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Evaluation of health concerns related to the consumption of European medium- and long-ripened cheese types with possibly high biogenic amine content

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List of abbreviations

AOC: Appellation d'Origine Contrôlée

AOP: Appellation d'Origine Protégée

BA: Biogenic amine

CAD: Cadaverine

COL: Colamine (ethanolamine)

CYS: Cystamine

DAO: Diamine oxidase

DMA: Dimethylamine

EA: Ethylamine

EFSA: European Food Safety Authority

HHP: High hydrostatic pressure

HIS: Histamine

MAO: Monoamine oxidase

MAOI: Monoamine oxidase inhibitor

MFFB: Moisture on fat-free basis

NOAEL: No-observed-adverse-effect level

PAO: Polyamine oxidase

PDO: Protected Designation of Origin

PEA: Phenylethylamine

PGI: Protected Geographical Indication

PUT: Putrescine

SPD: Spermidine

SPE: Spermine

TRY: Tryptamine

TYR: Tyramine

1. Introduction

Cheese is a diverse group of dairy products whose origin dates back several thousand years (Salque et al., 2013). Today, cheese consumption and manufacturing is seen worldwide, with Europe being the largest producing region in the world producing approximately 8.7 x 10^6 tons annually (McSweeney et al., 2017). Cheese is not only consumed for its nutritive properties, but also for its organoleptic qualities. For many, cheesemaking has become an art, and cheese has evolved to become a gourmet food paired with the finest of wines for those seeking gastronomic experiences. The differences in production, processing and ripening have given rise to a vast variety of cheese categories and subtypes. In many countries, cheesemaking has deep historical, cultural and geographical roots, in which tradition specifically dictates how each cheese type should be made. This can range from the specifics of the manufacturing process and length of ripening, to the type of milk used – whether it be cows', goats' or sheep's milk – and even to the specific regions or pastures on which these animals graze. Many such cheeses have been granted a protected status covered under the Regulation (EU) No 1151/2012 on quality schemes for agricultural products and foodstuffs (2012). This certification ensures that only products which meet the quality and geographical criteria are allowed to advertise their products as a given cheese type.

Many cheese types undergo a period of ripening, or aging, whether it be a few weeks or as long as two or more years. This is a process in which a series of biochemical and microbial changes lead to the development of the characteristic flavors, aromas, and textures typical for the different cheeses. The process is complex and involves a variety of enzymes – from milk, bacteria and rennet – responsible for the degradation and metabolism of lactose, lipids and proteins. The microbiota is influenced by the microbes naturally found in milk, the starter cultures and any secondary cultures added during processing, as well as by environmental contaminants. While these microbes play an essential role in the organoleptic and biochemical changes during ripening, they can also be responsible for the production and accumulation of undesirable substances, such as biogenic amines (BAs). BAs can be found in a variety of foods, most commonly in certain species of fish and fermented food products such as dairy, meat, vegetables, and alcoholic beverages (Doeun et al., 2017). In cheese, BAs are products of protein degradation, produced mainly by microbial decarboxylation of amino acids during ripening. A variety of factors influence the levels of BAs produced, including the presence of microorganisms possessing amino acid decarboxylating activity, conditions affecting the microbes' growth and activity, the

availability of free amino acids, pasteurization of milk, and ripening time and its conditions – temperature, pH, and NaCl concentration (Linares et al., 2012). Commonly detected BAs in cheese include histamine (HIS), tyramine (TYR), putrescine (PUT), cadaverine (CAD) and phenylethylamine (PEA), with members of the *Lactobacillus* and *Enterococcus* genera often being implicated in their production (Benkerroum, 2016). While BAs may affect the quality of the food itself, the main concern with BAs in foods is their known physiologic and toxic properties – and hence their potential health hazard – if consumed in high enough concentrations (Ruiz-Capillas & Herrero, 2019).

My aim is to evaluate what health concerns are scientifically based for the consumption of medium- and long-ripened cheese types. For the purpose of this thesis, I have grouped cheeses according to their country of origin, focusing on the largest cheese-producing regions of Europe. I wish to give a general overview based on the available data to help safe consumption of high-quality cheese products.

2. Materials and methods

I have used online academic databases such as ScienceDirect, Scopus, and PubMed, searching with keywords such as: biogenic amines, cheese, ripening, and Europe. I have filtered the results to literature regarding medium- and long-ripened cheeses with relatively high levels of BAs produced in Europe. In addition, I have researched the potential health concerns related to BA consumption and looked into the current legislation concerning limits of BA content in food. I have reviewed scientific papers from 1985-2020, but with the focus on using the most recent and relevant source material – the majority being from the last decade.

3. Literature review

3.1. Health concerns

BAs are naturally found in living organisms, including humans, animals, plants and microorganisms (Erdag et al., 2018). These are endogenous BAs formed in small amounts in various tissues as a result of normal metabolic processes. They are nitrogenous sources for the synthesis of various hormones, proteins, nucleic acids and other substances, and many play essential roles in physiological activities such as neurotransmission, blood pressure control and allergic response (Perin & Nero, 2019). The amounts of BAs present in foods are closely related to the availability of amino acids and the decarboxylating activity of microbes, and thus, foods containing high levels of BAs are associated with spoilage and fermentation processes (Özogul & Özogul, 2019). Foods containing low levels of BAs are normally not considered a health concern because the human gut will metabolize exogenous BAs by means of detoxifying enzymes: monoamine oxidase (MAO), diamine oxidase (DAO), and polyamine oxidase (PAO). If this protective mechanism is overwhelmed by excessive intake of BAs or by other means, it may lead to the accumulation of BAs within the body causing toxicological effects. In cases of insufficient amine oxidase activity, such as genetic predisposition, gastrointestinal disorders, or the use of mono and diamine oxidase inhibitory drugs (MAOI/DAOI), even low amounts of BAs can have a toxic effect (Ruiz-Capillas & Herrero, 2019). In addition, one must take into consideration any additive and possibly synergistic effects, making the total level of BAs consumed more important than the level of any one single amine from a single source (Del Rio et al., 2017). If a meal consists of more than one item containing high amounts of BAs, the total level consumed can potentially reach well above any limit considered safe.

Histamine and tyramine are considered the two most toxic biogenic amines, both having been implicated as the causative agents in several food poisonings (EFSA, 2011). Histamine poisoning, also commonly known as scombroid poisoning due to its association with the consumption of scombroid-type fish, is characterized by acute symptoms such as rash, skin flushing, gastrointestinal complaints, bronchospasm, tachycardia, hypotension and headache. Between 2010 and 2015, there were as many as 191 outbreaks and 1060 single cases of histamine poisonings reported in the EU – 107 of which were hospitalized cases (EFSA, 2017). Cheese is the second most commonly implicated food item associated with histamine poisoning after fish, with reported cases including the consumption of cheeses made from raw as well as from pasteurized milk (EFSA, 2011). The number of unrecorded

incidents may be high, as the symptoms can frequently be mistaken for allergic reactions or other more common forms of food poisonings.

Like histamine, tyramine has been identified as the culprit of similar food-related intoxications, commonly referred to as the "cheese reaction" for its association with the consumption of BA-containing cheese (Barbieri et al., 2019). Tyramine has indirect vasoactive effects, through its release of noradrenalin, leading to vasoconstriction, increased heart rate and hypertension. Other symptoms such as headache, nausea, vomiting, increased respiration, pupil dilatation and perspiration, among others, have been described (EFSA, 2011; Perin & Nero, 2019). The use of MAOI drugs – such as antidepressants or anti-Parkinsonian agents – have been known to contribute to hypertensive crisis and migraines when food rich in tyramine have been consumed. The MAOI drugs are aimed at inhibiting the actions of monoamine oxidase in the nervous system, but they also inhibit the detoxifying effect of the gut enzyme, resulting in the accumulation of tyramine within the body (McCabe-Sellers et al., 2006).

Phenylethylamine and tryptamine (TRY) have similar vasoactive effects to the ones elicited by tyramine, however their full toxic potential associated with the consumption of BA-rich foods has not been sufficiently characterized. Del Rio et al. (2020) found that both BAs caused cell necrosis and apoptosis in their in vitro studies, but only tryptamine showed cytotoxicity at concentrations found in BA-rich foods. Borah et al. (2013) described the potential neurological risk associated with long-term overexposure to phenylethylamine, due to its association with the endogenous BA being linked to Parkinson's disease.

Both putrescine and cadaverine are commonly detected BAs in foods, but the knowledge on their toxicity is still limited. Adverse effects such as hypotension, dilatation of the vascular system, bradycardia, increased cardiac output, lockjaw, paresis of the extremities, and possibly heart failure and cerebral hemorrhage have been described in the literature, however their role as potentiators may be more important (Benkerroum, 2016; del Rio et al., 2019; EFSA, 2011). It is thought that through competitive inhibition of the detoxifying enzymes as well as through disruption of the physical barrier function of the intestine, they enhance the toxicity of other BAs – especially histamine, but presumably also tyramine. In addition, putrescine and cadaverine can react with nitrite to form carcinogenic nitrosamines, and del Rio et al. (2019) found that both BAs showed cytotoxicity towards intestinal cell cultures at concentrations that can be found in BA-rich foods.

Spermidine (SPD) and spermine (SPE) arise from putrescine and together they belong to the polyamines. Their toxicological role is still not well-defined, but it is

reasonable to assume that the consumption of high levels of these BAs can have the potential to cause adverse effects. They are involved in cell proliferation and tumorigenesis, and in the literature, they have been linked to the risk of developing certain cancers such as colorectal cancer (del Rio et al., 2018; Wang et al., 2017).

3.2. Legislation and NOAE levels

Despite the known health concerns related to BA consumption, the current legislation regarding BAs in foods is not extensive. In the European Union, food safety limits are only set for histamine in certain fishery products as laid down in the Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (2005). The regulation covers those fishery products from species associated with high amounts of the amino acid histidine (fish of the *Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae*, and *Scombresosidae* families). The maximum limits of histamine are set between 100 mg/kg (m) and 200 mg/kg (M) (n=9, c=2), and in the case of fishery products that have undergone enzyme maturation in brine, it is set between 200 mg/kg (m) and 400 mg/kg (M) (n=9, c=2)[1].

In 2011, on request from the European Food Safety Authority (EFSA), a Scientific Opinion was released by the EFSA Panel on Biological Hazards (BIOHAZ) concerning BAs in fermented foods (EFSA, 2011). Prior to this, some Member States had reported an increase of BA content in fermented food products. A qualitative risk assessment of BAs in fermented foods was conducted, and several recommendations were made by the BIOHAZ panel. Among the recommendations were further research on: toxicity levels and concentrations of BAs in foods; the consumption data of fermented foods, especially cheese; and the need for the development of process hygiene criteria as well as food safety criteria for BAs in fermented foods other than fish – with the emphasis on histamine and tyramine, as they are considered the most toxic BAs.

Due to the number of factors that can affect the toxicity of BAs, toxicity levels are difficult to establish. Based on a limited number of studies, a no-observed-adverse-effect level (NOAEL) was set for histamine at 50 mg per meal for healthy individuals (EFSA, 2011; FAO/WHO, 2012). In patients with histamine intolerance, only levels below detectable limits can be considered safe. For the other biogenic amines, insufficient data is available to establish NOAELs, but a tyramine-level of 600 mg per meal has been proposed

[1] The result of sampling is deemed satisfactory when: n = number of samples; c = the maximum number of samples that can have values between m and M; the mean value is ≤ m; and no values obtained exceed M.

as it has been observed to not cause any adverse effects in healthy individuals. The EFSA (2011) reports that for individuals taking MAOI drugs, this level appears to be much lower: 50 mg for those taking third generation MAOI drugs and only 6 mg for those taking classical MAOI drugs. This means that consumption of foods containing high BA-levels, such as cheese, can lead to tyramine exposure well exceeding these levels.

3.3. Classification of cheese types

There is no one uniform way of classifying cheese types. Many attempts have been made, and several classification schemes have been proposed on the basis of a series of physical and technological parameters, such as: type of milk used; fat content; method of coagulation; processing technology and curd temperature; consistency and moisture content; ripening agents (indigenous or added enzymes, bacteria, yeasts and molds); and ripening duration (McSweeney et al., 2017).

Table 1 Categorization of cheeses based on moisture content and ripening time (Codex Alimentarius, 1978; Liberatore, 2015)

Moisture % (MFFB)	**Category**	**Typical ripening time**
<51	Extra hard	12-36 months
49-56	Hard	Min. 6 months
54-69	Semi-hard	Min. 1 month
>67	Soft	0-1 month

When considering the method of coagulation – either by rennet, acid or acid/heat – rennet-coagulated cheeses make up the biggest category representing approximately 75% of the total cheese production and almost all ripened cheeses (McSweeney et al., 2017). These cheeses can be further categorized based on their moisture content and consistency, as defined by the FAO/WHO Codex Alimentarius General Standard for Cheese (Codex Alimentarius, 1978), shown in Table 1. Cheese types of hard (e.g. Emmentaler, Cheddar) and extra-hard (e.g. Parmigiano Reggiano, Grana Padano) consistencies are defined as having values of moisture on fat-free basis (MFFB) lower than 56% and 51%, respectively. These cheeses typically have a long period of ripening going beyond 6 months and extending up to several years in the case of the extra-hard types (Liberatore, 2015). Semi-hard cheeses (e.g. Edam, Gouda) have a MFFB value ranging between 54-69% and a ripening period longer than 1 month. Soft cheeses (e.g. Camembert, Brie) are characterized by a high MFFB

value of >67% and a short or lacking ripening period. However, the categorization of cheeses based on their consistency and length of ripening is not clear-cut and may overlap. Several cheese varieties may be consumed at different lengths of ripening, and hence their texture will depend on the age of the cheese at consumption.

A number of cheese varieties have the EU Protected Designation of Origin (PDO) status or other similar country-specific certifications, such as the *Appellation d'Origine Contrôlée* (AOC) used in France. This contributes to the protection and promotion of authentic European high-quality agricultural products, and for a cheese to receive this status, it must be traditionally and entirely manufactured within a given region. Cheeses having gained PDO status, or a similar country-specific status, may be of special interest when investigating the BA content as many such cheeses are extensively exported and sold worldwide, and they are often produced by using long-standing traditional methods in spite of great changes in cheesemaking technology (McSweeney et al., 2017).

