Full title: Histamine accumulation in dairy products: microbial causes, techniques for the detection of histamine-producing microbiota, and potential solutions

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ABSTRACT

Histamine poisoning is a significant public health and safety concern. Intoxication from ingestion of food containing high amounts of histamine may cause mild or severe symptoms that can even culminate in cardiac arrest. Nonetheless, although histamine levels in dairy products are not subject to any regulation, important outbreaks and severe adverse health effects have been reported due to intake of dairy products with a high histamine content, especially ripened cheeses. Histamine, a biogenic amine, can accumulate in dairy products as a result of the metabolism of starter and nonstarter lactic acid bacteria, as well as yeasts that contribute to the ripening or flavoring of the final product, or even as a result of spoilage bacteria. The aim of this review is to describe the microbiological causes of the presence of histamine in fermented milk products, and to propose control measures and potential methods for obtaining histamine-free dairy products. Thus, this manuscript focuses on histamine-producing microbiota in dairy products, highlighting the detection of histamine-producing bacteria through traditional and novel techniques. In addition, this review aims to explore control measures to prevent the access of histamine-producing microbiota to raw materials, as well as the formation of histamine in dairy products such as a careful selection of starter cultures lacking the ability to produce histamine, or even the implementation of effective food processing technologies to reduce histamine-producing microbiota. Finally, the removal of histamine already formed in dairy products through histamine-degrading microorganisms or by enzymatic degradation will also be explored.

1 Introduction

As one of the most important biogenic amines, histamine is involved in immune system response, gastric acid secretion, and neurotransmission, among other processes. However, histamine is also associated with food intolerance and food poisoning. Strategies to prevent, detect, and overcome food safety problems caused by histamine accumulation will be presented in this review.

Histamine is an organic nitrogenous compound exclusively synthesized via oxidative decarboxylation of the amino acid L-histidine by L-histidine decarboxylase (HDC) enzyme. Fermentation in food (red wine, hard cheese, etc.) or improper preservation may result in the formation of high histamine concentrations. Histamine accumulated in food can cause symptoms such as nausea, headache, abdominal pain, diarrhea, and itching (Benkerroum, 2016; Gardini, Ozogul, Suzzi, Tabanelli, & Ozogul, 2016). An estimated 1% of the population could be histamine intolerant or hypersensitive: in such people, even lower intakes of histamine can lead to severe symptoms (Maintz & Novak, 2007). Fish and ripened cheese are the most common foods associated with histamine intoxication (EFSA, 2011). In fresh raw milk, histamine concentration is usually low; however, in fermented dairy products such as yogurt and especially ripened cheese, variable concentrations of histamine can be detected. A high concentration of nutrients, marked biochemical changes during extended ripening periods, along with complex microbiota make ripened cheese an ideal matrix for histamine accumulation; it is becoming an increasing health concern (Costa, Rodrigues, Frasao, & Conte-Junior, 2018; Linares, Martin, Ladero, Alvarez, & Fernandez, 2011). Many different genera and species of microorganisms are responsible for histamine production in dairy products. This manuscript reviews histamine-producing microbiota, which can be present in dairy products as starter cultures, usually lactic acid bacteria (LAB), or as non-starter cultures (naturally present in milk), as well as contaminant microorganisms (due to practices during dairy product manufacture or stemming from the processing environment), mainly members of the *Enterobacteriaceae* family. Some yeasts and

molds have been reported as histamine producers in dairy products as well (Barbieri, Montanari, Gardini, & Tabanelli, 2019; Linares et al., 2012).

In the food industry, the determination of histamine is a key aspect for food safety, in spite of the fact that its levels in dairy products are not subject to any regulation. Histamine can be detected and quantified in food by several techniques reviewed by Gagic et al. (2019). However, the detection of potential histamine-producing microbiota, reviewed in the present study, may help to determine whether the starter cultures of dairy products are potential histamine producers. Consequently, histamine accumulation in dairy food might be prevented.

It is important to find solutions for obtaining histamine-free dairy products, and to control histamine production through a series of measures. First of all, good hygienic practices must be implemented during manufacturing processes. Ripening and storage temperatures, pH and salt concentration, among others, are important factors that may also exert an influence on histamine production. Additionally, heat or high-pressure homogenization treatments applied to milk have been shown to prevent the production of histamine in dairy products (Benkerroum, 2016; Gardini et al., 2016; Linares et al., 2012).

Histamine degradation, on the other hand, is mainly performed by the diamine oxidase enzyme (DAO) or by histamine N-methyltransferase (HNMT) (Maintz & Novak, 2007). Thus, chemical or biological histamine degradation by DAO or the addition of strains with the ability to degrade histamine could also be a preventive measure (Benkerroum, 2016; Gardini et al., 2016; Linares et al., 2012).

This review focuses on providing an overview of previous studies related to histamine production in dairy products, highlighting the implication of the present microbiota. In addition, we review potential solutions designed either to prevent the formation of histamine in manufactured products, or its removal. The increased prevalence of histamine intolerance and food allergies in the general population make this issue an emergent worldwide public health care concern.

2 Histamine as a biogenic amine: consequences of its accumulation in dairy food

Biogenic amines (BAs) are low-molecular-weight nitrogenous compounds synthesized by enzymatic decarboxylation of their precursor amino acids, or by amination and transamination of aldehydes and ketones (Benkerroum, 2016; Linares et al., 2011; Pluta-Kubica, Filipczak-Fiutak, Domagała, Duda, & Migdał, 2020). A great variety of BAs exist, with different chemical structures classified as aliphatic (agmantine, putrescine, cadaverine, ethylamine, methylamine, isoamylamine, ethanolamine, spermine and spermidine), aromatic (tyramine, phenylamine, phenylethylamine) or heterocyclic (histamine, tryptamine, pyrrolidine), among others (Linares et al., 2011; Papageorgiou et al., 2018; Spano et al., 2010). From a physiological point of view, BAs are involved in the proper functioning of the human metabolism. On the other hand, histamine and other BAs can serve as indicators of quality and freshness of food and alcoholic beverages (Papageorgiou et al., 2018).

Despite the fact that putrescine and cadaverine have been recently reported as potentially cytotoxic (del Rio et al., 2019), it is well established that histamine and tyramine are the two most toxic BAs; they are the ones most frequently present in dairy products, and the ones which cause the most severe symptomatology. Notably, levels of histamine lower than tyramine appeared to cause typical symptoms in healthy people. This fact, together with the absence of detoxifying mechanisms for histamine in sensitive people who present intoxication symptoms even when exposed to small amounts thereof, makes this biogenic amine a major public health concern that needs to be addressed with the appropriate measures (Benkerroum, 2016).

Figure 1 provides an overview of histamine biosynthesis and degradation in the mammal cell. Histamine (2-[4-imidazolyl]ethylamine) is synthesized by oxidative decarboxylation of the amino acid L-histidine, catalyzed by the HDC enzyme. In humans, mast cells, basophils, platelets, histaminergic neurons, and enterochromaffin cells are responsible for synthesizing endogenous histamine, storing a heparin-histamine complex in secretory granules on an intracellular level,

and releasing it in response to various stimuli (Maintz & Novak, 2007). Other immune cells, such as T cells, dendritic cells, macrophages, and certain types of epidermal cells, have also been shown to synthesize lower amounts of histamine, which is released without having been stored (Huang, Li, Liang, & Finkelman, 2018).

Present in the brain, the lungs, the stomach, the intestine, the uterus, and the ureters, histamine is an important mediator of a number of biological processes (Ladero, Calles-Enriquez, Fernandez, & Alvarez, 2010). Figure 2 shows that histamine fulfills important physiological functions including neurotransmission, regulation of circadian rhythm, immunomodulation, hematopoiesis, gastric juice secretion, vessel permeability, wound healing, learning and memory, mucosa secretion, and regulation of temperature, as well as cell growth and differentiation (Ladero et al., 2010; Maintz & Novak, 2007; Schwelberger, Ahrens, Fogel, & Sánchez-Jiménez, 2014).

Once released, histamine binds one out of the 4 G-protein coupled receptors (H1, H2, H3 or H4) located in target cells, in order to produce those important physiological effects. Most of these effects are caused by the activation of H1 receptors, ubiquitously expressed, and they produce typical type 1 hypersensitivity reactions (allergic and asthma reactions). H2 receptors are involved in immunomodulation, gastric acid secretion, mucus secretion or vascular permeability. H3 receptors, exclusively expressed in neurons, participate in blood-brain barrier function. H4 receptors are mainly involved in pro-inflammatory responses (Thangam et al., 2018).

As shown in Figure 1, intracellular histamine can be inactivated by methylation of the imidazole ring, catalyzed by HNMT, a widely distributed enzyme. Conversely, extracellular histamine can be metabolized by oxidative deamination of the primary amino group, catalyzed by DAO, a copper-dependent amino oxidase also called histaminase, which is mainly produced by enterocytes, but also by placenta and kidney cells (Comas-Basté, Sánchez-Pérez, Veciana-Nogués, Latorre-Moratalla, & Vidal-Carou, 2020; Ladero et al., 2010; Schwelberger et al., 2014).

Figure 2 depicts the physiological equilibrium between histamine synthesis/intake and degradation or the consequences of a misbalance. When increased availability of histamine or decreased histamine degradation occurs, histamine accumulation causes unspecific gastrointestinal symptoms as well as extra-intestinal symptoms, mainly immediately after (few min) or even during meals, for a period up to 24 h (Comas-Basté et al., 2020; Tuck, Biesiekierski, Schmid-Grendelmeier, & Pohl, 2019). The toxicological effects of histamine include vascular disorders (dilation of arteries and increased capillary permeability producing headache, hypotension, edemas, urticaria, facial flushing, etc.), heart disorders (a stimulatory effect leading to palpitations, tachycardia, and arrhythmia), gastrointestinal disorders (contraction of smooth muscle cells causing cramps, diarrhea and vomiting), and neurological disorders (stimulatory effects resulting in pain and itching) (FAO/WHO, 2013; Ladero et al., 2010; Maintz & Novak, 2007; Schnedl et al., 2019).

Histamine can be expected to be present in all foods containing free histidine or proteins that can suffer proteolysis (Tuck et al., 2019); foods rich in histamine are detailed in Comas-Basté et al. (2020). Histamine may be present in fermented food as a consequence of the oxidative decarboxylation of L-histidine via the HDC enzymes from the microbiota of these products (Landete, Pardo, & Ferrer, 2008). A fairly efficient detoxification system, based on intestinal and liver amine oxidases, metabolizes the regular dietary intake of histamine (Schwelberger et al., 2014).

However, the presence of high amounts of this BA in food has been associated with histamine intolerance and intoxication (Maintz & Novak, 2007). Several studies of oral administration of histamine have shown that the same histamine dosage produces different effects and severity of symptomatology depending on each participant (EFSA, 2011). For that reason, it is well established that a percentage estimated in 1% of the population suffers from a great sensitivity to this compound, which is known as histamine intolerance (Comas-Basté et al., 2020). It is caused by the ingestion of moderate levels in food, and results from an imbalance between the

amount of accumulated histamine and the capacity for its degradation, mainly linked to a DAO deficit. The enzymatic activity and detoxification efficiency of DAO vary significantly among individuals. In some cases related to DAO deficiency, it can lead to hypersensitivity to histamine and subsequent variable symptomatology (Comas-Basté et al., 2020; Ozogul & Ozogul, 2020).

In relation to the etiology of histamine intolerance, several single-nucleotide polymorphisms (SNPs) in the DAO-encoding gene result in decreased activity of the enzyme, whereas other SNPs in the promoter region of that gene produce a diminished transcription level and thus a decreased level of the enzyme. However, DAO deficiency is not only due to a genetic background. It could also be related to impaired DAO activity, caused by inflammatory bowel pathologies or certain functional intestinal disorders, such as carbohydrate malabsorption or non-celiac gluten sensitivity. Finally, temporary and reversible DAO inhibition could also result from the presence of other BAs, alcohol, or even certain drugs as chloroquine, clavulanic acid, metamizol, etc. (Comas-Basté et al., 2020). The diagnostic criteria of histamine intolerance include low serum DAO values, two or more of the typical symptoms exposed above, and clinical improvement as a consequence of histamine-free or reduced diet, or of the intake of antihistaminergic medication (Schnedl et al., 2019; Tuck et al., 2019). Scientific publications referring to histamine intolerance or histaminosis have exponentially increased over the last two decades, thereby indicating the importance of this disorder (Comas-Basté et al., 2020).

On the other hand, histamine intoxication, caused by the ingestion of food containing high levels of histamine (Bodmer, Imark, & Kneubühl, 1999), is an immune system response that usually appears in the course of a short period (up to 24 h) after ingestion of contaminated food (Hungerford, 2010). The diagnosis is based on increased plasma histamine levels associated with the previous uptake of food with proved high histamine content (Comas-Basté et al., 2020).

Histamine is commonly found in dairy products such as cheese and yogurt, or raw, pasteurized, and UHT milks of different animal species, as well as reconstituted powdered milk (Benkerroum, 2016; Costa et al., 2018; Ladero et al., 2017; Linares et al., 2011; Spano et al., 2010). Amounts of

biogenic amines in milk, yogurt, cottage, and unripe cheeses can be expected to range from milligrams to tens of milligrams per kg (Linares et al., 2011; Spano et al., 2010). Histamine is present in higher amounts in fermented or ripened dairy products (Costa et al., 2018). In such products, variable amounts of histamine (7 mg/kg in sour cream, 13 to 21.2 mg/kg in yogurt, and 4 mg/kg in kefir) have been found (Bodmer et al., 1999; Özdestan & Üren, 2010). A drastic increase in histamine content often takes place in the course of cheese production, leading to histamine levels of up to 2500 mg/kg in aged cheese, a highly toxic amount. Histamine concentration varies among different types of ripened cheese and may differ within the same type of cheese, even within parts thereof, also depending on ripening time, manufacturing process conditions, and the bacterial starter culture used (Madejska, Michalski, Pawul-Gruba, & Osek, 2018; Novella-Rodríguez, Veciana-Nogués, Izquierdo-Pulido, & Vidal-Carou, 2003).

The first outbreak of histamine poisoning related to cheese was reported in 1967, involving Gouda and Swiss cheeses containing 850-2500 mg/kg, but other cheese varieties including Gruyere, Parmesan, Emmental, Suisse, and Provolone have also been involved in outbreaks (Fernandez-Garcia, Tomillo, & Nunez, 2000; Maintz & Novak, 2007). A study conducted by (Rauscher-Gabernig, Grossgut, Bauer, & Paulsen, 2009) concluded that tolerable limits for histamine in cheese would be 100–417 mg/kg on the basis of a supposed daily consumption of 60 g. Based on Austrian data for usual serving sizes and histamine concentration in foods, a proposed limit of 400 mg/kg is considered acceptable for cheeses (Rauscher-Gabernig et al., 2009). Given this threshold dose for histamine in cheese, Madejska et al. (2018) found that the amine content exceeded that value in Gorgonzola (400 and 730 mg/kg), and reached that level of toxicity in Camembert.

Maximum legal limits for histamine have been established for fresh fish (200 mg/kg) and for cured fish products (up to 400 mg/kg) by European Commission Regulation No. 2073/2005 (European Parliament, 2005). Despite the existing legal limits for fish, the histamine content in dairy products is not regulated by any type of legislation; maximum recommended

concentrations have only been suggested. For instance, the Netherlands Institute of Dairy Research sets a limit of 100-200 mg/kg on histamine in foods. In order to guarantee food safety and consumer health, legal histamine limits for dairy products should be established in regulations and enforced.

Overall, ripening cheeses are the most common candidates among dairy products for the potential accumulation of high contents of histamine; they are thus prone to cause significant adverse health effects and thereby constitute a notable health risk for consumers. Further insights into the inherent characteristics of dairy products, including composition, biochemical changes, and above all present microbiota, should enable our health systems to prevent, detect, and overcome the formidable safety issue constituted by histamine in dairy products.

3 Inherent characteristics of dairy products with potential impact on histamine production

3.1 Composition and biochemical changes in raw milk and fermented (cultured) dairy products

Milk is a secretion from mammary glands which serves as the basic food for neonates. It contains multiple nutrients whose proportion varies among animal species, explained in Table 1, as well as in the course of the lactation period in order to meet the varying nutritional needs of neonates. Protein content in the milk of several different dairy animals might vary from 3.4% in cow milk to 5.7% in sheep milk (Table 1). This can be of particularly importance because proteins are the main source of histidine in milk as a precursor of histamine. Apart from mother's milk, humans consume milk from certain domestic animals such as cows, goats, sheep, and buffalos, either in the form of fresh milk or as dairy products. Cow and buffalo milks are the most widely consumed milks in the world, although interest in goat and sheep milks has increased in recent years (OECD & FAO, 2020).

