Plant Archives Vol. 21, Supplement 1, 2021 pp. 1993-1997 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.324

DETERMINATION OF BIOGENIC AMINES IN EGYPTIAN SOFT CHEESE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

This study determined the quantity of six biogenic amines "histamine, tyramine, putrescine, cadaverine, tryptamine and phenylethylamine" in 250Egyptian soft cheese samples (Kariesh and Damietta) (125 of each) sold in retail markets in Delta region (Sharkia, Gharbia, Dakahlia, Minofya and Kaliobia Provinces) by using high performance liquid chromatography (HPLC) and correlation with chemical composition of cheese (pH, salt%, moisture% and acidity%). Our results showed that the mean values of pH, salt, moisture and acidity % were (4.89±0.05 and 4.95±0.06), (1.69±0.01 and 4.21±0.12 %), (75.79±0.25 and 56.84±0.46%) and(1.94 ±0.04 and 1.53±0.03%) in Kariesh and Damietta cheese samples, respectively. The mean values of total biogenic amines were 80.67± 13.46 and 36.30± 7.39 mg/100gin Kariesh and Damietta cheese samples, respectively. There wasa negative correlation between (pH value, salt % and moisture %) and biogenic amines production but the positive correlation was found between acidity% and biogenic amines production. On conclusion, optimization and standardization of milk quality and hygiene during cheeses manufacturing and storage should be taken for human safety.

Keywords: Biogenic amines, Kariesh and Damietta cheese, HPLC

Introduction

Cheese is considered the most consumed dairy product in the world as it is considered a good source of calcium, proteins, fatty acids, vitamins and minerals. Also it is considered a suitable food for patients who suffering from diabetes or lactose malabsorption, due to low lactose content(Kayagil, 2006). In Egypt, Kariesh and Damietta cheese varieties are the most popular types that are extensively manufactured in the rural areas, and are desired by a wide variety of consumers due to their organoleptic characters and economic prices (AbouDonia, 2008). This cheese is made of either cow or buffalo whole milk or a mixture of both (El-Baradei *et al.,* 2007). Biogenic amines (BAs) are organic compounds with basic character and low molecular weight formed via decarboxylation of precursor free amino acids or by amination and deamination of aldehydes and ketones and synthesized in the metabolism of plants, animals and microorganisms (Rodriguez *et al.,* 2014). Cheese is the main fermented dairy product possibly to contain harmful levels of BAs, especially histamine, tyramine and putrescine. Cheese is among foods containing high protein in which enzymatic and microbial activities cause the formation of BAs from amino acids decarboxylation (Tuba and Ebru, 2015). Presence of BAs in cheese may be extremely variable and depends on: the sort of cheese, type of milk, heat treatment of cheese milk, section of the cheese (edge/core),the ripening conditions, post ripening processing, kind of packaging, storage time and temperature, microbiota responsible for cheese making, availability of free amino acids, pH, water activity, bacterial density, and the presence of microorganisms owning amino acid decarboxylase activity (Martuscelli *et al.,* 2005).The interest in BAs determination is due to their ability to have a direct or indirect effect on human vascular and nervous systems. Indeed, a large amount of the BAs such as histamine (histamine intoxication) and tyramine (cheese syndrome) can cause rash, headache, nausea, hypo-or hypertension, cardiac palpitation, intracerebral haemorrhage and anaphylactic shock, especially if alcohols or monoamine oxidase inhibitor drugs (MAOI) are taken at the same time (Vinci and Antonelli, 2002). The most relevant issue of putrescine and cadaverine in relation to food is the potentiation of the toxicity of other amines, especially histamine and these diamines can react with nitrite to form carcinogenic nitrosamines (Flick *et al.,* 2001). There are two reasons for determination of BAs in foods: the first is their potential toxicity; the second is the possibility of using them as food quality marker (César and Carlos, 2013).The aim of this work was to determine the BAs content in Egyptian soft cheese and correlate their levels with pH, salt %, moisture % and acidity %.