3.4. Medium-ripened European cheese types with possibly high BA content

3.4.1. Northern European semi-hard cheeses

Gamalost is a traditional Norwegian mold-ripened cheese made from pasteurized skimmed cows' milk. Due to its high protein content, extensive proteolysis and relatively high amounts of amino acids, one could expect potentially high levels of BAs to be formed during ripening (Qureshi et al., 2013). Contradictory to its name – from Norwegian meaning "old cheese" – Gamalost is typically only ripened for up to 30 days. However, Qureshi et al. (2013) analyzed samples during ripening up to 60 days for the concentration of BAs. In addition, the semi-hard pasteurized cows' milk cheese Norvegia ripened for 90 days was analyzed for comparison. 60 percent of the cheeses consumed in Norway are ripened semi-hard or hard cheeses, with Norvegia being the most consumed cheese (Melk.no, n.d.). The more internationally well-known Norwegian semi-hard cheese Jarlsberg is the second most consumed cheese in Norway, but data on its BA content is lacking. In several samples of Gamalost, putrescine was the only detectable BA during ripening, with mean levels ranging from 11.9-25.2 mg/kg. The level increased up until day 20 of ripening, followed by a decrease to 16.2 mg/kg after 60 days. The standard deviation was high at 60 days, so this decrease could be batch dependent. Another explanation of this decrease could be that PUT was converted into SPE and SPD, but that their levels were below the detection limit. The Analysis of three Norvegia cheeses from different productions showed non-detectable levels

of PUT and very low levels of TYR (5.6 mg/kg), HIS (1.4 mg/kg) and SPE (1.1 mg/kg). With the levels found by Qureshi et al. (2013), neither Gamalost nor Norvegia contains sufficient concentrations of BAs to be considered a health hazard. The low levels of BAs found in Gamalost could be attributed to the cooking of the cheese at 90-95°C for 1-2 hours in whey, killing or inactivating BA-producing bacteria and enzymes.

Ladero et al. (2010) analyzed samples of different cheeses for the concentration of tyramine. In a sample of a semi-hard blue-veined Danish cheese made from pasteurized cows' milk, TYR was not detected. Mayer et al. (2010) also analyzed a similar Danish cheese, finding only low levels of BAs. The total concentration was measured at 55.5 mg/kg, with SPE at 16.5 mg/kg, PUT at 13.0 mg/kg and SPD at 11.1 mg/kg making up the majority. HIS was not detected. In contrast, relatively high levels of HIS at 490 mg/kg and TYR at 625 mg/kg were found in a Danish blue cheese in an older study by Ingles et al. (1985).

3.4.2. Southern European semi-hard cheeses

3.4.2.1. Portuguese and Spanish cheeses

Terrincho is a traditional Portuguese PDO cheese manufactured from raw ewes' milk and ripened for a minimum of 30 days. Pinho et al. (2004) monitored the changes of BAs in several samples of Terrincho cheese during ripening. Groups of six cheeses were analyzed at each step of the ripening, starting at day 0. A steady increase in the mean total BA content was observed, from 155.2 mg/kg at day 0 to 917.9 mg/kg at day 60 of ripening (Table 2). CAD, PUT and TYR were the predominant BAs, and they all increased during ripening reaching 349.7, 217.8 and 216.3 mg/kg at day 60, respectively. HIS was consistently low, while some variation was observed for TRY, PEA, ethylamine (EA), dimethylamine (DMA) and cystamine (CYS) – most of which reached their maximum at day 30 of ripening and decreased thereafter. SPE was not detected.

Pintado et al. (2008) researched the biogenic amine profiles of Terrincho cheese manufactured in five different dairy farms located throughout the PDO region. Altogether 19 samples (three or four from each dairy farm) were analyzed for BA content after 30 days of ripening. Some variability was seen among the different dairy farms, with a mean total ranging from 428.0 to 922.3 mg/kg (Table 2). However, the proportions of BAs were similar to those seen by Pinho et al. (2004), showing greater amounts of TYR, PUT and CAD compared to the other amines. HIS was also in this study found in only small amounts, and SPE was not detected. The differences seen in samples manufactured at different dairy farms

are likely related to different manufacturing practices, hygiene and the quality of the raw milk.

Espinosa-Pesqueira et al. (2018) analyzed the BA content in two artisanal cheese varieties manufactured in Spain and ripened for 60 days – one from raw goats' milk and the other from raw ewes' milk. In addition, samples from cheeses having undergone high hydrostatic pressure (HHP) treatment during their ripening were also analyzed to see if it would affect the level of BA production. The total BA content increased during ripening in both the untreated goats' and the ewes' milk cheeses, reaching mean levels of 1149.0 and 577.5 mg/kg, respectively (Table 2). TYR (491.9 mg/kg) and PUT (476.4 mg/kg) were the major BAs found in the goats' milk samples, while TYR (277.3 mg/kg) and CAD (105.9 mg/kg) were the predominant ones found in the ewes' milk samples. HHP is a non-thermal processing method used to extend the shelf-life of foods by decreasing microbiological counts. The application of HHP in the early stage of ripening (HHP1) significantly reduced the levels of BAs to around 75% lower than the non-treated samples. The application of HHP was also done in ewes' milk cheeses after 15 days of ripening (HHP15). However, while still reducing the level of BAs compared to the non-treated samples, it was not as effective as the early HHP1 treatment, as seen in Table 2.

Valdeón is a Spanish blue-veined cheese with Protected Geographical Indication (PGI) status. It is made from either raw or pasteurized cows' milk – or sometimes with a mix of cows', ewes' and/or goats' milk. Diezhandino et al. (2015) analyzed the BA content during ripening in 12 batches of Valdeón cheese. Eight were manufactured using pasteurized cows' and goats' milk (74°C, 20 s), while four were made from raw cows' and goats' milk. The cheeses were ripened for 120 days at different times of the year (summer, autumn, winter and spring) and sampled at different stages. Ripening time significantly influenced the mean total amount of BAs, almost doubling in concentration from 992.5 mg/kg after 60 days of ripening to 1829.5 mg/kg after 120 days of ripening (Table 2). At the end of ripening, spermine (1193.5 mg/kg) and tyramine (440.5 mg/kg) were the major BAs. When comparing the samples produced during the different seasons, no significant differences were observed. However, when comparing the samples produced from pasteurized and raw milk, the total concentration of BAs from the latter was almost double the amount found in those made from pasteurized milk.

Ladero et al. (2010) analyzed several types of Spanish commercial semi-hard cheeses for the detection of the BA tyramine and tyramine-producing microorganisms. A large variation in concentrations was found, ranging from non-detectable to 957.6 mg/kg.

Industrial and pasteurized cheese types generally showed the lowest concentrations. All the raw milk cheeses had detectable levels of tyramine ranging from 103.6 mg/kg in an industrial raw ewes' milk cheese, to 957.6 mg/kg in an artisanal blue raw cheese. The cheeses with the highest tyramine concentrations were also found to have the highest number of tyramine-producing microorganisms.

Table 2 BA content in some medium-ripened Portuguese and Spanish cheeses (in mg/kg)

Terrincho PDO (Pinho et al., 2004; Pintado et al., 2008) Portuguese, raw ewes' milk cheese. Five different dairy farms (a, b, c, d, e).										
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	EA	DMA	CYS	**Total**
0	nd	7.5	10.1	24.4	73.5	nd	29.0	10.7	nd	**155.2**
30	9.3	55.0	191.4	131.7	172.9	23.8	131.9	22.6	88.1	**826.7**
60	15.6	216.3	217.8	349.7	55.5	9.4	38.2	15.3	nd	**917.9**
30	nd[a]	61.4[a]	82.6[a]	186.3[a]	35.4[a]	23.5[a]	31.0[a]	7.8[a]	nd[a]	**428.0[a]**
30	10.9[b]	nd[b]	142.5[b]	48.6[b]	47.6[b]	237.8[b]	153.3[b]	50.7[b]	nd[b]	**691.4[b]**
30	nd[c]	nd[c]	446.5[c]	84.7[c]	46.9[c]	27.5[c]	143.9[c]	8.1[c]	nd[c]	**757.6[c]**
30	9.3[d]	54.9[d]	191.4[d]	131.7[d]	172.9[d]	28.3[d]	131.9[d]	22.6[d]	88.1[d]	**831.1[d]**
30	nd[e]	283.1[e]	247.4[e]	239.6[e]	35.5[e]	12.9[e]	90.9[e]	12.9[e]	nd[e]	**922.3[e]**

Artisanal Spanish Cheeses (Espinosa-Pesqueira et al., 2018) Made from raw goats' and ewes' milk								
Age (d)	Sample	HIS	TYR	PUT	CAD	TRY	PEA	**Total**
60	Goats' milk	15.4	491.9	476.4	70.5	63.7	31.1	**1149.0**
60	Goats' milk HHP1	4.9	28.9	79.8	44.2	89.1	25.6	**272.5**
60	Ewes' milk	91.0	277.3	74.9	105.9	15.7	12.7	**577.5**
60	Ewes' milk HHP1	7.1	32.7	5.2	28.5	11.1	4.4	**89.0**
60	Ewes' milk HHP15	57.7	147.6	26.1	80.3	11.7	13.3	**336.7**

Valdeón PGI (Diezhandino et al., 2015) Spanish, blue-veined cheese made from pasteurized and raw milk									
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	SPE	**Total**
60	39.3	200.4	22.3	6.4	11.6	18.3	48.6	645.6	**992.5**
90	42.6	295.4	23.4	11.0	12.4	21.6	65.9	905.3	**1377.6**
120	44.6	440.5	26.4	9.8	12.0	25.5	77.2	1193.5	**1829.5**

Values are expressed as mean; d = ripening age in days at the time of sampling; nd = not detected

3.4.2.2. Italian cheeses

Italy is one of the biggest cheese producing countries in Europe, and undoubtedly one of the countries with the longest and richest cheese-making traditions (Gobbetti et al., 2018). In 2019, 0.45 million tons of Italian cheeses were exported, 76% of which were to EU countries (Eurostat, 2020). Italy produces some of the most well-known, high-quality cheeses in the world, many of which have gained a Protected Designation of Origin (PDO) status.

Gosetti et al. (2007) analyzed three typical semi-hard PDO cheeses from the Piedmont region (North-West Italy) for the determination of six BAs: histamine, tyramine, cadaverine, tryptamine, spermidine, and spermine (Table 3). The cheeses Raschera, Toma Piemontese and Castelmagno, although similar in texture and taste, showed quite different results, with total levels of BAs measured in mg/kg at 1478.4, 1327.2, and 3464.0, respectively. HIS was the predominant BA measured in Raschera at 452.4 mg/kg, closely followed by TRY (389.9 mg/kg) and SPE (352.7 mg/kg). HIS was also the major BA in Toma Piemontese (587.6 mg/kg), but significant levels of TYR (282.3 mg/kg), TRY (255.5 mg/kg) and SPE (193.9 mg/kg) were also found. High levels of all BAs, except SPD, were found in Castelmagno, with TYR and TRY predominating at 1009.1 mg/kg and 1048.7 mg/kg, followed by HIS (645.8 mg/kg), SPE (449.6 mg/kg), and CAD (310.4 mg/kg). SPD was very low in all cheeses, with the highest value found in Raschera at only 10.6 mg/kg.

Table 3 BA content in some medium-ripened PDO cheeses from Italy (in mg/kg)

Raschera PDO (Gosetti et al., 2007) Made from raw or pasteurized cows' milk							
Age (d)	HIS	TYR	CAD	TRY	SPD	SPE	**Total**
min. 30*	452.4	153.9	118.9	389.9	10.6	352.7	**1478.4**
Toma Piemontese PDO (Gosetti et al., 2007) Made from raw or pasteurized cows' milk							
Age (d)	HIS	TYR	CAD	TRY	SPD	SPE	**Total**
min. 60*	587.6	282.3	1.3	255.5	6.6	193.9	**1327.2**
Castelmagno PDO (Gosetti et al., 2007) Made from raw cows' milk							
Age (d)	HIS	TYR	CAD	TRY	SPD	SPE	**Total**
min. 60*	645.8	1009.1	310.4	1048.7	0.4	449.6	**3464.0**
Montasio PDO (Innocente & D'Agostin, 2002) Made from raw or thermized cows' milk							
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	**Total**
60	28.1	37.8	192.3	32.4	2.1	4.7	**299.1**
90	37.0	51.9	11.5	4.9	1.4	4.2	**118.5**
120	67.4	61.2	6.4	2.2	1.2	4.8	**138.7**
150	216.6	248.9	363.6	13.0	4.0	14.2	**857.3**

Values are expressed as mean; d = ripening age in days at the time of sampling; * = as determined by the PDO requirements

Innocente & D'Agostin (2002) investigated the changes in concentrations of BAs during the ripening of the semi-hard Italian Montasio cheese. Histamine, tyramine,

putrescine, cadaverine, tryptamine, and phenylethylamine were measured in 30 samples taken at different times of the ripening process (Table 3). HIS and TYR were the major amines, both increasing during ripening from the means 28.1 and 37.8 mg/kg at day 60, to 216.6 and 248.9 mg/kg at day 150. TRY and PEA concentrations were very low at all stages of ripening. Although PUT showed high mean values, PUT along with CAD, were only present in significant amounts (up to 1104.6 and 167.5 mg/kg, respectively) in a few samples showing an anomalous fermentation process. This shows that the estimation of PUT and CAD may be important as indices of extended protein degradation and spoilage. If these anomalous samples are excluded, the total BA content increases with ripening time, from a mean of 45.9 mg/kg at day 60, to 755.0 mg/kg at day 150.

Pecorino, from the Italian *pecora* (sheep), is a common name given to Italian cheeses made from ewes' milk. Typically, Pecorino cheese varieties are semi-hard to hard cheeses, depending on their time of ripening. Many follow age-old traditional manufacturing methods, and numerous regions or provinces in Italy have their own Pecorino cheese with names reflecting the area in which they are produced (Figure 1) (Gobbetti et al., 2018; Schirone et al., 2012).

Figure 1 An example of a Tuscan Pecorino cheese ripened in a cave (Parmashop, n.d.)

Torracca et al. (2015) investigated the BA content in four different semi-hard Pecorino cheeses manufactured within the same dairy factory in Tuscany, Italy, but ripened under different conditions (Figure 2). Two of the cheese types were manufactured using pasteurized milk (70°C, 40 s) – one of which was ripened for 60 days in the ripening room

followed by 90 days in a "grotta" (natural cave), and the other was ripened for 90 days in the ripening room followed by 90 days in a traditional "fossa" (pit dug in tuff). The remaining two cheese types were manufactured using raw milk and ripened for 60 days in the ripening room, one of which underwent an additional 90 more days in an 18th-century tuff cave covered with straw. Tyramine was the predominant BA in all cheeses, ranging between 147 and 1132 mg/kg. PUT, CAD, PEA and TRY were also present in different degrees, while HIS, SPD and SPE were only present in very small or non-detectable amounts. The four types had very different total mean BA content, and there was also great variability even among the same type of cheese. The pasteurized cave-ripened cheese had a significantly lower BA level (274 mg/kg) compared to the one made from raw milk ripened under similar conditions (2161 mg/kg). However, other variables such as different starter cultures and different environments within the two caves cannot be ruled out. The pasteurized fossa-ripened cheese also had a high total level of BA at 1283 mg/kg, but this could be attributed to the anaerobic ripening conditions within the fossa promoting fermentative and proteolytic processes. The remaining cheese type manufactured from raw milk showed a total BA content at 274 mg/kg. This comparatively low level of BAs could be explained by the significantly shorter ripening period at only 60 days – without any additional cave- or fossa-ripening.