The proportions of chemical components in milk largely determine its nutritional, organoleptic, technological (i.e. chemical and physical reactions that can occur therein), and microbiological

254 (i.e. microbiological species and microbial load) properties (Walstra, Wouters, & Geurts, 2006).
255 Due to the importance of histidine in histamine formation, we will focus in milk proteins, as the
256 main source of amino acids. In cow milk, two groups of proteins can be differentiated according
257 to their pH stability. Caseins represent ~80% of total protein, while the remaining ~20% is
258 comprised of whey (serum) proteins. Caseins (a mixture of four heat-stable proteins: α_{s1}-, α_{s2}-,
259 β-, and κ-casein) are present in form of large colloidal particles, known as casein micelles (40-
260 600 nm diameter with an average of 5,000 casein molecules/micelle). Casein micelles precipitate
261 either at pH 4.6 or by action of rennet chymosin on κ-casein. Caseins are susceptible to
262 proteolysis due to their open structure (Fox & Kelly, 2006). On the other hand, whey proteins
263 (β-lactoglobulin, α-lactalbumin, blood serum albumin, and immunoglobulins) are globular, heat-
264 sensitive, soluble at pH 4.6, and very resistant to chymosin and proteolysis. In addition, non-
265 protein nitrogenous compounds represent 5% of total nitrogen in fresh milk, comprising
266 intermediate products of the animal's protein metabolism (e.g., ammonia, urea, creatine,
267 creatinine, and uric acid), amino acids and their derivatives, as well as small peptides that may
268 serve as essential nutrients for certain bacteria (Croguennec, Jeantet, & Shuck, 2016).
269 Milk additionally contains indigenous enzymes at trace levels, including proteinases, of which
270 the trypsin-like endopeptidases plasmin (alkaline proteinase) and cathepsin D (acid proteinase)
271 are the ones most relevant for this review. Plasmin is highly heat-resistant and contributes to
272 proteolysis in cheese during ripening. Cathepsin D is less heat-resistant than plasmin; due to its
273 low optimum pH (4.0), it displays a reduced activity in milk but causes proteolysis in cheese
274 (Walstra et al., 2006).
275 In Europe and North America, the consumption of processed dairy products is greater than that
276 of fresh dairy products. Furthermore, an increase of cheese consumption in those countries is
277 expected for the next decade (OECD & FAO, 2020). Fermentation was a key process for food
278 preservation in ancient times. Dairy products were central in Neolithic food cultures across
279 much of the Old World, and it is likely that milk was often fermented to obtain a safer and more

digestible product while avoiding seasonal or logistic fluctuations in the availability of fresh milk. Although it was previously assumed that food fermentation began with agriculture, it is now assumed that storage was and is widely practiced by non-sedentary foragers in order to have portable protein-rich foods at their disposal during travels (Sibbesson, 2019).

Due to its wide range of nutrients which allow the growth of many spoilage and pathogenic microorganisms. Microbial conversion of lactose is the basis for fermented milks. Microorganisms with lactase activity, such as LAB, metabolize lactose into glucose and galactose which are degraded to lactic acid. LAB can produce 1-2% of lactic acid leading to milk acidification (pH 4.0 - 4.6) that destabilizes dispersed elements and controls bacterial growth (Kelly & Fox, 2012).

Yogurt is obtained from pasteurized milk inoculated with starter cultures containing *Streptococcus salivarius* spp. *thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) (Hill, Ross, Arendt, & Stanton, 2017). The Codex Alimentarius Commission (CODEX STAN 243-2003) has established the sum of the specific microorganisms constituting the starter culture in the final product at ≥ 10^7 colony forming units per gram (CFU/g) (Commission, 2011). *Lb. bulgaricus* is required for acid production, whereas *S. thermophilus* is responsible for the flavor and texture of yogurt: the two bacteria have a synergistic relationship. After fermentation, yogurt is refrigerated to decelerate microbial metabolism and delay excessive microbial acidification or proteolysis (Walstra et al., 2006).

Kefir, on the other hand, is a creamy, aromatic, carbonated acid-alcohol milk beverage (0.7-1% lactic acid, pH 4.6) of Eastern European origin. It is prepared by adding “kefir grains” (composed of LAB, acetic acid bacteria and yeast in a polysaccharide matrix of semi-hard granules) to milk and incubating for 24 h at 25˚C (Guzel-Seydim, Kok-Tas, Greene, & Seydim, 2011). Volatile and non-volatile compounds generated upon fermentation via lipolysis, glycolysis, and proteolysis provide its characteristic flavor. After fermentation, grains are separated and kefir is refrigerated to attain a shelf life of 2-3 weeks (Farag, Jomaa, El-Wahed, & El-Seedi, 2020).

306 Cheese can be defined as the curd of milk that has been coagulated and separated from whey.
307 Basically, in the cheese manufacturing process, water and whey are removed from milk, and
308 casein and fat are concentrated. Figure 3 shows the basic process for cheese production
309 (Walstra et al., 2006), including the microbiota associated with each step. In brief, the steps
310 involved are the following:

311 1) the clotting of milk, consisting in the precipitation of casein micelles by acidification
312 (acid coagulation) and/or enzymatically (rennet coagulation), leading to gel formation.
313 2) removal of the whey: the separation of curd and whey is achieved by cutting and
314 stirring, and is facilitated by the spontaneous syneresis of the formed gel. Soluble
315 compounds, including whey proteins, small peptides, and most of the lactose, are
316 squeezed out and excluded from cheese. However, certain proteases, such as plasmin
317 and cathepsin D, tend to adsorb onto micelles, which are present during ripening,
318 thereby facilitating amino acid availability.
319 3) production of lactic acid by LAB before and/or after steps 1 and 2. After these 3 steps,
320 a fresh cheese is obtained. For a typical ripened cheese, the following two additional
321 steps are required.
322 4) curd fusion, assisted by pressing. A rind can be formed, shielding the interior of the
323 cheese, which contributes to the limitation of oxygen and water transfer for microbial
324 growth.
325 5) ripening or curing: a biochemical process determined by a number of factors (Kelly &
326 Fox, 2012), such as endogenous milk enzymes (e.g. plasmin or lipoprotein lipase), starter
327 and nonstarter LAB and their enzymes, thoroughly active secondary microbiota which
328 secrete proteases and lipases (e.g. *Penicillium roqueforti* in blue cheeses or *Leuconostoc*
329 spp. in Dutch-type cheeses), and storage conditions (e.g. temperature, time, and
330 humidity).

Salting (usually after step 2) is another key step designed to modify organoleptic characteristics and improve cheese preservation (by selecting growing microbiota). It involves the direct addition of salt crystals (in curd or rubbed onto surface) and/or immersion in a concentrated brine, in order to achieve a salt-in-water concentration ranging from 1% NaCl in cottage cheese up to 6% NaCl in Pecorino Romano cheese (Walstra et al., 2006). Further optional process steps can be mentioned, such as milk pasteurization (prior to step 1) with the purpose of inactivating pathogenic bacteria as well as microorganisms and enzymes that could be detrimental to ripening; and/or addition of microbial cultures (after steps 1 and/or 2), especially highly selected defined starters of LAB, and other microorganisms that are specific for certain cheese varieties.

Modifications in these steps allow for the achievement of more than 1,400 cheese varieties worldwide, with different shapes, flavors and textures (Kelly & Fox, 2012). During ripening, which can take from two weeks up to more than two years, three major biochemical reactions take place (Croguennec et al., 2016): a) fermentation of residual lactose and degradation of lactate to ethanol, acetaldehyde, CO_2, acetic acid or propionic acid; b) hydrolysis of lipids into fatty acids, and of proteins into peptides and amino acids, respectively; and c) flavor: the production of aroma by the degradation of fatty acids to methyl ketones, esters or lactones, and of amino acids to aldehydes, alcohols, acids, amines, phenolic compounds, indole, or NH_3.

Cheese can be considered a solid-like system in which bacteria are immobilized and molecules do not diffuse easily (Floury, Jeanson, Aly, & Lortal, 2010; Walstra et al., 2006). Therefore, microbial growth conditions fluctuate and vary as a function of time and localization in cheese. After production of lactic acid, bacterial metabolism and proteolysis create NH_3, which increases pH (Kelly & Fox, 2012). Water evaporation decreases water activity of cheese and facilitates the formation of rind around the cheese, thereby preventing microbial contamination and limiting oxygen diffusion. Oxygen is rapidly used by starter bacteria, favoring the creation of anaerobic conditions inside the cheese. All these physicochemical changes modify the environmental

conditions for bacterial development, thereby promoting a dynamic microbiota during cheese ripening.

3.2 Microbiota in dairy food

The detection of the main agents responsible for histamine production should be regarded as an important objective for dairy industries in order to avoid harmful outbreaks. Deciphering the microbiota present in dairy food can be regarded as a first step to elucidate which particular microorganisms are responsible for histamine production. Figure 3 displays the main microbiota involved in the cheese-making process from raw milk to ripened cheese, highlighting the final histamine producers both in cheese surface and core. The formation of this biogenic amine by histamine-producing microbiota is modulated by a series of factors that are detailed in Figure 4. In cheese, factors such as the type of starter cultures, salt content, ripening and storage temperatures and times, among others, may influence the production of histamine and the amounts of this biogenic amine in cheese.

The microbiota of raw milk is mainly composed of LAB (starter and non-starter), environmental microbiota or contaminants, putative spoilage bacteria, mostly stemming from the teat skin, but also from the farm environment, hygienic practices, or milking and storage equipment (Figure 3) (Irlinger, Layec, Helinck, & Dugat-Bony, 2015; Odeyemi, Alegbeley, Strateva, & Stratev, 2020; Yeluri Jonnala, McSweeney, Sheehan, & Cotter, 2018). The composition of milk microbiota is diverse, with a high abundance of LAB, and differs depending on the milk's origin: cow, goat, sheep, or buffalo milk (Agrimonti, Bottari, Sardaro, & Marmiroli, 2019; Quigley et al., 2013; Tilocca et al., 2020). In Regulation (EC) No 853/2004, the European Union established the total bacterial plate count limit in raw cow´s milk at ≤ 10^5 colony forming units per milliliter (CFU/ml) at 30°C, although this limit is allowed to increase to ≤ 1,5 x 10^6 CFU/ml for milk from other species (European Parliament, 2004). In general, bacteria present in cooled raw milk include gram-positive species such as spore-forming bacteria (*Clostridium, Bacillus*), non-starter LAB

(*Lactobacillus* (*Lb.*)*, Lactococcus* (*Lc.*)*, Streptococcus, Leuconostoc, Pediococcus*) and others (*Corynebacterium, Microbacterium, Staphylococcus*). Gram-negative bacteria are also present in cooled raw milk, usually as environmental or contaminant microbiota: the *Enterobacteriaceae* family and others (*Pseudomonas, Aeromonas, Alcaligenes, Achromobacter, Acinetobacter, Flavobacterium, Chryseobacterium*) (Odeyemi et al., 2020; Settanni & Moschetti, 2010). The *Pseudomonads* family has been reported to be the predominant spoilage bacteria found in cooled raw milk, reaching 70 – 90 % of the total microbial load (Odeyemi et al., 2020). Pathogenic foodborne bacteria such as *Listeria, Campylobacter, Yersinia, Mycobacterium, Escherichia, Salmonella*, *Coxiella*, and *Staphylococcus* have also been found in raw milk (Agrimonti et al., 2019; Tilocca et al., 2020). The yeasts most commonly present in raw milk are *Kluyveromyces, Yarrowia, Geotrichum, Candida, Debaryomyces*, and *Pichia* (Frohlich-Wyder, Arias-Roth, & Jakob, 2019; Irlinger et al., 2015). Bacteriophages or phages are viruses capable of infecting bacteria, and they can achieve entry into dairy products through raw milk (L. Fernandez et al., 2017). *Lc. lactis, Lb. helveticus, Lb. delbrueckii, Lactiplantibacillus plantarum* (formerly *Lb. plantarum*)*, Lb. acidophilus, Lacticaseibacillus casei* (formely *Lb. casei*)*, L. paracasei*, *S. thermophilus*, and *Leuconostoc* spp. can be infected by phages (del Rio et al., 2007; Marco, Moineau, & Quiberoni, 2012; Muhammed, Krych, Nielsen, & Vogensen, 2017).

Regarding yogurt, in addition to the aforementioned starter cultures *Lb. bulgaricus* and *S. thermophilus* used in adequate proportions to perform lactic fermentation, it can contain other beneficial or deleterious microorganisms. Probiotic bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp., which are not part of the starter cultures, can be found in probiotic fermented milks, namely bio-yogurts (Aryana & Olson, 2017; Hill et al., 2017). Flavor can be improved by adding further cultures as *S. diacetylactis* or *Leuconostoc* spp. Phages active against *S. thermophilus* or *Lb. bulgaricus*, and yeast such as *Torulopsis*, have also been reported for yogurt (Aryana & Olson, 2017). Additionally, viable *L. monocytogenes* and *S. enterica* cells have been detected in certain yogurts**,** as well as further pathogens including *Y. enterocolitica, M.*

409 *tuberculosis*, and *B. cereus*. However, the true hygienic state of yogurt has not been defined by
410 the presence of pathogenic species, but has been suggested to be controlled by monitoring the
411 *Enterobacteriaceae* family (Hervert, Martin, Boor, & Wiedmann, 2017). Other episodes of food
412 poisoning involving yogurts have been caused by *E. coli* O157:H7, *C. botulinum*, and *S.*
413 *typhimurium* (Aryana & Olson, 2017).
414 The microbiota of kefir and kefir grains comprises species of bacteria (*Lactobacillus, Lactococcus,*
415 *Streptococcus, Leuconostoc, Acetobacter, Enterococcus*) and yeasts (*Saccharomyces, Candida,*
416 *Kluyveromyces, Zygosaccharomyces, Debaryomyces, Issatchenkia, Pichia,* and *Torulopsis*)
417 (Guzel-Seydim et al., 2011; Singh & Shah, 2017; Tang et al., 2020). It should be noted that the
418 Codex Alimentarius Comission (CODEX STAN 243-2003) specifically mentions the presence of
419 *Lentilactobacillus kefiri* (formerly *Lb. kefiri*) and the yeasts *K. marxianus, S. unisporus, S.*
420 *cerevisiae*, and *S. exiguous*. It also establishes at ≥ 10^7 CFU/g the sum of the specific
421 microorganisms constituting the starter culture in the final product, and the sum of yeasts at ≥
422 10^4 CFU/g (Commission, 2011). Some species of probiotics such as *B. lactis, Lb. acidophilus*, or *L.*
423 *rhamnosus* (formerly *Lb. rhamnosus*), can also be added to kefir (Aryana & Olson, 2017).
424 The microbiota present in cheese is key for its organoleptic and physicochemical properties.
425 Cheese microbiota varies depending on starter and nonstarter cultures, and changes over time
426 (Figure 3). Bacterial communities present in cheese display an immense diversity, greater than
427 that of fungal communities, depending on cheese variety and manufacturing process (Afshari,
428 Pillidge, Dias, Osborn, & Gill, 2020; Rezac, Kok, Heermann, & Hutkins, 2018). LAB are definitely
429 the most important microorganisms present in cheese microbiota in view of their involvement
430 in the fermentation and maturation processes (Settanni & Moschetti, 2010). Starter LAB (SLAB),
431 including *Lactococcus*, *Streptococcus* and *Leuconostoc*, contribute to the initial ripening process,
432 due to the fermentation of lactose. Thus, SLAB are involved in coagulation of milk and acid
433 development. During cheese manufacture, the SLAB population comprises up to 10^8 to 10^9
434 CFU/g. The most common mesophilic SLAB is *Lc. lactis*, although strains of *Leuconostoc* spp. are

435 also used; whereas thermophilic SLAB usually consist of strains of *S. thermophilus, Lb.*
436 *delbrueckii*, *and Lb. helveticus* (Blaya, Barzideh, & LaPointe, 2018; Settanni & Moschetti, 2010).
437 However, the stresses and harsh conditions (high salt, low pH, low sugar availability, low
438 moisture…) that appear in the cheese matrix as a consequence of the cheese-making process
439 lead to a reduction in the population of SLAB due to autolysis (Gatti, Bottari, Lazzi, Neviani, &
440 Mucchetti, 2014; C. O. A. Møller, Christensen, & Rattray, 2021). Instead, adventitious non-
441 starter LAB (NSLAB), which mainly stem from raw milk, need to be present because they
442 contribute to the development of desirable flavor. NSLAB can grow and survive in more adverse
443 environmental conditions such as pH as low as 5.0 or energy depletion (Barbieri et al., 2019).
444 For that reason, an initial population of 10^2 to 10^3 CFU/g of NSLAB is found in cheese, but it can
445 reach up to 10^9 CFU/g during the onset of ripening (Blaya et al., 2018; Gatti et al., 2014). Among
446 the NSLAB *Lactobacillus* strains, the obligate homofermentative species *Companilactobacillus*
447 *farciminis* (formerly *Lb. farciminis*)*,* the facultative heterofermentative species *L. rhamnosus, L.*
448 *paracasei, L. casei*, *L. plantarum, L. pentosus* (formerly *Lb. pentosus*), and *Latilactobacillus*
449 *curvatus* (formerly *Lb. curvatus*), and the obligate heterofermentative species
450 *Limosilactobacillus fermentum* (formerly *Lb. fermentum*)*, L. buchneri* (formerly *Lb. buchneri*)*, L.*
451 *parabuchneri* (formerly *Lb. parabuchneri*) and *Levilactobacillus brevis* (formerly *Lb. brevis*)*,* are
452 considered to be the main NSLAB found in cheese. Other NSLAB found in cheese are *Pediococcus*
453 species (*P. acidilactici, P. pentosaceus), Enterococcus* species (*E. durans, E. faecalis, E. faecium*)
454 and *Leuconostoc* spp. (Settanni & Moschetti, 2010). Other microorganisms as enterococci,
455 micrococci, and yeasts are likewise important in cheese microbiota for maturation (Button &
456 Dutton, 2012; Gardini et al., 2006; Gobbetti, Minervini, Pontonio, Di Cagno, & De Angelis, 2016).
457 For instance, *B. linens* or *S. equorum* contribute to the development of flavor, aroma, and color
458 in cheese; even *Propionibacterium freundenreichii* causes the typical holes in Swiss cheeses by
459 producing CO_2 during fermentation (Button & Dutton, 2012; Yeluri Jonnala et al., 2018). On the
460 other hand, coliforms are considered indicative of non-hygienic conditions and thus regarded as

undesirable contaminants; *Pseudomonas* spp., *Serratia* spp., and *Kluyvera* spp. can reduce the sensory quality of cheese (M. Coton et al., 2012). Foodborne pathogens such as *L. monocytogenes, Salmonella* spp., *E. coli*, and *Campylobacter* spp. have been detected in soft cheese samples (Cremonesi et al., 2016). Cheese can also contain spoilage bacteria: in fact, the *Clostridium* spore might survive the entire cheese production process (Odeyemi et al., 2020). Bacteriophages active against *S. thermophilus* or *Lc. lactis,* for instance, are also present in cheese, thus helping to modulate the bacterial community (Gobbetti et al., 2016). Yeasts found in cheese participate in the ripening process, and contribute to its texture and organoleptic properties. *Debaryomyces*, *Yarrowia, Candida, Geotrichum, Kluyveromyces, Saccharomyces*, and *Pichia* are the most commonly described genera (Gardini et al., 2006; van den Tempel & Jakobsen, 2000). Some of them, like *D. hansenii* and *Y. lipolytica*, can be used as starter cultures due to their capacity to grow under hostile conditions and to improve the flavor and quality of cheese (Ferreira & Viljoen, 2003). *Penicillium*, *Scopulariopsis*, and *Fusarium* are important filamentous fungi found in cheese (Irlinger et al., 2015). Opportunistic pathogenic yeasts, mainly the *Candida* species, can also be present in cheese (Frohlich-Wyder et al., 2019). With regard to cheese localization, Figure 3 shows that the microbiota in the cheese rind differs from the microorganisms present in the core. Ripening bacteria (*Brevibacterium, Arthrobacter*, *Corynebacterium)* and psychrophilic and halophilic bacteria (*Psychrobacter*, *Halomonas, Proteus)* are mostly present on the cheese surface because they cope with the deacidification process. However, LAB are usually found in the cheese core, as well as anaerobic bacteria such as *Propionibacterium* that grow inside the wheel of cheese (Button & Dutton, 2012; M. Coton et al., 2012; Frohlich-Wyder et al., 2019). Only yeasts able to ferment carbohydrates, such as *K. marxianus*, *K. lactis*, and *P. fermentans*, can survive in the cheese core, while the yeast predominant on the surface are acid and salt tolerant: the most abundant ones are *D. hansenii, Y lipolytica*, and *G. candidum* (Frohlich-Wyder et al., 2019). In relation to molds, spores of *P. camemberti* are inoculated into milk of Brie and Camembert cheeses to develop bloomy rind,

while *P. roqueforti* grows in the core of blue cheese, producing its blue pigment during sporulation (Button & Dutton, 2012; Yeluri Jonnala et al., 2018).

