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A REVIEW ON BIOGENIC AMINES IN CHEESE

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Abstract

Biogenic amines fulfill different roles in the proper functioning of the human body. They result from oxidation processes in food. Biogenic amines are mainly found in fermented products such as wine, fish, meat and cheeses. Their existence in small quantities does not affect people's health, but in a larger volume leads to poisoning. The most common biogenic amine poisonings were found in tyramine and histamine. The main symptoms of poisoning are increased blood pressure and headaches. The concentration of biogenic amines in cheeses is influenced by several factors: temperature, baking time, pH, the culture used, environmental conditions, the origin of the raw material (raw or pasteurized milk and microbiological load. Compliance with hygienic conditions and the correct manufacturing process does not increase the amount of biogenic amines.

***Key words**: cheeses, control, health, methods, storage.*

INTRODUCTION

Biogenic amines have roles in blood pressure, synaptic transmission, and cell growth. Foods containing biogenic amines are broken down by the human body via monoamine oxidase and diamine oxidase (Broadley et al., 2010). This type of process can be different for each person, psychological factors, genetic factors, foods rich in biogenic amines or products that do not have beneficial effects on human health (drugs, tobacco and alcohol) called monoamine oxidase inhibitors (EFSA, 2011).

Biogenic amines can appear in fermented products at different stages, such as the hygiene of raw materials, the manufacturing process, the state of fermentation and the time of fermentation. Biogenic amines in food do not disturb human health if they are consumed in low quantities, but their existence in large amounts can cause health problems and poisoning (Doeun et al., 2017). Biogenic amines are formed as a result of more steps: microbial decarboxylation of amino acids with the help of microorganisms, reductive amination, and the last step is transamination of ketones and aldehydes (Wójcik et al., 2024). The main factor with impacts to composition of biogenic amines is the decarboxylase capacity of amino acids, followed by the pH level, free amino acids, water activity, temperature and the proportion of the microbial population. The presence of biogenic amines from cheeses is due to non-starter lactic bacteria *(Enterococcus, Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus*) and some Gram-negative bacteria (*Pseudomonadaceae* and *Enterobacteriaceae*) (Andic et al., 2010). The most common intoxications were found in histamine and tyramine, the presence of other biogenic amines (putrescine, cadaverine or phenylethylamine) can accentuate the negative effect of histamine (Omer et al., 2021). Therefore, every stage should be done correctly, and in hygienic conditions to reduce any risks that can affect the health of the consumers (Ruiz-Capillas & Herrero, 2019). The contamination process can appear in different stages, such as production, distribution, and consumption. The methods of prevention of biogenic amines can be: using high quality raw material, controlling the temperature, and using good manufacturing practices (Dapkevicius et al., 2000). Biogenic amines can influence food quality, with subsequent impact on people's health, that's why it's important to accord the necessary importance.

MATERIALS AND METHODS

To present a database for this study we conducted a systematic search of scientific publications in the Google Scholar, ScienceDirect PubMed and Scopus databases. In the tables below, the concentrations of the main biogenic amines (histamine, tyramine, putrescine, cadaverine, phenylethylamine) in milk from different animal species (cow, sheep and goat) and various types of cheese are highlighted.

RESULTS AND DISCUSSIONS

Tyramine

Cheese incorporates a large amount of free amino acids as a result of proteolysis, a favorable temperature, pH and water activity, it is an environment that contains the necessary characteristics for optimum biogenic amines development (Benkerroum et al., 2016). In the case of histamine, there are several cases of poisoning, the most frequent being in Ceddar cheese and Swiss cheese (Vale & Gloria, 1998). According to Linares et al. (2013) tyramine poisoning leads to a hypertensive crisis characterized by an increase in blood pressure of ≥180/120 mmHg and severe headaches. 150-750 mg/kg is the allowed concentration for tyramine. Levels greater than 1080 mg/kg are harmful to human health (Shalaby, 1996). EFSA (2011) recommends that the maximum level of tyramine should be between 110-750 mg/kg.

Mohamed et al. (2010) analyzed which are the levels of biogenic amines from cheeses (Cheddar, Ras and Gouda). The results indicated that tyramine recorded the highest tyramine content (93.33%), followed by cadaverine (80%).