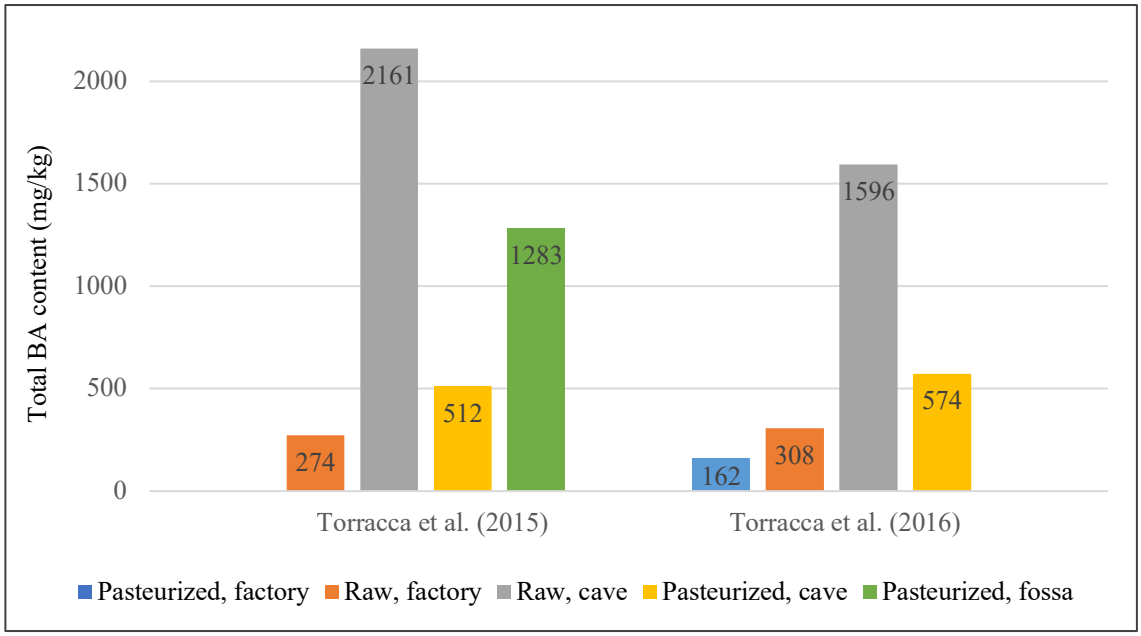

Figure 2 Mean total BA content (in mg/kg) in pasteurized and raw milk Pecorino cheeses ripened under different conditions

Similar to Torracca et al. (2015), Torracca et al. (2016) also investigated the influence of pasteurization and ripening conditions on the BA content in Tuscan Pecorino cheese (Figure 2). All samples were ripened for 120 days either in the factory or partly in a cave. Torracca et al. (2016) found that milk pasteurization significantly limited BA formation, however, substantial levels were still found in cave-ripened cheeses. The lowest level was seen in the pasteurized factory-ripened cheese with a mean of 162 mg/kg, followed by the raw factory-ripened cheese at 308 mg/kg. The highest mean levels were seen in the cave-ripened cheeses at 574 mg/kg and 1596 mg/kg for the pasteurized and raw milk varieties, respectively. The levels of each individual BA were similar to those found by Torracca et al. (2015) – with tyramine as the predominant amine at levels ranging between 149-871 mg/kg.

Forzale et al. (2011) evaluated the BA content in the traditional Tuscan cheese Pecorino del Parco di Migliarino-San Rossore (Pecorino del Parco). This is a raw milk cheese manufactured with traditional techniques that have remained unchanged over the years. The BA content was assessed at several points during the ripening period and measured in both the core and in the external part of the cheese. The total BA content increased during the ripening, from 591.9 mg/kg in the core and 393.1 mg/kg in the external part on day 91, reaching 1578.7 mg/kg in the core and 721.8 mg/kg externally on day 153. Similar to the other Tuscan Pecorino cheeses, tyramine was also the major BA found in Pecorino del Parco, showing concentrations at 1300.1 and 527.6 mg/kg on day 153 of ripening in the central and external part, respectively. HIS, PUT and CAD were also measured, but in much smaller concentrations.

Pecorino Abruzzese is a semi-hard or hard ewes' milk cheese produced in the Abruzzo region in Central Italy. It is made from raw or heat-treated milk, and often without the use of selected starter cultures. Martuscelli et al. (2005) compared the BA content produced in Pecorino Abruzzese cheeses from raw milk without starter culture and from pasteurized milk with added starter culture. Total BA content reached 697.0 mg/kg in the raw milk cheese after 60 days of ripening, with HIS (261.0 mg/kg), TYR (185.0 mg/kg) and PUT (80.0 mg/kg) being the most abundant amines. In the pasteurized cheese, total BA levels were measured at 1086.0 mg/kg with PEA (305.0 mg/kg), TYR (230.0 mg/kg), EA (180.0 mg/kg) and PUT being the most abundant (163.0 mg/kg). CAD, TRY, SPE and SPD were also detected, but in low or very low amounts. The presence of starter culture in the pasteurized cheese delayed the BA production by the secondary microflora in the first 2 weeks of ripening. However, a great production was seen from day 30 to 60 with a 77%

increase in total BA. As a result, the pasteurization did not reduce the quantity of BAs in Pecorino Abruzzese compared to the raw cheese.

Schirone et al. (2013) investigated the BA content in several Pecorino cheeses from the Abruzzo region. Four Pecorino d'Abruzzo cheeses produced from raw milk and ripened for 90 days were analyzed. Histamine was the predominant BA detected in all four samples ranging from 14.5-761.4 mg/kg. High levels of TYR (up to 702.4 mg/kg) and PUT (up to 377.7 mg/kg) were also detected in some samples. CAD (up to 172.4 mg/kg) and PEA (44.4 mg/kg) were detected in two and one samples, respectively, while SPD and SPE were not detected in any. The mean total level of BAs was measured to 824.7 mg/kg, with a range from 266.7-1483.1 mg/kg

Schirone et al. (2013) also analyzed the BA content in a Pecorino di Farindola cheese made from raw milk and ripened for 90 days. This is a cheese manufactured according to ancient local traditions using pig rennet. Unlike Pecorino d'Abruzzo, histamine content found in Pecorino di Farindola was quite low at 11.2 mg/kg. The predominating BA was TYR (397.7 mg/kg) followed by PUT (127.0 mg/kg) and CAD (110.6 mg/kg). PEA, SPD and SPE were not detected. The total level of BAs was measured at 678.5 mg/kg. Similar levels were found by Schirone et al. (2011) who examined the BA levels in ten samples of Pecorino di Farindola. The total mean level was measured at 957.7 mg/kg, however, four of the samples contained very high levels of total BAs at more than 1000 mg/kg – one of which contained as much as 2393.0 mg/kg. Because of the general trend of high tyramine concentration in all the Pecorino di Farindola cheeses (52.3-1171.3 mg/kg), Schirone et al. (2011) hypothesized the influence of proteolytic enzyme activity of pig rennet as a possible culprit.

A third type of Pecorino cheese from the Abruzzo region, Pecorino di grotta, was analyzed by Schirone et al. (2013). This cheese was made from a mix of pasteurized ewes' and cows' milk and cave-ripened for 120 days. The total level of BAs was measured at 582.4 mg/kg, with TYR (312.1 mg/kg) and HIS (235.4 mg/kg) being the major amines. Concerning other BAs, only small amounts of PEA and PUT were measured.

3.4.2.3. Bosnian Livno cheese

Livno cheese (Figure 3) is a raw cows' milk cheese from Bosnia and Herzegovina typically ripened for 60 days. Marijan et al. (2014) analyzed several samples of Livno cheese for the determination of BAs and compared the levels found in the core to those found in the rind.

Figure 3 Bosnian Livno cheese (Eko sir Puđa, n.d.)

Samples were taken at different points of the ripening, from day 0 to an extended ripening period of 105 days. Altogether 42 samples were analyzed – 21 from the cheese core and 21 from the rind. The total level of BAs progressively increased during ripening, both in the core and in the rind, from 9.2 and 3.6 mg/kg at day 0 to 184.1 and 76.2 mg/kg at day 105 (Table 4). Histamine was the predominant BA followed by tyramine. Lower levels of CAD, PEA, SPD, PUT and SPE were also measured. TRY was not detected in any of the samples. With the exception of SPD, all detectable BAs were found at a higher concentration in the core compared to the rind.

Table 4 BA content in Livno cheese at different stages of ripening (in mg/kg)

Livno cheese (Marijan et al., 2014) Made from raw cows' milk; core (c), rind (r)									
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	SPE	**Total**
0	4.3^{c}	0.4^{c}	nd^{c}	1.3^{c}	nd^{c}	nd^{c}	3.2^{c}	nd^{c}	$\mathbf{9.2^{c}}$
0	0.1^{r}	nd^{r}	nd^{r}	nd^{r}	nd^{r}	nd^{r}	3.5^{r}	nd^{r}	$\mathbf{3.6^{r}}$
29	22.4^{c}	5.2^{c}	1.7^{c}	2.6^{c}	nd^{c}	nd^{c}	nd^{c}	nd^{c}	$\mathbf{31.9^{c}}$
29	2.2^{r}	nd^{r}	nd^{r}	1.1^{r}	nd^{r}	nd^{r}	3.8^{r}	nd^{r}	$\mathbf{7.1^{r}}$
50	33.1^{c}	11.3^{c}	2.9^{c}	7.7^{c}	nd^{c}	5.0^{c}	nd^{c}	nd^{c}	$\mathbf{60.0^{c}}$
50	2.3^{r}	2.4^{r}	nd^{r}	2.0^{r}	nd^{r}	1.1^{r}	7.0^{r}	nd^{r}	$\mathbf{14.8^{r}}$
60	30.4^{c}	12.6^{c}	0.8^{c}	11.7^{c}	nd^{c}	6.3^{c}	0.2^{c}	nd^{c}	$\mathbf{62.0^{c}}$
60	5.1^{r}	5.0^{r}	nd^{r}	3.5^{r}	nd^{r}	1.9^{r}	10.9^{r}	1.0^{r}	$\mathbf{27.4^{r}}$
105	81.0^{c}	70.4^{c}	nd^{c}	9.8^{c}	nd^{c}	17.6^{c}	5.3^{c}	nd^{c}	$\mathbf{184.1^{c}}$
105	24.1^{r}	24.0^{r}	nd^{r}	5.3^{r}	nd^{r}	4.4^{r}	17.6^{r}	0.8^{r}	$\mathbf{76.2^{r}}$

Values are expressed as mean; d = ripening age in days at the time of sampling; nd = not detected

3.4.3. Western European semi-hard cheeses

3.4.3.1. French cheeses

France is one of the top producers and exporters of cheese in the world. In 2019, nearly 0.7 million tons of French cheese were exported, 83% of which were to EU countries (Eurostat, 2020). As of 2020, more than 40 types of French cheeses are protected under AOC/PDO certification ensuring the protection of their geographical origin and their traditional methods of manufacturing (INAO, 2020). Data on BA content in traditional French cheese types is however sparse as of 2020.

Mayer et al. (2010) analyzed the BA content in several types of French semi-hard cheeses (Table 5). Two samples of the raw cows' milk cheese Cantal – a cheese AOC-protected since 1956 and considered one of the oldest French cheeses – were analyzed. Total BA values of 472.3 mg/kg in sample one and 727.7 mg/kg in sample two were found, with tyramine as the predominant BA in both samples at 323.4 and 486.4 mg/kg, respectively. Histamine was measured at 94.7 and 165.6 mg/kg, followed by putrescine at 40.8 and 28.0 mg/kg. Only small or non-detectable amounts were measured of the remaining BAs CAD, TRY, PEA, SPD, EA and colamine (COL).

Another AOC/PDO raw cows' milk cheese analyzed by Mayer et al. (2010) was Comté. A much lower total BA level was measured in this cheese at 105.6 mg/kg. COL (40.6 mg/kg), TYR (39.1 mg/kg), SPE (16.9 mg/kg) and SPD (9.0 mg/kg) were the only amines detected.

Figure 4 The blue-veined French Roquefort cheese (Thesupermat, 2015)

Low amounts of BAs were also detected by Mayer et al. (2010) in the blue-veined cheese Roquefort produced with raw ewes' milk (Figure 4). This is one of the most well-

known French cheeses, and it was the very first recipient of the AOC certification in 1925 (INAO, 2020). The total BA level was measured at 51.0 and 98.3 mg/kg in two samples. In the first sample, only CAD (22.1 mg/kg), COL (16.4 mg/kg), HIS (10.6 mg/kg) and PUT (1.9 mg/kg) were detected. In the second sample, COL (22.6 mg/kg), PUT (18.3 mg/kg) and SPE (18.1 mg/kg) were detected, while HIS, CAD, EA, PEA and SPD were measured at even smaller amounts. TYR was not detected in either of the samples.

Table 5 BA content in some medium-ripened French cheese types (in mg/kg)

Cantal AOC/PDO (Mayer et al., 2010) Made from raw cows' milk										
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	COL	EA	**Total**
min.	94.7	323.4	40.8	2.1	nd	nd	6.8	2.7	1.8	**472.3**
30*	165.6	486.4	28.0	2.1	13.0	14.5	10.6	7.5	nd	**727.7**
Comté AOC/PDO (Mayer et al., 2010) Made from raw cows' milk										
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	SPE	COL	**Total**
min. 120*	nd	39.1	nd	nd	nd	nd	9.0	16.9	40.6	**105.6**
Roquefort AOC/PDO (Mayer et al., 2010) Blue-veined cheese made from raw ewes' milk										
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	COL	EA	**Total**
min.	10.6	nd	1.9	22.1	nd	nd	nd	16.4	nd	**51.0**
90*	9.9	nd	18.3	8.9	7.7	4.6	18.1	22.6	8.2	**98.3**
Fourme d'Ambert AOC/PDO (Sawilska-Rautenstrauch et al., 2010) Semi-hard, blue-veined cheese made from raw cows' milk										
Age (d)	HIS		TYR		PUT		CAD			**Total**
min. 28	1.5		2.1		1.8		2.7			**7.9**
French blue-veined cheese (Ladero et al., 2010) Industrial semi-hard cheese, made from raw cows' and ewes' milk										
Age (d)	TYR									**Total**
-	1051.1									**-**

Values are expressed as mean; d = ripening age in days at the time of sampling; nd = not detected; - = not measured; * = as determined by the AOC/PDO requirements

Sawilska-Rautenstrauch et al. (2010) analyzed the BA content in a different type of French blue-veined cheese, the AOC/PDO certified Fourme d'Ambert. This is a semi-hard cheese made from raw cows' milk. HIS, TYR, PUT and CAD were detected in very small amounts ranging from 1.5 to 2.7 mg/kg, with a total BA content at only 7.9 mg/kg (Table 5).

