	
			nd	50	nd	25	2000	nd	18	Rabie et al. (2011)	
			2101.4	376.6	39.7	2572	1585.4	–	–	Novella-Rodríguez et al. (2003)	
		Mixture	756.78	1041.81	875.8	1051.98	–	–	–	Fernández et al. (2007b)	
		489.4	127.2	–	237.56	526.63	–	–	Fernández et al. (2007b)		
Kefir			1.8	4.0	nd	12.1	12.8	nd	4.5	Özdestand and Üren (2010)	
			2.2	1.6	nd	1.4	9.8	nd	1.5	Özdestand and Üren (2010)	

Cad, cadaverine; His, histamine; Phe, 2-phenylethylamine; Put, putrescine; Tyr, tyramine; Spn, spermine; Spd, spermidine; nd, undetected; –, not determined.

ROLE OF BA-PRODUCING MICROORGANISMS

The presence of BA-producing microorganisms is an indispensable condition for the biosynthesis of these toxic compounds. However, their accumulation in high concentrations depends not only on the presence of these BA-producing microorganisms but also if they reach a minimum number, and the coincidence of different factors during the elaboration and storage of the dairy products (Joosten and Northolt, 1987; Ladero et al., 2008). In general, it was difficult to find a correlation between the presence of high concentrations of BA in cheeses with and increment of a specific group or population of LAB. This was due to two main causes: (i)

the fact that the capability to produce BA is mostly related to strain rather than to specie (Novella-Rodríguez et al., 2002) and (ii) the use of non-specific methods for the identification of these bacteria.

Certainly, the presence of the capability to produce BA in LAB has been described as a characteristic acquired through horizontal gene transfer associated to plasmids, mobile elements, or acidic resistance islands (Lucas et al., 2005, 2007; Marcobal et al., 2006b). However, in certain cases this trait can be considered as species-characteristic, such as the production of tyramine in *Enterococcus* (Ladero et al., 2012a) or that of putrescine in *Lactococcus* (Ladero et al., 2011a). In this latter case, it seems that the adaptation to the

Table 2 | BA-producing microorganisms from dairy products.

Biogenic amine	Producer microorganisms	Reference	
Histamine	Molds and yeast	<i>Debaryomyces hansenii</i> , <i>Geotrichum candidum</i>	Roig-Sagués et al. (2002), Gardini et al. (2006)
	Gram negative	<i>Enterobacteriaceae</i> , <i>Morganella morganii</i>	Marino et al. (2000), Coton et al. (2011)
	Gram positive	<i>Lactobacillus buchneri</i> , <i>Lactobacillus curvatus</i> , <i>Lactobacillus helveticus</i> , <i>Lactobacillus parabuchneri</i> , <i>Streptococcus thermophilus</i>	Martín et al. (2005), Burdychova and Komprda (2007), Calles-Enríquez et al. (2010)
Tyramine	Molds and yeast	<i>Yarrowia lipolytica</i>	Gardini et al. (2006)
	Gram negative	–	
	Gram positive	<i>Enterococcus casseliflavus</i> , <i>Enterococcus durans</i> , <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> , <i>Enterococcus hirae</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus curvatus</i> , <i>Streptococcus thermophilus</i> .	Fernández et al. (2007b), Lucas et al. (2007), Bonetta et al. (2008), Bunková et al. (2009), La Gioia et al. (2011), Ladero et al. (2012a)
Putrescine	Molds and yeast	<i>Debaryomyces hansenii</i> , <i>Yarrowia lipolytica</i>	Wyder et al. (1999)
	Gram negative	<i>Enterobacteriaceae</i> , <i>Proteus</i> .	ten Brink et al. (1990), Coton et al. (2011)
	Gram positive	<i>Enterococcus faecalis</i> , <i>Enterococcus hirae</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus curvatus</i> , <i>Lactococcus lactis</i>	Llacer et al. (2007), Lucas et al. (2007), Ladero et al. (2011a,b)
Cadaverine	Molds and yeast	<i>Yarrowia lipolytica</i>	Wyder et al. (1999)
	Gram negative	<i>Enterobacteriaceae</i> , <i>Halomonas</i> sp., <i>Morganella morganii</i> , <i>Pseudomonas putida</i>	Meng and Bennett (1992), Marino et al. (2000), Coton et al. (2011)
	Gram positive	–	
Tryptamine	Molds and yeast	–	
	Gram negative	<i>Morganella morganii</i> , <i>Proteus</i> , <i>Serratia</i>	Marino et al. (2000), Coton et al. (2011)
	Gram positive	–	
Phenylethylamine	Molds and yeast	<i>Yarrowia lipolytica</i>	Wyder et al. (1999)
	Gram negative	<i>Halomonas</i> , <i>Serratia</i>	Marino et al. (2000)
	Gram positive	<i>Enterococci</i>	Marcobal et al. (2006c)

