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Biogenic amines levels in old Kareish cheese and ripened Domiati cheese, Qena, Egypt

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Abstract

Cheeses are among high-protein containing foodstuffs in which enzymatic and microbial activities cause the formation of the biogenic amines from amino acid decarboxylation. A total of 80 samples of old Kareish cheese and ripened Domiati cheese (40 samples each) were collected from local dairy shops and vendors and dairy cattle rural house in Qena city, Egypt. The concentration of biogenic amines (Tyramine, Histamine, Cadaverine and Putrescine) were assayed via using high performance liquid chromatography (HPLC). In old Kareish cheese samples, histamine had the highest concentration followed by tyramine, cadaverine and putrescine with means of 835±32.9, 564±37.1, 556±27.4 and 537±37.5, respectively. Also, histamine (513±29.1) was the highest among the detected biogenic amines in ripened Domiati cheese followed by putrescine (492±32.2), tyramine (478×±25.3) and cadaverine (470±30). In conclusion our data showed higher levels of tyramine, histamine, putrescine and cadaverine in old Kareish and ripened Domiati cheese. The highest levels were detected in old Kareish cheese in compare with ripened Domiati cheese.

Keywords:
Biogenic amines, Cadaverine, Histamine, HPLC, Putrescine, Tyramine.

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Introduction

Dairy products have frequently been linked to foodborne intoxications as a result of contamination with formed microbial toxins, such as bacterial exotoxins, mold mycotoxins, and biogenic amines (BAs) (Benkerroum, 2016). BAs are low molecular weight basic nitrogenous compounds in which group of alkyl or aryl replace one, two, or three hydrogen atoms in ammonia. Four enzymatic reactions produce these chemicals: (I) decarboxylation, (II) transamination, (III) reductive amination, (IV) degradation of specific precursor amino compounds. The decarboxylation occurs as a result of the activity of microorganisms that can decarboxylase amino acids (Bunková et al., 2010; Feddern et al., 2019).

Biogenic amines are classified to (I) monoamines such as phenylethylamine and tyramine; (II) diamines such as histamine, putrescine, serotonin, cadaverine, and tryptamine; (III) polyamines such as agmatine, spermine, and spermidine depending on the number of amine groups (Santos, 1996; Shalaby, 1996; Ruiz-Capillas and Jiménez-Colmenero, 2004). Histamine (HIM), tyramine (TYM), putrescine (PUT), cadaverine (CAD), β-phenylethylamine, agmatine, tryptamine, serotonin (SRT), spermidine, and spermine are the most important BAs found in food.

BAs are commonly found in foods and beverages (milk, cheese, meat, fish, vegetables, and wine). Its existence and frequent detection at elevated levels in dairy products particularly in ripened cheeses continues to be an issue of concern require the increased awareness of its existing or perceived adverse health effects. Furthermore, BAs are complicated to be controlled by traditional methods because they are produced not only by microbial dairy contaminants of diverse origins, but also by some starters utilized in the fermentation and/or ripening of dairy products, such as lactic acid bacteria, yeasts, and molds (Benkerroum, 2016).

As milk provides a favorable medium for mostly all microorganisms growth, including those which produce toxic metabolites owing to its richness and having balanced chemical components (Linares et al., 2011). The amount of BAs in fermented milk can be utilized as a quality indicator and a factor for starter and probiotic culture selection (Linares et al., 2011), Bacterial strains known to be capable of biogenic amines production include *Clostridium perfringens*, *Enterobacter*, *Escherichia*, *Lactobacillus*, *Leuconostoc*, *Pseudomonads*, *Salmonella*, *Shigella*, and *Streptococcus* (Santos, 1998). Several extrinsic and intrinsic features (for example, NaCl, water activity, pH, temperature), as well as the hygienic conditions of manufacturing processes, all have a role in the production of biogenic amines in meals (Naila et al., 2010).

The methods proposed for detection and determination of biogenic amines either in dairy products and other food categories include high-performance liquid chromatography (HPLC), HPLC-tandem mass spectrometry (HPLC-MS/MS), gas chromatography, gas chromatography-mass spectrometry (Slemr and Beyermann, 1984; He et al., 2016; Plotka et al., 2016; Ngapo and Vachon, 2017).

In comparison to the other approaches, HPLC with UV detection is considered to be the most suited and is thus the most often used technology for the determination of biogenic amines in foods (Liu et al., 2018). The present study aimed to explore the presence of the biogenic amines including TYM, HIM, PUT and CAD, in some cheese samples in Qena city, Egypt using high-performance liquid chromatography (HPLC) considering their permissible limits and acceptable daily intakes (ADI) for human taking the age into consideration.