4 Environmental conditions applied to dairy foods may influence histamine accumulation

The amount of histamine in dairy food, and even the presence or absence thereof, is determined by a number of factors, shown in Figure 4, which include available precursors or cofactors, environmental conditions such as acidic pH, ripening and storage temperatures, water activity, and salt concentration (Costa et al., 2018). Furthermore, microbiological factors, such as microbial competition or the presence of microbiota capable of degrading histamine, could also contribute to modify the amount of histamine present in dairy food (M. Coton et al., 2012). All these factors should be carefully controlled in order to obtain histamine-free dairy products.

The availability of histidine, the precursor amino acid for the synthesis of histamine during the ripening of cheese, is a limiting factor on histamine formation (Linares et al., 2011). Although histidine can be naturally present in milk in a free state, the proteolysis of casein or other milk proteins is the main cause of the presence of this substrate amino acid in milk and dairy products (Benkerroum, 2016). Since the rate of proteolysis increases with ripening time, long-ripened cheeses present higher concentrations of histamine. Ripening time also contributes to the proteolysis rate, so that long-ripened cheeses have a higher proteolysis rate and thus a higher level of histamine (M. Fernandez, del Rio, Linares, Martin, & Alvarez, 2006). The addition of exogenous proteinases to milk with the aim of accelerating cheese ripening significantly increases the amount of histamine in a wide variety of cheeses (Linares et al., 2011).

NSLAB are known to survive and grow under very harsh conditions such as an acidic pH. Since amino acid decarboxylases in bacteria are known to contribute to their adaptation to acidic environment (because the decarboxylation process results in an increase of environmental pH), an acidic pH in the final dairy product could also promote the synthesis of histamine (Barbieri et

al., 2019; Linares et al., 2012). The HDC enzyme of *S. thermophilus* seems to be much more active at pH 4.5 than at pH 8 (Tabanelli, Torriani, Rossi, Rizzotti, & Gardini, 2012). It has also been reported that acidic pH may induce structural changes in the HDC from *Lactobacillus* sp. 30a (ATCC 33222) required for the protein to be active (Schelp, Worley, Monzingo, Ernst, & Robertus, 2001). At pH 8.0, however, histamine accumulation was also observed in a culture of *Tetragenococcus halophilus* (Satomi, Furushita, Oikawa, Yoshikawa-Takahashi, & Yano, 2008).

Sodium chloride concentrations higher than 5% (w/v) seem to notably decrease the amount of histamine, probably due to an inhibitory effect on the growth rate of histamine producers (Tabanelli et al., 2012). However, the halophilic bacterium *Tetragenococcus* can produce histamine even at up to 20% (w/v) NaCl (Kimura, Konagaya, & Fujii, 2001; Satomi et al., 2008).

The carbon source could also be a factor that influences bacterial histamine formation, depending on the histamine producer. High concentrations of glucose or lactose have been reported to inhibit the production of histamine, although a recent study showed no effect of the presence of up to 2% glucose on the synthesis of histamine for *L. parabuchneri* and *L. paracasei,* but completely inhibiting histamine formation by *P. pentosaceus* (Calles-Enriquez et al., 2010; C. O. A. Møller, Ucok, & Rattray, 2020).

High storage temperatures and prolonged ripening time increase the microbial production of histamine. For instance, the concentration of histamine was 10-fold higher at 42°C than at 4°C in a culture of *S. thermophilus* grown in milk after 24 hours, due to the activity of the enzyme rather than to a variation in its gene expression (Calles-Enriquez et al., 2010). *L. parabuchneri*, isolated from cheese, has also been reported to grow and produce histamine at refrigeration temperatures (4-8°C), but this characteristic seems to be strain-dependent (Díaz et al., 2018).

On the other hand, as mentioned above, the *hdc* genes in some bacteria such as *T. muriaticus*, *T. halophilus*, *Oenococcus oeni* and *L. hilgardii* (formerly *Lb. hilgardii*) are codified in unstable plasmids (P. M. Lucas, Claisse, & Lonvaud-Funel, 2008; P. M. Lucas, Wolken, Claisse, Lolkema, & Lonvaud-Funel, 2005; Satomi et al., 2008). In these cases, the instability of the plasmid depends

on the bacterial culture conditions, since a poor and acidic medium seems to favor the maintenance of the plasmid and thus the expression of the gene.

5 Techniques for the detection of histamine-producing microbiota

A series of techniques for the study of microbial communities in food have been developed in recent years. High-throughput sequencing applications have provided detailed knowledge concerning food-associated microbiota and microbiomes. Not only metagenomics and metatranscriptomics, but also metaproteomics and metabolomics have been thoroughly exploited to decipher the composition and functionality of microbiota, thereby contributing to the improvement of food quality and safety. The expansion of our knowledge of food-associated microbiota by meta-omics technologies would allow us to control their main drivers along with the influence of environmental or technological factors over them. Monitoring food spoilage organisms or even pathogens could also help to improve hygienic practices in food production plants (De Filippis, Parente, & Ercolini, 2018). This multi-omics approach applied to cheese has been recently called “Cheesomics”, focusing on the ripening process and promoting the identification of biomarkers and bioactive metabolites to improve the attributes of cheese (Afshari et al., 2020). In addition, if we learn to consider the core microbiota of cheese as a super-organism comprising all microbial metabolisms and interactions among individual microbes, we can gain a better understanding of the complex metabolic network of dairy products on the whole (Gobbetti et al., 2016).

Techniques aimed at detecting a putative histamine intoxication in food are currently based on direct analysis of the metabolite, e.g. on the detection and quantification of histamine. Nevertheless, it is interesting to highlight the interest in detecting and quantifying the microbiota responsible for synthesizing the metabolite, since putative outbreaks can thereby be prevented or detected even before they cause harmful effects to human health.

For that reason, this review focuses on describing techniques designed to detect histamine-producing bacteria (HPB), which can be classified into three types: culture-based, electroanalytical, and molecular methods. The advantages and disadvantages of these techniques are summarized in Figure 5.

5.1 Culture-based methods

Techniques using chromogenic agar or broth media were implemented in the 80s and 90s as useful tools for the identification of HPB. Several methods were developed to detect histamine accumulation during the growth of bacteria, which is evident in a change of color in the growth medium as a consequence of change in pH. Møller´s group and, many years later, Niven and collaborators developed chromogenic agar media supplemented with L-histidine using bromocresol purple to reveal the change in pH during histamine production (V. Møller, 1954; Niven, Jeffrey, & Corlett, 1981). Niven´s agar medium was later modified to differentially support bacterial growth (Chen, Wei, Koburguer, & Marshall, 1989) and to be used with increased selectivity for the enumeration of HPB in fish products (Mavromatis & Quantick, 2002). That medium has also been used as a basis for the development of other media adapted to cheese (Joosten & Northolt, 1989) or meat (Maijala, 1993). A liquid decarboxylase medium using bromocresol green and chlorophenol red was also described by Yamani & Untermann (1985) for use in pure or mixed cultures, avoiding solid media that could prevent the growth of certain HPB. A leucocrystal violet detection method was also developed to detect high-histamine-producing lactobacilli in cheese (Sumner & Taylor, 1989). A comparative analysis of the composition of some of these published decarboxylase media was reported in Bover-Cid & Holzapfel (1999). Also, an improved decarboxylase medium was proposed by these authors, which proved itself sensitive and suitable for screening the ability not only of LAB but also enterobacteria to produce different BAs. The main problem of these indicator media is the occurrence of false positives, caused by the simultaneous production of alkaline metabolites that lead to a pH-related color change (Bover-Cid & Holzapfel, 1999). For instance, a *P.*

590 *pentosaceus* isolate from cheese was able to produce ornithine from arginine causing the
591 release of ammonium ion to the medium, which raised the pH and rendered a false positive
592 result when tested in the indicator medium (C. O. A. Møller et al., 2020).
593 As shown in Figure 5, ease of use, availability, and low cost are some of the advantages of
594 culture-based methods, whereas false positives, the great amount of time required, and the
595 inability of growth of some HPB due to the conditions of the chromogenic medium are important
596 disadvantages. Furthermore, such methods are not able to detect low histamine producers:
597 thus, other methods might be required to confirm the detection of HPB (Bjornsdottir-Butler,
598 Jones, Benner, & Burkhardt, 2011; Chen et al., 1989; Landete, de Las Rivas, Marcobal, & Munoz,
599 2007).
600 In order to solve the time length problem involved in the methods exposed above, a rapid
601 technique has been recently described involving a two-layer membrane filtration assay and a
602 subsequent bacterial culture on agar plates with histidine and bromothymol blue as pH
603 indicator, requiring only 5 hours to analyze HBP in liquid samples as well as in seafood (Tao,
604 Sato, Abe, Yamaguchi, & Nakano, 2009).

605 **5.2 Electroanalytical methods**

606 Many methods based on measurements of potential (volts) and/or current (amperes) have been
607 described in the literature to quantify histamine in food, as reviewed in Yadav, Nair, Sai, & Satija
608 (2019). However, only few studies have applied electroanalytical techniques to reveal HPB,
609 which are difficult to detect since they constitute a minority among the present microbiota. In
610 the late 80s, Klausen & Huss (1987) developed a potentiometric method for the detection of
611 HPB by measuring conductance produced by the histidine-decarboxylase activity of HPB using a
612 histidine-decarboxylase medium: the method was validated in spoiled mackerel. It seems to be
613 highly effective in the detection of high-histamine producers, but is ineffective with low-
614 histamine-producing bacteria (Figure 5).

615 Recently, Trevisani et al. (2019) reported an enzyme-based amperometric biosensor designed
616 to detect histamine and HPB in tuna, based on measurements of HDC activity in a histidine
617 decarboxylase broth. However, to our knowledge, no electroanalytical methods for the
618 detection of histamine-producing microbiota in dairy foods have yet been reported.

619 **5.3 Molecular methods**

620 Culture-based as well as potentiometric techniques are nowadays being substituted by modern
621 molecular methods that enhance sensibility and reliability, even involving the implementation
622 of nucleic acid hybridization techniques. Molecular methods for the detection of biogenic
623 amine-producing bacteria in food were reviewed some years ago (Landete et al., 2007), but,
624 from our point of view, an update of that review, focusing on histamine, is required.

625 Molecular methods are based on the polymerase chain reaction (PCR), a useful and rapid
626 technique that allows the exponential amplification (the increase of number of copies) of target
627 DNA fragments or amplicons from a template by using a DNA polymerase enzyme and a series
628 of cycles of different temperature. To perform this reaction, two short single-strand DNA
629 fragments called oligonucleotides or primers are required. The primers are composed by the
630 complementary sequence of the ends of target DNA (Erlich, 1989). These methods are rapid,
631 specific, and sensitive, although they are unable to distinguish whether the HPB are dead, alive
632 or even viable but not cultivable (Figure 5) (Landete et al., 2007).

633 PCR methods to detect HPB are commonly based on the amplification of a fragment of the
634 histidine decarboxylase (*hdc*) gene, sometimes named *hdcA* (Landete et al., 2007; Linares et al.,
635 2011). Bacteria capable of producing histamine exhibit the *hdc* gene in the genome, which is
636 mainly located in the chromosome, but can sometimes be found in an unstable plasmid (Landete
637 et al., 2008). Figure 6 compiles the routes involved in the bacterial histamine metabolism,
638 depicting *hdcA* and other genes involved in the production of histamine such as *hdcC* (codifying
639 for a histidine/histamine antiporter), *hdcB* (involved in HDC maturation) or *hisS* (codifying for a
640 histidyl-t-RNA synthase like protein) that are usually present in gram-positive bacteria,

641 constituting the typical so-called *hdc* cluster (Benkerroum, 2016; Linares et al., 2011). The
642 genomic structure of the gene responsible for the synthesis of histamine in yeasts or molds has
643 not yet been described.
644 Two HDC enzyme families have been identified with completely different sequential and
645 biochemical characteristics: in gram-positive bacteria, in which the enzyme requires a pyruvoyl
646 moiety, and in gram-negative bacteria, which contain pyridoxal phosphate-dependent HDC
647 enzymes (Landete et al., 2008). Nucleotide sequences of enzymes from one or the other group
648 share high similarity (Wuthrich et al., 2017); the nucleotide sequence alignment of the *hdc* gene
649 in gram-positive bacteria was published some years ago (Diaz, Ladero, Redruello, et al., 2016a).
650 To our knowledge, no genomic studies regarding the putative *hdc* gene in yeasts have been
651 published to date. Taking advantage of this high similarity of the nucleotide sequence of *hdc*
652 genes among groups of bacteria, the design of primers that align in conserved regions within
653 the *hdc* gene would allow for the amplification of the gene from whichever bacteria are present
654 in food. Additionally, to better amplify the same gene from different microorganisms,
655 degenerated primers (a mixture of similar but not identical oligonucleotides) could also be used.
656 For these reasons, different pairs of primers for the amplification of the *hdc* gene in food through
657 a unique PCR reaction using only a pair of primers to detect each microorganism individually
658 (uniplex PCR) are reported in literature. However, only few of those studies refer to dairy
659 products. Primers designed to amplify the *hdc* gene of bacteria from dairy products are detailed
660 in Table 2. Specifically, STDEC-F and STDEC-R primers were designed to detect histamine-
661 producing *S. thermophilus* (Rossi et al., 2011) and degenerated HIS1-F and HIS1-R primers were
662 used in cheese to detect gram-positive bacteria (de Las Rivas, Marcobal, Carrascosa, & Munoz,
663 2006). Some authors adapted the pair of primers HDC3 and HDC4 to detect gram-positive HPB
664 in cheese or in home-made yogurt, which had been initially applied to smoked salmon by E.
665 Coton & Coton (2005) (Berthoud et al., 2017; Burdychova & Komprda, 2007; Gezginc, Akyol,
666 Kuley, & Ozogul, 2013; O'Sullivan et al., 2015). Primers CL1, CL2, JV16HC and JV17HC, initially

667 published by Le Jeune, Lonvaud-Funel, ten Brink, Hofstra, & van der Vossen (1995), were used
668 by other authors to highlight LAB containing the *hdc* gene in ripened or artisan cheeses (del
669 Valle, Ginovart, Gordún, & Carbó, 2018; Ladero et al., 2015; C. O. A. Møller et al., 2020). Primers
670 HIS2-F and HIS2-R, initially described by de Las Rivas et al. (2006), were used to detect gram-
671 negative HPB in cheese, although no amplification was obtained in any cheese sample
672 (O'Sullivan et al., 2015). Figure 7 shows the regions of *hdc* genes from the alignment of different
673 bacteria where the primers align. As observed, the high similarity among the *hdc* genes allows a
674 good alignment. It is also noteworthy the great sequence similarity of most primers results in
675 alignments in the same regions.
676 Several multiplex PCR methods (combining multiple pairs of primers in a single and optimized
677 PCR reaction to detect several microorganisms simultaneously) have been reported to detect
678 BAs in food. E. Coton & Coton (2005) described a PCR method for the simultaneous detection of
679 histamine- and tyramine-producing gram-positive bacteria using HDC3-HDC4 and TD2-TD5
680 primers directly on bacterial colonies in a single reaction. Some years later, these authors
681 incorporated other pairs of primers to additionally detect ornithine-producing bacteria from
682 wine and cider (M. Coton et al., 2010). Another multiplex PCR was published for the
683 simultaneous detection of LAB-producing histamine (primers JV16HC and JV17HC), tyramine
684 (primers P1-rev and P2-for, first described by P. Lucas & Lonvaud-Funel (2002)), and putrescine
685 (primers 3 and 16) in food, specifically in wine and grape must (Marcobal, de las Rivas, Moreno-
686 Arribas, & Munoz, 2005). These pairs of primers, together with an extra pair (106 and 107
687 primers) aimed to detect harmful gram-negative HPB, were used in an improved multiplex PCR
688 validated with DNA mixtures of several HPB (de Las Rivas, Marcobal, & Munoz, 2005). It is
689 noteworthy that those multiplex PCR methods are mainly applied for the detection of BA-
690 producing bacteria in wine and its derivatives, but not in dairy foods.
691 Methods that combine PCR with other techniques have also been used to determine HPB in
692 food. For instance, a PCR-denaturing gradient gel electrophoresis (PCR-DGGE) method for the

identification of HPB in cheese on the species level has been recently described. This is a useful and effective method that allows the separation of the *hdc* amplicons with the same size but different sequences, in order to distinguish among different *hdc* variants present in complex microbial communities. The pair of primers used in that study (hdcDG-F and hdcDG-R) aligns in the conserved regions of *hdc*, flanking a variable region, and renders a 250-base pair PCR products that are subsequently subjected to DGGE analysis (Diaz, Ladero, Redruello, et al., 2016b).