Histamine

The main food with the most histamine poisoning is fish, followed by cheese. Cheddar and Swiss cheese contain high amounts of histamine and are responsible for producing more outbreaks (Stratton et al., 1991). Lehane & Olley (2000) state that for sensitive people, an amount of 5-10 mg of ingested histamine is dangerous, a limit of 10 mg of histamine is stated to be tolerated by the human body, a concentration of 100 mg presents a degree of toxicity average, and the 1000 mg level is considered toxic. EFSA (2011) recommends that the maximum level of histamine be between 50-100 mg/kg, 20-28 mg/kg for phenylethylamine, 250-950 mg/kg for the total concentration of biogenic amines allowed for consumption.

Among the three types of analyzed cheeses, the highest concentration of biogenic amines was found in Ras cheese histamine (86.67%), followed by Cheddar cheese (73.33%), and Gouda (53.33%). The mean value of histamine was 12.98 for Cheddar cheese, 17.43 for Ras cheese and 7.65 for Gouda cheese (Mohamed et al., 2010).

Table 1. Biogenic amines from cheeses (mg/kg)

	Histamine	Tyramine	Putrescine	Cadaverine	Reference
Blue cheese	20.3-62.5	63.1-9.9	20.7-61.5	8.30-11.8	EFSA (2011)
Blue pasteurized milk cheese	0-127.02	0-526.63	0-237.56	40-89.4	Fernandez et al. (2007)
Blue raw milk cheese	0-1041.81	0-1051.98	0-875.8	0-756.78	Fernandez et al. (2007)
Brie	30.9-40.0	-	-	-	Ladero et al. (2009)
Camembert	0-40.0	0-4.9	0-82.2	0-266.8	Ladero et al. (2009)
Cheddar	25.4-40.0	-	48	-	Valsamaki et al. (2000)
Curd	1.0	2.0	0.9	0.7	Novella-Rodriguez et al. (2002)
Emmental	23.5-117.5	52.5-64.5	3.9-38.0	98.3	Ladero et al, 2009; Mayer et al. (2010)
Feta cheese	84.6	246	193	82.8	Valsamaki et al. (2000)
Fresh cheese	3.1-37.5	12.5-48.0	5.3-40	10.5-44.7	EFSA (2011)
Gorgonzola	23.7-255.3	13.2-247.6	3.2-31.3	33.7-748.2	Mayer et al. (2010)
Mozzarella	39.7	-	-	-	Ladero et al. (2009)
Parmesan	10.9-40.0	6.4-29.9	1.8-75.9	3.2-15.6	Innocente, N. & D'Agostin (2002)
Pasteurized goat's milk cheese	6.2	10.7	14.4	32.5	Novella-Rodriguez et al. (2004)

Table 2. Biogenic amine composition from different species (mg/kg)

Product type	Type of milk	Cadaverine	Histamine	Phenylethylamine	Putrescine	Reference
Raw milk	cow	-	-	-	-	Novella-Rodriguez et al. (2000)
Cheeses (raw milk)	sheep	123.0	50	-	107.6	Mercogliano et al. (2010)
	sheep	26.7	-	28.5	394.0	Schirone et al. (2011)
	goat	349.6	15.5	9.2	217.7	Pinho et al. (2004)
	goat	88.6	88.3	11.6	191.7	Novella-Rodriguez et al. (2002)
	goat	93.5	116	159	940	Galgano et al. (2001)
	cow	-	-	-	-	Fernandez et al. (2007b)
Cheese (raw milk) Parmigiano	cow	3.2	10.9	-	1.8	Mayer et al. (2010)
	cow	15.4	28.4	9.3	75.7	Innocente & D'Agostin (2002)
Extra hard grana	cow	-	248	-	-	Mayer et al. (2010)
Feta	goat	82.7	84.5	4.8	192	Valsamaki et al. (2000)
Emmental hard	cow	98.2	23.2	-	37	Mayer et al., (2010)
Cheddar	cow	-	25.3	-	4.7	Mayer et al. (2010)
Semi hard	cow	-	-	-	-	Mayer et al. (2010)
Gouda	cow	-	-	-	-	Mayer et al. (2010)
Edam	cow	-	3.1	-	-	Mayer et al. (2010)
Gorgonzola	cow	-	23.6	-	-	Mayer et al. (2010)
Gorgonzola	cow	33.6	255.2	-	3.1	Mayer et al. (2010)