Materials and methods

Materials:

Tyramine hydrochloride (98%), Putrescine (99%), Histamine dihydrochloride (99%), Cadaverine dihydrochloride (99%), Saturated NaHCO3 solution, Dansyl chloride (99%), 1,7-diamino-heptane (98%), NaOH, Ammonia solution and Acetonitrile of HPLC grade (99.9%) were purchased from sigma-aldrich (St. Louis, MO, USA). Ultrapure water was generated by a Milli-Q system (Millipore, Bedford, MA).

Collection of samples:

A total of 80 samples of different varieties of cheese represented as old Kareish cheese and Ripened Domiati cheese (40 samples each) were collected from local dairy shops and vendors and dairy cattle rural house in Qena city, Egypt.

Preparation of standard amine solution:

Tyramine hydrochloride (126.7 mg), Putrescine dihydrochloride (182.9 mg), histamine dihydrochloride (165.7 mg) and cadaverine dihydrochloride (171.4 mg) were dissolved separately in 10 ml of ultrapure water. The final concentration of free base for each amine was 10 mg/ml of solution. A series of diluted standard solutions were prepared from the standard stock solutions.

Determination of Biogenic amines

Four biogenic amines including TYM, PUT, HIM and CAD were determined in all examined samples according to the protocol recommended by (Martuscelli et al., 2005).

A. Extraction of biogenic amine

• Two g of each sample were mixed with 20 ml of 0.1 M Hydrochloric acid (HCL) containing the internal standard (1,7-diamino-heptane, 100 mg/l) and then homogenize.
• The suspension was centrifuged at 1400 g for 20 min at 4 ˚C and collect supernatant.
• Another extraction was done with 20 ml of 0.1 M HCl.
• The two acid extracts obtained were mixed and made up to 50 ml with 0.1M HCl.
• The final acid extract was filtered through Whatman™ Grade 54 Quantitative Filter Paper.

B. Derivatization step

• Each sample acid extract (0.5 ml) was mixed with 150 µl of a saturated NaHCO3 solution and the pH was adjusted to 11.5 with about 150 µl of 1.0M NaOH.
• Dansyl chloride solution (2 ml of 10 mg/ml dansyl chloride/acetone) was added to the alkaline amine extract.
• The mixture was incubated at 40 °C under agitation for 60 min.
• The residual dansyl chloride was removed by adding 200 µl of 300 g/l ammonia solution.
• The solution was kept for 30 min at 20 °C and protected from light.
• Each sample was brought up to 5 ml with acetonitrile and filtered through a 0.22 mm PTFE filter.

C. HPLC analysis:

C.1. Apparatus: High-performance liquid chromatography (HPLC) analyses used the Agilent 1100 series (Waldbronn, Germany). The separations were performed on a reverse-phase C18 Waters Spherisorb ODS-2 (150 by 4.60 mm, particle diameter 3 µm) HPLC column (Milford, MA) with a matching guard cartridge of the same type.

C.2. Separation of biogenic amines by HPLC: The High-performance liquid chromatography was used to analyze the prepared samples. The HPLC parameters are clarified in (Table 1). Estimation of biogenic amines were operated as previously decorated by Martuscelli et al.

(2005) and Magwamba et al. (2010), with minor modifications. Mobile phase consisted of acetonitrile of HPLC grade (eluent A) and ultrapure water (eluent B). The mobile phases were filtered and degassed before use. Chromatographic separation made use of an isocratic elution method with acetonitrile of HPLC grade (eluent A) and ultrapure water (eluent B) in the ratio 60:40, respectively. The flow rate was 1.2 ml/min for 6.5 min to ensure full separation. Derivatized amines were detected with a UV detector at 254 nm. A set of biogenic amine standards and their mixtures were analyzed together with test samples. During analysis, a standard solution was also injected intermittently between test samples to check chromatographic consistency. Each sample was injected twice. The peak heights of the biogenic amine standard solutions were used to prepare standard curves and then to determine the amine concentrations in the test samples by comparing the peak area retention durations of the samples to the standard curves obtained from HPLC analysis of standard solutions. The results of the biogenic amines were compared to the permitted limit of the guidance level of global organizations.