In order to prevent misclassification, only those species which identification is based on the sequencing of 16S rRNA gene or other gene with taxonomical relevance have been included in the table.

milk environment and/or the empirical selective pressure exerted because of the use of the strains as fermentation starters resulted in the inactivation or even the loss of the capability to produce putrescine, a compound that confers undesirable flavor.

The development of molecular methods toward the specific detection and quantification of BA producers in dairy products (Fernández et al., 2006a; Ladero et al., 2010b,c, 2012b) allowed to establish a direct relationship between the number of BA-producing microorganisms and the final BA concentration (Ladero et al., 2008, 2010b, 2012b). A minimum number of these BA producers has been proposed to reach high concentrations of BA in the final product (Joosten and Northolt, 1987; Ladero et al., 2008, 2010b).

STARTER CULTURES

In most industrial dairy fermentations, starter cultures are used to ensure the standard quality of the final products. Some LAB generally used as starter cultures, may have specific amino acid decarboxylase activities and thus, the potential to synthesize BA that could be accumulated in the dairy products. Belonging to this group are lactococci, lactobacilli and streptococci, as mentioned in the previous section.

The risk of incorporating BA-producing strains can be minimized by using well-characterized starter cultures, excluding those strains with the undesirable potential to produce BA. Several strains of *Streptococcus thermophilus* (Calles-Enríquez et al., 2010; La Gioia et al., 2011), *Lactobacillus brevis* and *Lactobacillus curvatus* (Ladero et al., 2011b), and *Lactococcus lactis* (Ladero et al., 2011a) obtained from dairy sources have been identified as histamine, tyramine, or putrescine producers. This becomes particularly relevant if we consider that strains belonging to those species are frequently used as starter cultures for cheese production. Burdychova and Komprda (2007) have isolated a histamine-producing *Lactobacillus helveticus* strain from a starter used for cheese production. Similarly, other *Lactobacillus* spp. isolated from ripened cheese, have been shown to produce BA such as tyramine (Straub et al., 1994; Arena et al., 2007; Ladero et al., 2011b) and also putrescine (Ladero et al., 2011b).

These facts led the recommendation to include the non-production of BA as a condition of strains intended to be used as starter cultures (Crow et al., 2001; Linares et al., 2011; EFSA Panel on Biological Hazards (BIOHAZ), 2011) or even raise the question about the addition of absence of BA production and BA

production associated genes as qualification criteria in the “*Qualified Presumption of Safety*” (QPS) assessment scheme introduced by the European Food Safety Agency (EFSA). For this assessment, molecular methods for the identification and quantification of BA producers are currently available (Fernández et al., 2006b; Torriani et al., 2008; Ladero et al., 2010b, 2012b).

Another approach to reduce the accumulation of BA in dairy products, could be the use of adjunct cultures that include bacteria capable to degrade BA (Leuschner and Hammes, 1998; Naila et al., 2010) as is proposed for fish sauce, sausage, or wine fermentation (Martuscelli et al., 2000; Fadda et al., 2001; Gardini et al., 2002; García-Ruiz et al., 2011; Zaman et al., 2011). In the case of cheeses it was described the use of *Brevibacterium linens* to catabolize histamine and tyramine during the elaboration of Munster cheese (Leuschner and Hammes, 1998; Leuschner et al., 1998).

