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Biogenic amines and microbiological profile of egyptian cheeses

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Cheeses are among those high-protein-containing foodstuffs in which enzymatic and microbial activities cause the formation of biogenic amines (BAs) from amino acids decarboxylation. Most of the methods for amine determination in these products involve acid extraction followed by a liquid-liquid purification step to selectively separate amines and amino acids. This study aimed to describe the development of biogenic amines in Egyptian cheeses during ripening and storage regimes. Biogenic amines content in Mish, Ras and Blue cheeses were 270-1300, 340-980 and 210-700 mg/kg, respectively. The dominant biogenic amines were different. This work confirms that the main biological feature influencing amines formation is the extent of growth of microorganisms, like *Enterococci*, characterized by decarboxylase activity. It is important to note that the presence of biogenic amines due to the activities of these microorganisms is maintained within safe levels. In Egypt, reports dealing with the Egyptian cheeses (Mish, Ras and Blue) are scanty. So, the present work was carried out to fill the gap in our knowledge on its microbiological and biochemical features, focusing on hygiene and consumer health aspects.

Key words: Biogenic amines, food safety, proteolysis, ripening, *Enterococci* spp.

INTRODUCTION

Milk and milk products are very important in human nutrition and, among them; cheese is considered a good source of proteins, vitamins and minerals. However, cheese is one of the most fermented foods commonly associated with biogenic amines (BAs) contamination. These compounds are basic nitrogenous compounds formed by series of microorganisms, mainly by decarboxylation of amino acids or "*in vivo*" also by de-amination and trans-amination of aldehydes and ketones (Loizzo et al., 2012, 2013). Biogenic amines are compounds commonly present in living organisms in which they are responsible for many essential functions. They can be naturally present in many foods such as fruits and vegetables, meat, fish, chocolate and milk, but they can also be produced in high amounts by microorganisms through the activity of amino acid decarboxylases (Ten Brink et al., 1990). Excessive consumption of these amines can be of health concern because when there is are no equilibrate assumption in human organism, can generate different degrees of diseases determined by their action on nervous, gastric and intestinal systems and blood pressure (Suzzi and

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Gardini, 2003). Biogenic amines are low molecular weight nitrogenous bases, they were found in fermented foods and cheese (Mohamed et al., 2013).

Also, biogenic amines are low-molecular nitrogenous compounds that are formed in foodstuffs mainly by microbial decarboxylation of the precursor amino acids (Alberto et al., 2002). The importance of observing BAs content lies in potential toxicity to human, mainly when the concentration is up to 100 mg/kg (or up to 100 mg/L). Thus, the presence of BAs significantly influences the food quality and safety (Smit et al., 2005).

The presence of relevant amounts of BAs in cheeses has been documented (Martuscelli et al., 2005; Kung et al., 2007; Pintado et al., 2008; Ladero et al., 2009; Mercogliano et al., 2010). In cheeses, BAs formation is caused by curdling and cheese decarboxylase-positive microorganisms. Histamine (HIS), tyramine (TYR), putrescine (PTR), cadaverine (CAD), spermidine (SPD), spermine (SPR), tryptamine (T), and β-phenylethylamine (PE) are frequently found in these products. Cheese is one of the fermented foods most commonly associated with BAs poisoning; mainly HIS, TYR, PTR and CAD. Indeed, the term ‘‘cheese reaction” to refer to it (Ten Brink et al., 1990). Tyramine and histamine are the most abundant and frequent BAs in cheese (Fernández et al., 2007). Consumption of food containing high levels of BAs is considered undesirable since it can be associated with several toxicological problems such as respiratory distress, headache, hyper- or hypo-tension or allergies (Ladero et al., 2010). These problems are especially severe in consumers with low levels of the enzymes involved in the detoxification system (mono and di-amine oxidases), either by genetic disorders (Caston et al., 2002) or medical treatments (Halász et al., 1994). The content of biogenic amines and polyamines significantly differed according to the technology of ripening. The cheeses unwashed during ripening had much higher contents of all observed amines and polyamines in comparison with the washed-rind cheeses. The mean content of putrescine, cadaverine and tyramine exceeded 100 mg/kg in unwashed-rind cheeses, while the other amines occurred at lower levels. The content of all detected amines was very low in washed-rind cheeses; no tryptamine, phenylethylamine and histamine were found. The effect of storage on the amine formation was not confirmed (Samková et al., 2013).