Table 1: HPLC conditions

Parameter	Optimized conditions
Column	C18 Waters Spherisorb ODS-2 (150 by 4.60 mm, particle ∅ 3 µm)
Mobile Phase	Acetonitrile: ultrapure water (60:40)
Flow Rate	1.2 ml/min
UV Detector	254 nm.
Injection Volume	20 µl
Column Temperature	ambient

Estimation of human daily and weekly intakes of biogenic amines from examined milk products

Estimated Daily and Weekly Intakes (EDI and EWI) of the examined samples were obtained according to the equations described before elsewhere (Ahmed et al., 2020; Diab et al., 2021). Moreover, the results were matched to the international standards organization's Acceptable Daily Intake (ADI) (Diab et al., 2020).

Statistical analysis

Experiments were carried in duplicate measurements and mean values were used for the statistical analysis. Data obtained in this study were statistically analyzed using Excel's data analysis tools. Data shows mean values and standard deviations.

Results and Discussion

BAs are a class of toxicants that have conventionally been collaborated with seafood (Shalaby, 2000); and their presence in dairy products tends to raise concerns about food safety. BAs are low molecular weight compounds that are chemically equivalent to alkaloids and are mimics of physiologically significant amines found in animals and plants (Smith 1971; Medina et al., 2003). Cell development and gene expression, protein synthesis, membrane division and stability, tissue repair, and modulation of intracellular signalling pathways and ion channels are all bioregulated by "natural BAs" (Kusano et al., 2008; Galgano et al., 2012).

The incidence of a specific BAs in food products varies among studies according to the type of BAs formed and the food. Biogenic amines biosynthesis in foods requires the availability of amino acids, the existence of microorganisms synthesizing the amino acid decarboxylases and favorable conditions which promotes their growth and decarboxylation process activity. The type and amounts of biogenic amines formed in foods is highly influenced by the intrinsic food characteristics such as (microbiological population, water activity, pH, food composition) and by extrinsic parameters such as (temperature, storage time) (EFSA, 2011). Fermentation and formation of biogenic amines promoted by pH, as in an acidic medium (an optimum pH ranged around 5), the amino acid decarboxylation activity is highly elevated. Furthermore, bacterial proliferation raises the manufacture of the decarboxylase enzyme, which increases the amounts of biogenic amines (Lazaro et al., 2013). Cheese is a good environment for amine production, although the amount of amine produced varies greatly depending on age, cheese variety, and microflora (Joosten and van Boekel, 1988). Moreover, Biogenic amines existence in food indicates the raw materials hygienic quality during both manufacturing and processing (Hernandez-Jover et al., 1997). Biogenic amines are thermostable and not affected by processing or cooking heat (EFSA, 2011). Novella-Rodriguez (2004) reported that raw milk cheese showing elevated level of biogenic amines in compare with pasteurized milk cheese due to its higher microbial contamination especially with Enterobacteriaceae and *Enterococci*.

Histamine poisoning is a chemical intoxication induced via consuming foods having high histamine level (Taylor et al., 1989). Our data as shown in (Table 2) revealed that the highest histamine levels were higher in old Kareish cheese than in Ripened Domiati cheese with mean value representing 835 ± 32.9 and 513 ± 29.1 mg/kg respectively. Our data agreed with the data recorded by Doeglas et al. (1967) and Taylor et al. (1985). But in contrast with Dardir and Abeer (2007); Rabiae et al. (2011); Ibrahim and Mehanna (2016); Amine et al. (2007) and Eleiwa et al. (2013), which recorded low levels of histamine in Ripened Domiati cheese. Moreover, Chang (1985) explained that the lower or undetectable level of histamine may be due to catabolism by microorganisms or enzymes. Furthermore, our data supported by FDA guidelines (FDA, 2001), which reported that histamine levels in old Kareish and Ripened Domiati cheese exceeded the maximum permissible limits (< 200 mg/kg).

Our data in (Table 2) showed that TYM levels was detected in all examined cheese samples with elevated level in old Kareish cheese than Ripened Domiati cheese with mean values representing 564±37.1 and 478±25.3 mg/kg, respectively. The same results in old Kareish cheese was concluded by (Rabiae et al., 2011), and low levels of tyramine in Ripened Domiati cheese was reported by Ibrahim and Mehanna (2016) and Amine et al. (2017). These elevated levels of biogenic amines revealed that is due to proteases activity derived from microbial contamination originated from bad hygienic conditions (Abo El-Makarem and Amer, 2016). Furthermore, high levels of tyramine and histamine in foods correlated microorganism and their enzymatic activities (Fonberg-Broczek and Sawilska-Rautenstrauch, 1995).