PASTEURIZATION

Milk is a rich organic fluid that provides the necessary nutrients for the growth of different microorganisms. Indeed, microbial loads of up to 10^7 cfu ml⁻¹ may be reached if storage conditions are not adequate (Varnam and Sutherland, 1994). The main microbiological groups present in raw milk are mesophilic LAB (enterococci, lactococci, lactobacilli, or leuconostoc), enterobacteriaceae, and psychotropic microorganisms such as *Pseudomonas* or *Acinetobacter* (Varnam and Sutherland, 1994; Muir and Banks, 2003; Martuscelli et al., 2005; Serio et al., 2007). Members of all these groups have been described as BA producers (Linares et al., 2011).

Pasteurization is a heat treatment that reduces the microbial load of raw milk. It has long been used by dairy industry to extend the shelf-life of the dairy products by reducing the presence of spoilage bacteria, pathogens, and those microorganisms with the capacity to produce toxic compounds that would render products unsafe for consumption (Lewis, 2003). The aim of the pasteurization is not the absolute elimination of the bacteria present on the milk, but the reduction of the bacterial load to levels safe for health and to ensure that the fermentative process is not on risk. The pasteurization reduces the bacterial load present in milk, including the BA producers. Thereby, cheeses elaborated with pasteurized milk use to have lower BA concentrations than those made with raw milk (Novella-Rodríguez et al., 2002; Fernández et al., 2007a; Naila et al., 2010). However, it is possible to detect BA in cheeses elaborated with pasteurized milk and sometimes at elevated concentrations (Fernández et al., 2007a; Pircher et al., 2007). The contamination with BA-producing microorganisms during the manufacture of cheese, due to deficient hygienic conditions, has been proposed as explanation (Pircher et al., 2007; Ladero et al., 2009). Moreover, some BA-producing *Lactobacillus* and *Enterococcus* are resistant to pasteurization and could develop as secondary microbiota after the thermal treatment, resulting in the apparition of BA in the final product (Ladero et al., 2011b). Both facts explain the existence of cheeses made with pasteurized milk and relatively high levels of BA. Consequently, pasteurization itself is not the ultimate solution to the problem of BA. Some authors have suggested that hurdle technology combining pasteurization with other treatments, such as high pressure, might reduce the presence of BA in cheeses, although no results were obtained yet (Novella-Rodríguez et al., 2002; Ladero et al., 2011b). Finally,

it is important highlight that even using milk of good microbiological quality, if it is inoculated with BA-producing starters, the problem would persist. Therefore, a good starter selection – taking into account the ability to produce BA – is essential.

RIPENING PROCESS AND PROTEOLYSIS

Cheese ripening involves a complex variety of biochemical processes that include degradation of the lactose, lipolysis, and the most complex process, the catabolism of the proteins or proteolysis. The ripening process is carried out by starter LAB and the secondary microbiota, which comprises non-starter LAB, propionic acid bacteria, molds, and yeasts (Beresford and Williams, 2004). Starter bacteria contribute to the protein break down (Lane and Fox, 1996; Lynch et al., 1997) while the non-starter LAB are responsible of the peptidolysis and the release of free amino acids (Muehlenkamp-Ulate and Warthesen, 1999). Some blue cheeses elaborated with raw milk show high levels of proteolytic activity – due to the action of the fungi – that correlates with a high accumulation of BA (Fernández et al., 2007a).

Ripening and proteolysis are very important factors affecting the accumulation of BA in cheeses (Fernández-García et al., 2000). The proteolysis rate increases with the ripening time, leading the accumulation of free amino acids that serve as substrate of decarboxylic activities, which ultimately derives in BA accumulation (Fernández et al., 2007a). In general, the longer the aging process, the higher the content of BA (Fernández et al., 2007a). There are several studies showing that long-ripened cheeses have high proteolysis rate, which contributes to the high level of BA found in these cheeses in comparison with short-ripened ones (Arlorio et al., 2003; Bunková et al., 2010; Ladero et al., 2010b). In the particular case of histamine, it is also common to find the highest concentrations in long-ripened cheeses, which suggest that proteolysis occurring during the ripening period increases the production and accumulation of histamine in cheeses (Fernández et al., 2006b; Ladero et al., 2008). The same trend was observed for tyramine accumulation (Fernández et al., 2007a; Ladero et al., 2010b).