Ladero et al. (2010) analyzed commercially available cheese types for the concentration of tyramine (Table 5). In a sample of a semi-hard blue-veined French cheese made from raw cows' and ewes' milk, TYR was measured to a significantly high level at 1051.1 mg/kg. This number considerably differs from the level of TYR found in the other blue-veined cheeses by Mayer et al. (2010) and Sawilska-Rautenstrauch et al. (2010). No other BAs were measured in this cheese.

3.4.3.2. Dutch cheeses

Gouda is today used as a general term encompassing cheeses produced in the traditional Dutch manner, hence it is not restricted to cheeses of Dutch origin. It is made from either pasteurized or raw cows' milk – the latter being more common for smaller producers of the traditional and protected *boerenkaas* (farmer's cheese) variety (Harbutt, 2015). Mayer et al. (2010) analyzed several semi-hard Gouda varieties for the content of BAs. Three Dutch varieties showed variable amounts with total concentration ranging from 49.3 to 305.5 mg/kg. Two of the cheeses, with unknown ripening time, showed high levels of tryptamine at 104.4 and 243.4 mg/kg, and lower levels of HIS, TYR, SPD and SPE. Very low or non-detectable levels of PUT, CAD, PEA and COL were also reported. The cheese ripened for 360 days showed the lowest BA content of the Dutch varieties at 49.3 mg/kg. TYR (24.3 mg/kg), SPE (17.3 mg/kg), SPD (5.0 mg/kg) and COL (2.7 mg/kg) were the only BAs detected.

3.4.4. Central European semi-hard cheeses

3.4.4.1. Austrian cheeses

Edam, a cheese originating in the Netherlands, is a semi-hard pasteurized cows' milk cheese manufactured all over the world as there are no geographical restrictions. Mayer et al. (2010) analyzed a sample of an Austrian variety. It only contained low levels of BAs at a total concentration of 31.7 mg/kg. COL (22.5 mg/kg), EA (6.0 mg/kg) and HIS (3.2 mg/kg) were the only BAs detected. A sample of an Austrian Gouda cheese also showed to contain low levels of BAs at a total concentration of 33.0 mg/kg.

Two traditional semi-hard Austrian cheeses made from pasteurized cows' milk, Moosbacker and Bergbaron, were also analyzed by Mayer et al. (2010). Moosbacker (Figure 5) showed a total BA level of 112.9 mg/kg – the majority of which was histamine at 68.4 mg/kg. Bergbaron showed a much higher level of BAs with a total level of 687.2 mg/kg.

TRY (312.2 mg/kg), TYR (192.3 mg/kg) and PUT (64.0 mg/kg) made up the majority, while low or very low levels of CAD, HIS, SPE, SPD, COL and PEA were detected.

Figure 5 Austrian Moosbacker cheese (Landwirt.com, n.d.)

3.4.4.2. Czech cheeses

Komprda et al. (2008) analyzed the Czech blue-veined cheese Niva for the detection of BAs. Altogether six batches in three consecutive months (October, November, December) were manufactured using pasteurized cows' milk and ripened for 49 days (Figure 6). Samples were taken from both the edge and the core of the cheese during and at the end of ripening. Total levels of BAs ranged from 97 to 649 mg/kg in the edge and 89 to 1366 mg/kg in the core after 49 days of ripening. The cheese samples produced in October had the highest mean BA content both in the edge and the core, while the samples produced in December had the lowest. All, except one batch, showed a significantly higher total concentration of BAs in the core compared to the edge of the cheese. High levels in the core compared to the edge were also found in the Bosnian Livno cheese by Marijan et al. (2014), the Italian Pecorino del Parco cheese by Forzale et al. (2011), and a semi-hard cheese by Garmiene et al. (2012), as seen in Figure 6. Tyramine was the predominant BA in Niva cheese, ranging from 10 to 875 mg/kg, and its concentration in the core increased considerably during ripening. Cadaverine was the second most prevalent BA with concentrations ranging from 3 to 491 mg/kg. In contrast to tyramine, cadaverine was found in the highest concentration in the edge of the cheese in all but one batch. Low or very low levels of HIS, PUT, SPD, SPE and TRY were found. PEA was not detected in any of the samples.

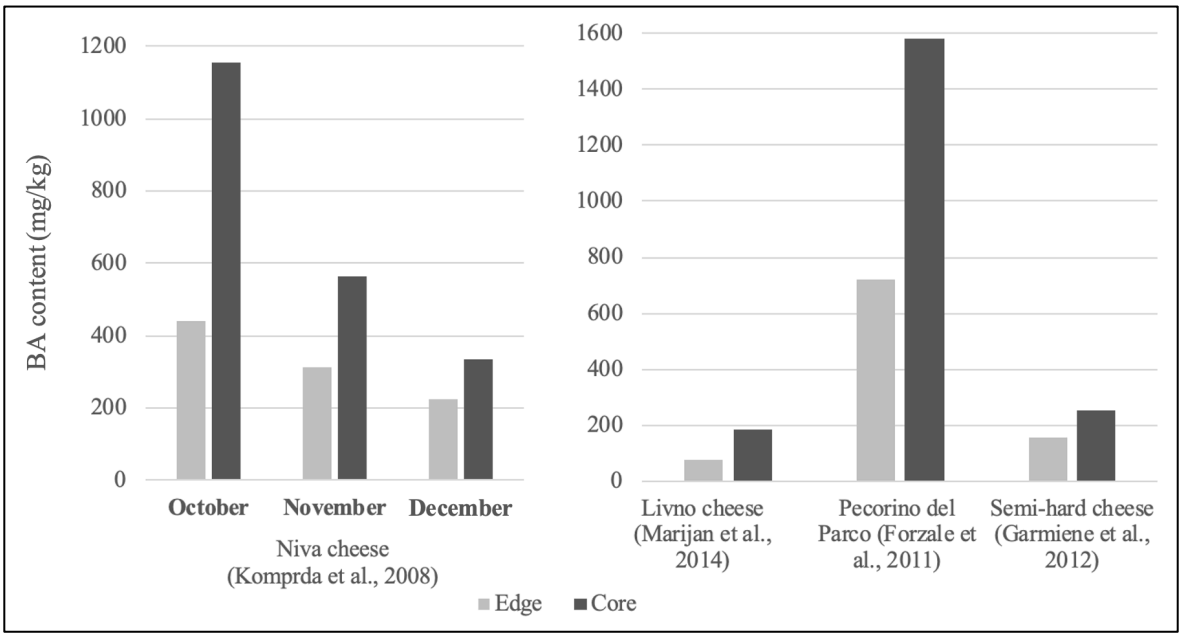

Figure 6 Comparison of BA concentrations in edge/rind and core of Niva, Livno, Pecorino del Parco and a semi-hard cheese at the end of ripening (in mg/kg)

Burdychová (2009) studied enterococci isolates and their relation to tyramine production in semi-hard cheeses produced in the Czech Republic. Cheese samples from two different producers (I and II) were analyzed during 176 days of ripening. As many as 361 suspected enterococci isolates were obtained during this period. Ten isolates from producer I and five isolates from producer II had the gene coding for tyrosine decarboxylase, and tyramine production was confirmed in all of these. As a result, higher levels of tyramine were detected in the cheese samples from producer I in comparison with producer II. The most frequent tyramine-producing *Enterococcus* species was *E. faecium*, followed by *E. durans* and *E. faecalis*.

3.4.4.3. Swiss cheeses

In 2009, the Agroscope Liebefeld-Posieux Research Station (ALP) released a report on the importance of biogenic amines and their presence in Swiss cheeses (Wechsler et al., 2009). The report presents a summary of the then-current knowledge and concludes that the BA content in high-quality Swiss cheeses made under optimal conditions is generally low (less than 500 mg/kg). There are however exceptional cases in which very high levels of BA have been found. Sporadically, total levels as high as 1000-1500 mg/kg have been measured in several semi-hard varieties. Typically, the BAs with the highest levels found, are histamine and tyramine, but a sample of Schabziger with a very high total BA level at 4010.0 mg/kg also showed very high levels of cadaverine, as well as high levels of putrescine,

phenylethylamine and tryptamine. However, the ALP reports that a large part of the cheeses showing high BA content was sampled due to quality defects – such as structural anomalies and atypical tastes. Such defects are normally observed during quality controls and can often be prevented by selecting only first quality cheeses for ripening. Thus, Wechsler et al. (2009) reported that many Swiss cheeses with potentially high BA content will not be marketed and reach the consumer.

Table 6 BA content in some medium-ripened Swiss cheese types (in mg/kg)

Schabziger (Wechsler et al., 2009) Made from raw cows' milk					
Age (d)	HIS	TYR	PUT	CAD	**Total**
min. 60	511 (71-760)	392 (135-867)	351 (90-552)	1126 (123-1712)	**2613** (490-4010)
Tilsiter (Wechsler et al., 2009) Made from pasteurized (Tilsiter green, a) and raw (Tilsiter red, b) cows' milk					
Age (d)	HIS	TYR	PUT	CAD	**Total**
35-56	<2 (<2-26)[a]	22 (<2-35)[a]	17 (<2-49)[a]	63 (3-133)[a]	**127** (22-217)[a]
91-126	53 (10-765)[b]	91 (17-578)[b]	<2 (<2-59)[b]	32 (<2-657)[b]	**275** (48-1018)[b]
Appenzeller (Wechsler et al., 2009; Mayer et al., 2010) Made from thermized cows' milk					
Age (d)	HIS	TYR	PUT	CAD	**Total**
135-165 90-120 120-180 >180	173 (10-500) 105 (60-435) 103 (50-456) 186 (46-571)	57 (10-800) 25 (<2-68) 53 (11-174) 93 (16-744)	2 (nd-100) <2 (<2-8) 2 (<2-18) 5 (<2-30)	18 (10-1300) 24 (2-90) 56 (5-121) 35 (<2-383)	- **227** (91-473) **281** (137-572) **420** (134-1185)

Age (d)	HIS	TYR	PUT	CAD	TRY	SPD	SPE	COL	EA	**Total**
-	51.9	375.5	8.2	8.3	9.2	6.2	16.9	0.9	3.5	**480.6**

Raclette (Wechsler et al., 2009) Made from thermized milk							
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	**Total**
- - 300	227 532 945	541 407 454	- - <2	- - 21	- - <2	- - 86	**820** **1010** **1506**

Values are expressed as median or mean and, where applicable, range (min-max concentration); d = ripening age in days at the time of sampling; - = not measured/specified by reference

Schabziger, a traditional cheese and Switzerland's oldest protected brand, is a raw cows' milk cheese with a pungent and spicy taste (Kulinarisches Erbe der Schweiz, n.d.). Wechsler et al. (2009) reported high levels of BAs found in 11 samples, with a median of

2613 mg/kg and a range between 490-4010 mg/kg (Table 6). CAD at 1126 mg/kg showed the highest number, followed by HIS (511 mg/kg), TYR (392 mg/kg) and PUT (351 mg/kg). It is thought that the specific manufacturing process of Schabziger puts the cheese at particular risk of developing BAs. While the acidification process limits vegetative microbes, the subsequent use of added culture in combination with the low salt content promotes the growth of contaminating *Enterobacteriaceae*. However, due to Schabziger's particular sensory properties, it is unlikely that this is a cheese consumed in large quantities, and thus it might not pose a risk even at high BA concentrations.

A comparison between BA content in samples of pasteurized (72°C, 15-40 s) and raw Tilsiter – a semi-hard Swiss cows' milk cheese – was made. Wechsler et al. (2009) reported that the spectrum and total content of BAs in 7 samples of green-labeled Tilsiter (pasteurized) and 23 samples of red-labeled Tilsiter (raw) show clear differences (Table 6). The total BA content was lower overall in the pasteurized samples with a median of 127 mg/kg and a range between 22-217 mg/kg. Cadaverine was the major amine with a median of 63 mg/kg. In the raw samples, a total BA content median of 275 mg/kg and a range between 48-1018 mg/kg was found. TYR (91 mg/kg), HIS (53 mg/kg) and CAD (32 mg/kg) were the major BAs.

The BA levels measured in Tilsiter were in a range comparable to the ones found in Appenzeller – a thermized cows' milk cheese with a technologically similar manufacturing process. Wechsler et al. (2009) reported the BA content in several samples of Appenzeller with different lengths of ripening (Table 6). An older study from 1988, with 50 samples with a ripening age between 135-165 days, showed high levels of BAs in several analyzed samples. Particularly high levels of cadaverine were reported above the 75th percentile (at 521-1300 mg/kg), but also HIS (357-500 mg/kg) and TYR (372-800 mg/kg) showed high levels. More recent measurements (from years 2001-2007) showed much lower concentrations, especially for cadaverine and tyramine. A change in the manufacturing process with regards to hygiene and the quality of milk as well as the use of thermization (65-68°C, 15-25 s) could explain this difference (Wechsler et al., 2009; Wechsler et al., 2013). A median of 227 mg/kg for total BA content was measure in 10 samples ripened for 90-120 days. An increase in BAs was found when analyzing 10 samples ripened for 120-180 days and 22 samples ripened for >180 days, at 281 mg/kg and 420 mg/kg, respectively. Histamine was the major BA, followed by tyramine and cadaverine. Putrescine was detected at small or non-detectable levels. Mayer et al., (2010) reported a similar total BA content level in a sample of Appenzeller at 480.6 mg/kg. Tyramine was the predominant BA at 375.5

mg/kg, followed by histamine (51.9 mg/kg) and then smaller amount of SPE, TRY, CAD, PUT, SPD, EA and COL.