Microorganisms that produce biogenic amines

A greater possibility of the appearance of biogenic amines is found in fermented foods. Biogenic amines can appear from lactic acid bacteria type even if they don't represent a potential for toxicity commonly. Big quantities of biogenic amines were found in streptococci, lactococci, pediococci and enterococci (Özogul & Hamed, 2018). Following genetic studies, it was found that many of these strains harbor genes or operons that encode decarboxylating enzymes or other pathways involved in the biosynthesis of biogenic amines (Marcobal et al., 2012; Wunderlichová et al., 2014). Next, the main non-starter lactic acid species associated with fermented products and involved in the production of biogenic amines are described.

Enterococci

They have impressive ecological adaptability and an ability to grow even under adverse conditions. The enterococci are characterized by their resistance to salt and low pH, the reason why they represent an ideal substrate for the fermentation of traditional cheeses (Foulquié et al., 2006). Analyses made by different sources from fermented products, such as cheeses, fish, meat, human feces and wine showed that the presence of enterococci from these is correlated with the production of biogenic amines (the most representative biogenic amine was tyramine) (Bover et al., 2001; Ladero et al., 2012). The enterococci were also found in artisanal cheeses due to their role in natural microbiota, as well as demonstrated that they can be present in bigger quantities than lactobacilli and lactococci (Suzzi et al., 2000). Starter culture species collected from cheeses represent a high risk of contamination and may co-exist with biogenic amines even though enterococci are generally not found in starter cultures. In dairy products, for example in the milk were detected many strains of *Enterococcus faecalis, E. durans* and *E. faecium* (Giraffa, 2003). Some species of this strain in cheeses have been shown to generate tyramine production (Sarantinopoulos et al., 2001). Rea et al. (2004) analyzed how the production of biogenic amines (tyramine) in Cheddar cheese was affected by the manufacturing and ripening processes of different strains of *E. casseliflavus, E. durans, E. faecium* and *E. faecalis*. The results demonstrated that, after 9 months of ripening, *E. durans* produced the highest concentration of tyramine, and *E. casseliflavus* is the only strain that does not produce tyramine compared to all strains analysed.

Lactobacilli

Lactobacilli are found in various fermented products and are an important category of biogenic amine producers (Spano et al., 2010). *Lactobacillus* species such as *Lactobacillus* *helveticus, Lactobacillus curvatus* or *Lactobacillus buchneri* as well as Gram-negative bacteria have the ability to produce biogenic amines in cheeses (Ladero et al., 2008). At the same time, the presence of histamine was detected in several strains of *L. parabuchneri* isolated from cheeses (Wüthrich et al., 2017). Pachlová et al. (2018) analysed the effect that have *L. curvatus* subsp. *curvatus* and *L. paracasei* on cheeses with these types of strains. Following the study, it was proven that these strains can accumulate tyramine up to 190 mg/kg in dairy products over a maturation period of 90 days.

Streptococci

Although several species of streptococci are important for human health, some of them cause diseases such as endocarditis, bacterial pneumonia, meningitis. In fermented products, such as cheeses, one of the most used streptococci as an initial culture is *S. thermophilus* due to its valuable components, due to the fact that it is present in most stages of their manufacture, due to its baking relationship together with biogenic amines (Delorme, 2008). The presence of tyramine was found in *Streptococcus macedonicus* species from Greek Kasseri cheese (Georgalaki et al., 2000).

Lactococci

The most important lactic bacteria involved in the milk industry, as well as in the manufacture of fermented milk products, are lactococci and lactic species: *Lactococcus* subsp. *lactis, Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis* biovar *diacetylactis* (Fox et al., 2004). The main putreiscin producing species in dairy products are *E. hirae, E. faecalis* and *Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis, L. curvatus* and *L. brevis* represents another category of species that can produce putreiscine from agmatine (Ladero et al., 2011; Ladero et al., 2012).