Physiologically, histamine is one of the most effective BAs; it has vasoactive and psychoactive effects (Repka-Ramirez and Baraniuk, 2002). Moreover, it is the main BAs involved in food poisoning and it is limited in some foodstuffs by law. At non-toxic doses, food borne histamine can cause intolerance symptoms such as diarrhoea, hypotension, headache, pruritus and flushes. Just 75 mg of histamine, a quantity commonly present in some meals, can induce symptoms in the majority of healthy persons with no history of histamine intolerance (Wöhrl et al., 2004).

The ability of microorganisms to decarboxylate amino acid is highly variable. Due to strain-specific, it is important to count decarboxylase-positive microorganisms to estimate; the risk of BAs food content and to prevent BAs accumulation in food products. Presence and accumulation of BAs depends on many factors such as presence of specific bacteria (*Enterococci, Micrococci, Enterobacteriaceae* and *Lactobacilli*) and enzymes, availability of free amino acids, presence of suitable cofactors, that is, pH level, water activity, temperature and salt content, type of cheese, ripening and storage period (Galgano et al., 2001). Some controversial results have been reported on the contribution of *Enterococci* sp. in BAs production in cheeses, and in particular in histamine (Sumner and Taylor, 1989). *Enterococci* have a long history of use as artisanal cultures for preparation of various types of cheeses (Izquierdo et al., 2009), they are sometimes associated with pathogenicity (Khan et al., 2010), can cause endocarditic, bacteraemia, and several infections, as well as multiple antibiotic resistances (Kayser, 2003). Although, the chemical composition and microbiological quality of cheeses in Egyptian markets have been studied extensively, little data is available on the occurrence of biogenic amines in Egyptian cheeses. Therefore, this survey was undertaken to determine the presence of BAs in commercially available cheeses during ripening and storage, also to make an assessment of the health hazard arising from the consumption of these products especially by susceptible individuals. Other studies such as Lorencová et al. (2012) and Buňková et al. (2010) deal with selection and study of microorganism (such as lactic acid bacteria), which are major producer of biogenic amine. However, these works explore the particular biogenic amine production in growth medium, where the concentration of biogenic amine could be biased optimal environment for the bacteria metabolisms. Moreover, some strains of the starter lactic acid bacteria (such as *Lactococcus lactis* subsp. *lactis*) have decarboxylase activity that was observed in model environment of growth broth. Behaviour of these strains has not been investigated in real system of the cheese and can be different in comparison with condition in growth broth.

The objective of our pilot study was to compare the BAs content and other selected parameters in Egyptian cheese and to review hypothesis that the BAs content developed during the ripening and storage period is related to the presence of decarboxylase positive strain of *Enterococci* sp.

MATERIALS AND METHODS

Cheese samples

A total 85, 49 and 44 of Mish, Ras and Blue cheeses samples were

purchased from different Egyptian retail markets and small scale factories. The samples collected were 6-48 months old. They were kept in sterile plastic bags and transported to the laboratory of Food Science Department, Zagazig University (Egypt), then stored at 4 ±1°C until analyzed.

Chemical analysis

Cheeses were analyzed in triplicates for moisture by the oven drying method at 102°C (IDF, 1993), salt by titration with $AgNO_3$, and fat by Gerber method (AOAC, 2002). For pH measurement, grated cheese (10 g) was macerated with 10 mL of distilled water and the pH of the resultant slurry was measured using a digital pH meter (pH 211, Hanna Instruments, Vila do Conde, Portugal). Titratable acidity was determined as g lactic acid/100 g cheese is using the method of AOAC (2002). Total volatile fatty acids and total nitrogen (TN) were determined using the methods of AOAC (2002). All analyses were carried out in triplicates.