Materials and Methods

Collection of samples

250 random samples of Egyptian soft cheese (Kariesh and Damietta) (125 of each) were collected from retail markets in Delta region (Gharbia, Sharkia, Dakahlia, Kaliobia and Minofya Provinces). All samples were collected and transported aseptically to National Research Centre, Cairo, Egypt in a 4°C vehicle-mounted refrigerator.

Preparation of sample

25 g of each cheese sample was cut into small parts and placed into a sterile plastic bag with 225 mL of sterile distilled water and mashed with automatic stomacher for 2 minutes.

Determination of pH

The pH of each prepared sample`s solution was measured by using digital pH meter (Crison pH meter 507) via inserting pH meter electrode directly into previously prepared sample`s solution.

Chemical analysis

Samples were analyzed chemically for estimation of salt%, moisture % and acidity% according to AOAC, (2016).

Determination of biogenic amines

Six BAs including "histamine, tyramine, cadaverine, putrescine, trypyamine and phenylethyleamin" were determined in all examined cheese samples in National Research Center in Egypt according to the method adopted by (Moret and Conte, 1996).

Chemicals

Histamine, tyramine, cadaverine, putrescine, trypyamine, 2-phenylethylamine, 1,7-diamminoeptane and dansyl chloride were purchased from Fluka (Buchs, Switzerland). For HPLC analysis, Acetonitrile Super Gradient Lab Scan and water purified with a Milli-Q system, were used throughout.

Standard solutions

A Stock standard solutions of the tested amines were prepared by adding weighed amount of each amine (100mg) to a 100ml volumetric flask and dissolved in required volume of distilled water individually.

Sample preparation

A precisely weighed 10 g sample was homogenized with a Polytron homogenizer in an acidic medium. Two extractions with 20 ml of 0.1 *M* HCI were carried out.1,7-diaminopentane(1,7-De) was used as the internal standard. The organic extracts were saturated with NaCl (to avoid turbidity) and the pH was adjusted to 11.5 by automatic titrator. The extraction with 5 ml butanol, an organic solvent was then performed. This was carried out either in a test tube on 5 ml of acid extract, with three portions of 5 ml each (Vortex agitation) or in a separator funnel, with blender agitation, for 30 min. The derivatization was then performed in a test tube as follows: 1 ml organic extract was dried under vacuum (UNIEQUIP), after 2 drops of 1 *M* HC1 had been added. Then 1 ml of 0.1 *M* HC1, 0.5 µl saturated solution of NaHCO3 and 1 ml dansyl chloride solution (5 mg/ ml) were added. The reaction vessel was incubated at 40°C for 1 h, then the solution was dried under vacuum, acetonitrile was added and HPLC injection followed.

Results and Discussion

Several chemical factors such as the pH, salt concentration, moisture content and acidity can affect the BAs producing microorganisms' growth and the decarboxylase activities during the production and fermentation of dairy products (Linares *et al.,* 2011). The pH is a crucial element for fermentation and formation of BAs, as amino acid decarboxylase activity is higher in an acidic environment, this may explain why decarboxylase enzymes have an optimum pH of around 5.0. Furthermore, the bacterial growth also increases the amount of BAs, by raising the production of the decarboxylase enzyme (Lazaro *et al.,* 2013). Table (1) showed that the mean values of pH in the examined Kariesh and Damietta cheese samples were4.89±0.05 and 4.95±0.06, respectively. The effects of salt include control of microbial growth and activity, control of various enzyme activities in cheese, reduction of cheese moisture content, and physical changes in cheese proteins that can influence cheese texture, flavour development and formation of BAs from free amino acids (Hayaloglu *et al.,* 2002). It is evident from table (1) that the mean values of salt % in the examined Kariesh and Damietta cheese samples were1.69±0.01 and 4.21±0.12 %, respectively. Retaining moisture is very essential in cheese making to attain desired texture and manipulate flavor development via the beneficial bacteria (Lee *et al.,* 2007). Mean values of moisture % in Kariesh and Damietta cheese samples were 75.79±0.25 and 65.84±0.46 %, respectively. Mean values of acidity %in Kariesh and Damietta cheese samples were 1.94±0.04 and 1.53±0.03 %, respectively. Table (3) illustrated the correlation coefficient (r) between (pH, salt%, moisture% and acidity %) and BAs formation in the examined cheese samples, in which pH value was a negatively correlated (at 0.05 level) with tryptamine with r value -0.481. Similar results were obtained by Morad (2018). As at low pH value, the microorganisms are stimulated to produce decarboxylase enzyme as a part of their defense mechanism against acidity (Bover-Cid *et al.,* 2000). Also, there was a negative correlation between salt % and BAs production (histamine and putrescine) (at the 0.05 level) with r value -0.355 and -0.398, respectively, and negative correlation with tryptamine (at the 0.01 level) with rvalue0.681. High salt content has an inhibitory effect on the growth of BA producing bacteria and/or amino acid decarboxylation activities (Gardini *et al.,* 2001). There was a negative correlation (at the 0.01 level) between moisture content and BAs production (tryptamine and PEA) with r value -0.634 and -0.726, respectively. But a positive correlation was found (at the 0.01 level) between acidity % and BAs production (histamine, tyramine and putrescine) with r value 0.636, 0.473 and 0.648, respectively. These results were supported by Maijala, (1995) which said that the formation of BAs is considered a protective mechanism of bacteria against acidic environments.