It has been shown that in addition to rotting *Lc. lactis* is capable of producing other types of biogenic amines (2-phenylethylamine and tyramine) which was found in strains of bacteriocinogenic lactococci isolated from raw goat milk (Coton et al., 2011). Two types of strains (*Lc. lactis* subsp *strains*) were detected during the ripening process (90 days) in an assortment of Dutch cheese (Flasarová et al., 2016). Lactococci are a key element for the development of biogenic amines, as the results also demonstrated: the levels of biogenic amines in the control group were very low compared to the control group, where the amounts of biogenic amines obtained were between 500 mg/kg tyramine and 800 mg/kg putrescine (Santos et al., 2003).

The presence of biogeneous amines depending on the period of storage

10 types of commercial Kashar cheese were randomly analyzed, 5 of them were matured and the other 5 were fresh. The values of the main biogenic amines in cheeses (histamine, cadaverine, tyramine, phenylethylamine, etc.), pH and moisture content were evaluated. The experiment lasted 3 weeks, the cheeses were kept at a temperature of 4 ± 1°C. At the end of the study, it was found that matured Kashar cheese has a higher content of biogenic amines compared to fresh Kashar cheese. Regarding the degree of toxicity, toxic levels of histamine were detected in matured cheeses, compared to fresh cheeses that contained no risk of toxicity (Şahin et al., 2019).

The production of biogenic amines was monitored in blue cheese at the beginning of ripening process (12 days) in the following order: γ-irradiated (6, 4 and 2 kGy) and non-irradiated during storage at 5°C. Following irradiation treatment, the cheese's dry weight resulted and biogenic amine content descended from 977 to 430 mg/kg after the irradiation process at 6 kGy, but increased from 1022 to 2311 mg/kg at 90 days in non-irradiated cheeses.

Tyramine is a powerful biogenic amine due to its ability to withstand radiation, it was found that 95% of all non-irradiated cheeses were made up of tyramine. Histamine decreased from 35 mg/kg unirradiated cheese (90 days of processing) to 29 mg/kg after irradiation, then was not detected at all. This procedure is useful for the safety process and detect biogenic amine values in cheeses (Rabie et al., 2011).

The degree of evolution of amino acids and biogenic amines over 4 months of ripening in Feta cheese was investigated. At 60 days of storage, the value of biogenic amines was 330 mg/kg, followed by increasing values to 617 mg/kg at 120 days. During the ripening period, the lowest levels of biogenic amines were identified in phenylethylamine and tryptamine compared to the other predominant amine types, tyramine and putrescine, which recorded values of 69.7% at 60 days and 71.2% at 120 days of storage. During the ripening period, the content of biogenic amines reached 615 mg/kg at 120 days and from 1 to 15 days and from 60 to 120 days the maximum levels were reached. Feta cheese has a high salt content and a pH at low levels, this is the reason why biogenic amines cannot develop under optimal conditions and the decarboxylation process of amino acids cannot take place (Valsamaki et al., 2000). The total content of biogenic amines is 10 to 2000 times higher in ripened cheeses compared to unripened ones (Novella-Rodríguez et al., 2003).

The presence of biogenic amines depending on cultures

Unpasteurized low-sodium Cheddar cheeses were analysed with products containing commercial probiotics (*Lactiplantibacillus* and *Bifidobacterium animalis* ssp. *lactis*) and biogenic amine which produce strains (*Levilactobacillus brevis, L. sacrimbacillus ATCC 362CC; L. sacrimbacillus ATCC 362CC*) to evaluate the content of biogenic amines according to the cultures used over a ripening period of 125 days at different temperatures of 15 or 4°C. The results highlighted the fact that for 125 days maintained at a temperature of 4°C, in the cheese samples inoculated with probiotics the amount of biogenic amines was reduced more compared to those inoculated with biogenic foods. Probiotic cheeses ripened at 15°C had the highest content of biogenic amines (1332 mg/kg). After exceeding the 13-day ripening period, it was observed that the populations of probiotics decreased significantly, and their ability to degrade biogenic amines decreased. To limit the production of biogenic amines in Cheddar cheese, probiotic cultures can be used as an additional option (Gentès et al., 2024).