Assessment of proteolysis

Water-soluble nitrogen fraction (WSN) of cheese was prepared according to Kuchroo and Fox (1982) and a cheese to water ratio of 1:5 was used. 12% trichloroacetic acid soluble nitrogen-fraction (TCA-SN, that is, NPN) was obtained by mixing equal volumes of water-soluble fraction and 24% (w/w) TCA solution, followed by filtration through a white ribbon filter paper (Schleicher and Schuell, Dassel, Germany). The nitrogen content of both fractions WSN and TCA-SN, respectively, was determined by Kjeldahl method (AOAC, 2002) and expressed as percentage of TN.

Free amino acids and biogenic amines

Free amino acids (FAA) and BAs were assayed according to the method of Krause et al. (1995), modified by Pinho et al. (2001). In brief, a 4 g cheese sample was suspended in 15 mL of 0.2 M aqueous perchloric acid; the mixture was homogenized in an Ultra Turrax blender (Sotel, Warsawa, Poland) for 2 min, then kept in an ultrasonic bath (Heraeus, Osterode, Germany) for 30 min, and finally centrifuged at 4000 xg for 20min. Derivatization was carried out via dansyl chloride, at 70°C per 15 min. The reaction was quenched by placing the vials in an ice bath for 5 min. High performance liquid chromatography (HPLC, Waters 600) was used to dansylamines determination. The system was equipped with delivery system, reverse phase CI8 Nucleosil column 250 x 4 mm, 10 µm packing (Macherey - Naggl). The detection was performed using U.V detector (Waters 486) at wavelength of 254 nm using linear program of 25 min period and 1 ml/min constant solvent flow rate. Data were integrated and recorded using a Millennium Chromatography (Waters, Milford MA 01757). Elution was carried out at a flow rate of 1 mL/ min, using a volumetric gradient of solution A, 9 mM aqueous sodium dihydrogenophosphate, 4% (w/v) dimethyl formamide and 0.1% (w/v) triethylamine (adjusted to pH 6.55 with phosphoric acid), and solution B, 80% (v/v) aqueous acetonitrile. Detection was performed by measuring absorbance at 436 nm. Quantification was carried out based on a mixture of amino acid standards: aspartic acid, glutamic acid, serine, threonine, glycine, alanine, arginine, proline, valine, methionine, isoleucine, leucine, lysine, histidine, tyrosine, cystine, tryptophan and phenylalanine; and biogenic amine standards: ornithine, tryptamine, phenyl ethylamine, putrescine, cadaverine, histamine, tyramine and spermine (Sigma Chemical). All determinations were performed in triplicates (Figure 1).

Microbiological analysis

For each cheese sample, 10 g was weighed and dispersed aseptically in 90 mL of citrate buffer (2%,w/v) and homogenized in a sterile polyethylene bag using a Stomacher (Seward Laboratory Blender Stomacher 400 Lab Blender UK) for 1.5 min. Serial dilutions were made in 0.1% sterile peptone water and all determinations were made in triplicates (Messer et al., 1985). The enumeration of total mesophilic bacteria (Plate Count Agar, Merck, Germany) at 30°C/48 h, total coliform groups (Violet Red Bile Agar, Merck, Germany) at 37°C/48 h, yeasts and moulds (Potato Dextrose Agar, Merck, Germany) at 21°C/7 days, *Lactobacilli* (MRS agar, Merck, Germany), *Lactococcus* sp. (M_{17} agar, Merck, Germany) and *Enterococci* (Azide Dextrose agar, Merck, Germany) at 28°C/48 h (Frank et al., 1993) were performed.