The addition of proteinases to cheese milk or curd has shown to accelerate the cheese ripening process but also the availability of small peptides and amino acids (Núñez et al., 1991; Mohedano et al., 1998), which has a direct effect on the BA production (Fernández-García et al., 1999).

CHEMICO-PHYSICAL FACTORS

Many chemico-physical factors such as the pH, salt concentration, and temperature can affect the BA-producing microorganism growth and the decarboxylic activities during the production of fermented dairy products. Some studies have investigated the effect of these factors on BA production, but little is known about the consequences of their interaction.

pH

Dairy fermentations are intrinsically associated to a low pH environment caused by the fermentation of lactose to lactic acid. Although the physiological role of BA biosynthesis may differ depending of the BA and the producing microorganism, it has been proposed as a system for neutralization of low extracellular pH that increases survival under acidic stress condition (Meng

and Bennett, 1992; Rhee et al., 2002). In LAB, the theory most widely extended is that those strains with the specific amino acid decarboxylases, produce BA to counteract the acidic stress of some environments, as that of dairy fermentations (Bearson et al., 1997; Wolken et al., 2006). This theory is supported by the relation between the pH decrease and BA production observed in LAB (Marcobal et al., 2006a; Fernández et al., 2007b). Certainly, it is well known that low pH is a crucial factor for the activity of some amino acid decarboxylases (Chander et al., 1988; Teodorovic et al., 1994). There are in the literature different works describing an acid pH to be optimum for tyrosine decarboxylase (Moreno-Arribas and Lonvaud-Funel, 1999, 2001) and other bacterial amino acid decarboxylases (Gale, 1946; Schelp et al., 2001). Moreover, the genes encoding for the decarboxylases can be induced at low pH. Linares et al. (2009) revealed that the increased production of tyramine by *Enterococcus durans* under low pH conditions is prompted by a significant induction of the decarboxylase (*tdcA*) and transporter (*tyrP*) genes expression, whereas they are not expressed at neutral pH. Similarly, the *tdc* and *aguA1* genes (involved in tyramine and putrescine production, respectively) of *L. brevis* are transcriptionally induced by low pH (Arena et al., 2010).

Overall, although some authors indicate that rapid acidification could reduce the levels of BA production related to a decrease in the growth of the decarboxylating microorganisms (Gardini et al., 2001), low pH is a key parameter which represents a potential risk of BA accumulation in the final product. However, it is difficult to act on this parameter, since it is inherent to the fermentation process.

Temperature

Temperature is a key parameter in the elaboration of cheeses and affects the accumulation of BA particularly during maturation and storage steps. In general, the production and accumulation of BA increases with the temperature during production and storage of cheese. The storage of the product includes the period between the last step of production – the ripening – until consumption. A number of studies show that low ripening and storage temperatures (e.g., 5°C) decrease the accumulation of BA such as histamine, tyramine, putrescine, and cadaverine, while their content increases with higher temperatures (Stratton et al., 1991; Gardini et al., 2001; Pinho et al., 2001; Gennaro et al., 2003; Santos et al., 2003; Martuscelli et al., 2005; Bunková et al., 2010). A multifactorial study of factors influencing tyramine production by LAB revealed that high temperature play a role on tyramine production by *L. brevis* and *Enterococcus faecium* (Marcobal et al., 2006a). In addition, a histamine-producing *S. thermophilus* strain produces lower amounts of histamine when it is stored at low temperatures (4°C) after its growth in milk. This reduction was attributed to a reduction in the activity of the histidine decarboxylase itself rather than a reduction in gene expression or the presence of a lower cell number (Calles-Enríquez et al., 2010).

It has been described that even if the cheese is kept in refrigeration, the BA accumulation could rise above the safe limits for consumption (Bunková et al., 2010). A step further, frozen temperature (e.g., –18°C) impairs the increase on the BA accumulation,

most likely because the microbial activity is stopped (Andiç et al., 2010). However, more affordable solutions have to be found.