Determination of BAs is very important due to their frequent detection at excessive levels in various kinds of cheeses, and to increased awareness of their potential adverse health effects. Also, the fact that BAs are produced not only by microbial dairy contaminants of different origins but also via the technological microbiota used in the fermentation and/or ripening of dairy products, including LAB, yeasts, and moulds, complicates their manipulation by using traditional means (EFSA, 2011). Dietary histamine is rapidly detoxified by amine oxidases, but may develop severe symptoms of histamine intoxication as a result of its high amounts ingested with food such as cheese. Impairment of DAO activity either due to gastro intestintal diseases or due to medication with DAO inhibitors results in high histamine level and intoxication (Maintz and Novak, 2007). The results recorded in Table (2) revealed that the mean values of histamine levels in the examined Kariesh and Damietta cheese samples were 20.41 ±2.86 and 12.42 ± 2.02mg/ 100 g with an incidence of

80 and 70.4 %, respectively. Histamine level was found higher in Kariesh cheese samples than Damietta cheese samples may be due to manufacturing under poor hygienic conditions, using poor quality raw materials, microbial contamination or using unpasteurized milk (Al-Adawy, 2019). Tyramine is the predominating BA in dairy products and the most frequently associated etiological agent with BA-mediated dairy borne intoxications designated as "cheese reaction" (Brink *et al.,* 1990). Mean values of tyramine in the examined Kariesh and Damietta cheese samples were 30.54 ±4.80 and 11.46 ± 3.03 mg/100g with an incidence of 80 and 72 %, respectively. In this study we found that tyramine is the most commonly detected BA in examined dairy products samples this is due to many lactic acid bacteria can produce microbial tyrosine decarboxylase enzyme (Bunkova *et al.,* 2010). The presence of cadaverine in food could pose an indirect risk to consumers, since they may potentiate the toxicity of other BAs such as tyramine and histamine, by inhibiting the detoxifying enzymes. (Flick *et al.,* 2001). Mean values of cadaverine in the examined Kariesh and Damietta cheese samples were11.06 ±1.66 and 4.80 ± 1.20 mg/100g with an incidence of 60 and 52 %, respectively. Puterscine may react with nitrite to form carcinogenic nitrosamines, it can be also considered as spoilage indicators. Probably, the most relevant issue of Puterscine is related with its role as enhancer of other BAs toxic effects due to the inhibition of detoxifying enzymes (Valsamaki *et al.,* 2000). Mean values of puterscine in the examined Kariesh and Damietta cheese samples were14.88±3.36 and 5.60 ± 0.88 mg/100gwith an incidence of 64 and 60 %, respectively. The concentrations of tyramine, putrescine, and cadaverine, increased during the processing and storage of dairy products, therefore their amounts have been proposed as an index of the hygienic conditions of raw materials and/or manufacturing practices since their amount increase during microbial fermentation or spoilage (Latorre-Moratalla *et al.,* 2008).