Statistical analysis

The effect of time of ripening on all parameters of proteolysis and on total FAA and BAs content of the cheese was assessed by analysis of variance (ANOVA) using the SPSS 10.0 for Windows software (Liu et al., 2003).

RESULTS AND DISCUSSION

Evaluation of physico-chemical parameters

The chemical compositions of Egyptian cheeses are presented in Table 1. The total solids content of cheese samples varied from 30.5 to 46.5, 47.2 to 58.3 and 41.2 to 48.8% in Mish, Ras and Blue cheeses, respectively. A significant variation of fat content was observed, 17.8-30.4; 33.8-48.3 and 25.3-38.4% in Mish, Ras and Blue cheeses, respectively. The salt content of the cheese samples fell within the range, 6.1-10.5; 5.6-6.8 and 4.5-5.7% in Mish, Ras and Blue cheeses, respectively. Whereas, the pH of cheeses ranged from 4.2 to 5.3; 4.5-5.2 and 4.9-5.8 respectively, which agrees with those reported for good quality Egyptian cheeses (Kebary et al., 1999; Ibrahim and Amer, 2010). Total nitrogen content in cheese samples was slightly higher in Ras cheese as compared to Mish and blue cheeses. Whilst, the water soluble nitrogen was lowest in Blue cheese as compared to other cheeses (Table 1). The WSN/TN ratio showed differences in the degree of ripening of the component cheeses. NPN represented more than 50% of the WSN of the tested cheeses; this may have originated from the component cheeses. WSN and NPN have been classically used as a measure of the extent of secondary proteolysis, that is, formation of small sized peptides (2–20 residues) and free amino acids (Furtado and Partridge, 1988). Total volatile fatty acids showed a significant variation among the tested cheeses, 33.5-55.4; 62.7-92.7 and 45.6-74.5 as 0.1 N NaOH/100 g, in Mish, Ras and Blue cheese, respectively. These variations indicated large differences in quality and degree of ripening in Egyptian cheeses. Production of BAs has frequently been

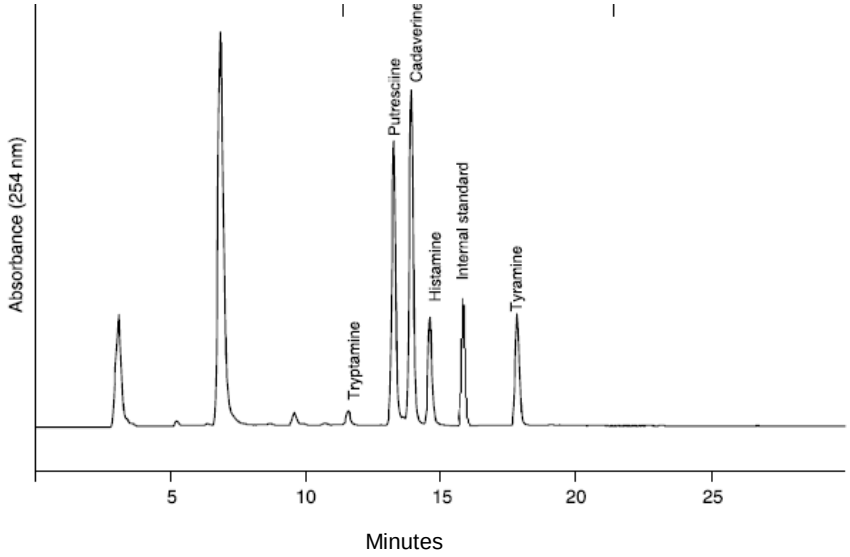

Figure 1. Chromatograms present the areas of biogenic amine standard solution derivatized with incubation at 40°C for 40 min.

Table 1. Chemical composition and proteolysis indices of Egyptian cheese samples ripened/stored at different periods.