The main disadvantage presented by end-point PCR methods is the impossibility of quantifying DNA template: thus, real-time quantitative PCR methods (RT-qPCR) have been developed to detect HPB in food, mainly in wine, fish, and cheese (Bjornsdottir-Butler et al., 2011; P. M. Lucas et al., 2008; Nannelli et al., 2008). Particularly, in cheese, primers hdc1 and hdc2 were used to detect and quantify gram-positive HPB (M. Fernandez et al., 2006; Ladero, Linares, Fernandez, & Alvarez, 2008; C. O. A. Møller et al., 2020; Tofalo et al., 2019). A RT-qPCR assay has also been developed in raw milk and cheese to detect and enumerate *L. parabuchneri*, one of the main histamine producers in dairy food, although this method is not based on the analysis of the *hdc* gene but on the unique locus *tmp,* not present in other species (Berthoud et al., 2017).

Finally, genomic-based tools for the rapid and accurate assessment of microbial communities have been developed in recent years. Target metagenomics is based on the sequencing of selected target genes: it provides variable information depending on the studied gene, for instance 16S rRNA or biogenic amine synthetic genes (Ruiz & Alvarez-Ordoñez, 2019). As an example, high-throughput DNA sequencing has been implemented to assess the presence of bacterial histidine and tyrosine decarboxylases in cheeses. This method consists in amplifying the *hdc* and *tdc* genes with primers HIS2-F and HIS2-R or TD2 and TD5, and then cloning the PCR amplicons to subsequently perform high-throughput sequencing of the created amplicon libraries. Finally, the obtained *hdc* and *tdc* sequences are compared with a nucleotide database to identify bacteria with histaminogenic or tyraminogenic potential (O'Sullivan et al., 2015).

719 Another example of the application of next-generation sequencing techniques combining
720 sequencing and quantification of DNA has also been described in fish: the correlation of the
721 histamine content with the presence of gram-negative harmful bacteria, based on the
722 amplification of the 16S rRNA gene (de Lira et al., 2020; Tsironi et al., 2019). Unlike selected
723 target gene sequencing which only targets 16S rRNA or another key gene, shotgun
724 metagenomics sequences all given genomic DNA from a sample. As an example, several *L.*
725 *parabuchneri* species isolated from cheese or raw milk were genomically characterized by
726 sequencing their whole genomes to study the *hdc* cluster in profound detail and to conclude
727 that it was gained by horizontal gene transfer among different lactobacilli species (Wuthrich et
728 al., 2017).
729 In spite of the above-exposed advantages offered by modern molecular methods and
730 summarized in Figure 5 (such as high sensitivity and reliability or rapidity), important
731 disadvantages should be noted. One of the most important drawbacks is the impossibility of
732 identifying *hdc* genes of novel strains with emerging ability of histamine formation by using
733 traditional primers, as explained in Table 2. For instance, C. O. A. Møller et al. (2020) highlighted
734 that, in cheese, the *hdc* genes of *P. pentosaceus* isolates capable of producing histamine could
735 not be detected with use of both JV16HC/JV17HC and Hdc1/Hdc2 primer pairs, described in the
736 literature and useful for traditional histamine producers such as *L. parabuchneri* and *L.*
737 *paracasei*. Alternative methods should therefore be developed to allow the identification of all
738 HPB in food. Among them, whole genome sequencing of emerging histamine producers and
739 subsequent metagenomics annotation, or the search for new potential decarboxylase genes
740 based on nucleotide sequencing or tridimensional protein similarity, could yield good results.
741 Once all the putative histidine decarboxylase genes have been identified, the design of new
742 matching primer sets is indispensable.
743

744 **6 Histamine producers in dairy products**

745 The use of the aforementioned techniques in dairy products has allowed the identification of a
746 great variety of microorganisms with the ability to produce histamine (i.e., with histidine
747 decarboxylase activity). Histamine-forming microbiota in dairy products could be classified
748 according to different criteria. For instance, based on their origin and purpose, histamine
749 producers could be divided in 1) NSLAB (naturally present in milk), 2) SLAB (intentionally added
750 to dairy products) and 3) contaminants (due to practices during obtaining and handling the milk
751 through dairy products manufacture, as well as from the processing environment - including
752 insufficient cleaning-disinfection practices and biofilm formation). However, the traditional
753 classification of microorganisms allows to divide histamine-producing microbiota present in
754 dairy products in gram-positive bacteria, gram-negative bacteria, or yeasts and molds. Specific
755 genera, species and strains of microorganisms capable of synthesize histamine are detailed in
756 Table 3. Additionally, in bold type, Figure 3 highlights the species of histamine producers present
757 on cheese surface and in cheese core among the total microbiota that can be found in cheese.
758 It is key to consider that histamine formation is influenced by a series of factors, as exposed in
759 Figure 4, which should be carefully controlled during the cheese-making process. Some of these
760 factors directly focus on modulating the growth of histamine producers among total cheese
761 microbiota; for instance, environmental conditions such as salt content or water activity or even
762 bacterial competition processes.

763 **6.1 Gram-positive bacteria**

764 LAB are the main histamine producers in dairy products; *Lactobacillus* species such as *L.*
765 *parabuchneri, L. buchneri, Lb. helveticus*, and *L. curvatus,* among others, seem to be responsible
766 for histamine accumulation in cheese (Barbieri et al., 2019). Some of these species can be
767 present in cheese because they were either already contained in milk (above all, NSLAB), or
768 because they took part as contaminants or starter cultures in the course of the cheese
769 production process (Linares et al., 2012). Notably, *L. buchneri* and *L. parabuchneri*, present as
770 contaminants in fermented dairy products and closely related with one another

771 phylogenetically, have been reported to be the major histamine producers in cheese, capable
772 of synthesizing high amounts of histamine even at low temperatures (Berthoud et al., 2017; Díaz
773 et al., 2018; O'Sullivan et al., 2015; Wuthrich et al., 2017). *L. parabuchneri* has been reported to
774 produce histamine in a wide variety of cheese samples, even at low refrigeration temperatures
775 (Díaz et al., 2018; Diaz, Del Rio, et al., 2016; Diaz, Ladero, Del Rio, et al., 2016; C. O. A. Møller et
776 al., 2020). From several cheeses containing histamine, Berthoud et al. (2017) isolated certain *L.*
777 *parabuchneri* strains with the *hdc* gene, and developed a molecular method to detect and
778 enumerate *L. parabuchneri* in raw milk and cheese. Later on, the same authors investigated the
779 genome variability of these strains and concluded that the *hdc* cluster is located in a genomic
780 island that can be transferred within the *L. parabuchneri* species. Some strains have lost that
781 island and thus the capacity to synthesize histamine (Wuthrich et al., 2017). Relative to *L.*
782 *buchneri*, one isolate of a histamine-forming strain was detected in Spanish traditional cheeses,
783 and was shown to be the predominant LAB with histaminogenic potential in 10 different cheese
784 varieties, as evidenced by high-throughput DNA sequencing (O'Sullivan et al., 2015; Roig-
785 Sangüés, Molina, & Hernández-Herrero, 2002). On the other hand, Diaz et al. (2015) isolated (for
786 the first time from cheese) and typified several *L. vaginalis* (formerly *Lb. vaginalis*) strains
787 capable of producing histamine, as well as a number of histamine-producing isolates identified
788 as *L. reuteri* (formerly *Lb. reuteri*). Burdychova & Komprda (2007) also studied the histamine-
789 producing potential displayed by certain bacterial communities in a Dutch-type semi-hard
790 cheese. Among the histamine-producing strains isolated from the cheese, the authors found
791 that *Lb. delbrueckii* subsp. *lactis* and *L. curvatus* played a role as contaminants, whereas *Lb.*
792 *helveticus* originated from a starter culture used for cheese production. Other species such as *L.*
793 *brevis*, *L. casei*, and *L. plantarum* were found to contain the *hdc* gene in cheeses prepared with
794 raw milk, and some of those species had not been added as starter cultures (del Valle et al.,
795 2018). The *Lb. delbrueckii* species was also reported as a histamine producer (Roig-Sangüés et
796 al., 2002). *L. hilgardii*/*L. sakei* may present histaminogenic potential as well; the

797 indistinguishable *hdc* genes of these species have been detected in two Cabrales cheeses and
798 even highlighted by high-throughput DNA sequencing (Diaz, Ladero, Del Rio, et al., 2016;
799 O'Sullivan et al., 2015). An *L. paracasei* isolate from cheese was also shown to be a fast producer
800 of high levels of histamine, together with several isolates of *L. parabuchneri* (C. O. A. Møller et
801 al., 2020).
802 In addition to *Lactobacillus* species, the *Streptococcus* genus is also an important histamine
803 producer in cheese, although the source of this microorganism in the product is unclear
804 (O'Sullivan et al., 2015). However, not all of the strains actually contain the *hdc* gene. A recent
805 study classifies most *S. thermophilus* strains into two major clusters: Cluster A and Cluster B.
806 Strains belonging to Cluster A present larger genomes or complete histidine biosynthesis gene
807 clusters, among other characteristics. The *hdc* cluster is also present in all *S. thermophilus* strains
808 pertaining to Cluster A, supporting the hypothesis of acquisition by horizontal gene transfer
809 from a satellite phage (Alexandraki et al., 2019). In fact, up to 6% of *S. thermophilus* strains
810 isolated from natural sources contain the *hdc* gene, and some of them are able to produce
811 histamine in milk under conditions relevant to cheese-making, or even at low temperatures
812 (Calles-Enriquez et al., 2010; Gardini et al., 2012; Rossi et al., 2011). However, certain *S.*
813 *thermophilus* strains isolated from cheeses or home-made natural yogurt were also shown to
814 contain the *hdc* gene, although only a low amount or even no histamine at all was found in the
815 supernatant in culture media (Diaz, Ladero, Del Rio, et al., 2016; Gezginc et al., 2013; Ladero et
816 al., 2015).
817 Apart from *Lactobacillus* and *Streptococcus*, other LAB genera have been shown to synthesize
818 histamine in dairy products. Recently, C. O. A. Møller et al. (2020) reported *P. pentosaceus* for
819 the first time as a histamine producer in cheese. *T. halophilus* has been previously described as
820 a histamine producer in fish or soy sauces, although it was reported for the first time as a
821 histamine-producing species in certain Cabrales and Manchego cheeses (Diaz, Ladero, Del Rio,
822 et al., 2016; Satomi et al., 2008). The *hdc* gene was also amplified in a *Leuconostoc* sp. strain

isolated from raw goat milk cheese (del Valle et al., 2018). Potential histamine formation by *E. faecium* or *E. casseliflavus* in cheese has also been reported, but the contribution of enterococci to the level of histamine in cheese is probably irrelevant (Roig-Sangüés et al., 2002; Tham, Karp, & Danielsson-Tham, 1990).

6.2 Gram-negative bacteria

On the other hand, common contaminants of milk or spoilage bacteria such as the microbial families *Enterobacteriaceae* or *Pseudomonads* could also be responsible for histamine production in food. Many members of the *Enterobacteriaceae* family can act as histamine producers in cheese, but they only produce low amounts thereof, usually in early steps of the cheese-making process (Barbieri et al., 2019; Costa et al., 2018).

Several studies have isolated gram-negative bacteria present in different cheese varieties, and some of them have also quantified the amount of histidine that every bacterial isolate was able to produce *in vitro* or even in cheese model. M. Coton et al. (2012) obtained gram-negative bacterial isolates from French cheeses or milk, and then evaluated their ability to produce histamine *in vitro*. Many of the isolates were able to produce histamine in a culture medium, but only few of them produced more than 1000 mg/kg of histamine, namely *Morganella morganii* and *Serratia* sp. Additionally, *H. alvei, C. freundii, Halomonas* spp., *Raoultella planticola*, and *Providencia heimbachae* also produced more than 500 mg/kg of histamine (M. Coton et al., 2012). Many isolates of enterobacteria obtained from Montasio cheeses produced low amounts of histamine (<300 mg/kg), but only four isolates, two corresponding to *E. cloacae* and two more to *C. freundii*, produce more than 1000 mg/kg (Maifreni et al., 2013). According to another study, more than 50% of the 104 bacterial isolates from blue-veined cheeses were able to form histamine; although the histamine production was very low (< 20 mg/kg), isolates corresponding to *Enterobacter gorgoviae, S. liquefaciens, E. coli, H. alvei, E. cloacae, E. aerogenes, C. freundii, Arizona* spp., and *Klebsiella oxytoca* were confirmed to produce histamine (Marino, Maifreni, Moret, & Rondinini, 2000). The analysis of isolates of

849 enterobacteria obtained from Pecorino cheese resulted in the production of very low amounts
850 of histamine by all the strains (< 3 mg/kg), namely *E. coli, S. enterica* spp*. Arizonae, E. sakazakii,*
851 *C. braakii, Kluyvera* spp., and *S. odorifera* (Chaves-Lopez et al., 2006).
852 Other studies have also analyzed the presence of histamine-producing microbiota but have
853 failed to obtain quantitative results. For instance, Roig-Sangüés et al. (2002) isolated total
854 microbiota from certain Spanish cheeses: most of the gram-negative isolates, identified as
855 enterobacteria, displayed histamine-forming activity. The authors detected *H. alvei, E. coli, E.*
856 *sakazakii, Edwarsiella* spp., and *Serratia* spp. as histamine producers in cheese. Additionally, one
857 isolate of *Cedecea* spp., a genus genetically very close to *Serratia*, was reported for the first time
858 to produce histamine (Roig-Sangüés et al., 2002).
859 On the other hand, *Psychrobacter* sp. was reported for the first time to produce histamine *in*
860 *vitro* in a culture medium containing histidine*,* and even in a cheese model with the yeast *D.*
861 *hansenii* as co-culture (Helinck, Perello, Deetae, de Revel, & Spinnler, 2013).
862 **6.3 Yeasts and molds**
863 Certain yeasts and molds can also produce histamine in food, although few studies have
864 analyzed that production specifically in cheese. The major histamine producer in cheese
865 belonging to this group is *D. hansenii*, but this seems to be a strain-specific characteristic (Gardini
866 et al., 2006). In a cheese model, *D. hansenii* was able to produce histamine only in the presence
867 of the bacterium *Psychrobacter* (Helinck et al., 2013). *G. candidum* was also mentioned as a
868 histamine-forming mold in Cabrales cheese (Roig-Sangüés et al., 2002).
869
870 **7 Potential solutions to counteract histamine accumulation in dairy food: from**
871 **prevention to histamine degradation**
872 In order to avoid the release of dairy products with high levels of histamine to the market, the
873 main measure the food industry could take would be the reduction of HPB in dairy products by
874 a) preventing their access to raw materials, b) inactivating them, and/or c) controlling

875 environmental conditions. If those measures are not effective, d) microbial or enzymatic
876 degradation of histamine is the alternative. Figure 8 compiles the potential strategies for
877 obtaining histamine-free dairy products, aimed at preventing histamine formation or promoting
878 histamine degradation.
879 The promotion of hygienic conditions during milking and during food processing could decrease
880 and even inactivate histamine-producing microbiota. Additionally, the selection of suitable
881 starter cultures unable to synthesize histamine is an appropriate alternative for the reduction
882 of histamine production in dairy products, although it is necessary to assess whether the
883 organoleptic characteristics of the final product are eventually thereby altered.
884 To obtain a safe product with an extended shelf life, it is necessary to apply food preservation
885 treatments designed to reduce the microbial load and guarantee milk safety in the cheese-
886 making process (Quigley et al., 2013; Tilocca et al., 2020). Heat treatment (sterilization or
887 pasteurization) is currently the most commonly applied process for the preservation of liquid
888 milk (Walstra et al., 2006). Nevertheless, non-thermal technologies such as high-pressure
889 homogenization, or irradiation, have also been proposed as alternative technologies to
890 preservation of milk, although these methodologies are not currently being used industrially for
891 this purpose (Ramaswamy, Ahn, Balasubramaniam, Rodriguez Saona, & Yousef, 2019).
892 As mentioned above, the production and quantity of histamine synthesized in dairy products
893 such as cheese depends on a number of factors such as histidine availability, ripening and
894 storage temperatures, pH, sodium concentration, decarboxylation potential of the HPB, and
895 carbon source (Benkerroum, 2016; Linares et al., 2012). These factors can be occasionally
896 modified to prevent or reduce the rate of histamine production. In case the strategies for the
897 prevention of histamine formation in dairy products fail, the degradation of histamine can be
898 considered as a crucial alternative (Linares et al., 2012). Figure 8 summarizes the main strategies
899 aimed at preventing or reducing histamine content in dairy products.
900 **7.1 Measures aimed to prevent histamine formation during processing of dairy products**

One of the most important measures aiming to reduce histamine production is the overall improvement of hygiene during production and storage of dairy food. Other changes in food processing designed to inhibit or reduce HPB in dairy products include selection of *hdc*-negative starters, pasteurization, high-pressure homogenization, and control of physicochemical factors during dairy processing (Linares et al., 2012; Naila, Flint, Fletcher, Bremer, & Meerdink, 2010).