Raclette is a semi-hard Swiss cheese made from raw, thermized or pasteurized milk, with a ripening time that can vary considerably. Unlike many other cheese types, Raclette is commonly used for melting and is generally consumed in relatively large quantities in the Swiss dish with the same name (Raclette), making high levels of BAs potentially problematic. Wechsler et al. (2009) reported the BA content in three samples of Raclette made from thermized milk (Table 6). All samples were analyzed due to customer complaints or visible quality defects (Figure 7), showing total BA levels between 820 and 1506 mg/kg. The samples contained significant levels of histamine (227-945 mg/kg) and tyramine (407-501 mg/kg).

Figure 7 Raclette with opening defects, a pungent taste and a very high content of BAs at 1506 mg/kg (Wechsler et al., 2009)

3.4.4.4. Hungarian cheeses

Simon et al. (2013) studied the effect of selected *Lactobacillus* strains with different decarboxylase activity on the formation of BAs in cheese. Pasteurized cows' milk from Dabas, Hungary was used in the production of semi-hard cheeses. Four different starter cultures were used, three of which contained different *Lactobacillus* strains, and the fourth being the industrial starter culture Choozit MA 4001 (*Lactococcus lactis* subsp. *lactis* biovar diacetylactis, *Streptococcus thermophilus*). The cheeses were stored at 13±2°C in a cooling cabinet, and samples for BA measurements were taken every week for six weeks. Total BA levels ranged between 25-42 mg/kg, 25-85 mg/kg, 14-112 mg/kg and 3-207 mg/kg in cheese samples inoculated with *Lactobacillus curvatus, Lactobacillus fermentum, Lactobacillus*

paracasei and Choozit industrial starter culture, respectively (Figure 8). Whereas cadaverine and putrescine were the predominant BAs detected in the cheeses with selected *Lactobacillus* strains, tyramine and histamine were predominant in the cheese manufactured using Choozit culture. The result of this study showed that cheeses fermented with selected *Lactobacillus* strains contained lower levels of BAs, in particular tyramine and histamine.

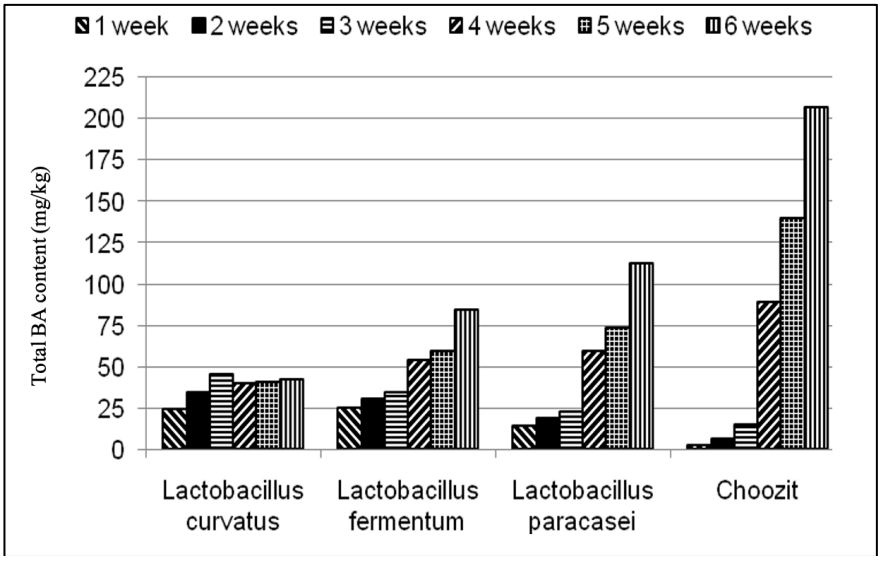

Figure 8 BAs in Hungarian cheeses inoculated with different starters (Simon et al., 2013)

3.5. Long-ripened European cheese types with possibly high BA content

3.5.1. Northern European hard cheeses

Tham et al. (1990) investigated histamine formation by enterococci in Swedish goat cheese. Six cheeses were made using raw goats' milk inoculated with *Lactococcus lactis* subsp. *lactis.* Based on an earlier study by Tham (1988), two of the cheeses were additionally inoculated with a histamine-producing strain of *Enterococcus faecium,* and two cheeses were inoculated with a non-histamine-producing strain of *Enterococcus faecalis.* Each cheese was sampled for bacterial and histamine analyses every second week during 91 days of ripening. The numbers of enterococci remained high throughout the whole ripening period, but the histamine level was comparatively very low. The highest levels of histamine were found in the two cheeses inoculated with *E. faecalis* at 8.2 mg/kg and 6.1 mg/kg. In the earlier study by Tham (1988), only 1.9% of the *E. faecalis* strains isolated from home-made goat cheese produced histamine when grown in an artificial medium (Trypticase Soy Broth Histidine medium). On the other hand, 31.5% of the *E. faecium* strains produced histamine in an artificial medium, but produced only very small amounts in the goat cheeses. None of

the strains produced sufficient histamine to cause histamine intoxication in healthy individuals.

3.5.2. Southern European hard cheeses

3.5.2.1. Spanish cheeses

Fernández et al. (2007) analyzed the BA content in 61 commercial cheeses produced and ripened under different conditions. 48 of the samples were from different Spanish regions, while the remaining 13 were imported from different European countries. The highest BA concentrations were found in long-ripened cheeses made from raw milk. 66.7% of the long-ripened cheeses contained BAs, while only 33.3% of the short-ripened cheeses were BA-positive. The average concentration of BAs in the long-ripened types (at 453.8 mg/kg) was much higher than the concentration found in the shorter ripened cheeses (at 233.3 mg/kg). In addition, the BA concentration was highest in those long-ripened cheeses made from raw milk, with mean tyramine and histamine levels at 122.0 and 59.5 mg/kg, compared with 33.2 and 13.2 mg/kg found in the pasteurized long-ripened cheeses. Overall, tyramine was the predominant BA, followed by HIS, PUT and CAD. CAD and PEA were only detected in raw milk cheeses.

Manchego is a Spanish PDO cheese manufactured from raw or pasteurized ewes' milk from the Manchega breed (Figure 9). Poveda et al. (2015) analyzed the BA content in Manchego cheese manufactured with two different autochthonous starter cultures and compared them with those of commercial starter and raw milk cheese manufactured without starter. Samples were analyzed at day 60, 150 and 240 of ripening. Only low levels of BAs were found in the samples made from pasteurized milk (72°C, 15-20 s), and the use of different starter cultures did not seem to have a significant impact. Histamine, tyramine and cadaverine were the only BAs detected, and none of the samples contained a total BA content exceeding 90 mg/kg. The samples made from raw milk had significantly higher contents of BAs, with total levels measured at 674.7, 1565.7 and 1881.7 mg/kg at day 60, 150 and 240 of ripening, respectively. In these samples, CAD was the predominant amine (289.9-803.1 mg/kg), followed by PUT (215.4-601.9 mg/kg), TYR (109.4-326.8 mg/kg) and HIS (66.0-100.1 mg/kg). Low levels of PEA were also detected in the samples ripened for 150 and 240 days.

Ladero et al. (2010) examined the tyramine content in several samples of Manchego cheese with different ripening times. Tyramine was not detected in the pasteurized milk sample, but concentrations comparable to those reported by Poveda et al. (2015) were found

in raw milk samples ripened for 240 and 300 days at 269.9 and 203.2 mg/kg, respectively. The longest ripened sample, at 360 days, only contained small amounts of tyramine at 28.5 mg/kg.

Figure 9 Manchego cheese with the Protected Designation of Origin (PDO) logo in red and yellow (The Spanish Hamper, n.d.)

Zamorano is a PDO-protected ewes' milk cheese manufactured using either pasteurized or raw milk from the Churra and Castellana breeds. Combarros-Fuertes et al. (2016) analyzed and compared the concentrations of BAs found in pasteurized milk (72°C, 20 s) samples and raw milk samples of Zamorano cheese aged for 300 days. The total BA content increased during ripening reaching a mean of 243.6 mg/kg in the pasteurized samples, with TYR and SPE as the predominant amines. In the raw milk samples, total mean BA content reached 574.5 mg/kg, and PEA, PUT, SPE and TYR were the main BAs representing around 80% of the total level. HIS only showed low levels, never exceeding 40 mg/kg in any of the samples.

Renes et al. (2014) analyzed the BA content in 40 ewes' milk cheeses made from pasteurized milk and four different starter cultures. Overall, the mean total BA content increased notably throughout ripening from 115.7 to 819.1 mg/kg at day 210 of ripening – a number which is significantly higher than the ones reported for other pasteurized ewes' milk cheeses by Poveda et al. (2015), Ladero et al. (2010) and Combarros-Fuertes et al. (2016). PEA, SPE and TRY were the predominant amines found, representing 81% of the total BA content. The concentrations of BAs were similar in the four batched manufactured using different starter cultures up until 30 days of ripening. After that point, differences became

significantly marked. Starter cultures with certain autochthonous strains having a lower proteolytic and decarboxylase activity – in this case *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* – gave rise to lower levels of BAs. The batch manufactured with commercial starter culture had the second highest level of BAs at the end of ripening.

3.5.2.2. Italian Pecorino cheeses

Zazzu et al. (2019) measured the amount of BAs in the long-ripened, hard ewes' milk cheese Fiore Sardo. This is a PDO cheese exclusively produced in Sardinia by traditional manufacturing techniques and the use of raw milk from the Sarda breed. A total of 36 samples produced by four different cheesemakers were analyzed for eight BAs (Table 7). The average total was measured at 700 mg/kg, with a range between 170 and 1100 mg/kg. A great variability was seen in the total BA content among the different samples, but TYR (350 mg/kg), PUT (150 mg/kg) and HIS (80 mg/kg) were the predominant BAs in most samples. The exception was samples from one of the four cheesemakers in which SPE was the major amine reaching up to 160 mg/kg. However, the overall BA content was in the lower range for these samples compared to the other cheesemakers, with a mean at 170 mg/kg. CAD, PEA and TRY were measured at relatively low levels in all samples, and SPD was always found to be below its limit of detection.

Manca et al. (2020) examined a total of 37 samples of Fiore Sardo cheese produced by 19 dairy farms (Table 7). The mean total BA content was 1270 mg/kg, significantly higher than the 700 mg/kg found by Zazzu et al. (2019). However, TYR (820 mg/kg), followed by PUT (210 mg/kg) and HIS (80 mg/kg) were also the predominant BAs in this case. PEA, CAD and TRY were found in smaller amounts, while SPD and SPE were never detected. Large variability was seen in samples from the different manufacturers, with a total BA level ranging from 60 to 3660 mg/kg.

In a different variety of Pecorino cheese – the PDO cheese Pecorino Sardo – Manca et al. (2015) examined the BA content during ripening and compared them to the levels found in a type of farmhouse Pecorino (Table 7). The manufacturing of Pecorino Sardo is limited to Sardinia, and it is produced from thermized ewes' milk inoculated with a starter culture and then ripened under controlled conditions. In contrast, the Sardinian farmhouse Pecorino was manufactured from raw milk without a starter culture and then ripened in rooms with no control over humidity and temperature. 13 samples of Pecorino Sardo and 12 samples of farmhouse Pecorino were examined with ripening time ranging from 30-360

days. The samples from Pecorino Sardo showed a mean total value at 81.5 mg/kg for cheeses ripened for 30 days, and 279.0 mg/kg for cheeses ripened for 360 days. However, this increase was not consistent as there was large variability among the samples with several of the longer ripened samples showing low levels. Tyramine (7.0-163.0 mg/kg) was the predominant BA in most samples of Pecorino Sardo, with the other BAs (HIS, PUT, CAD, TRY, SPD and SPE) showing great variability. PEA was not detected in any of the samples.

Table 7 BA content in some long-ripened Sardinian Pecorino cheeses (in mg/kg)

Fiore Sardo PDO (Zazzu et al., 2019; Manca et al., 2020) Made from raw ewes' milk									
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	SPE	**Total**
min. 105	80 (<0.7-250)	350 (<0.5-800)	150 (<0.2-730)	30 (1.0-94)	3 (<0.2-16.9)	10 (<0.3-42)	<0.23	50 (10-160)	**700** (170-1100)
180-360	80 (nd-650)	820 (30-2000)	210 (nd-950)	60 (nd-330)	30 (nd-80)	70 (nd-460)	nd	nd	**1270** (60-3660)
Pecorino Sardo PDO (Manca et al., 2015) Made from thermized ewes' milk									
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	SPE	**Total**
30	4.0	25.5	1.0	13.5	8.0	nd	5.0	24.5	**81.5**
60	21.0	65.8	6.0	35.5	46.3	nd	11.5	20.0	**206.1**
90	27.0	44.0	7.0	9.0	24.0	nd	72.0	10.0	**193.0**
150	10.5	46.5	0.5	18.5	16.0	nd	30.5	6.0	**128.5**
180	19.5	7.0	3.0	8.0	8.5	nd	5.0	2.5	**53.5**
270	43.0	64.0	nd	7.0	nd	nd	111.0	12.0	**237.0**
360	53.0	163.0	8.0	26.0	nd	nd	5.0	24.0	**279.0**
Farmhouse Pecorino (Manca et al., 2015) Made from raw ewes' milk									
Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	SPD	SPE	**Total**
60	nd	52.0	22.5	103.5	14.5	15.0	nd	nd	**208.0**
90	122.0	259.0	477.0	740.0	20.5	43.0	nd	nd	**1661.5**
150	nd	252.5	82.0	27.5	nd	nd	nd	nd	**362.0**
180	493.0	544.0	43.3	92.7	37.0	33.7	nd	nd	**1210.0**
270	nd	42.0	nd	52.0	nd	nd	nd	nd	**94.0**
360	167.0	187.5	193.5	334.0	94.5	50.5	nd	nd	**1027.0**

Values are expressed as mean and, where applicable, range (min-max concentration); d = ripening age in days at the time of sampling; nd = not detected; * = as determined by the PDO requirements

When comparing these findings to the ones for the farmhouse Pecorino, the latter showed a significantly higher level of BAs with mean total levels ranging from 94.0 to 1661.5 mg/kg. As with Pecorino Sardo, the levels of BAs in the farmhouse variety did not

seem to correlate with the time of ripening. The highest total mean level was found for those ripened for 90 days (1661.5 mg/kg), followed by 180 days (1210.0 mg/kg) and then 360 days (1027.0 mg/kg). CAD (27.5-740.0 mg/kg) was the major amine followed by TYR (42.0-544.0 mg/kg) and PUT (nd-477 mg/kg). HIS (nd-493 mg/kg) was detected in high amounts in those samples showing a high total BA content, but it was not detected in those with a low total value. TRY and PEA were detected in low concentrations, while SPD and SPE were not detected in any of the samples. The results found by Manca et al. (2015) supports the importance of environmental and hygienic conditions in limiting the concentration of BAs. Whereas Pecorino Sardo was produced using thermized milk and ripened under controlled temperature and humidity, the high content of BAs in farmhouse Pecorino could be related to the less than optimal conditions during its manufacturing and ripening process.