Cheese type	Ripening/storage period (mon)	Physico-chemical parameter						Proteolysis indices			
		Total Solid (%)	Fat in solid (%)	Protein in solid (%)	Acidity *	pH	Salt in solid (%)	Total nitrogen (%)	Non-protein nitrogen (%)	Water soluble nitrogen (%)	TVFA**
Mish	6	30.5±2.0^{d}	17.8±2.5^{d}	20.1±1.07^{a}	3.31±0.71^{a}	4.2±0.1^{d}	6.1±1.5^{b}	3.15±0.98^{a}	0.22±0.4^{b}	0.29±0.5^{d}	33.5±2.2c
	12	35.6±2.1^{c}	20.8±2.2^{cd}	18.5±1.15^{b}	3.01±0.65^{b}	4.4±0.1^{cd}	7.2±1.6^{ab}	2.91±0.78^{a}b	0.31±0.6^{a}	0.34±0.6^{cd}	40.5±2.5b
	24	37.0±1.9^{b}	22.3±3.2^{bc}	17.23±1.01^{b}	2.8±0.52^{b}	4.5±0.2^{c}	8.3±2.4^{ab}	2.71±0.73^{b}c	0.33±0.6^{a}	0.41±0.4^{bc}	47.9±2.9a
	36	39.4±2.5^{b}	25.6±5.2^{b}	14.35±0.8^{c}	2.5±0.53^{c}	4.9±0.2^{b}	9.2±2.4^{ab}	2.24±0.56^{cd}	0.36±0.7^{a}	0.49±0.4^{b}	52.3±3.1a
	48	46.5±2.7^{a}	30.4±4.5^{a}	13.33±0.5^{c}	2.2±0.44^{c}	5.3±0.2^{a}	10.5±2.9^{a}	2.19±0.57d	0.39±0.7a	0.55±0.3^{a}	55.4±3.2a
Ras	6	47.2±2.9d	33.8±1.5d	24.88±1.8a	2.2 ±0.43a	4.5±0.2b	5.6±1.4b	3.91±1.29ab	0.19±0.1ns	0.31±0.6ns	62.7±3.5d
	9	50.01±3.1c	37.8±2.0c	24.44±1.3b	2.1±0.42ab	4.6±0.1b	6.7±1.5ab	3.81±1.17a	0.20±0.2ns	0.32±0.5ns	74.0±3.8c
	12	52.5±3.6b	42.5±2.4b	23.6±1.4b	2.0±0.34bc	4.8±0.2b	7.3±1.7a	3.71±1.35b	0.21±0.2ns	0.33±0.5ns	85.8±4.3b
	24	58.3±3.8a	48.3±3.1a	21.05±1.0c	1.85±0.23c	5.2±0.1a	6.8±1.9a	3.31±1.42ns	0.25±0.4ns	0.39±0.7ns	92.7±4.5a
Blue-viened	6	41.2±2.2c	25.3±2.1c	22.64±1.2a	1.9±0.22b	4.9±0.3b	4.5±1.2b	3.55±1.47ns	0.20±0.2b	0.25±0.3b	45.6±2.7c
	9	44.5±2.4b	32.1±2.9b	21.69±1.1b	1.7±0.22c	5.1±0.5b	4.8±1.5ab	3.2±1.36ns	0.21±0.3ab	0.24±0.4b	66.6±3.7b
	12	48.8±2.8^{a}	38.4±3.6^{a}	20.41±1.1^{c}	2.8±0.34^{a}	5.8±0.2^{a}	5.7±1.7^{a}	3.4±1.38^{ns}	0.24±0.4^{a}	0.34±0.5^{a}	74.5±3.9a

Averaged data of analyzed cheese samples in triplicate; Mean value ± a standard deviation ***** Acidity is expressed as lactic acid. ** Total volatile fatty acids is expressed as 0.1 N NaOH/100 g cheese.

Table 2. Levels of biogenic amines (mg/100 g cheese) of Egyptian cheese samples ripened/stored at different periods.