The impact of different types of starter cultures and ripening periods on biogenic amines (putrescine, tryptamine, phenylethylamine, histamine, spermine and spermine) in cheeses produced from pasteurized sheep's milk were analysed (Renes et al., 2014). The study was based on the analysis of four batches of cheese in duplicate, matured for 7 months. Cheese samples were analyzed by High-performance liquid chromatography (HPLC). Has been demonstrated that tryptamine, phenylethylamine and spermine represent 80% of all biogenic amines studied.

The production of biogenic amines was reduced as a result of the application of starter cultures. The content of biogenic amines was significantly lower ($p < 0.001$) in the batches consisting of autochthonous starter cultures (*Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*) also in combination with *Lactobacillus plantaru*.

Butor et al. (2023) set out to investigate the factors responsible for degrading the content of biogenic amines (cadaverine, histamine, putrescine, tyramine, putrescine and phenylethylamine) in foods using *Bacillus subtilis* from Gouda cheeses.

Aerobic and anaerobic conditions combined with different temperatures (30°C, 8°C, 23°C) correlated with medium pH (from 5.0 to 8.0) led to a decrease in biogenic amines.

Cultivation of *Bacillus subtilis* was in an environment consisting of biogenic amines. Cultivation temperature and pH of the medium had a significant effect on the degradation of biogenic amines by *Bacillus subtilis*. At the end of the cultivation period, the content of studied biogenic amines decreased by 60-90%. Due to the positive effects that the strain has it can be used in the food industry to maximize consumer safety.

The presence of biogeneous amines depending on thermal treatments

The levels of biogenic amines were monitored by Novella et al. (2002) in goat cheese under two different conditions: pasteurization or under high hydrostatic pressure. Tyramine was found in both types of cheese, then of histamine, cadaverine and putreiscin. For some of the amines, no changes were observed in the type of treatment applied to the milk (pressurization or pasteurization).

The ripening process can be reduced by using high-pressure treatment, once with their application can change the structure of biogenic amines. The most representative results were observed for tyramine, reaching three times higher levels compared to control samples at a pressure of 50 MPa (MegaPascals). By increasing the high pressure to 400 MPa for a shorter time duration of 5 minutes, the heat treatment mechanism acted differently, resulting in lower tyramine values compared to control cheeses (Novella et al., 2002).

Methods of analysis ion exchange chromatography coupled with tandem detection mass spectrometry

The profile of biogenic amines can be established using chromatography with ion spectrometric detection; therefore 10 biogenic amines were determined from different cheeses. The biogenic amines taken were tyramine, 2-phenylethylamine, spermine, spermidine, trimethylamine, histamine, tryptamine and agmatine. Compared to other existing methods in the literature, an advantage of this method is that the extraction of biogenic amines is determined only with water, without the need for another sample cleaning procedure. At the end of the study, it was illustrated that this method is useful for determining the profile of biogenic amines in cheeses and can also be used for determining food products (Ščavničar et al., 2018).

Salt-assisted liquid-liquid extraction

The next stages of analysis are based on following steps: soluble pre-extraction (using hydrochloric acid), followed by derivatization, salinization and biogenic amine profile analysis by HPLC.

At this time, the extraction time of the hydrochloric acid improved, the reduction from the influence of the average pH on the sample as well as the dose of the reagent. Histamines, dimethylamine and putrescine recorded the most significant levels among the biogenic amines. The method stands out for the simplification of the preparation of the sample and the general improvement of the process, being an agreeable alternative in relation to the existing methods.

They can also be used to analyze the structure of biogenic amines from the food matrix or fish meat (Ramos et al., 2020).