7.1.1 Preventing access of HPB to raw materials

7.1.1.1. Improving hygienic conditions along the dairy food chain

Hygienic conditions during milking are a very important factor for the dairy industry. The milk of healthy animals produced under hygienic conditions should contain less than 5 x 10^5 CFU/mL (Bereda, Yilma, & Nurfeta, 2012). The initial microbial load of milk varies between 10^3 and 10^5 CFU/ml, rising to 10^6-10^7 CFU/ml before processing (depending on its handling), and increasing during cheese ripening to up to 10^8 CFU/g in the final product (Benkerroum, 2016; Mlejnkova et al., 2016; Schirone, Tofalo, Visciano, Corsetti, & Suzzi, 2012). The microbiological quality of milk is clearly influenced by the way in which milk is handled from milking to consumption. The environment, handlers, equipment, and packaging materials can all be a reservoir for microbial contamination of milk and dairy products (Pal, Devrani, & Pinto, 2018). Lack of hygiene in the handling of milk, the misuse of milking equipment, and the lack of drinking water for cleaning purposes can contribute to the poor hygienic quality of milk. Strict hygienic measures must be applied during preparation, storage, and delivery of a variety of dairy products for human consumption. It is thus necessary to educate food handlers regarding the basic principles of hygiene and manufacturing of dairy products, which ensure their quality and safety for consumption.

On the other hand, histamine-producing microorganisms are likely to appear in the food chain in the form of food contaminant microbiota or NSLAB contained in the raw material (Linares et al., 2012). Pintado et al. (2008) indicates that the production of BAs in cheese made from raw milk depends, among other variables, on the level of enterobacteria, enterococci, and

927 lactobacilli present in raw milk, which can attain levels of 10^7 CFU/g. This level of contamination
928 in raw milk appears to be frequently associated with a high histamine content in raw milk
929 cheeses. The number and diversity of histamine-producing microorganisms increases as the
930 total count in raw milk rises (Benkerroum, 2016). Ascone et al. (2017) reported repeated
931 contamination of *L. parabuchneri* in milk from providers, capable of forming biofilms on stainless
932 steel surfaces in dairy processing equipment, and thus constituting a reservoir and a source of
933 contamination of post-ripening-processed cheeses (Diaz, Del Rio, et al., 2016). To reduce the
934 histamine content in such cheeses, it would be necessary to perform routine screening of
935 provided milks and to control the formation of biofilms containing HPB in the dairy food
936 processing industry (Diaz, Del Rio, et al., 2016). This would allow the identification and exclusion
937 of contaminated raw milk in order to prevent the production of contaminated raw milk cheeses
938 (Ascone et al., 2017).
939 On the other hand, in the final histamine content, contamination stemming from food
940 processing seems to be more important than contamination stemming from the raw material.
941 Ladero, Fernández, & Álvarez (2009) studied the effect of post-ripening processing of different
942 types of cheese on the presence of HPB and on the average histamine concentration of the final
943 product. The highest concentrations of histamine (734 mg/kg) were reported in grated cheese
944 samples in comparison with whole Emmental cheeses (115 mg/kg). In this case, the presence of
945 HPB during cheese manufacturing was due to poor hygiene practices in product processing: the
946 contact of the cheese with equipment surfaces increased the risk of microbiological
947 contamination.
948 Thus, in sum, it is necessary to control and improve microbiological and hygienic conditions
949 along the entire production chain (i.e. from farm to fork) in order to reduce the amounts of
950 biogenic amines or to avoid their presence altogether in dairy products (Benkerroum, 2016).
951 **7.1.1.2. Selection of cheese starters unable to synthesize histamine**

To guarantee the quality of dairy products and minimize the adverse health effects of histamine, starter cultures must be carefully selected on the basis of their inability to produce histamine and their capacity to degrade it (Naila et al., 2010; Spano et al., 2010).

Raw milk cheeses are particularly vulnerable to the formation of histamine, favored by high levels of secondary proteolysis as a consequence of the action of starter and non-starter cultures, along with a higher microbial load and, in some cases, long ripening times (Guarcello et al., 2016; Linares et al., 2011; O'Sullivan et al., 2015; Schirone et al., 2013). The addition of proteinases to milk or curd has been widely used with the purpose of accelerating cheese ripening (Fernandez-Garcia et al., 2000). The effect of the selection of starter cultures on the proteolytic pattern and thus on histamine production in cheese was demonstrated by Gardini et al. (2012) by using a histaminogenic *S. thermophilus* strain (PRI60) and, alternatively, a non-histamine-producing strain (PRI40) as starter cultures. Nieto-Arribas, Poveda, Seseña, Palop, & Cabezas (2009) suggested *L. plantarum* and *L. paracasei,* isolated from an artisan cheese, as possible starter cultures for cheese production due to their inability to produce BAs and, at the same time, because they do not alter the sensory characteristics of cheeses.

As a promising approach to the strain selection procedure, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas technique, commonly used for gene editing (Jiang, Bikard, Cox, Zhang, & Marraffini, 2013; Jinek et al., 2012), could also be applied either to inactivate the *hdc* gene and thus to obtain fermentative *hdc*-negative strains, or to ensure a greater phage resistance to starter LAB (Roberts & Barrangou, 2020). By generating these kinds of strains, fermented foods could be developed with similar sensory characteristics to those obtained with traditional strains, but with no histamine content or a greater phage resistance. CRISPR/Cas systems are present in many LAB, predominantly in *Streptococcus*, *Lactobacillus*, and *Bifidobacterium*. However, to our knowledge, no approaches based on CRISPR/Cas techniques in dairy products have been published to date, since in the European Union, CRISPR/Cas methods are considered as genetically modified organisms (GMO) and thus

regulatorily restricted. Consumers, and specifically those in the European Union, do not accept the use of GMOs. The United States, for instance, have recently allowed the use of CRISPR-Cas9 edited plants (Plavec & Berlec, 2020). Therefore, although the CRISPR/Cas technique is currently not approved for the production of starters in the European market, it could serve as an alternative for other international markets.

7.1.2 Treatments for microbial inactivation in milk

7.1.2.1 Heat

Heat treatment is an important step in the manufacturing of most dairy products, since high temperature can inactivate the bacterial species responsible for histamine formation (Naila et al., 2010).

-Sterilization virtually inactivates all present microbiota. Sterile milk is microbiologically stable, even at room temperature. Its shelf-life is usually limited by age-gelation (Deeth & Lewis, 2016), a progressive increase in viscosity leading to gel formation that can be associated with the action of heat-resistant proteases (e.g. plasmin or proteases of *Pseudomonas*) or other physicochemical factors (e.g. changes in micelles, availability of calcium ions, etc.).

-Pasteurization inactivates vegetative pathogenic microbiota. However, bacterial spores and vegetative spoilage microbiota (e.g. heat-resistant micrococci and thermophilic streptococci) might survive heat treatment, thus limiting shelf-life. Subsequent bacterial growth to 10^6 CFU/mL causes noticeable undesirable changes, such as acid production, protein breakdown, and lipolysis. Thus, it is necessary to refrigerate pasteurized milk in order to limit bacterial growth, allowing for up to 2-3 weeks of storage at 4°C, depending on the milk's hygienic properties. As mentioned for sterile milk, heat-resistant proteases can also be active in pasteurized milk.

In artisanal dairies, a thermization process is applied to milk at 57–68°C for 15 s or more, whereas in industrialized dairies, the milk is pasteurized at 72°C for 15 s (Martuscelli et al., 2005). In general, bacterial counts in cheeses made from pasteurized milk are lower than raw milk cheeses (Novella-Rodríguez et al., 2003). The decrease of the initial microbial load by pasteurization can lead to lower levels of BAs detected in dairy products obtained from pasteurized milk compared to those obtained from raw milk (Benkerroum, 2016). In this regard, Novella-Rodríguez, Veciana-Nogués, Roig-Sagués, Trujillo-Mesa, & Vidal-Carou (2004) reported lower levels of BAs in pasteurized milk cheeses in relation to raw milk cheeses. Tabanelli et al. (2012) determined that the inactivation of the HDC enzyme of *S. thermophilus* required a heat treatment of at least 75°C for 2 min.

However, once histamine is formed, high-temperature treatment could not destroy it, since biogenic amines appeared to be stable and difficult to degrade (McCabe, Frankel, & Wolfe, 2003).

Milk pasteurization thus contributes to reduce the risk of histamine content in the final cheese. However, survival of HPB or their HDC enzymes to the thermal treatment, and/or contamination with HPB in the subsequent steps of cheese formation (see Section 3.1), might be responsible for histamine outbreaks reported even in pasteurized cheeses (EFSA, 2011).

7.1.2.2 High-pressure homogenization

Currently, the food industry is particularly interested in non-thermal techniques for the inactivation of microorganisms, including foodborne pathogens. These techniques allow to increase shelf life while achieving a “fresh-like” product presentation. High-pressure homogenization (HPH) treatment is one of the most promising food preservation strategies that can help to inactivate microorganisms while likewise avoiding traditional thermal treatments (Lanciotti et al., 2007). In milk, for instance, an HPH treatment in pressure ranges between 100 and 1200 MPa helps to maintain flavor, body, texture, and nutrients while improving rennet or

acid coagulation. Pressure treatment also improves the preservation and rheological properties of yogurt (Chawla, Patil, & Singh, 2011).

HPH treatment can promote histamine synthesis because it produces a higher proteolysis rate than pasteurization, thereby leading to a higher availability of histamine precursors (Novella-Rodríguez, Veciana-Nogués, Saldo, & Vidal-Carou, 2002). Both aminopeptidase activity and free amino acid concentration of ripening cheeses are significantly increased by treatment at 400 MPa or 600 MPa for 21 and 35 days. However, HPH can also inhibit BA formation in cheese depending on the level of pressure applied (Novella-Rodríguez et al., 2002). Total BA formation decreased by about 50% in cheeses treated at 600 MPa compared to untreated cheeses, thus suggesting that HPH exerts an antimicrobial effect (Calzada, Olmo, Picon, Gaya, & Nuñez, 2013). Lower doses of 100 MPa applied to milk before cheese-making also resulted in decreased microbial counts and a lower histamine concentration at the end of the ripening process (Lanciotti et al., 2007).

Therefore, HPH could be regarded by the dairy industry as a suitable treatment aiming to decrease the population of potentially histamine-producing microorganisms and, consequently, to inhibit BA production. This technique is also useful in the development of innovative dairy foods without harmful effects on safety and milk coagulation, as well as for the improvement of cheese yields (Lanciotti et al., 2007). Moreover, HPH can help to achieve improved nutritional and sensory quality combined with longer shelf life, while maintaining a food's original texture (Chawla et al., 2011).

7.1.3. Control of physicochemical factors during processing of dairy products

During the production of fermented dairy products, decarboxylase activities and the growth of BA-producing microorganisms are affected by a number of physicochemical factors such as pH and salt concentration (see Section 4 and Figure 4) (Linares et al., 2012). If good hygiene conditions, controlled pH, and high salt content are achieved, the formation of BAs in cheese is decreased (Valsamaki, Michaelidou, & Polychroniadou, 2000).

Although some authors have proposed that low pH inhibits the accumulation of BAs in ripened cheese (Pintado et al., 2008; Valsamaki et al., 2000), most studies have suggested that acidic pH can encourage the formation of BAs in the course of cheese production (E. Coton, Rollan, & Lonvaud-Funel, 1998; Ladero et al., 2017; Landete et al., 2008; Marcobal, De Las Rivas, Moreno-Arribas, & Muñoz, 2006). The fermentation of lactose to lactic acid produces a low pH that is difficult to modify, since it is inherent to the milk fermentation process (Linares et al., 2012). In order to neutralize acid stress caused by dairy fermentation, it is assumed that specific amino acid decarboxylases produce BAs (Linares et al., 2012); in fact, the optimal pH for certain amino acid decarboxylases has been reported to be acid. Furthermore, histamine-producing NSLAB are able to survive and grow at low pH, and even produce high amounts of histamine at acidic pH (Barbieri et al., 2019; Frohlich-Wyder et al., 2015). Since formation of BAs raises pH (Barbieri et al., 2019), monitoring of pH could detect increases in pH which might be associated with histamine production. This change of pH could be used as a decision-making tool, e.g. for determining the period allotted to the ripening of the monitored cheese.

On the other hand, high salt content seems to reduce BA-producing microbiota and amino acid decarboxylase activity (Linares et al., 2012; Pintado et al., 2008). Salt has been conventionally added to prevent spoilage and food poisoning, while indirectly inhibiting the production of histamine in the final product (Linares et al., 2012). Gardini et al. (2001) demonstrated that a concentration of 5% NaCl minimizes the production of biogenic amines in culture medium and milk by inhibiting microbial growth. However, excessive addition of NaCl should be avoided (Dotsch-Klerk, Goossens, Meijer, & Van het Hof, 2015), since a limited intake of NaCl is recommended (less than 5 g per day) in order to avoid health issues.

Additional preventive measures that could be adopted during processing include low temperatures for ripening. It has been shown that refrigeration can help to reduce the final BA concentration (Calles-Enriquez et al., 2010). Thus, cheese ripening in cold storage and the freezing of cheese samples can reduce the rate of histamine production, probably due to a

reduction or inhibition of microbial growth, as well as to a decrease in enzymatic activity of HDC at low temperatures (Martuscelli et al., 2005; Santos, Souza, Cerqueira, & Glória, 2003). However, it is noteworthy to mention that low temperatures could not always be an effective preventive measure, since it has been described that *L. parabuchneri* is capable of producing histamine even in refrigerated cheese (Díaz et al., 2018).

7.2 Histamine degradation: addition of histamine-catabolizing strains or enzymatic degradation

The food preservation measures expounded above can be useful in preventing the production of histamine, but are in fact unable to eliminate accumulated histamine. As explained in Figure 6, histamine can be biologically catabolized by histamine-degrading microbiota through the activity of DAO enzyme (in the same or a different cell), which breaks down histamine to produce aldehyde, ammonia (which contributes to raise pH) and hydrogen peroxide (Pugin et al., 2017). Thus, to degrade histamine already formed in dairy food, the addition of histamine-degrading bacteria (biological degradation) or of degrading enzymes such as DAO (enzymatic histamine degradation) should also be considered (Naila et al., 2010).

7.2.1 Addition of histamine-degrading microbiota

Histamine-degrading microbial strains can be used as starter cultures to reduce histamine content in dairy products (Benkerroum, 2016; Dapkevicius, Nout, Rombouts, Houben, & Wymenga, 2000). Guarcello et al. (2016) identified the enzymatic activities responsible for BA degradation in LAB isolated from Italian cheeses. They selected 431 isolates unable to synthesize histamine (*hdc*-negative); 94 of them were also able to degrade histamine during culture in chemically defined medium. Those isolates belonged to the *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, Streptococcus, Enterococcus*, and *Weissella* genera. Among them, *L. paracasei* subsp. *paracasei* CB9CT exhibited the highest histamine-degrading activity. These results pointed toward a useful strategy to improve safety while maintaining the sensory

1106 characteristics of traditional cheeses. Tittarelli, Perpetuini, Di Gianvito, & Tofalo (2019) studied
1107 24 isolates of a raw ewe's cheese unable to produce histamine and, at the same time, able to
1108 degrade it. The most interesting strains appeared to be *L. casei* A422 and *E. casseliflavus* A143,
1109 with degradation rates higher than 50%; thus, they were proposed to be used as starter cultures
1110 to reduce the concentration of histamine in raw milk cheeses. Herrero-Fresno et al. (2012) also
1111 identified 17 histamine-degrading isolates of *L. casei* from cheese, among which two strains (*L.*
1112 *casei* 4a and 5b) with the highest histamine degradation rates (over 40%) were tested in a
1113 Cabrales-like mini-cheese manufacturing model. Due to their validated ability to degrade
1114 histamine during cheese ripening, those two *L. casei* strains are proposed as adjunct cultures for
1115 the reduction of histamine content in cheese. Leuschner & Hammes (1998) observed a
1116 degradation of 55% histamine content during a 4-week ripening period by the *B. linens* strains
1117 LTH456 and LTH3686 in a phosphate buffer. A reduction in histamine content was observed
1118 throughout the fermentation period of Munster cheese with both strains. Regarding yeasts, the
1119 strains of *D. hansenii* H525 and *Y. lipolytica* H446 were demonstrated to degrade several BAs,
1120 including histamine, when cultivated in red grape juice with each amine and in phosphate buffer
1121 (Baumlisberger, Moellecken, Konig, & Claus, 2015). It is interesting to once more point out the
1122 ability of *D. hansenii* to produce histamine as well, but in a strain-dependent manner (Gardini et
1123 al., 2006). Physicochemical and sensorial characteristics of dairy products should nevertheless
1124 be carefully assessed to guarantee their quality.

1125 **7.2.2 Addition of histamine-degrading enzymes**

1126 Apart from histamine-degrading strains, the addition of the DAO enzyme represents another
1127 strategy for the degradation of preformed histamine (Naila et al., 2012). Although the ability of
1128 DAO to degrade histamine has not yet been studied in dairy products, Dapkevicius et al. (2000)
1129 and Naila et al. (2012) analyzed the use of DAO to degrade histamine in buffer and in fish
1130 products. Dapkevicius et al. (2000) concluded that in fish slurry, the addition of DAO was more
1131 effective than histamine-degrading bacteria. Histamine degradation by DAO is pH- and

temperature-dependent, whereas the addition of sucrose or NaCl does not affect histamine degradation. Naila et al. (2012) also evaluated the action of DAO in a tuna soup, corroborating that it is more efficient than histamine-degrading microorganisms in the removal of histamine from food. Enzymatic degradation of histamine by DAO might be considered a safe strategy in raw milk, since the enzyme would be inactivated by heat treatment before its consumption.