Cheese type	Ripening/storage period (month)	Biogenic amines (mg/100 g)								
		TYR	T	PTR	HIS	CAD	SPD	SPR	PE	Total
Mish	6	6±0.62^{e}	4±0.51^{d}	4±0.45^{d}	9±0.69^{e}	3±0.3^{e}	1±0.05^{d}	Nd^{c}	Nd^{d}	27±2.2
	12	12±1.5^{d}	10±0.85^{c}	10±0.85^{c}	14±0.98^{d}	10±0.5^{d}	1±0.11^{d}	Nd^{c}	Nd^{d}	57±3.2
	24	14±0.42^{c}	17±1.54^{b}	18±1.5^{b}	27±2.4^{c}	18±1.72^{c}	2±0.21^{c}	1±0.08^{b}	4±0.53^{c}	101±4.3
	36	15±0.46^{b}	21±1.79^{a}	19±1.7a^{b}	29±2.3^{b}	20±2.1^{b}	3±0.42^{b}	1±0.09^{b}	7±0.64^{b}	115±4.6
	48	19±1.55^{a}	22±2.15^{a}	20±2.16^{a}	31±2.5^{a}	22±1.97^{a}	4±0.63^{a}	2±0.16^{a}	12±1.15^{d}	132±5.3
Ras	6	3±0.28^{d}	10±0.93^{b}	6±0.42^{d}	12±0.96^{d}	Nd^{d}	1±0.08^{b}	Nd^{b}	3±0.19^{b}	34±2.6
	9	4±0.54^{c}	11±1.05^{c}	8±0.62^{c}	14±0.91^{c}	8±0.71^{c}	1±0.11^{b}	Nd^{b}	3±0.21^{b}	49±2.8
	12	5±0.61^{b}	13±1.13^{b}	13±0.54^{b}	23±2.05^{b}	13±1.26^{b}	Nd^{c}	1±0.08^{a}	5±0.41^{a}	73±3.7
	24	14±1.07^{a}	20±1.86^{a}	16±1.25^{a}	26±2.4^{a}	20±2.1^{a}	2±0.21^{a}	Nd^{b}	Nd^{c}	98±4.1
Blue-viened	6	Nd^{c}	11±0.45^{c}	1±0.15^{c}	4±0.34^{c}	4±0.22^{c}	Nd^{b}	Nd^{b}	1±0.11^{c}	21±2.1
	9	1±0.1^{b}	15±0.87^{b}	2±0.31^{b}	9±0.94^{b}	7±0.65^{b}	Nd^{b}	Nd^{b}	2±0.25^{b}	36±2.6
	12	8±0.72^{a}	17±1.26^{a}	9±0.93^{a}	14±1.26^{a}	11±1.13^{a}	3±0.41^{a}	2±0.17^{a}	6±0.62^{a}	70±3.5

Averaged data of analyzed cheese samples in triplicates, nd: not detected.

referred to as the proteolytic activity of microorganisms present in cheese during manufacture and ripening. Increases in the non-protein nitrogen fractions (WSN and NPN) often means level increase of free amino acids, which are precursors of BAs.

Amino acids and BAs

BAs content of cheese can be extremely variable and depends on the type of cheese, the ripening time, the manufacturing process and the microorganisms present (Ordonez et al., 1997). The Egyptian cheeses (Mish, Ras and Blue) examined confirmed this variability in the total content of BAs ranging from 21.0 to 130.0 mg/100 g cheese (Table 2 and Figure 2). There are significant differences among contents of the eight BAs assayed. Only the Mish cheese contained more than 100 mg/100 g cheese of the total BAs, as affected by increasing the storage period. According to Taylor (1985), the threshold of risk is 100 mg/kg total amines of cheese, if ingestion is associated with such potentiating co-factors as amine oxidase-inhibiting drugs or alcohol, or else if there are pre-existing gastrointestinal diseases (Stratton et al. 1991). Production of BAs in cheese has often been associated with non-starter lactic acid bacteria and *Enterobacteriaceae* (Joosten and Northolt, 1987), so it may be a toxicological risk associated with consumption of raw milk cheese, especially by sensitive individuals. Spanjer and van Roode (1991) suggested that the total concentration of tyramine, histamine, putrescine and cadaverine in cheese should not exceed 900 mg/kg DW^{-1}, but no upper limit for BAs in cheese has been legally enforced.