Sodium chloride

Another factor that might have an effect on BA accumulation is the concentration of salt in the fermented product. Traditionally salt has been employed to control the growth of pathogens during the fermentation and ripening process of dairy products with the last aim of preventing the spoilage and food-poisoning. Another consequence of the reduction on the growing rate of bacteria – including BA producers – would be the decrease of the BA concentration in the final product.

Most of the cheeses made with raw milk reach high numbers of *Enterococcus* spp., which is one of the main important BA producers in artisanal and traditional cheeses. The addition of high concentration of sodium chloride (5%) to milk inoculated with *Enterococcus faecalis*, reduced to the minimum the amount of 2-phenylethylamine and tyramine production (Gardini et al., 2001). The decrease on the BA production could be explained by the inhibitory effect of high salt content on the growth rate of BA-producing bacteria (Gardini et al., 2001) and/or on the amino acid decarboxylation activities (Chander et al., 1989). A similar effect of sodium chloride concentration has been observed in cheeses made of milk inoculated with *Lactobacillus bulgaricus* (Chander et al., 1989) or *Lactobacillus buchneri* (Sumner et al., 1990).

POST-RIPENING TECHNOLOGICAL PROCESSES

In the last decades, the market has reacted to customer requirements and it has evolved to sell ready-to-eat products (cut, sliced, grated. . .) instead of traditional formats such as the entire cheese. This ulterior processing implies more manipulation and the consequent increase of risk for microbial contamination (Reij and Den Aantrekker, 2004). The post-ripening process appears to have a direct impact in the presence of BA and BA producers. This could be due to the development of BA-producing bacteria present on the raw milk or to the contamination during the technological process (Custódio et al., 2007; Ladero et al., 2009).

Ladero et al. (2009), reported the effect of post-ripening process in the histamine-producing microbiota and the histamine content on a variety of cheeses. In some cases, histamine-producing bacteria were not found in the entire piece, but an increase on the number of histamine producers and histamine content was detected after grating. This result suggests an external contamination due to the post-ripening process. Thus, cheese grating seems to facilitate microbiological contamination due to the manipulation and the increase in the surface/volume ratio of the cheese fragments, which facilitates the presence of BA-producing bacteria and the subsequent accumulation of BA.

CONCLUSION

Safety is a basic requirement that must be always satisfied in food production. BA levels are unevenly distributed within cheese, where they can reach concentrations that represent a health risk, at least for those consumers with diminished detoxification system. Currently, there is no legislation defining the limits of BA tolerance in fermented foodstuff. However, cheese is the food that reach the highest BA concentrations, and therefore, a more severe control should be exercised.

Greater knowledge of the factors involved in the synthesis and accumulation of BA should lead to a reduction in their incidence in dairy foods. The production of BA in cheese is a complex phenomenon that depends on several variables, such as the presence of BA-producing microorganisms, their proteolytic and decarboxylase activities, ripening time, ripening and storage temperature, etc. In this sense, acidic pH is described to enhance BA accumulation *in vitro*. However, similarly to other chemico-physical conditions (such as amino acid availability, temperature, or salt concentration), it becomes difficult to modify these parameters, since they are inherent to the fermentation process. This clearly indicates that key actions to prevent BA accumulation must be addressed toward a reduction of the numbers of BA-producer microorganisms during cheesemaking. So far, control of BA-producing microorganisms by adequate thermal treatment of milk is one of the most important factors for reducing BA accumulation in dairy products. Moreover, encouraging a more responsible production by improving hygienic conditions, selection of starters

without BA synthesis capability, and low storage temperatures, would contribute to reduce BA accumulation and to produce healthier cheeses. For this purpose, a number of quantitative PCR-based methods allowing detection and quantification BA-producer microorganisms either in starters, raw material, or even during cheesemaking and ripening processes have been described (Ladero et al., 2010b,c, 2012b).

In addition, more research is needed to increase our knowledge in two directions: (i) the effects of BA on the health of the consumers and (ii) the factors involved in the synthesis of BA in order to develop new strategies to prevent their accumulation.

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