HIM and TYM are the most toxic effects and related to food poisoning (Świder et al., 2019). It is well established that TYM is the predominant BAs in dairy products and the most commonly associated etiology with dairy borne intoxications known as "cheese reaction" or “cheese effect” as it is mainly associated with the consumption of cheese rich in this amine (Ten Brink et al., 1990; Komprda et al., 2008; Pegg, 2013; Costa et al., 2015; Ruiz-Capillas and Herrero, 2019). However, BAs such like putrescine, HIM, and CAD are frequently detected in cheeses, and occasionally in amounts that exceed those of TYM (Novella-Rodrguez et al., 2003; Martuscelli et al., 2005; Custodio et al., 2007; Bunkova et al., 2013). In agreement with Rabiae et al. (2011), the highest levels of cadaverine were detected in old Kareish cheese followed by Ripened Domiati cheese representing mean value 556±27.4 and 470 ±30 mg/kg, respectively as represented in (Table 2), while in contrast with Eleiwa et al. (2013), whom reported lower levels of cadaverine. Marino et al. (2000) noticed that the high count of Enterobacteriaceae promoted the formation of cadaverine via lysine decarboxylation activity.

Furthermore, almost all assessed tyramine, putrescine, histamine and cadaverine levels were above the international regulation permitted limits. On light of above, 100% of old cheese samples contaminated with all estimated pyogenic amines and 100, 95, 100 and 100% of Ripened Domiati cheese samples contaminated with tyramine, putrescine, histamine and cadaverine, respectively (Nout, 1994; FDA, 2001; Halász et al., 1994). Putrescine and cadaverine can induce unfavorable health conditions due to its toxic effects (Flick et al., 2001). In this study putrescine was detected exceeding the permissible limit that revealed by (FDA, 2001), as our data showed higher levels in old Kareish cheese followed by Ripened Domiati cheese representing a mean value of 537±37.5 and 492±32.2 mg/kg, respectively as concluded in (Table 2 and 3). But our data disagreed with Rabiae et al. (2011), who reported lower levels in old Kareish cheese.

Table 2: Incidence and Statistical analytical results of biogenic amines (mg/kg) in examined samples (N=40)

Biogenic amines	Old Kareish Cheese				Ripened Domiati Cheese			
	Positive samples No. (%)	Min.	Max.	Mean±SD	Positive samples No. (%)	Min.	Max.	Mean±SD
Tyramine	40 (100)	180	1130	564±37.1	40 (100)	144	778	478±25.3
Putrescine	40 (100)	114	1050	537±37.5	40 (100)	70.4	1040	492±32.2
Histamine	40 (100)	514	1360	835±32.9	40 (100)	191	1410	513±29.1
Cadaverine	40 (100)	197	902	556±27.4	40 (100)	172	876	470±30.0

Table 3: Incidence of biogenic amines levels exceeding the Maximum Permissible Limit (mg/kg) in examined samples (N=40)

Biogenic amines	MPL (mg/kg)	Old Kareish Cheese		Ripened Domiati Cheese	
		>MPL		>MPL	
		No. of samples	%	No. of samples	%
Tyramine	50[a]	40	100	40	100
Putrescine	100[b]	40	100	38	95
Histamine	100[c]	40	100	40	100
Cadaverine	100[b]	40	100	40	100

a: Nout (1994); b: FDA (2001); c: Halász et al. (1994).

The acceptable intake (AI) and the estimated intake of biogenic amines (EI) for adult and children were reported in relation to time, either per day or per week (Table 4). The estimated daily (EDI) of biogenic amines (tyramine, putrescine, histamine and cadaverine) in old Kareish cheese and Ripened Domiati cheese samples were evaluated by using the consumed amount of old and Ripened Domiati cheese and estimated biogenic amines mean concentrations in each sample type, taking into account the average body weight of different groups (children and adult) in 100% of old Kareish cheese samples contaminated with all estimated biogenic amines, the EDI values were substantially higher the recommended daily intake (ADI) of children and adult; 100, 17.95, 50 and 21 mg/kg bw, respectively. Moreover, in 100% of Ripened Domiati cheese samples contaminated with all estimated biogenic amines, the EDI values were substantially higher the recommended daily intake (ADI) of children and adult; 100, 17.95, 50 and 21 mg/kg bw, respectively (Ten Brink et al., 1990; Elmadfa, 2003 and EFSA, 2011) (Table 4).

Table 4: Comparative analysis of the AI with EI (mg/kg b.w.) of biogenic amines from examined samples for children and adult.