Even if no significant differences were observed in the final amounts of BAs in Blue and Ras cheeses, the dynamics of accumulation were not the same. Overall, histamine was the most prevalent amine, being found in all analyzed cheese samples. It was followed by T (98%), PTR (97%), CAD (95%), TYR (89%), SPD (73%), PE (72%) and SPR (37%). In spite of being the most frequently detected amine, SPR was present at low levels, below 2.5 mg/ 100 g cheese. Spermidine and PE were also detected at low levels (below 4 and 12mg/100 g cheese, respectively). However, HIS, CAD, T, PTR and TYR were detected at levels up to 30, 21, 20, 19 and 18 mg/ 100 g, respectively. HIS was the most prevalent amine, it was found in all analyzed cheese samples (Table 2). Higher means levels were detected for Mish, Ras and Blue cheeses (9-31, 12-26 and 4-14 mg/ 100 g, respectively). HIS levels capable of causing histamine poisoning were detected in all cheese samples. However, taking into account the concomitant presence of polyamines, it is likely that a higher percentage of cheese samples could cause HIS poisoning. TYR was present in 100% of Mish and Ras cheeses and in 60% of Blue cheese. Mish (53%) and Ras cheese (18%) contained TYR at levels capable of causing hypertensive crisis (Komprda et al., 2008). Overall, T was detected sporadically, at lower amounts as compared to HIS. Similar results were observed by Chang et al. (1985). Higher means levels of tryptamine were observed in Mish and Ras cheeses. The toxic threshold of tryptamine is not known (Joosten, 1988). PE, another amine of health significance was detected 100% in Blue, 82% in Ras and 53% in Mish cheese. The prevalence of this amine was

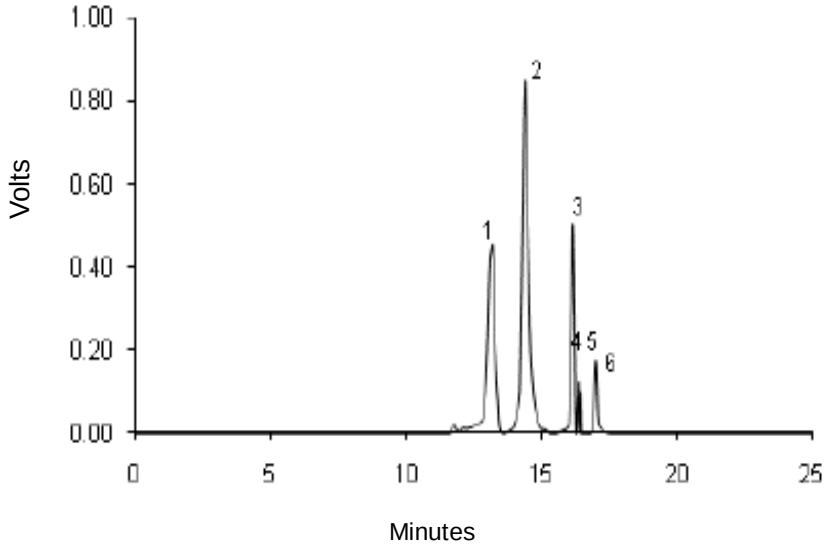

Figure 2. Chromatograms presenting the concentration of histamine, tryptamine, putrescine, tyramine, phenyl ethylamine and cadaverine in Ras cheese.

high, however the levels detected were low (≤12 mg/100 g), below its toxic threshold. The rate of CAD and T accumulation were similar for the traditional Mish and Ras cheeses and that described for the total BAs formation. The accumulation of amines increased remarkably later in the ripening and storage periods. The rate of PE accumulation was similar for Ras and Blue cheeses, whereas HIS and T values were higher in the Ras cheese and reached concentrations of 12 - 26 mg/100 g and 10-20 mg/100 g, respectively.