Schirone et al. (2013) investigated the BA content in several Pecorino cheeses from the Abruzzo region. From the long-ripened varieties (Table 8), the total BA content in the pasteurized cheeses Pecorino Canestrato di Castel del Monte (10.3 mg/kg), Pecorino di Atri (10.6 mg/kg), and Pecorino sotto fieno (304.1 mg/kg) showed the lowest levels regardless of ripening time. In Pecorino sotto fieno, histamine was the major amine measured at 200.0 mg/kg, followed by lower levels of TYR (58.9 mg/kg), CAD (36.9 mg/kg) and PUT (8.3 mg/kg). Higher levels of total BA content were found in the raw milk cheeses Pecorino d'Abruzzo and Pecorino abruzzese sott'olio. The sample of Pecorino d'Abruzzo showed a total BA level at 446.0 mg/kg after 300 days of ripening. This level is lower than the mean level of 824.7 mg/kg detected in samples of the same cheese type ripened for 90 days. The proportions of the different BAs were however similar, showing high levels of TYR (140.9 mg/kg), HIS (130.7 mg/kg) and PUT (128.1 mg/kg) compared to low levels of CAD (33.6 mg/kg) and PEA (12.7 mg/kg). SPD and SPE were detected in neither the medium- nor the long-ripened variety of Pecorino d'Abruzzo. Tyramine was the predominant BA in Pecorino abruzzese sott'olio at 394.5 mg/kg, followed by lower levels of PEA (62.1 mg/kg), CAD (57.3 mg/kg), PUT (36.3 mg/kg) and HIS (13.1 mg/kg). SPD and SPE were not detected.

Tofalo et al. (2019) analyzed several samples of Pecorino di Farindola for the detection of BAs during ripening. The cheese samples were made according to the traditional protocol using raw ewes' milk and pig rennet. Measurements were taken at several points during the ripening process, showing a steady increase in the three BAs putrescine, cadaverine and tyramine. PUT showed the highest concentration at 732.0 mg/kg on day 270 of ripening, followed by TYR at 450.0 mg/kg and CAD at 111.0 mg/kg (Table 8). HIS was

not detected. The total mean value at 1293.0 mg/kg on day 270 was higher than the levels found by Schirone et al. (2011) at 678.5 mg/kg and Schirone et al. (2013) at 957.7 mg/kg for the 90-day medium-ripened Pecorino di Farindola.

Table 8 BA content in some long-ripened Abruzzian Pecorino cheeses (in mg/kg)

Pecorino di Atri (Schirone et al., 2013) Made from pasteurized ewes' milk								
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	**Total**
150	nd	10.6	nd	nd	nd	nd	nd	**10.6**
Pecorino sotto fieno (Schirone et al., 2013) Made from pasteurized ewes' milk								
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	**Total**
180	200.0	58.9	8.3	36.9	nd	nd	nd	**304.1**
Pecorino abruzzese sott'olio (Schirone et al., 2013) Made from raw ewes' and cows' milk								
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	**Total**
240	13.1	394.5	36.3	57.3	62.1	nd	nd	**563.2**
Pecorino Canestrato di Castel del Monte (Schirone et al., 2013) Made from pasteurized ewes' milk								
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	**Total**
240	10.3	nd	nd	nd	nd	nd	nd	**10.3**
Pecorino d'Abruzzo (Schirone et al., 2013) Made from raw ewes' milk								
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	**Total**
300	130.7	140.9	128.1	33.6	12.7	nd	nd	**446.0**
Pecorino di Farindola (Tofalo et al., 2019) Made from raw ewes' milk								
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	EA	**Total**
270	nd	450	732	111	-	-	-	-	**1293**

Values are expressed as mean; d = ripening age in days at the time of sampling; nd = not detected; - = not measured

3.5.2.3. Italian Grana cheeses

Most of the extra-hard cheese varieties, whose ripening time is particularly long, are produced in Italy, such as the renowned Parmigiano Reggiano (Figure 10) commonly referred to as Parmesan (McSweeney et al., 2017). Both Parmigiano Reggiano and Grana Padano are PDO Grana cheeses, defined by their distinctive hard, granular texture, often used for grating. These high-quality cheeses are manufactured from raw cows' milk only in

restricted geographical areas in Northern Italy following traditional and strict manufacturing protocols (Gobbetti et al., 2018).

Figure 10 Parmigiano Reggiano cheese with identification mark, date and quality stamp encircling the entire cheese confirming authenticity (Gobbetti et al., 2018)

Rivoira et al. (2019) analyzed the presence of BAs in Grana Padano and Parmigiano Reggiano after 12 months of ripening (Table 9). COL, EA, PUT, CAD, HIS, SPD, and SPE were investigated in untreated samples and samples having been subjected to consumer handling (grating, unrefrigerated storage). Colamine was the only amine detected, with low levels ranging from 19.0-43.9 mg/kg. Grana Padano was analyzed untreated and after home grating, and Parmigiano Reggiano was analyzed after proper storage in the refrigerator and after an improper storage period of three days unrefrigerated after opening. Manipulation of the cheeses resulted in an almost doubling of the amount of colamine, confirming microbiological recontamination of the cheese.

Similarly, Mayer et al. (2010) also found very low levels of BAs (with one exception) when analyzing the same two types of Grana cheeses (Table 9). The grated Grana Padano cheese (mean 117.4 mg/kg) also here showed a doubling of BAs compared to the non-grated variety (mean 60.5 mg/kg). The cheese sample with the highest total BA level at 285.3 mg/kg, had a histamine concentration at 249.0 mg/kg. Several samples of Parmigiano Reggiano with different ripening age were analyzed. The two cheeses with the longest ripening time at 720 days showed the lowest and the highest total BA level at 26.9 and 116.2 mg/kg, respectively, indicating that factors other than ripening time are more important for the BA production. The predominant BA was histamine in both Grana cheeses, followed by

colamine. Only very small or non-detectable amounts were measured for TYR, PUT, CAD, SPD, SPE and EA.

Table 9 BA content in some extra-hard, long-ripened Italian cheese types (in mg/kg)

Grana Padano PDO (Rivoira et al., 2019; Mayer et al., 2010) Non-grated (a) and grated (b) raw cows' milk cheese									
Age (d)	HIS	TYR	PUT	CAD	SPD	SPE	COL	EA	**Total**
360	nd[a]	-	nd[a]	nd[a]	nd[a]	nd[a]	19.0[a]	nd[a]	**19.0[a]**
360	nd[b]	-	nd[b]	nd[b]	nd[b]	nd[b]	37.6[b]	nd[b]	**37.6[b]**
min. 270*	16.3[a] (nd-23.7)	2.2[a] (nd-6.2)	0.4[a] (nd-1.7)	3.1[a] (nd-10.0)	10.5[a] (nd-37.6)	15.3[a] (nd-61.0)	8.2[a] (nd-14.7)	4.5[a] (nd-9.5)	**60.5[a]** (37.5-123.3)
min. 270*	98.9[b] (4.0-249.0)	5.45[b] (nd-18.0)	nd[b]	nd[b]	nd[b]	nd[b]	8.7[b] (0.5-12.2)	4.4[b] (nd-9.8)	**117.4[b]** (4.5-285.3)
Parmigiano Reggiano PDO (Rivoira et al., 2019; Mayer et al., 2010) Made from raw cows' milk. Proper storage (c) and improper storage (d).									
Age (d)	HIS	TYR	PUT	CAD	SPD	SPE	COL	EA	**Total**
360	nd[c]	-	nd[c]	nd[c]	nd[c]	nd[c]	26.9[c]	nd[c]	**26.9[c]**
360	nd[d]	-	nd[d]	nd[d]	nd[d]	nd[d]	43.9[d]	nd[d]	**43.9[d]**
min. 360*	29.3	5.9	nd	nd	nd	nd	8.0	16.1	**59.3**
min. 360*	58.7	nd	nd	nd	nd	nd	12.6	13.1	**84.4**
540	23.2	nd	nd	nd	4.8	16.7	22.3	nd	**67.0**
720	79.6	nd	nd	nd	4.4	16.4	15.8	nd	**116.2**
720	10.9	6.4	1.8	3.2	4.6	nd	nd	nd	**26.9**

Values are expressed as mean and, where applicable, range (min-max concentration); PDO = Protected Designation of Origin; d = ripening age in days at the time of sampling; nd = not detected; - = not measured; * = as determined by the PDO requirements

3.5.3. Western European hard cheeses

The hard cows' milk cheese Cheddar originated in the English village of Cheddar. Today, this popular cheese is produced in several regions around the world. Mayer et al. (2010) analyzed a sample of Irish Cheddar Rot for the detection of BAs. A total content of 83.2 mg/kg was measured, with HIS (25.4 mg/kg), TRY (22.1 mg/kg) and SPE (18.2 mg/kg) being the predominant BAs. Benkerroum (2016) reported similar HIS levels in a Cheddar cheese at 29.4 mg/kg. TYR was however the major BA at 44.5 mg/kg, with lower levels of SPD, SPE, CAD, PUT and PEA also measured. O'Brien et al. (2004) reported a wider range of BA levels in Cheddar cheese, with values of HIS and TYR ranging from nd-2120 mg/kg and nd-1530 mg/kg, respectively. TRY and PEA were reported in the range nd-300 mg/kg.

3.5.4. Central European hard cheeses

3.5.4.1. Austrian cheeses

Vorarlberger Bergkäse and Tiroler Almkäse – both PDO certified – are Austrian mountain cheeses traditionally manufactured from raw cows' milk. Mayer et al. (2010) analyzed several samples for the concentration of BAs (Table 10).

Table 10 BA content in some long-ripened Austrian cheeses (in mg/kg)

Vorarlberger Bergkäse PDO (Mayer et al., 2010) Made from raw cows' milk										
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	COL	EA	**Total**
180	11.7	19.0	nd	nd	nd	5.9	22.5	5.8	nd	**64.9**
300	42.6	85.6	nd	nd	nd	14.5	36.4	6.5	nd	**185.6**
360	397.2	25.6	2.0	7.6	nd	nd	nd	nd	15.9	**448.3**
Bergkäse (Mayer et al., 2010) Made from raw cows' milk										
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	COL	EA	**Total**
-	16.4	nd	nd	nd	nd	5.2	28.4	5.8	nd	**55.8**
300	80.0	134.4	nd	27.0	15.8	18.9	25.1	8.8	nd	**310.0**
-	65.2	253.2	1.7	2.0	61.3	nd	16.2	9.0	nd	**412.2**
Tiroler Almkäse PDO (Mayer et al., 2010) Made from raw cows' milk										
Age (d)	HIS	TYR	PUT	CAD	PEA	SPD	SPE	COL	EA	**Total**
min.	820.6	189.9	27.4	9.3	nd	nd	nd	2.9	10.1	**1060.2**
135*	1159.7	475.2	152.9	59.9	37.5	4.4	nd	17.8	28.4	**1938.2**

Values are expressed as mean; d = ripening age in days at the time of sampling; nd = not detected; - = not measured/specified by reference; * = as determined by the PDO requirements

Increasing levels of BAs were found in three Vorarlberger Bergkäse (PDO) cheeses with different ripening times. The total content of BAs ranged from 64.9 mg/kg to 448.3 mg/kg. In the cheeses ripened for 180 and 300 days, TYR (19.0 and 85.6 mg/kg), SPE (22.5 and 36.4 mg/kg) and HIS (11.7 and 42.6 mg/kg) were the predominant BAs, with lower levels of SPD and COL also found. In the longest-ripened cheese at 360 days, histamine made up the vast majority at a concentration of 397.2 mg/kg. Tyramine was measured at 25.6 mg/kg and the remaining BAs were either not detected or detected at very low levels. Three other varieties of Bergkäse cheese were also analyzed showing total BA values ranging from 55.8-412.2 mg/kg. Their ripening time was not specified, except one which was ripened for 300 days with a total BA value of 310.0 mg/kg. TYR, HIS and SPE were

also the predominant amines in these, although some degree of variability was seen among the three different samples.

Tiroler Almkäse had significantly higher concentrations of BAs compared to Vorarlberger Bergkäse, with total levels reaching as high as 1938.2 mg/kg. The two samples that were analyzed both contained high levels of histamine at 820.6 and 1159.7 mg/kg. High levels of tyramine were also found at 189.9 and 475.2 mg/kg, while putrescine was high in one of the samples at 152.9 mg/kg. Smaller amounts of CAD, PEA, EA, COL and SPD were also measured in one or both samples. SPE was not detected.

3.5.4.2. Swiss cheeses

Switzerland, which is not an EU member, protects its cheeses with the Appellation d'Origine Protégée (AOP) certification, which is similar to the PDO certification used in the EU. In addition to Swiss semi-hard cheese types, Wechsler et al. (2009) also reported the BA content found in some hard and extra-hard cheeses – all of which are AOP certified cheeses made from raw cows' milk: Emmentaler, Gruyère, Sbrinz, and Berner Hobelkäse.

In 2006, 197 samples of Emmentaler from 16 different manufacturers ripened from 3-12 months were analyzed. In general, the total BA content was low with only exceptional cases exceeding 200 mg/kg. The total BA content in samples from the different manufacturers showed however great variability, ranging from a total average of 411 mg/kg from one manufacturer to 23 mg/kg from another. Histamine ranging from 4 to 269 mg/kg and tyramine ranging from 16 to 139 mg/kg were the only BAs of significance found in these samples. In 2008, ten samples of Emmentaler ripened for 12 months from different manufacturers were analyzed. Two of the samples showed very high levels of histamine at 576 and 911 mg/kg. When subsequently analyzing four more samples from the manufacturer with the highest levels, levels of histamine ranging from 400 to 1364 mg/kg were found. This further validates that the amount of BAs can be highly influenced by the manufacturing process and any hygienic or quality shortcomings by different manufacturers. Cadaverine and putrescine were for the most part not detected in Emmentaler, except in some samples with quality problems as shown in Table 11. A sample of Emmentaler ripened for 165 days with a visible quality defect showed a total BA level of 1637 mg/kg (Figure 11). The major amines were HIS (630 mg/kg), TYR (505 mg/kg), and an atypical high concentration of PUT (481 mg/kg).