Biogenic amines	ADI	PTWI	Samples		Children				Adult			
					DI		WI		DI		WI	
			Type of sample	Mean Conc. mg/kg	EDI	>ADI	EWI	>PTWI	EDI	>ADI	EWI	>PTWI
Tyramine	100[a]	700	K	564	1020	40 (100%)	7110	40 (100%)	390	40 (100%)	2730	40 (100%)
			D	478	860	40 (100%)	6020	40 (100%)	331	40 (100%)	2320	40 (100%)
Putrescine	17.95[b]	125.65	K	537	967	40 (100%)	6770	40 (100%)	372	40 (100%)	2600	40 (100%)
			D	492	885	40 (100%)	6190	40 (100%)	340	40 (100%)	2380	40 (100%)
Histamine	50[c]	350	K	835	1500	40 (100%)	10500	40 (100%)	578	40 (100%)	4040	40 (100%)
			D	513	924	40 (100%)	6470	40 (100%)	355	40 (100%)	2490	40 (100%)
Cadaverine	21[b]	147	K	556	1000	40 (100%)	7000	40 (100%)	385	40 (100%)	2690	40 (100%)
			D	470	852	40 (100%)	5960	40 (100%)	328	40 (100%)	2290	40 (100%)

a: Ten Brink et al. (1990); b: Elmadfa (2003); c: EFSA (2011); K: Old Kareish Cheese; D: Ripened Domiati Cheese

Foods with high quantities of BAs may constitute a substantial hazard to public health from a safety standpoint, as BAs can have severe toxicological effects. Intoxications caused by BAs have been connected to various types of cheese in various nations (Rauscher-Gabernig et al., 2009; EFSA, 2011). BAs perform a variety of important roles in humans at physiological quantities, including acting as neurotransmitters (psychoactive) and vasoactive chemicals in the neurological and vascular systems, among other things. Continued high-dose ingestion of BAs from external sources, on the other hand, might cause toxicological effects ranging from a moderate headache to organ failure and death. It is widely known that putrescine and CAD have a role in cancer progression by interacting with nitrite to generate carcinogenic nitrosamines in heat-treated foods (Medina et al., 2003; Koutsoumanis et al., 2009). In fact, GIT bacteria can convert polyamines like putrescine and CAD into stable carcinogenic N-nitroso compounds (such nitrosopyrrolidine) in vivo, promoting the formation of chemically induced aberrant crypt foci in the intestine (Paulsen et al., 1997; Eliassen et al., 2002). BAs have also been linked to many health disorders including Alzheimer's disease, cystic fibrosis, depression, epilepsy, hepatic encephalopathy, immunopathologies, ischemia, muscular dystrophy, oxidative stress, Parkinson's disease, psoriasis, and schizophrenia, (Medina et al., 2003). BAs may have chronic or subchronic effects after repeated exposure, in addition to being well-established causal agents of acute health concerns. As a result, evaluating the risk associated with BAs food consumption should take into account the chronic and subchronic health impacts that these toxicants may have, and have been previously overlooked because they are not thought to be related to BAs dietary intake. The results of this study show that the optimal conditions for the formation of BAs were satisfied in the dairy products tested. Ingesting too much BAs will almost certainly result in acute illnesses because the BAs will not be fully metabolized, and any unmetabolized BAs will quickly enter the bloodstream and travel to various parts of the body, including the central nervous system, where they can cause serious health problems (Medina et al., 2003). Children, the elderly, women during pregnancy or menstruation, people with allergies or gastrointestinal diseases (gastritis, inflammatory bowel disease, and gastric ulcers), or those taking monoamine or DAO inhibitors (such as antidepressants and anti-disease Parkinson's drugs) are among those at risk. Alcohol consumption and smoking were also reported (Santos 1996; McCabe-Sellers et al., 2006; EFSA, 2011; Wunderlichova et al., 2014).

Conclusion

In conclusion, our data showed higher levels of tyramine, histamine Putrescine and cadaverine in both old Kareish and ripened Domiati cheese. The highest levels were detected in old Kareish cheese in compare with ripened Domiati cheese which can be explained due to the bad hygienic condition including longer storage period, processing, and manufacturing processes, which promotes the enzymatic activity (decarboxylation and protease activity) due to high microbial contamination. Although underreported and largely ignored, the presence of BAs in dairy products is a pretty common cause of foodborne intoxication. Controlling the prevalence of these toxins in dairy products will undoubtedly help to reduce the global impact of foodborne disease. To fulfil such an objective, a new food safety approach should be implemented in light of the changing world and consumers' rising demands for minimally processed and safe food products. Such a challenge necessitates extensive scientific knowledge of these toxins, their contamination sources, conditions for their formation and/or inactivation, toxicological effects, and possible interactions between them to enhance or reduce such toxicological effects.

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Conflict of interest

The authors declare that there is no conflict of interest.

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