Ultra-High Performance Liquid Chromatography

By using the UHPLC analysis method, the content of biogenic amines in different types of cheeses, namely in 151 samples, was evaluated. Of the cheeses analyzed, only 5% presented total concentrations of biogenic amines higher than 90 mg/100 g, and some samples had values from 150 mg/100 g cheese to 313 mg/100 g cheese. Histamine concentrations were 116 mg/100 g, in a few cases (5%) histamine levels were higher than 17 mg/100 g. The other types of amines in cheeses were detected at different values: tyramine 72%, putrescine 70%, cadaverine 48%, tryptamine 15% with an average concentration of 0.3 mg/100 g (Mayer & Fiechter, 2018). In the case of HPLC-type analysis, each sample is allocated between 20 and 60 minutes, which means a long time, thus UHPLC analysis improves resolution, sensitivity and contributes to decreasing the analysis time for the studied probe. (Loizzo et al., 2013). Mayer et al. (2010), propose a method for the analysis of amines by UHPLC by separating amines in 9 minutes. The results had the following values: 13.5% of the samples had a histamine content above 10 mg/100 g, 22.4% recorded a tyramine content higher than 10 mg/100 g, 8.6% of the samples had a concentration of cadaverine or putrescine greater than 10.2 mg/100 g.

HPLC

A series of cheese samples were analyzed by HPLC analysis methods to determine the content of biogenic amines in them. The raw material from which the cheeses were made had two sources: raw milk or pasteurized milk, at the same time the content of biogenic amines was also evaluated depending on the ripening period. The levels of biogenic amines in cheeses ripened for a short period were lower compared to cheeses ripened for a longer period. At the same time, in terms of milk composition, raw milk recorded a higher composition in biogenic amines compared to pasteurized milk. The most significant result in the composition of biogenic amines was detected in blue cheeses from raw milk. At the same time, among the biogenic amines, tyramine was the most frequently encountered. Through the PCR method, the bacteria that produce tyramine were determined. Following this analysis, there was an affirmative link between the results of the detection of bacteria that produce tyramine by PCR or by the HPLC method.

To reduce the development process of tyramine in cheeses it is recommended to use both methods of analysis (HPLC and PCR) (Fernández et al., 2007).

Kandasamy et al. (2021) obtained a total content of biogenic amines in the analyzed cheeses ranging from 11 to 62 mg/kg. The other biogenic amines had the following values: putrescine (3.45 mg/kg), histamine (13.45 mg/kg), 2-phenylethylamine (with values between 24.20 and 48.02 mg/kg), respectively spermine (11.0-20.45 mg/kg). The highest content of biogenic amines was 62.09 mg/kg and the lowest content was 11.21 mg/kg. Novella et al. (2003), found a similar histamine content with an average value of 18.3 mg/kg.

Sensory changes depending on storage period

In traditional Czech cheese, the influence of storage time and temperature on the development of biogenic amines was monitored (Standarová et al., 2009). Over 7 weeks, the samples were subjected to different temperature treatments: 5°C and 20°C. Biogenic amines were analyzed by the RP-HPLC method. By means of the Czech national standard, analyses related to sensory (color, smell, texture and aroma) and physical and chemical properties were carried out.

The concentrations of biogenic amines are described in order of concentration: cadaverine (120-2400 mg kg^{-1}), tyramine (110-1050 mg kg^{-1}), putrescine (70-760 mg kg^{-1}), histamine (75-41 mg kg^{-1}). The lowest values among biogenic amines were found in spermine, spermidine and tryptamine. After 17 days of storage, the total content of biogenic amines in cheese samples stored at 5°C was 900 mg kg^{-1} in contrast to cheese stored at 20°C Concentrations of biogenic amines had an upward trend during the evolution of the storage period ($P < 0.01$).

In the cheese stored at 5°C, no significant changes were observed in the content of biogenic amines at the end of the storage period (4600 mg kg^{-1}) compared to the cheese stored at 20°C where the concentration of amines tripled. Samples stored at 5°C retain their appropriate sensory characteristics for the duration of safe storage. Storage conditions and the development of biogenic amines are of particular importance in reducing the risk of consuming cheese with toxic concentrations of biogenic amines.

CONCLUSIONS

Safety it's the first requirement who must to be accomplish in food industry and the peoples health. The highest amounts of BA are found in cheeses, the reason why the control should be very rigorous. A greater potential for diseases that can affect the health of consumers is found in cheeses that contain non-uniformly distributed biogenic amines. To minimize the negative effects of biogenic amines, more careful control of the processing steps is recommended: pasteurization, irradiation, hydrostatic pressure, temperature and the starter culture used. It is advisable to respect the limits of biogenic amines in cheeses to avoid the harmful effects they have on human health. Increased attention to the influencing factors of biogenic amines should reduce their occurrence in the dairy category.

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