Figure 11 Emmentaler cheese showing quality defects and a high concentration of BAs after 165 days of ripening (Wechsler et al., 2009)

Ten samples of Gruyère ripened for 240 days and 12 samples ripened for 360 days were analyzed for BAs, as reported by Wechsler et al. (2009). The levels of BAs were generally low, with median total concentrations at 234 mg/kg and 65 mg/kg. Contrary to expectations, the shorter ripened variety at 240 days showed a higher concentration of all BAs. Tyramine was the predominant BA, followed by lower levels of CAD, HIS and PUT. A few exceptional cases with very high levels of BAs were also found. In a sample ripened for 240 days, histamine was measured at 2265 mg/kg, but also TYR (744 mg/kg) and CAD (307 mg/kg) showed significantly high levels (Table 11). Mayer et al. (2010) also analyzed a sample of Gruyère. EA, TYR, PUT, HIS, CAD and COL were all measured at very low levels, and a total BA concentration of 37.8 mg/kg was found. The low levels of BAs seen in Gruyère may be explained by the targeted selection of only the best quality cheeses for a longer maturation.

With the exception of some samples showing quality defects (Table 11), the cheese types Sbrinz and Berner Hobelkäse were also reported to contain generally low levels of BAs by Wechsler et al. (2009). All but one of the 45 samples of Sbrinz showed a total BA level below 100 mg/kg. Histamine and tyramine were the BAs detected in the highest concentrations with a median of 27 mg/kg and 14 mg/kg, respectively. Ten samples of Berner Hobelkäse taken from the top-ranked cheeses at the Alpine Cheese Championship were analyzed. These high-quality cheeses ripened for 750 days had very low levels of BAs with a median at 34 mg/kg and a range between 17-84 mg/kg, in which tyramine was the predominant BA.

A very high level of BAs was detected in a Berner Alpkäse cheese at 2538 mg/kg (Table 11). This was a cheese ripened for 990 days and recognized by the manufacturer to have quality defects. When selecting cheeses for a long period of ripening, only the best are chosen. However, despite this, some exceptional cases with defects are ripened and these can potentially end-up having very high levels of BAs.

Table 11 Samples of hard and extra-hard Swiss cheeses with some degree of quality defect and a very high content of BAs (in mg/kg) (Wechsler et al., 2009)

Cheese	Age (d)	HIS	TYR	PUT	CAD	TRY	PEA	**Total**
Emmentaler AOP	165	630	505	481	4	<2	17	**1637**
Emmentaler AOP	360	1364	<2	<2	<2	47	<2	**1411**
Gruyère AOP	240	2265	744	67	178	<2	30	**3284**
Sbrinz AOP	360	1289	42	<2	<2	<2	<2	**1331**
Berner Alpkäse AOP	390	304	720	214	7	<2	108	**1353**
Berner Hobelkäse AOP	990	1823	566	134	<2	<2	15	**2538**

3.5.4.3. Polish cheeses

Bonczar et al. (2018) assessed the BA content in some hard cheeses available on the Polish market. Three hard cheeses with eyes (of emmental type) and three hard smooth cheeses (of cheddar type) were analyzed. Only the former contained significant levels of BAs with a total mean at 153.0 mg/kg. Tyramine at 67.6 mg/kg and putrescine at 67.0 mg/kg made up the majority. Small or very small amounts of PEA, CAD, HIS, SPE, SPD and TRY were detected. Only low levels of BAs were detected in the hard smooth cheese varieties with a mean total level at 21.1 mg/kg.

Madejska et al. (2018) investigated the effect of storage temperature on histamine levels in the Polish hard cheeses Salami and Mlekdamer. Each cheese sample was divided into two portions, one of which was stored at 4 ± 2°C for up to 133 days, and the other was kept at room temperature at 22 ± 2°C for up to 42 days. The histamine level was highest in Mlekdamer cheese stored at room temperature, showing an increase from day 0 and up until day 35 reaching 35.8 mg/kg at the highest (Figure 12). In Mlekdamer cheese stored at 4 ± 2°C, the histamine level ranged from 10 to 15 mg/kg during the first 91 days and decreased thereafter. Only small amounts of histamine were measured in Salami cheese kept at room temperature, and in the samples stored at 4 ± 2°C the levels were below the limit of detection.

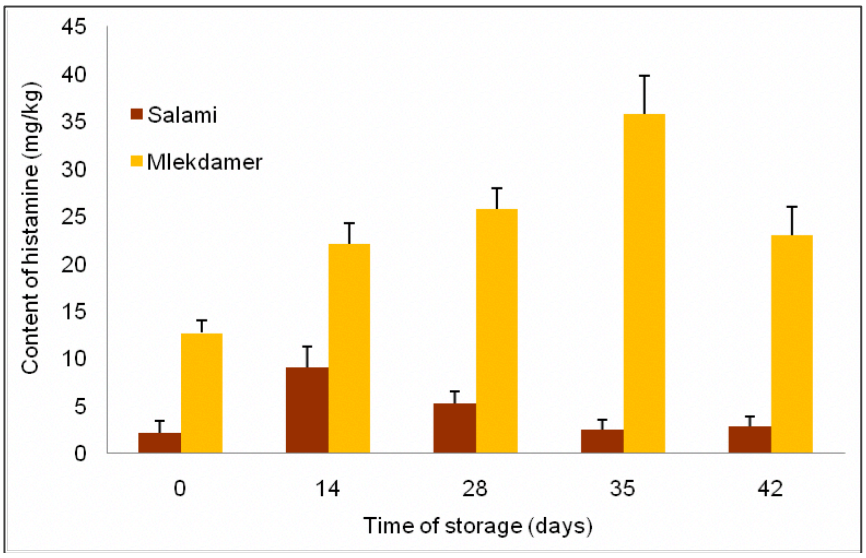

Figure 12 Concentration of histamine (mean ± SD in mg/kg) in hard cheeses during storage at 22 ± 2°C (Madejska et al., 2018)

4. Discussion and conclusion

The data presented in this thesis shows that high levels of BAs in some types of cheeses have been well documented. However, the BA concentrations are highly variable, not only among the different types, but also among cheeses of the same type. Numerous factors have been shown to contribute to the formation and accumulation of BAs – some more than others. While a longer period of ripening leads to a more extensive proteolysis and the potential for higher amount of BAs to accumulate, it is not the one and only decisive factor. When looking at the data collected from the different types of cheese, some common parameters can be seen in cheeses with higher concentrations of BAs. Cheeses manufactured using ancient traditional methods, or ripened under non-controlled environmental conditions, such as in a cave or fossa, often showed very high levels of BAs. The type of milk used, its origin and microbiological composition can be significant contributing factors. Many of the ewes' milk cheeses included in this thesis have shown to contain relatively high amounts of BAs – even the shorter-ripened varieties. Some samples of Italian Pecorino cheese contained levels well above 1000 mg/kg, with one sample ripened for 90 days reaching as high as 2393.0 mg/kg. Milk pasteurization almost consistently showed to minimize the formation of BAs in cheese when compared to their raw milk counterpart. The traditional medium-ripened cheeses Castelmagno (PDO), Schabziger and Valdeón (PGI) are examples manufactured from raw milk that showed very high levels of BAs.

When studying the same types of cheese during ripening, the total BA content had a tendency to increase over time, such as with Montasio (PDO), Appenzeller, Valdeón (PGI), and Manchego (PDO). However, the ripening time itself is not a good indicator for estimating BA content – as was evident in the cheeses with the longest ripening time stretching up to 750 days. Many of these long-ripened hard and extra-hard cheeses contained relatively low concentrations of BAs. The Grana cheeses Parmigiano Reggiano (PDO) and Grana Padano (PDO), and the Swiss Sbrinz (AOP) and Berner Hobelkäse (AOP), generally showed total concentrations well below 100 mg/kg, with only a few samples reaching above this level. A targeted selection of only the best quality cheeses for long-term ripening, in addition to quality-controls to remove cheeses showing or developing quality defects, are important contributing factors to the low levels of BAs seen in these cheeses.

The formation of BAs is typically not uniform throughout the cheese, as shown in several samples of Pecorino del Parco, Livno and Niva. The presence, activity and growth of different BA-producing microorganisms vary and will thus influence the pattern of BA formation. The total BA content was significantly higher in the core compared to the external

part or rind. While TYR always showed the highest concentration in the core, some BAs, like HIS, CAD, SPD and SPE, showed the opposite with higher concentration towards the external parts of some cheeses.

With the current knowledge and data on BAs, it is reasonable to assume that the consumption of certain types of ripened cheeses can represent a health risk. The NOAEL level for HIS at 50 mg per meal for healthy individuals can easily be exceeded when eating cheeses with high levels of BAs. For consumers with diminished detoxification capacity due to gastrointestinal conditions or MAOI drugs, even small amounts of cheese can contain BAs above any limit considered safe. In view of the lacking legislation for BAs in cheese and the known potential health risks associated with the consumption of high levels of BAs, efforts should be made to control and limit their occurrence in cheese. More research on the various factors involved in BA formation and accumulation and on how to limit them should be prioritized. With greater knowledge of the microbiota of milk and bacterial decarboxylase activity, certain strains with reduced tendencies to produce BAs can be selected as starter cultures. Enterococci in milk and cheese are linked to poor bacteriological quality and hygienic conditions during manufacturing, thus good hygienic practices should be implemented wherever lacking and pasteurization should be considered in raw milk cheeses with high levels of BAs.

5. Summary

In cheese, BAs are products of protein degradation, produced mainly by microbial decarboxylation of amino acids during ripening. The human gut will normally metabolize BAs by means of detoxifying enzymes (MAO, DAO, PAO). However, this protective mechanism can be overwhelmed by excessive BA intake through fermented foods or in people suffering from gastrointestinal disorders or insufficient amine oxidase activity – either due to genetic predisposition or MAO/DAO inhibitory drugs.

Histamine (HIS) and tyramine (TYR) are considered the two most toxic biogenic amines, causing acute symptoms such as rash, gastrointestinal complaints, bronchospasm, tachycardia, hypotension, hypertensive crisis, headache and vomiting. In EU legislation, food safety limits are only set for HIS in fish. Based on studies, NOAELs at 50 mg per meal for HIS and 600 mg per meal for TYR have been proposed. However, the EFSA (2011) reports that much lower levels are considered safe for sensitive individuals (HIS levels below detectable limits; TYR levels at 6-50 mg in people taking MAOI drugs).

High total BA levels have been detected in different cheeses all across Europe (medium-ripened: 7.1-4010.0 mg/kg; long-ripened: 4.5-3284.0 mg/kg), but often with varying levels even within a given cheese type (as for e.g. Fiore Sardo and Gruyère). Cheeses manufactured using ancient traditional methods (such as handwork, farmhouse production, usage of pig rennet), or ripened under non-controlled environmental conditions (cave, fossa), often showed very high levels of BAs. Milk pasteurization (72-76°C for 15-40 seconds) almost consistently showed to minimize the formation of BAs in cheese to a level half (or less) of that seen in their raw milk counterpart (as in e.g. Tilsiter, Zamorano and Valdeón).

With the current knowledge and data on BAs, it is reasonable to assume that consumption of certain types of popular medium- and long-ripened cheeses (such as Manchego, Cantal, Fiore Sardo and Emmentaler) can represent a potential health risk. The NOAEL levels can easily be exceeded when eating cheeses with high levels of BAs. Efforts should be made to control and limit their occurrence in cheese. A targeted selection of only the best cheeses showing no quality defects for long-term ripening is important in limiting BA formation. Selected strains of *Lactobacillus* or other starter bacteria with reduced tendencies to produce BAs should be used as starter cultures, and good hygienic practices should be implemented (during milking, production, ripening and storage) wherever lacking. Pasteurization should be considered in medium- and long-ripened cheeses made from raw milk, especially in high producing and exporting countries such as France and Italy.

6. Bibliography

Barbieri, F., Montanari, C., Gardini, F., Tabanelli, G., 2019: Biogenic amine production by lactic acid bacteria: A review. *Foods*, 8(1). 27 p.

Benkerroum, N., 2016: Biogenic Amines in Dairy Products: Origin, Incidence, and Control Means. *Comprehensive reviews in food science and food safety*, 15. p. 801-826.

Bonczar, G., Filipczak-Fiutak, M., Pluta-Kubica, A., Duda, I., Walczycka, M., Staruch, L., 2018: The range of protein hydrolysis and biogenic amines content in selected acid- and rennet-curd cheeses. *Chemical Papers*, 72. p. 2599-2606.

Borah, A., Paul, R., Mazumder, M. K., Bhattacharjee, N., 2013: Contribution of β-phenethylamine, a component of chocolate and wine, to dopaminergic neurodegeneration: Implications for the pathogenesis of parkinson's disease. *Neuroscience Bulletin*, 29(5). p. 655-660.

Burdychová, R., 2009: Study of enterococci during cheese manufacture and ripening and evaluation of their role in tyramine production. *Acta univ. agric. et silvic. Mendel. Brun*., LVII, No. 5. p. 49-56.

Codex Alimentarius, 1978: General Standard for Cheese CXS 283-1978. URL: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B283-1978%252FCXS_283e.pdf. Accessed: 22 July 2020

Combarros-Fuertes, P., Fernández, D., Arenas, R., Diezhandino, I., Tornadijo, M.E., Fresno, J.M., 2016: Biogenic amines in Zamorano cheese: factors involved in their accumulation. *J Sci Food Agric*, 96. p. 295-305.

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *OJ*, L 338, 22.12.2005. p. 1-26.

del Rio, B., Redruello, B., Fernandez, M., Martin, M. C., Ladero, V., Alvarez, M. A., 2020: The biogenic amine tryptamine, unlike β-phenylethylamine, shows in vitro cytotoxicity at concentrations that have been found in foods. *Food Chemistry*, 331. 7 p.

del Rio, B., Redruello, B., Linares, D.M., et al., 2017: The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture. *Food Chemistry*, 218. p. 249-255.

del Rio, B., Redruello, B., Linares, D. M., Ladero, V., Ruas-Madiedo, P., Fernandez, M., Martin, M. C., Alvarez, M. A., 2018: Spermine and spermidine are cytotoxic towards intestinal cell cultures, but are they a health hazard at concentrations found in foods*? Food Chemistry*, 269. p. 321-326.

del Rio, B., Redruello, B., Linares, D.M., Ladero, V., Ruas-Madiedo, P., Fernandez, M., Martin, M.C., Alvarez, M.A., 2019: The biogenic amines putrescine and cadaverine show in vitro cytotoxicity at concentrations that can be found in foods. *Scientific Reports,* 9: 120. 7 p.

Diezhandino, I., de Abajo, M., Renes, E., Arenas, R., Fernández, D., Fresno, J.M., 2015: Evolution of Free Amino Acids and Biogenic Amines contents during ripening of a Spanish blue cheese (Valdeón cheese). *Caracterización tecnológica, química, bioquímica y sensorial del queso de Valdeón con I.G.P. durante la maduración.* p. 157-177.