Although DAO is presented as an innovative and promising alternative for the degradation of histamine in food, important drawbacks are also associated with its use, especially in dairy products. Firstly, as mentioned above, the enzymatic activity of DAO strongly depends on pH, temperature, and other environmental conditions. Thus, these parameters need to be adjusted and maintained within the enzyme's optimum ranges of activity, which can turn out to be extremely complicated in certain dairy products since yogurts, for instance, have a very acidic pH and must be stored in refrigerated condition. Secondly, DAO can be easily added to liquid or semi-liquid dairy products such as milk, yogurt, or kefir without any inconvenience. It would be quite complicated, however, to add DAO to a complex and heterogeneous matrix as cheese, mainly because of putative problems and limitations of enzyme diffusion. The composition, heterogeneity, and microstructure of the cheese matrix would condition the diffusion pattern of the enzyme (Floury et al., 2010; Silva, Peixoto, Lortal, & Floury, 2013), and subsequently its ability to migrate and find the substrate histamine. Finally, although most dairy products are regarded as basic consumer goods, DAO is an expensive commercial product, and its addition would significantly increase retail prices. The production of greater amounts of DAO at a competitive price could represent an interesting challenge to help promote the implementation of this effective solution for the degradation of histamine from dairy products.

8 Conclusion

Histamine in dairy products constitutes an important safety and health concern, specifically in fermented and ripened products. This biogenic amine is produced by present microbiota

(gram-positive and gram-negative bacteria, as well as yeasts and molds) from the precursor amino acid histidine via oxidative decarboxylation by the HDC enzyme. It is important to detect and quantify histamine-producing microbiota, particularly through the *hdc* gene, which is responsible for the synthesis of histamine. The accumulation of histamine in dairy products can be additionally prevented by controlling specific environmental and microbiological conditions (pH, temperature, salt concentration, etc.) when preparing dairy products, and/or by applying milk treatments (pasteurization, HPH, etc.). The use of starter cultures unable to produce histamine is another strategy designed to prevent histamine from dairy products. Finally, once histamine is accumulated, it could be necessary to implement its biological or enzymatic degradation through the addition of histamine-degrading microbiota or DAO. Obtaining histamine-free dairy food is a formidable challenge: if met, it would improve the quality of life of histamine-intolerant individuals, but also of the rest of the population, since it would prevent histamine outbreaks that cause significant harmful health effects on the public at large.

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Author Contributions

M. Moniente collected test data and drafted the manuscript. D. García-Gonzalo designed the study, drafted and reviewed the manuscript. I. Ontañón reviewed the manuscript. R. Pagán designed the study, reviewed the manuscript and carried out project administration and funding

acquisition. L. Botello-Morte designed the study, collected test data, interpreted the results and drafted, reviewed and edited the manuscript.

Conflicts of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Nomenclature

Biogenic amines (BAs)

Base pairs (bp)

Colony forming units per gram (CFU/g)

Colony forming units by milliliter (CFU/ml)

Companilactobacillus farciminis (*C. farciminis*)

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

Diamine oxidase enzyme (DAO)

Food and Agriculture Organization (FAO)

European Food Safety Authority (EFSA)

Genetically modified organisms (GMO)

High-performance liquid chromatography (HPLC)

High-pressure homogenization (HPH)

Histamine N-methyltransferase enzyme (HNMT)

Histamine-producing bacteria (HPB)

Histidine decarboxylase enzyme (HDC)

Histidine decarboxylase gene (*hdc*)

Lactic acid bacteria (LAB)

Lacticaseibacillus casei (*L. casei*)

Lacticaseibacillus paracasei (*L. paracasei*)

Lacticaseibacillus rhamnosus (*L. rhamnosus*)

Lactiplantibacillus pentosus (*L. pentosus*)

Lactiplantibacillus plantarum (*L. plantarum*)

Lactobacillus (*Lb.*)

Lactobacillus acidophilus (*Lb. acidophilus*)

Lactobacillus delbrueckii subsp*. bulgaricus* (*Lb. bulgaricus*)

Lactobacillus delbrueckii subsp*. delbrueckii* (*Lb. delbrueckii*)

Lactobacillus delbrueckii subsp*. lactis* (*Lb. delbrueckii* subsp*. lactis*)

Lactobacillus helveticus (*Lb. helveticus*)

Lactococcus (*Lc.*)

Latilactobacillus curvatus (*L. curvatus*)

Latilactobacillus sakei (*L. sakei*)

Lentilactobacillus buchneri (*L. buchneri*)

Lentilactobacillus hilgardii (*L. hilgardii*)

Lentilactobacillus kefiri (*L. kefiri*)

Lentilactobacillus parabuchneri (*L. parabuchneri*)

Levilactobacillus brevis (*L. brevis*)

Limosilactobacillus fermentum (*L. fermentum*)

Limosilactobacillus reuteri (*L. reuteri*)

Non-starter LAB (NSLAB)

Polymerase chain reaction (PCR)

PCR-denaturing gradient gel electrophoresis (PCR-DGGE)

Real-time quantitative PCR (RT-qPCR)

Single-nucleotide polymorphisms (SNPs)

1235 Starter LAB (SLAB)

1236 World Health Organization (WHO)

1237

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Tables

Table 1. Average composition in % w/w and range (in parentheses) of milk from different species (Ballard & Morrow, 2013; Becskei et al., 2020; Jenness, 1980; Recio, de la Fuente, Juárez, & Ramos, 2009; Walstra, Wouters, & Geurts, 2006).

	HUMAN	COW	GOAT	SHEEP	BUFFALO
FAT	3.4 (3.2-3.6)	3.7 (2.5-5.5)	4.7 (3.0-7.8)	7.1 (5.1-8.7)	6.0 (4.3-7.2)
PROTEIN	1.2 (0.6-1.4)	3.4 (2.3-4.4)	3.6 (2.9-5.0)	5.7 (4.8-6.6)	4.6 (4.1-5.6)
LACTOSE	7.2 (6.7-7.8)	4.8 (3.8-5.3)	4.9 (1.0-6.3)	4.6 (4.1-5.0)	5.4 (5.1-5.6)

Table 2. List and characteristics of primers aimed to amplify the *hdc* gene of bacteria from dairy products. Reference highlighted in bold is the original manuscript that described the primers for the first time.

PRIMER NAME	PRIMER SEQUENCE 5´→ 3´	AMPLICON SIZE	MICROORGANISMS AND REFERENCES	DAIRY PRODUCT SOURCES
STDEC-F **STDEC-R**	GAATTACCGATCTATGATGC ACACCTTTGTTAGCACAAAC	121 bp	*Streptococcus thermophilus* **(Rossi et al., 2011)**	Grana-type and mozzarella cheeses Traditional yogurts
HIS1-F **HIS1-R**	GGNATNGTNWSNTAYGAYMGNGCNGA ATNGCDATNGCNSWCCANACNCCRTA	372 bp	*Lactobacillus* sp. 30a (ATCC 33222) and *Lentilactobacillus buchneri* StA2 **(de Las Rivas, Marcobal, Carrascosa, & Munoz, 2006)** Other bacterial genera as *Micrococcus, Clostridium, Oenococcus* **(de Las Rivas et al., 2006)** *Streptococcus thermophilus* (Rossi et al., 2011)	Foodborne bacterial strains

HDC3 **HDC4**	GATGGTATTGTTTCKTATGA CAAACACCAGCATCTTC	435-440 bp	*Lentilactobacillus parabuchneri* (Berthoud et al., 2017), *Lentilactobacillus parabuchneri* DSM 5987 and *Lentilactobacillus parabuchneri* B301 (Diaz, Ladero, Del Rio, et al., 2016), *Lentilactobacillus buchneri* DSM 5987, *Lactobacillus* sp. 30a (ATCC 33222), *Latilactobacillus sakei* LTH 2076 and *Lentilactobacillus hilgardii* IOEB 0006 **(E. Coton & Coton, 2005),** *Lentilactobacillus buchneri* and *Latilactibacillus* (O'Sullivan et al., 2015), *Latilactibacillus curvatus, Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp*. lactis* (Burdychova & Komprda, 2007), *Limosilactobacillus vaginalis* (Diaz et al., 2015) *Tetragenococcus muriaticus* LMG 18498 **(E. Coton & Coton, 2005)**	Dutch-type semi-hard, Cabrales, Emmental, Reblochon, Irish Artisanal, Morbier, Pecorino Sardo, Ossau-Iraty, Emmental, Tête de Moine, Mont Soleil, Tilsit, Alpine and Raclette cheeses. Traditional home-made yogurts Foodborne bacterial strains

			Oenococcus oeni IOEB 9204 **(E. Coton & Coton, 2005)** *Streptococcus thermophilus* (Gezginc, Akyol, Kuley, & Ozogul, 2013)	
CL1 **CL2**	CCWGGWAAWATWGGWAATGGWTA GAWGCWGTWGTCATATTWATTTGWCC	150 bp	*Leuconostoc oenos* IOEB 9203 and *Leuconostoc oenos* IOEB 9204 **(Le Jeune, Lonvaud-Funel, ten Brink, Hofstra, & van der Vossen, 1995)** *Lactobacillus* sp. 30a (ATCC 33222) **(Le Jeune et al., 1995),** *Lentilactobacillus buchneri, Levilactobacillus brevis, Lacticaseibacillus casei, Lactiplantibacillus plantarum* and *Lactobacillus delbrueckii* subsp. *lactis* (del Valle, Ginovart, Gordún, & Carbó, 2018) *Lactococcus* sp. (del Valle et al., 2018)	Ripened raw goat milk cheeses Foodborne bacterial strains
HIS2-F	AAYTSNTTYGAYTTYGARAARGARGT	531 bp	*Morganella morganii* CECT 173^{T} (de Las Rivas et	Foodborne bacterial strains

HIS2-R	TANGGNSANCCDATCATYTTRTGNCC		al., 2006) *Photobacterium phosphoreum* CECT 4192^{T} and *Photobacterium damselae* CECT 626^{T} (de Las Rivas et al., 2006) *Proteus vulgaris* CECT 484^{T} **(de Las Rivas et al., 2006)** Other bacterial genera as *Enterobacter, Pseudomonas* **(de Las Rivas et al., 2006)**	
JV16HC **JV17HC**	AGATGGTATTGTTTCTTATG AGACCATACACCATAACCTT	367 bp	*Lactobacillus* sp. 30a (ATCC 33222), *Lentilactobacillus buchneri* StA2 and *Lentilactobacillus hilgardii* BIFI-87 (Marcobal, de las Rivas, Moreno-Arribas, & Munoz, 2005), *Lentilactobacillus buchneri* B301 (Ladero et al., 2015), *Lentilactobacillus buchneri* StA2, *Lentilactobacillus buchneri* NZHD1,	Danish Gouda-type and artisanal cheeses Foodborne bacterial strains

Lentilactobacillus buchneri NZHD2,

Lentilactobacillus buchneri NZHD3,

Lentilactobacillus buchneri NZHD4,

Lentilactobacillus buchneri NZHD5 and

Lentilactobacillus buchneri ClVO29 **(Le Jeune et al., 1995),** *Lentilactobacillus buchneri,*

Levilactobacillus brevis, Lacticaseibacillus casei,

Lactiplantibacillus plantarum and *Lactobacillus delbrueckii* subsp. *lactis* (del Valle et al., 2018),

Lentilactobacillus parabuchneri KUH1,

Lentilactobacillus parabuchneri KUH2,

Lentilactobacillus parabuchneri KUH8 and

Lacticaseibacillus paracasei KUH3 (Moller, Ucok, & Rattray, 2020)

			Clostridium perfringens ATCC 13124 **(Le Jeune et al., 1995)** *Leuconostoc oenos* IOEB 9203 and *Leuconostoc oenos* IOEB 9204 **(Le Jeune et al., 1995)** *Staphylococcus* sp. (de Las Rivas, Marcobal, & Munoz, 2005) *Streptococcus thermophilus* (Ladero et al., 2015) *Lactococcus* sp. (del Valle et al., 2018)	
106 **107**	AAYTCNTTYGAYTTYGARAARGARG ATNGGNGANCCDATCATYTTRTGNCC	534 bp	*Morganella morganii* CECT 173^{T} **(de Las Rivas et al., 2005)** *Photobacterium phosphoreum* CECT 4192^{T} **(de Las Rivas et al., 2005)** *Proteus vulgaris* CECT 484^{T} **(de Las Rivas et al., 2005)** *Klebsiella planticola* CECT 843 **(de Las Rivas et al.,**	Foodborne bacterial strains

			2005)	
HDCDG-F **HDCDG-R**	CCTGGTCAAGGCTATGGTGTATGGTC GGTTTCATCATTGCGTGTGCAAA	250 bp	*Lentilactobacillus parabuchneri* and *Latilactobacillus sakei/Lentilactobacillus hilgardii* **(Diaz, Ladero, Redruello, et al., 2016)** *Tetragenococcus halophilus* **(Diaz, Ladero, Redruello, et al., 2016)** *Streptococcus thermophilus* **(Diaz, Ladero, Redruello, et al., 2016)**	Cabrales, Manchego-type, Idiazabal, Casín and Gamoneu cheeses
HDC1 **HDC2**	TTGACCGTATCTCAGTGAGTCCAT ACGGTCATACGAAACAATACCATC	174 bp	*Lentilactobacillus parabuchneri* KUH1, *Lentilactobacillus parabuchneri* KUH2, *Lentilactobacillus parabuchneri* KUH8 and *Lacticaseibacillus paracasei* KUH3 (Moller et al., 2020), *Lentilactobacillus buchneri* B301, *Lentilactobacillus buchneri* B302, *Lentilactobacillus buchneri* B303, *Lentilactobacillus buchneri* DSM	Danish Gouda-type and Cabrales cheeses

		5987 and *Lentilactobacillus hilgardii* 321 **(Fernandez, del Rio, Linares, Martin, & Alvarez, 2006)** *Enterococcus* 15A **(Fernandez et al., 2006)** *Oenococcus oeni* 206 and *Oenococcus oeni* 212 **(Fernandez et al., 2006)** *Pediococcus parvulus* 276 **(Fernandez et al., 2006)**

Y = C or T; K = G or T; R = A or G; S = C or G; W = A or T; M = A or C; D = G, A or T; N = A, C, G or T

Table 3. Histamine-producing microbiota present in different dairy products.

MICROORGANISMS		REFERENCES	DAIRY PRODUCT SOURCE	TECHNIQUES APPLIED FOR IDENTIFICATION (I) AND FOR CONFIRMING (C) HISTAMINE FORMING ABILITY
GRAM-POSITIVE BACTERIA	*Lentilactobacillus buchneri* (formerly *Lb. buchneri*)	(O'Sullivan et al., 2015)	Reblochon, Irish artisanal, Morbier, Tête de Moine and Pecorino Sardo cheeses	High-throughput DNA sequencing of total metagenomic DNA extracts (I) and HPLC quantification (C)
	Lentilactobacillus buchneri	(Roig-Sangüés, Molina, & Hernández-Herrero, 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)

	Lentilactobacillus parabuchneri (formerly *Lb. parabuchneri*) KUH8, KUH1, KUH2	(Moller et al., 2020)	Vintage Danish Gouda cheese	Histidine decarboxylase activity and PCR analysis (I) and UPLC quantification (C)
	Lentilactobacillus parabuchneri FAM21731, FAM21809, FAM21823, FAM21829, FAM21834, FAM23163, FAM23164, FAM23165, FAM23166, FAM23167, FAM23168, FAM23169	(Wuthrich et al., 2017)	Emmental, Tête de Moine, Mont Soleil and Tilsit cheeses	Whole-genome sequencing and HPTLC quantification (C)
	Lentilactobacillus parabuchneri	(Berthoud et al., 2017)	Emmental, Tête de Moine, Mont Soleil, Tilsit, Alpine and Raclette cheeses Raw milk	Histidine decarboxylase activity and qPCR analysis (I) and HPLC quantification (C)

	Lentilactobacillus parabuchneri	(Diaz, Ladero, Redruello, et al., 2016)	Cabrales, Gamoneu, Manchego-type, Casín and Idiazabal cheeses	PCR-DGGE analysis (I) and HPLC quantification (C)
	Lentilactobacillus parabuchneri IPLA 11118, IPLA 11119, IPLA 11120, IPLA 11121, IPLA 11122, IPLA 11123, IPLA 11124, IPLA 11125, IPLA 11126, IPLA 11127, IPLA 11128, IPLA 11129, IPLA 11130, IPLA 11131, IPLA 11132, IPLA 11133, IPLA 11134, IPLA 11135, IPLA 11136, IPLA 11137, IPLA 11138	(Diaz, Ladero, Del Rio, et al., 2016)	Emmental cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Lentilactobacillus parabuchneri IPLA 11122, IPLA 11117, IPLA 11150	(Díaz et al., 2018)	Different types of commercial cheeses	HPLC quantification (C)
	Lentilactobacillus parabuchneri	(Diaz et al., 2015)	Cabrales cheese	Histidine decarboxylase activity (I)

	Lactobacillus delbrueckii subsp. *lactis* (formerly *Lb. lactis*)	(Burdychova & Komprda, 2007)	Dutch-type semi-hard cheese	PCR analysis (I) and HPLC quantification (C)
	Lactobacillus helveticus	(Burdychova & Komprda, 2007)	Dutch-type semi-hard cheese	PCR analysis (I) and HPLC quantification (C)
	Lactobacillus delbrueckii	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Latilactobacillus sakei/Lentilactobacillus hilgardii (formerly *Lb. sakei* group)	(Diaz, Ladero, Redruello, et al., 2016)	Cabrales cheeses	PCR-DGGE analysis (I) and HPLC quantification (C)
	Latilactobacillus gen.	(O'Sullivan et al., 2015)	Ossau-Iraty, Irish Artisanal, Morbier and Pecorino Sardo cheeses	High-throughput DNA sequencing of total metagenomic DNA extracts (I) and HPLC quantification (C)