Tryptamine is formed by decarboxylation of tryptophan. Once is formed, it's difficult to be destroyed either by pasteurization or cooking. Its hazardous effects on humans: rash, migraine hypertension and hypotension (Gnog *et al.,* 2014). Mean values of tryptamine in examined Kariesh and Damietta cheese samples were 1.26 ± 0.26 and 2.02 ± 0.26 mg/100g with an incidence of 61.6 and 32 %, respectively. Phenylethylamine (PEA) ingestion has sometimes been associated with symptoms such as headache, dizziness and discomfort. In addition, PEA has been proposed as the initiators of hypertensive crisis in certain patients and of dietary-induced migraine. Clinical signs appear between 30 min to a few hours following BA consumption and usually disappear within few hours; recovery is usually complete within 24 h. (EFSA, 2011). In Kariesh cheese samples the mean value of PEA was 2.52 ± 0.52 mg/100g with an incidence of 60 %, but not detected in Damietta cheese samples. From the previous results we found that the low content of PEA may be due to degradation effect of some lactic acid bacteria on BAs by oxidase enzymes (Tosukhowong, 2011).Our results showed that all examined dairy products samples were not exceeded the toxicity threshold of PEA which is 3 mg/100g that reported by Brink *et al.* (1990). Although in Damietta cheese processing, the milk is subjected to heat treatment to provide the desirable conditions for rennet enzymes activities. Many studies indicated that Damietta cheese may contain considerable quantities of BAs. It was reported that heat treatment had little impact on the content of BAs in foods. Therefore, processed cheese may include BAs. Also, found that BAs and decarboxylase enzymes are thermo stable once formed within the food will remain El-Aswad (2001).

Conclusion

The assessment of results obtained allowed to conclude that BAs were detected in Damietta and Kariesh cheese samples in variable amounts depending on several factors such as moisture content, pH and salt % as well as presence of various microorganisms that have the capacity to produce decarboxylase enzymes which convert amino acids to BAs. Whereas the Kariesh cheese samples were higher that contain total BAs than Damietta cheese samples. So, Monitoring of raw materials and products at multiple factors alongside the food chain is vital to evaluate the relevance of several factors contributing to BAs formation and accumulation in fermented foods.

Table 1 : Statistical analytical results of pH, salt%, Moisture% and Acidity% in the examined Kariesh and Damietta cheese samples (125=20 each).

Cheese type	Mean ±SEM*			
	pH value	Salt%	Moisture%	Acidity %
Karieh	4.89±0.05	1.69±0.01	75.79±0.25	1.94±0.04
Damietta	4.95±0.06	4.21±0.12	65.84±0.46	1.53±0.03

*Standard error mean

Table 2 : Statistical analytical results of BAs mg/100g in examined Kariesh and Damietta cheese samples (n=125 each).

Biogenic amines mg/100g	Cheese types					
	Karieh cheese			Damietta cheese		
	No.	%	Mean ± SEM*	No.	%	Mean ± SEM*
Histamine	100	80	20.41 ±2.86	88	70.4	12.42±2.02
Tyramine	100	80	30.54 ±4.80	90	72	11.46±3.03
Cadaverine	75	60	11.06 ±1.66	65	52	4.80±1.20
Putrescine	80	64	14.88±3.36	75	60	5.60±0.88
Tryptamine	77	61.6	1.26 ± 0.26	40	32	2.02±0.26
PEA	75	60	2.52 ± 0.52	ND*		

*Not detected

Table 3 : Correlation between chemical analysis and biogenic amines production in examined softcheese samples.

	Histamine	Tyramine	Cadaverine	Putrescine	Tryptamine	PEA
pH	-0.017	0.073	0.242	-0.183	-0.481*	0.140
Salt%	-0.355*	-0.224	-0.195	-0.398*	-0.681**	-0.391
Moisture%	0.003	0.334	-0.213	-0.216	-0.634**	-0.726**
Acidity %	0.636**	0.473**	0.268	0.648**	0.280	0.083

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Acknowledgment

We would like to thank Milk Hygiene Department, Faculty of Veterinary Medicine, Zagazig University and our supervisor in Animal Health Research Institute for their financial and technical support. This work represents a part of the doctoral research by AUTHOR for the ph. D. degree at the Zagazig University.

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