Doeun, D., Davaatseren, M., Chung, MS., 2017: Biogenic amines in foods. *Food Science and Biotechnology*, 26. p. 1463-1474.

EFSA, 2017: Assessment of the incidents of histamine intoxication in some EU countries. *EFSA supporting publications 2017*, 14(9):EN-1301. 37 p. URL: https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2017.EN-1301. Accessed: 24 June 2020.

EFSA, 2011: Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal 2011*, 9(10):2393. 93 p. URL: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2011.2393. Accessed: 24 June 2020.

Eko sir Puđa, n.d.: Livanjski sir. URL: https://livanjskisir.com/galerija/. Accessed: 22 October 2020.

Erdag, D., Merhan, O., Yildiz, B., 2018: Biochemical and Pharmacological Properties of Biogenic Amines. URL: https://www.intechopen.com/books/biogenic-amines/biochemical-and-pharmacological-properties-of-biogenic-amines. Accessed: 30 August 2020.

Espinosa-Pesqueira, D., Hernández-Herrero, M.M., Roig-Sagués, A.X., 2018: High Hydrostatic Pressure as a Tool to Reduce Formation of Biogenic Amines in Artisanal Spanish Cheeses. *Foods*, 7, 137. p. 88-103.

Eurostat, 2020: EU trade since 1988 by HS2,4,6 and CN8. URL: http://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do. Accessed: 11 October 2020.

FAO/WHO, 2012: Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products: Meeting Report. 112 p. URL: http://www.fao.org/fileadmin/user_upload/agns/news_events/Histamine_Final_Report.pdf. Accessed: 30 August 2020.

Fernández, A., Linares, D.M., del Río, B., Ladero, V., Alvarez, M.A., 2007: HPLC quantification of biogenic amines in cheeses: correlation with PCR-detection of tyramine-producing microorganisms. *Journal of Dairy Research*, 74. p. 276–282.

Forzale, F., Giorgi, M., Pedonese, F., Nuvoloni, R., D'Ascenzi, C., Rindi, S., 2011: Contenuto Di Amine Biogene Nel "Pecorino Del Parco Di Migliarino-San Rossore". *Italian Journal of Food Safety*, 1. p. 149-153.

Garmiene, G., Salomskiene, J., Stankiene, J., Januškeviciene, G., Zaborskiene, G., 2012: Changing of biogenic amine content in cheese during its manufacture. *Milchwissenschaft*, Vol 67(3). p. 308-311.

Gobbetti, M., Neviani, E., Fox, P., Varanini, G.M., 2018: The Cheeses of Italy: Science and Technology. Springer. 274 p.

Gosetti, F., Mazzucco, E., Gianotti, V., Polati, S., Gennaro, M.C., 2007: High performance liquid chromatography/tandem mass spectrometry determination of biogenic amines in typical Piedmont cheeses. *Journal of Chromatography A*, 1149. p. 151-157.

Harbutt, J. (Ed), 2015: World Cheese Book. London, DK. 352 p.

INAO, 2020: Institut national de l'origine et de la qualité. URL: https://www.inao.gouv.fr/Les-signes-officiels-de-la-qualite-et-de-l-origine-SIQO/Appellation-d-origine-protegee-controlee-AOP-AOC. Accessed: 25 August 2020.

Ingles, D.L., Black, J.F., Gallimore, D., Tindale, R., Shaw, K.J., 1985: Estimation of biogenic-amines in foods. *J. Sci. Food Agric*, 36. p. 402-406.

Innocente, N., D'Agostin, P., 2002: Formation of Biogenic Amines in a Typical Semihard Italian Cheese. *Journal of Food Protection*, Vol. 65, No. 9. p.1498-1501.

Komprda, T., Dohnal, V., Závodníková, R., 2008: Contents of some biologically active amines in a Czech blue-vein cheese. *Czech J. Food Sci*., 26. p. 428-440.

Kulinarisches Erbe der Schweiz, n.d.: Schabziger. URL: https://www.patrimoineculinaire.ch/Produkte?text=Schabziger&canton=&categorie=#1. Accessed: 26 August 2020.

Ladero, V., Fernández, M., Cuesta, I., Alvarez, M.A., 2010: Quantitative detection and identification of tyramine-producing enterococci and lactobacilli in cheese by multiplex qPCR. *Food Microbiology*, 27(7). p. 933-939.

Landwirt.com, n.d, URL: https://www.landwirt.com/Kaese-Praemierung-fuer-Schaerdinger-und-Tirol-Milch,,16175,,Bericht.html. Accessed: 22 October 2020.

Liberatore, L., 2015: Merceologia degli alimenti. Milano, Franco Angeli. p.106-107.

Linares, D. M., del Río, B., Ladero, V., Martínez, N., Fernández, M., Martín, M. C., Álvarez, M.A., 2012: Factors influencing biogenic amines accumulation in dairy products. *Frontiers in Microbiology,* 3, 180.

Madejska, A., Michalski, M., Pawul-Gruba, M., Osek, J., 2018: Histamine content in rennet ripening cheeses during storage at different temperatures and times. *J Vet Res*, 62. p. 65-69.

Manca, G., Porcu, A., Ru, A., Salaris, M., Franco, M.A., De Santis, E.P.L., 2015: Comparison of γ-aminobutyric acid and biogenic amine content of different types of ewe's milk cheese produced in Sardinia, Italy. *Italian Journal of Food Safety*, 4(2). p. 123-128.

Manca, G., Ru, A., Siddi, G., Maria, M. A., Murittu, G., De Santis, P. L., 2020: Biogenic amines content in Fiore Sardo cheese in relation to free amino acids and physicochemical characteristics. *Italian Journal of Food Safety*, 9(1). p. 48-53.

Marijan, A., Džaja, P., Bogdanović, T., Škoko, I., Cvetnić, Ž., Dobranić, V., Zdolec, N., Šatrović, E., Severin, K., 2014: Influence of ripening time on the amount of certain biogenic amines in rind and core of cow milk Livno cheese. *Mljekarstvo*, 64(3). p. 159-169.

Martuscelli, M., Gardini, F., Torriani, S., Mastrocola, D., Serio, A., Chaves-Lopez, C., Schirone, M., Suzzi, G., 2005: Production of biogenic amines during the ripening of Pecorino Abruzzese cheese. *International Dairy Journal,* 15. p. 571-578.

Mayer, H. K., Fiechter, G., Fischer, E., 2010: A new ultra-pressure liquid chromatography method for the determination of biogenic amines in cheese. *Journal of Chromatography A*, 1217(19). p. 3251-3257.

McCabe-Sellers, B.J., Staggs, C.G., Bogle, M.L., 2006: Tyramine in foods and monoamine oxidase inhibitor drugs: A crossroad where medicine, nutrition, pharmacy, and food industry converge. *Journal of Food Composition and Analysis*, 19. p. 58-65.

McSweeney, P.L.H., Fox, P.F., Cotter, P.D., Everett, D.W. (Eds.), 2017: Cheese: Chemistry, Physics and Microbiology. 4th ed. London, Academic Press. 1302 p.

Melk.no, n.d., URL: https://www.melk.no/Melkekilden/Meieriprodukter/Ost/Hvilke-oster-spiser-vi-mest-av-i-Norge. Accessed: 27 September 2020.

O'Brien, N.M., O'Connor, T.P., O'Callaghan, J., Dobson, A.D.W., 2004: Toxins in Cheese. *Cheese: Chemistry, Physics and Microbiology,* 3rd ed., volume 1: General Aspects. p. 561-571.

Parmashop, n.d., URL: https://www.parmashop.com/dutch/organic-pecorino-toscano-seasoned-in-grotta.html. Accesses: 22 October 2020.

Perin, L.M., Nero, L.A., 2019: Chapter 7. Occurrence of Biogenic Amines in Cheese: An Overview. *Biogenic Amines in Food: Analysis, Occurrence and Toxicity*. p. 119-132.

Pinho, O., Pintado, A.I.E., Gomes, A.M.P., Pintado, M.M.E., Malcata, F.X., Ferreira, I.M.P.L.V.O., 2004: Interrelationships Among Microbiological, Physicochemical, and Biochemical Properties of Terrincho Cheese, with Emphasis on Biogenic Amines. *Journal of Food Protection*, Vol. 67, No. 12. p. 2779-2785.

Pintado, A.I.E., Pinho, O., Ferreira, I.M.P.L.V.O., Pintado, M.M.E., Gomes, A.M.P., Malcata, F.X., 2008: Microbiological, biochemical and biogenic amine profiles of Terrincho cheese manufactured in several dairy farms. *International Dairy Journal,* 18. p. 631-640.

Poveda, J.M., Chicón, R., Cabezas, L., 2015: Biogenic amine content and proteolysis in Manchego cheese manufactured with *Lactobacillus paracasei* subsp. *paracasei* as adjunct and other autochthonous strains as starters. *International Dairy Journal*, 47. p. 94-101.

Qureshi, T.M., Vermeer, C., Vegarud, G.E., Abrahamsen, R.K., Skeie, S., 2013: Formation of biogenic amines and vitamin K contents in the Norwegian autochthonous cheese Gamalost during ripening. *Dairy Sci. & Technol*., 93. p. 303-314.

Regulation (EU) No 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs, *OJ*, L 343, 14.12.2012. p. 1-29.

Renes, E., Diezhandino, I., Fernández, D., Ferrazza, R.E., Tornadijo, M.E., Fresno, J.M., 2014: Effect of autochthonous starter cultures on the biogenic amine content of ewe's milk cheese throughout ripening. *Food Microbiology*, 44. p. 271-277.

Rivoira, L., Castiglioni, M., Bruzzoniti, M.C., 2019: Chromatographic determination of biogenic amines in four typical Italian cheeses: correlations with processing and nutritional characteristics through a chemometric approach. *J Sci Food Agric*, 99. p. 4963-4968.

Ruiz-Capillas, C., Herrero, A., 2019: Impact of Biogenic Amines on Food Quality and Safety. *Foods*, 8(2), 62.

Salque, M., Bogucki, P.I., Pyzel, J., Sobkowiak-Tabaka, I., Grygiel, R., Szmyt, M., Evershed, R.P., 2013: Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature,* 493. p. 522-525.

Sawilska-Rautenstrauch, D., Fonberg-Broczek, M., Gawarska, H., Starski, A., Jedra, M., Karłowski, K., 2010: Wystepowanie amin biogennych w serach dojrzewajacych pochodzacych z rynku warszawskiego [Occurrence of biogenic amines in ripening cheeses taken from the Warsaw market]. *Rocz Panstw Zakl Hig*, 61(4). p. 361-365.

Schirone, M., Tofalo, R., Mazzone, G., Corsetti, A., Suzzi, G., 2011: Biogenic amine content and microbiological profile of Pecorino di Farindola cheese. *Food Microbiology*, 28. p. 128-136.

Schirone, M., Tofalo, R., Visciano, P., Corsetti, A., Suzzi, G., 2012: Biogenic amines in Italian Pecorino cheese. *Frontiers in Microbiology,* 3, 171. 9 p.

Schirone, M., Tofalo, R., Fasoli, G., Perpetuini, G., Corsetti, A., Manetta, A.C., Ciarrocchi, A., Suzzi, G., 2013: High content of biogenic amines in Pecorino cheeses. *Food Microbiology*, 34. p. 137-144.

Simon, P., Pásztor-Huszár, K., Dalmadi, I., Kiskó, G., Simon-Sarkadi, L., 2013: Biogenic amine content in cheese produced with different selected lactic acid bacterial strains. *Studia Universitatis Babes-Bolyai Chemia,* Vol 2013(3). p. 43-48.

Tham, W., 1988: Histamine formation by enterococci isolated from home-made goat cheeses. *International Journal of Food Microbiology*, 7. p. 103-108.

Tham, W., Karp, G., Danielsson-Tham, M-L., 1990: Histamine formation by enterococci in goat cheese. *International Journal of Food Microbiology*, 11. p. 225-229.

The Spanish Hamper, n.d., URL: https://www.thespanishhamper.co.uk/wp-content/uploads/2013/12/Manchego-Semi-Cured-1Kg-PGI-The-Spanish-Hamper-v3.jpg. Accessed: 14 September 2020.

Thesupermat, 2015: Du Roquefort, fromage de l'Aveyron. URL: https://en.wikipedia.org/wiki/Roquefort#/media/File:Wikicheese_-_Roquefort_-_20150417_-_003.jpg. Accessed: 14 September 2020.

Tofalo, R., Perpetuini, G., Battistelli, N., Pepe, A., Ianni, A., Martino, G., Suzzi, G., 2019: Accumulation γ-Aminobutyric Acid and Biogenic Amines in a Traditional Raw Milk Ewe's Cheese. *Foods*, 8, 401. 13 p.

Torracca, B., Nuvoloni, R., Ducci, M., Bacci, C., Pedonese, F., 2015: Biogenic Amines Content of Four Types of "Pecorino" Cheese Manufactured in Tuscany. *International Journal of Food Properties*, 18:5. p. 999-1005

Torracca, B., Pedonese, F., López, M.B., Turchi, B., Fratini, F., Nuvoloni, R., 2016: Effect of milk pasteurisation and of ripening in a cave on biogenic amine content and sensory properties of a pecorino cheese. *International Dairy Journal*, 61. p. 189-195.

Wang, C., Ruan, P., Zhao, Y., et al., 2017: Spermidine/spermine N1-acetyltransferase regulates cell growth and metastasis via AKT/β-catenin signaling pathways in hepatocellular and colorectal carcinoma cells. *Oncotarget*, 8(1). p. 1092-1109.

Wechsler, D., Walther, B., Jakob, E., Winkler, H, 2009: Importance Des Amines Biogènes Dans L'alimentation Et Présence Dans Les Différents Fromages. *ALP forum*, 73. 25 p.

Wechsler, D., Winkler, H., Guggisberg, D., 2013: Optimierung der teigeigenschaften von Appenzeller mit festem und zu trockenem teig. *ALP forum*, 98. 20 p.

Zazzu, C., Addis, M., Caredda, M., Scintu, M. F., Piredda, G., Sanna, G., 2019: Biogenic amines in traditional fiore sardo pdo sheep cheese: Assessment, Validation and Application of an RP-HPLC-DAD-UV Method. *Separations*, 6, 11. 17 p.

Özogul, Y., Özogul, F., 2019: Chapter 1. Biogenic Amines Formation, Toxicity, Regulations in Food. *Biogenic Amines in Food: Analysis, Occurrence and Toxicity*. p. 1-17.

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