	Latilactobacillus curvatus (formerly *Lb. curvatus*)	(Burdychova & Komprda, 2007)	Dutch-type semi-hard cheese	PCR analysis (I) and HPLC quantification (C)
	Levilactobacillus brevis (formerly *Lb. brevis*)	(del Valle et al., 2018)	Raw goat milk cheese	PCR analysis (I) histamine formation assessment and HPLC quantification (C)
	Lacticaseibacillus casei (formerly *Lb. casei*)	(del Valle et al., 2018)	Raw goat milk cheeses	PCR analysis (I) histamine formation assessment and HPLC quantification (C)
	Lacticaseibacillus casei	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Lacticaseibacillus paracasei KUH3 (formerly *Lb. paracasei*)	(Moller et al., 2020)	Vintage Danish Gouda cheese	Histidine decarboxylase activity and PCR analysis (I) and UPLC quantification (C)

	Lactiplantibacillus plantarum (formerly *Lb. plantarum*)	(del Valle et al., 2018)	Raw goat milk cheese	PCR analysis (I) histamine formation assessment and HPLC quantification (C)
	Limosilactobacillus vaginalis (formerly *Lb. vaginalis*) IPLA11140, IPLA11141, IPLA11142, IPLA11143, IPLA11144, IPLA11145, IPLA11147, IPLA11050, IPLA11051, IPLA11052, IPLA11053, IPLA11054, IPLA11055, IPLA11056, IPLA11057, IPLA11058, IPLA11060, IPLA11062, IPLA11064, IPLA11065, IPLA11067, IPLA11068, IPLA11069, IPLA11070 and IPLA11075.	(Diaz et al., 2015)	Cabrales cheese	Histidine decarboxylase activity and PCR analysis (I) and HPLC quantification (C)

	Limosilactobacillus reuteri (formerly *Lb. reuteri*)	(Diaz et al., 2015)	Cabrales cheese	Histidine decarboxylase activity (I)
	Lactococcus lactis subsp. *lactis*	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Streptococcus thermophilus PRI60	(Gardini et al., 2012)	Dairy products	HPLC quantification (C)
	Streptococcus thermophilus	(Gezginc et al., 2013)	Home-made natural yogurts	Histidine decarboxylase activity and PCR analysis (I) and HPLC quantification (C)
	Streptococcus thermophilus	(Ladero et al., 2015)	Artisanal raw milk cheeses	PCR analysis (I) and (U)HPLC quantification (no histamine) (C)
	Streptococcus thermophilus PRI17, PRI18, PRI21, PRI60, PRI74	(Rossi et al., 2011)	Mozzarella and Grana-type cheeses.	PCR analysis (I) and HPLC quantification (C)

			Traditional yogurts	
	Streptococcus thermophilus	(Diaz, Ladero, Redruello, et al., 2016)	Idiazabal cheeses.	PCR-DGGE analysis (I) and HPLC quantification (no histamine) (C)
	Pediococcus pentosaceus KUH5, KUH6, KUH7	(Moller et al., 2020)	Vintage Danish Gouda cheese	Histidine decarboxylase activity (I) and UPLC quantification (C)
	Tetragenococcus halophilus	(Diaz, Ladero, Redruello, et al., 2016)	Cabrales and Manchego-type cheeses	PCR-DGGE analysis (I) and HPLC quantification (C)
	Leuconostoc sp.	(del Valle et al., 2018)	Raw goat milk cheese	PCR analysis (I) histamine formation assessment and HPLC quantification (C)
	Enterococcus faecium	(Tham, Karp, & Danielsson-Tham, 1990)	Goat milk cheese	Fluorimetric histamine determination (C)

	Enterococcus faecalis	(Tham et al., 1990)	Goat milk cheese	Fluorimetric histamine determination (C)
	Enterococcus casseliflavus	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Microbacterium foliorum C45	(Helinck, Perello, Deetae, de Revel, & Spinnler, 2013)	French cheeses	HPLC quantification (C)
GRAM-NEGATIVE BACTERIA	*Citrobacter freundii*	(Marino, Maifreni, Moret, & Rondinini, 2000)	Blue-veined cheeses	HPLC quantification (C)
	Citrobacter freundii	(Maifreni et al., 2013)	Montasio cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Citrobacter freundii UCMA 4217	(M. Coton et al., 2012)	Livarot cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Citrobacter braakii CtT 6, CtT 10, CtT 29, CtT 60, CtT 61	(Chaves-Lopez et al., 2006)	Pecorino Abruzzese cheeses	HPLC quantification (C)
Hafnia alvei	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Hafnia alvei	(Maifreni et al., 2013)	Montasio cheeses	HPLC quantification (C)
Hafnia alvei 1 B16	(M. Coton et al., 2012)	Livarot cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Hafnia alvei	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Hafnia paralvei 920	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Halomonas sp. nov. B39	(M. Coton et al., 2012)	Livarot cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Halomonas venusta 3D7M	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Halomonas venusta/alkaliphila/hydrothermalis 4C1A	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Morganella morganii 3A2A, 3A5A, 3D4A	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Providencia heimbachae GR4	(M. Coton et al., 2012)	Epoisses cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Serratia liquefaciens	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Serratia liquefaciens 1B4F	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Serratia liquefaciens	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Serratia marcescens 448	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Serratia proteomaculans 1C2F	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Serratia odorifera CtT 28, CtT 57, CtT 58, CtT 74	(Chaves-Lopez et al., 2006)	Pecorino Abruzzese cheeses	HPLC quantification (C)
Serratia odorifera	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Serratia grimesii UCMA 3895	(M. Coton et al., 2012)	Livarot cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Serratia sp. (close *S. grimesii*) GB3	(M. Coton et al., 2012)	Epoisses cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Serratia spp.	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)

Chryseobacterium shigense PCA1 B2.3	(M. Coton et al., 2012)	Cow milk Salers cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Chryseobacterium sp. (close *C. bovis*) Pi 18	(M. Coton et al., 2012)	St. Nectaire cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Enterobacter hormaechei 380, 272, INRA 1439	(M. Coton et al., 2012)	Munster and Salers cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Enterobacter cloacae	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Enterobacter cloacae	(Maifreni et al., 2013)	Montasio cheeses	HPLC quantification (C)
Enterobacter gergoviae	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Enterobacter aerogenes	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Enterobacter sakazaki CtT 9, CtT 23, CtT 29	(Chaves-Lopez et al., 2006)	Pecorino Abruzzese cheeses	HPLC quantification (C)

Enterobacter sakazakii	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Enterobacter spp.	(Maifreni et al., 2013)	Montasio cheeses	HPLC quantification (C)
Pseudomonas grp *putida* CV 30.6, VRBG 37.3, CFC25.4	(M. Coton et al., 2012)	Milk	Histidine decarboxylase activity (I) and HPLC quantification (C)
Pseudomonas lundensis PCAi D2.2	(M. Coton et al., 2012)	Cow milk Salers cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Pseudomonas stutzeri UCMA 3883	(M. Coton et al., 2012)	Livarot cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Psychrobacter celer 91	(M. Coton et al., 2012)	Camembert raw milk cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Psychrobacter sp. 580	(Helinck et al., 2013)	French cheeses	HPLC quantification (C)
Raoultella planticola 924	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Raoultella ornithinolytica	(Maifreni et al., 2013)	Montasio cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Sphingobacterium sp. (close *S. faecium*) PCAi F2.5	(M. Coton et al., 2012)	Cow milk Salers cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Acinetobacter sp. (close genospecies 3) PCA E6.10	(M. Coton et al., 2012)	Cow milk Salers cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Alcalingenes faecalis 1 904	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Proteus sp. (close *P. hauseri*) UCMA 3780	(M. Coton et al., 2012)	Livarot cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Proteus heimbachae 945	(M. Coton et al., 2012)	Munster cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
Providencia sp. nov. GB1	(M. Coton et al., 2012)	Epoisses cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)

Escherichia coli	(Maifreni et al., 2013)	Montasio cheeses	HPLC quantification (C)
Escherichia coli	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Escherichia coli CtT 1, CtT 24, CtT 43, CtT 75	(Chaves-Lopez et al., 2006)	Pecorino Abruzzese cheeses	HPLC quantification (C)
Escherichia coli	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Escherichia fergusonii	(Maifreni et al., 2013)	Montasio cheeses	HPLC quantification (C)
Klebsiella oxytoca	(Maifreni et al., 2013)	Montasio cheeses	HPLC quantification (C)
Klebsiella oxytoca	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)
Klebsiella pneumoniae	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
Arizona spp.	(Marino et al., 2000)	Blue-veined cheeses	HPLC quantification (C)

	Salmonella enterica spp. *arizonae* CtT 31, CtT 33, CtT 37 CtT 50	(Chaves-Lopez et al., 2006)	Pecorino Abruzzese cheese	HPLC quantification (C)
	Kluyvera spp. CtT 3, CtT 26, CtT 49, CtT 53	(Chaves-Lopez et al., 2006)	Pecorino Abruzzese cheese	HPLC quantification (C)
	Cedecea spp.	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Edwarsiella spp.	(Roig-Sangüés et al., 2002)	Spanish traditional cheeses	Histidine decarboxylase activity (I) and HPLC quantification (C)
YEASTS AND MOLDS	*Geotrichum candidum*	(Roig-Sangüés et al., 2002)	Cabrales cheese	Histidine decarboxylase activity (I) and HPLC quantification (C)
	Debaryomyces hansenii LM21, LM24, LM26	(Gardini et al., 2006)	Pecorino Crotonese cheese	Histidine decarboxylase activity (I)

Debaryomyces hansenii 304	(Helinck et al., 2013)	French cheeses	HPLC quantification (C)

Figures

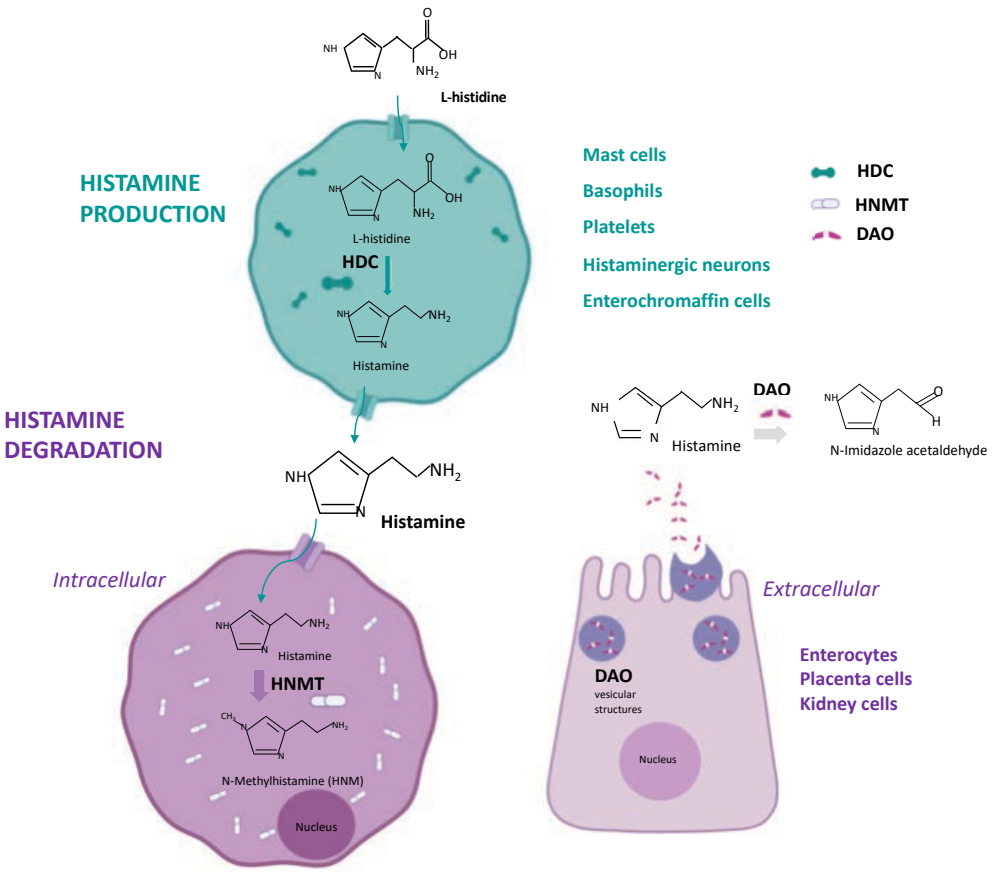


Figure 1. Overview of the main mechanisms of histamine production and degradation in the mammal cell. Histamine is intracellularly synthesized by L-histidine decarboxylase (HDC) from the amino acid histidine by certain mammal cells (mast cells, basophils, platelets, histaminergic neurons, and enterochromaffine cells). Conversely, histamine is intracellularly degraded by histamine N-methyltransferase (HNMT), ubiquitously expressed, and extracellularly by secreted diamine oxidase (DAO), mainly produced in enterocytes.

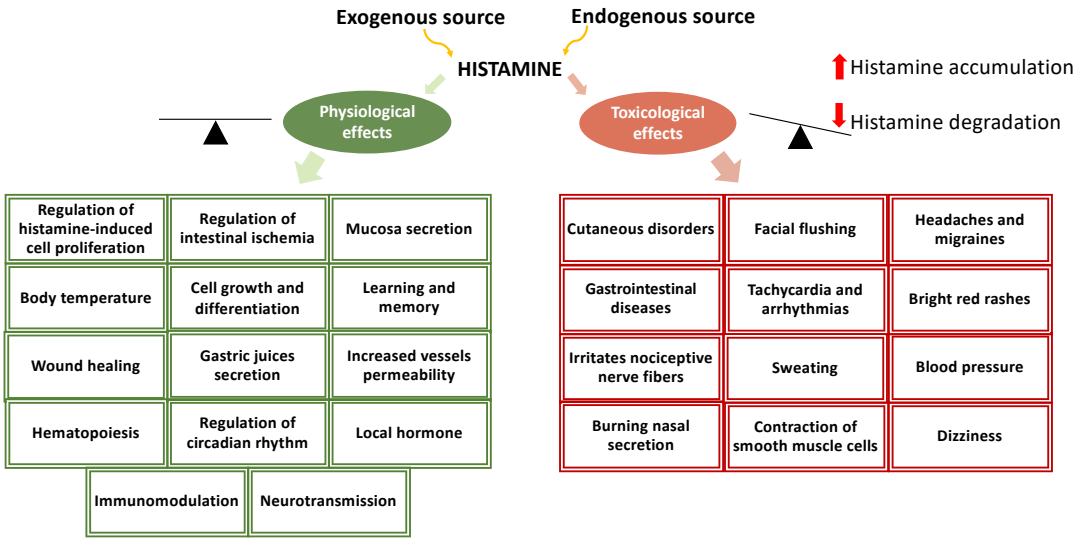
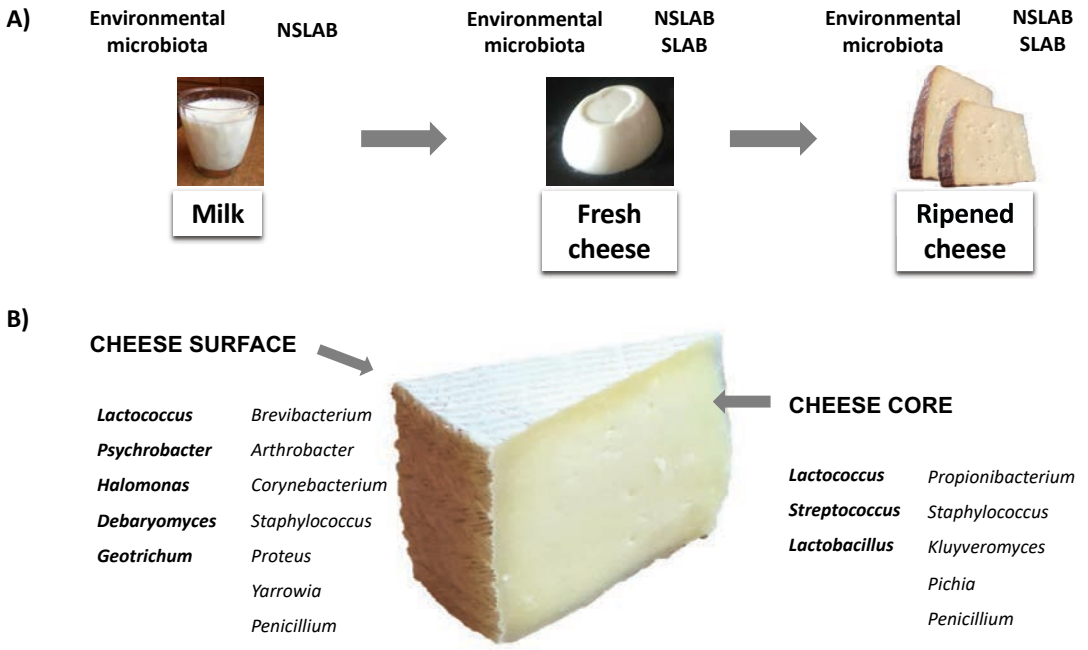


Figure 2. Physiological equilibrium between histamine synthesis/intake and degradation or the consequences of a misbalance due to an increase in histamine accumulation or a decrease in histamine degradation. The physiological and toxicological effects of histamine on the human metabolism are also shown.



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16 Figure 3. Cheese microbiota. A) Source of histamine-producing microbiota in cheese making. B)

17 Microorganisms present in cheese surface versus core: those able to synthesize histamine are

18 highlighted in bold.

19

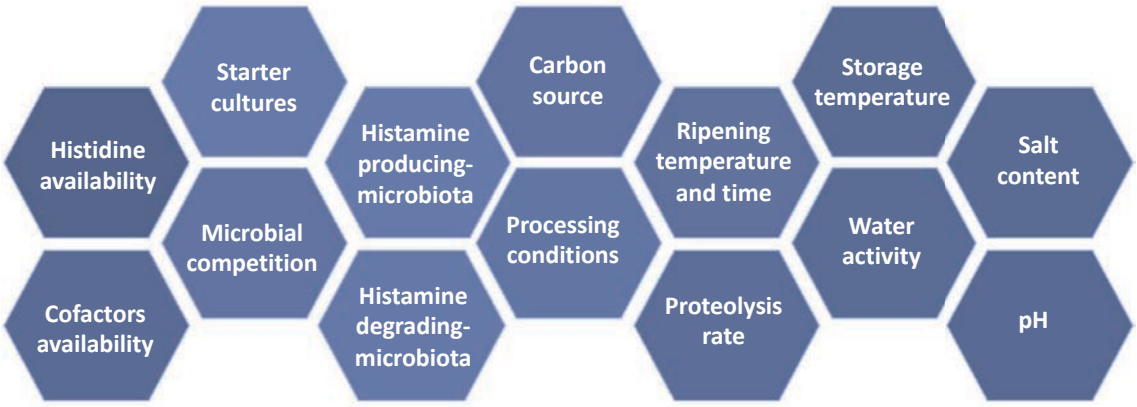

Figure 4. Factors related to histamine production in dairy products, including availability of precursors, environmental conditions, and microbiological factors. All these factors should be carefully controlled in dairy products to avoid histamine accumulation.

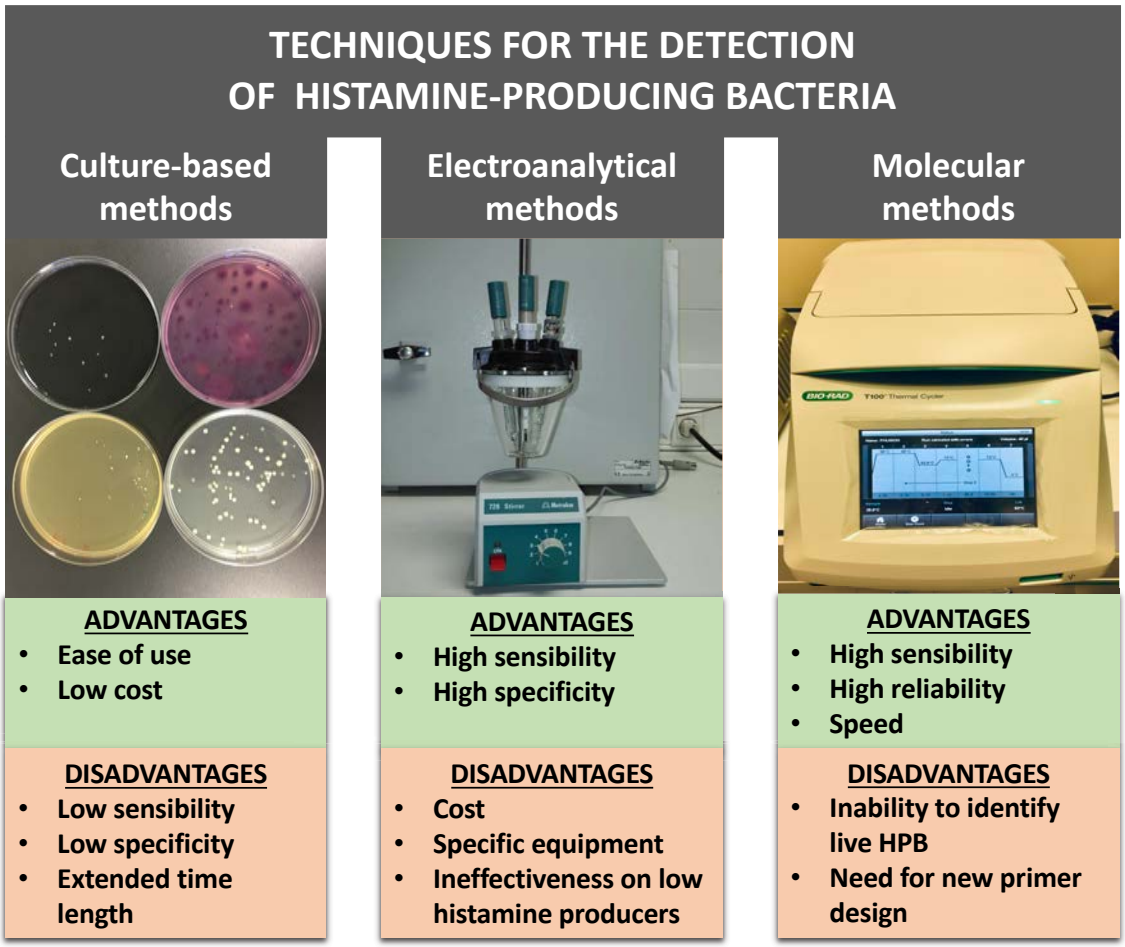

Figure 5. Techniques for the detection of histamine-producing bacteria in dairy products. Their main advantages and disadvantages are listed.

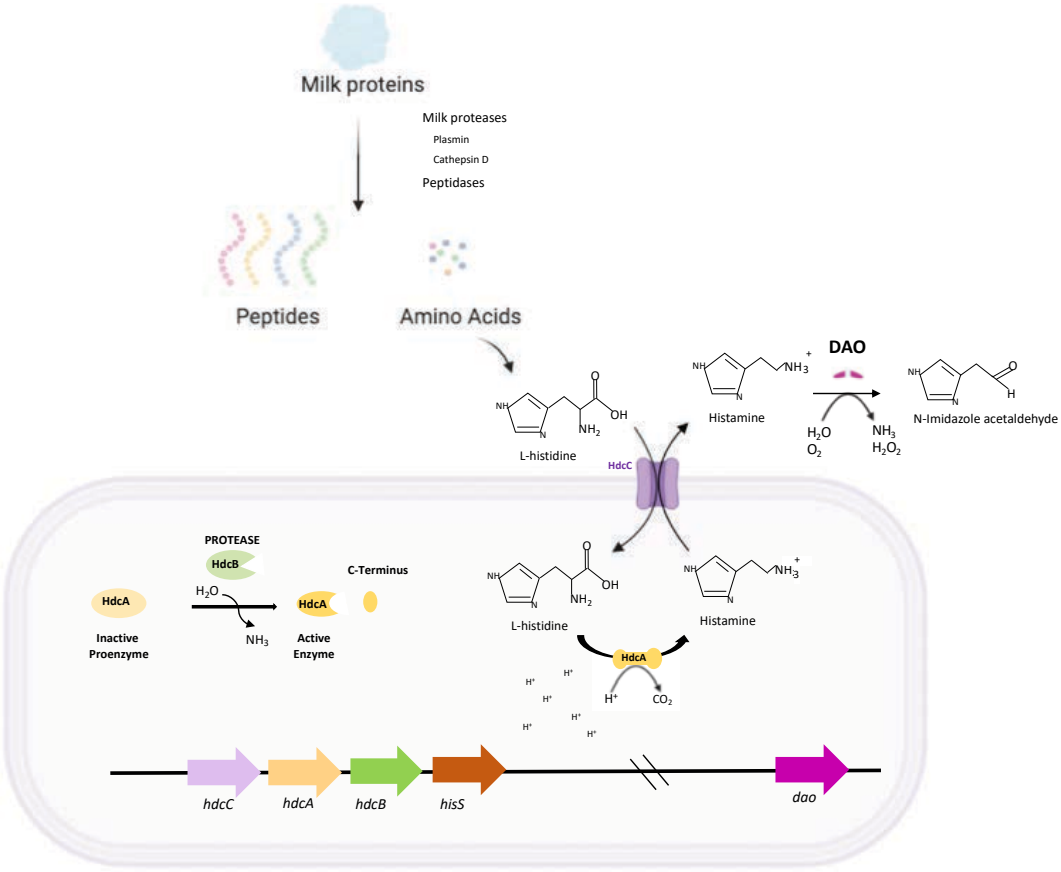

Figure 6. Overview of the mechanisms of histamine formation and degradation by microorganisms. The synthesis of histamine is mediated by the enzyme histidine decarboxylase (HDC or HdcA), codified by the *hdc* (or *hdcA*) gene. In some gram-positive bacteria, this gene takes part in the so-called *hdc* cluster, together with genes codifying for a histidine/histamine antiporter (*hdcC*), a histamine decarboxylase enzyme (*hdcA*), an enzyme involved in proenzyme HdcA cleavage and maturation (*hdcB*), and a protein similar to a histidyl tRNA synthetase (*hisS*). The HdcA enzyme is synthesized as a proenzyme, which requires the proteolysis of the C-terminus, mediated by HdcB, to be an active enzyme. In dairy products, breakdown of milk proteins by plasmin, cathepsin D and other milk proteases and peptidases results in the formation of free peptides and amino acids, such as histidine, precursor of histamine. When the survival mechanism inducing histamine (no sugar available, low pH…) is activated in histamine-producing bacteria, the antiporter HdcC allows histidine to enter the cell in order to be decarboxylated by HdcA, to form histamine, with the consumption of a proton, that contributes

to raise pH. When histamine needs to be metabolized, the same antiporter HdcC secretes this metabolite to be degraded by the enzyme DAO. Since a net positive charge is transported out of the cell by the electrogenic antiport, it results in the generation of proton motive force and energy generation (Molenaar, Bosscher, ten Brink, Driessen, & Konings, 1993).

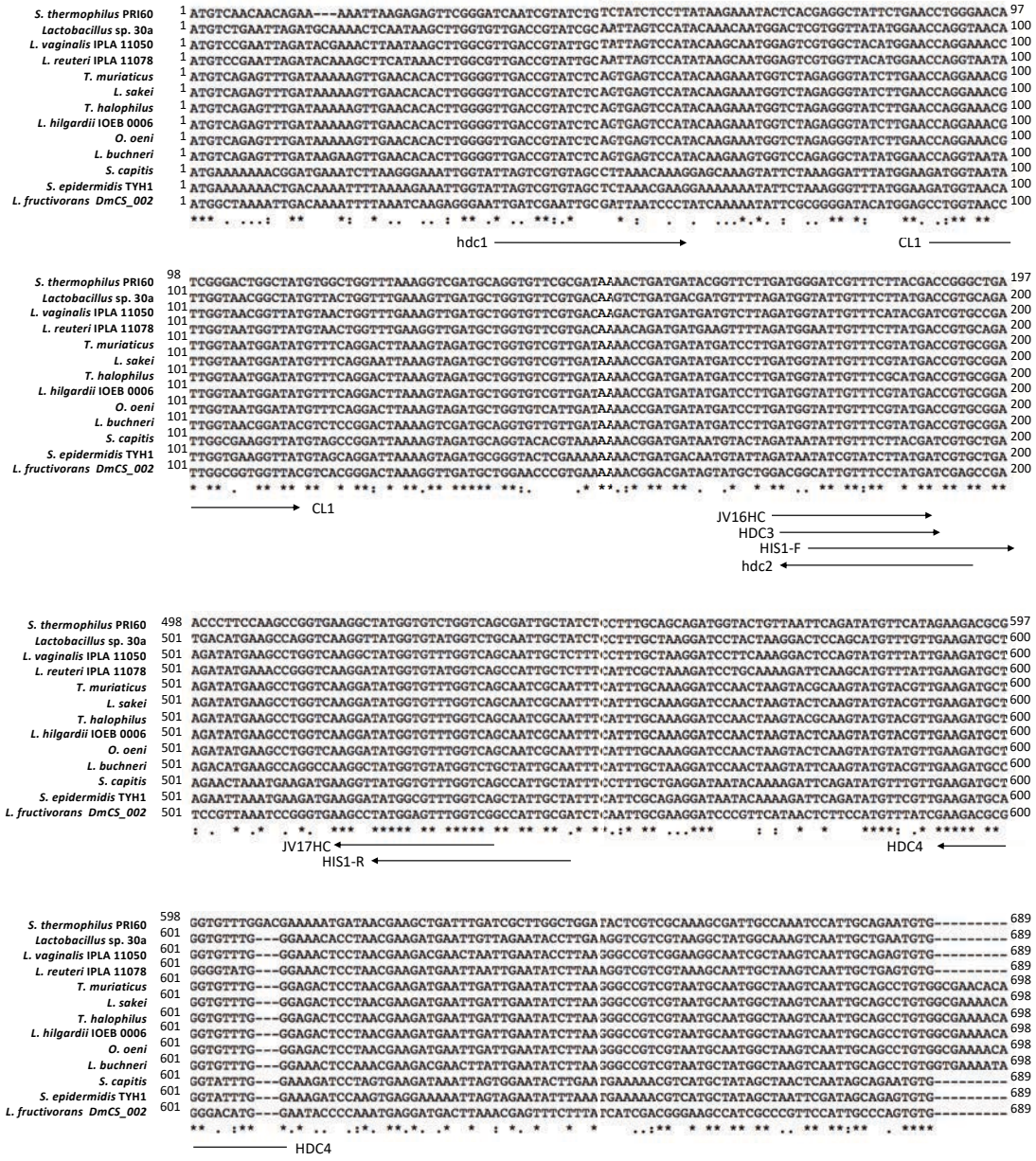

Figure 7. Partial nucleotide alignment of the *hdc* genes from representative gram-positive bacteria, using ClustalW software. The bacterial species, shown at the left of each sequence, as well as the GenBank accession numbers and the references they were taken from (in parentheses), are *S. thermophilus* strain PRI60 (FR693807.2, (Rossi et al., 2011)), *Lactobacillus* sp. 30a (AAB59151.1, Schelp, Worley, Monzingo, Ernst, & Robertus, 2001), *L. vaginalis* strain IPLA11050 (LN828720.1, Diaz et al., 2015), *L. reuteri* strain IPLA11078 (LN877767.1, Diaz et al., 2016b), *T. muriaticus* (DQ132889.1, Kimura, Konagaya, & Fujii, 2001), *L. sakei* (DQ132888.1, Diaz et al., 2016b), *T. halophilus* (AB362339.1, Satomi, Furushita, Oikawa, Yoshikawa-Takahashi, &

Yano, 2008), *L. hilgardii* strain IOEB 0006 (AY651779.1, P. M. Lucas, Wolken, Claisse, Lolkema, & Lonvaud-Funel, 2005), *O. oeni* (DQ132887.1, P. M. Lucas, Claisse, & Lonvaud-Funel, 2008), *L. buchneri* (DQ132890.1, Diaz et al., 2016b), *S. capitis* (AM283479.1, de Las Rivas, Rodríguez, Carrascosa, & Muñoz, 2008), *S. epidermidis* strain TYH1 (AB583189.1, Yokoi et al., 2011), and *L. fructivorans* strain DmCS_002 (NZ_JOJZ01000009.1, Diaz et al., 2016a). Regions where the primers used in dairy products align are indicated by arrows. Numbers indicate the nucleotide position in the sequence of the *hdc* gene.

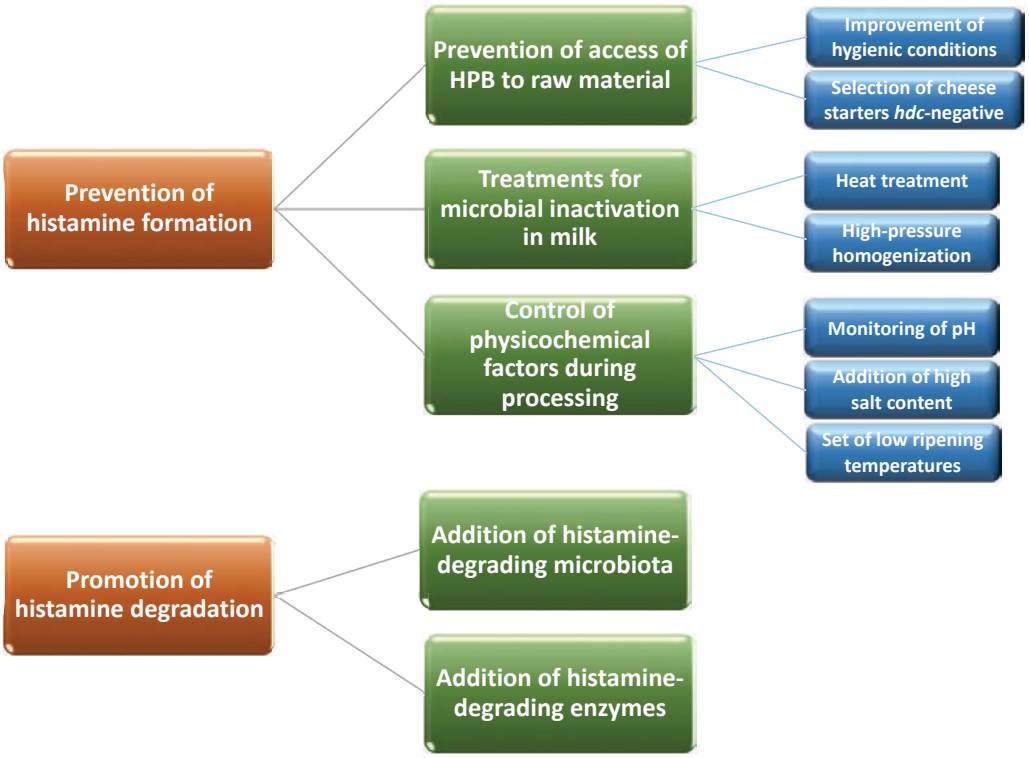

Figure 8. Strategies aimed at preventing histamine formation or promoting histamine degradation in dairy products.