Similar results were obtained with heat treatment or bactofugation of the milk used for emmental production and had little effect on the TYR content (Krause et al., 1997). The rates of SPR and SPD accumulation were similar for Mish and Blue cheeses, whereas T and HIS were higher in the Mish cheeses and reached concentrations of 4-22 and 9-31 mg/100 g, respectively. According to Halász et al. (1994), Gouda cheese along with Swiss and Cheddar cheeses, which contain high levels of BAs are the most frequently incriminated cheese in histamine poisoning episodes.

Amino acid levels in cheeses types were extremely variable (Table 3). This fact was attributed to an accelerated amino acid release at the manufacturing day, when cheeses were incubated at temperatures favorable for microorganism development and activity (Bütikofer and Fuchs, 1997). Any food with free amino acids, especially tyrosine and phenylalanine, are subject to BAs formation if poor sanitation and low quality foods are used or if the food was subjected to temperature abuse or extended storage time (Schirone et al., 2011).

High variability was observed in pH, acidity, moisture and fat contents of the different analyzed cheese types. Several analyzed samples did not meet the standard of identity and quality established by Egyptian legislation. With regard to quality parameters, pH, moisture and fat content and acidity correlated significantly (P≤ 0.05) with formation and accumulation of some BAs. These results suggest that, among quality parameters evaluated, acidity influenced amine formation in several cheese types. These results are supported by the theory that the formation of BAs is a protective mechanism of bacteria against acidic environments (Maijala, 1994). The presence of micro-organisms with high decarboxylase activity has been reported as the main factor for BAs production in cheese. Moreover, some strains have proteolytic activity, which can affect the accumulation of BAs in cheese (Galgano et al., 2001). For the production of amines, the enzymatic activity of proteases derived from micro-organisms, or from another origin, is important from a qualitative point of view, that is, in relation to the type of amino acids provided to the amino acid decarboxylating microflora. The bacteriological composition of milk could be critical to define the amine profile in cheese; therefore, large amounts of amines in cheese could indicate unsuitability from a hygienic point of view, and the milk used for cheese making. Moreover, the results emphasize the necessity of controlling the indigenous bacterial population responsible for high production of BAs and the use of competitive adjunct cultures is suggested.

Microbiological evaluation

Microbiological analyses of the Egyptian cheeses were examined throughout ripening/storage period (Table 4). Lactic acid bacteria did not show any substantial change

Table 3. Total amount of free amino acids content (mg/g dry weight of cheese) of Egyptian cheese ripened/stored at different periods.

Amino acid	Mish cheese					Ras cheese				Blue cheese		
	Ripening/storage period (mon)											
	6	12	24	36	48	6	9	12	24	6	9	12
Threonine	1.38±0.1	1.36±0.13	1.38±0.13	1.20±0.11	1.18±0.1	1.18±0.1	1.32±0.1	1.38±0.1	1.28±0.1	1.28±0.1	1.18±0.1	1.12±0.1
Serine	2.26±0.2	2.09±0.23	2.06±0.23	1.96±0.23	1.70±0.2	1.62±0.2	1.88±0.2	1.88±0.3	1.81±0.3	1.79±0.2	1.72±0.2	1.44±0.1
Glutamic	8.52±1.3	8.21±1.2	8.19±1.2	6.50±1.1	5.76±1.2	9.96±1.6	6.63±1.1	5.54±0.9	5.46±1	8.66±1.3	8.60±1.3	5.34±1
Proline	3.34±0.3	3.62±0.34	3.6±0.34	2.86±0.12	2.80±0.2	2.68±0.24	2.60±0.3	3.04±0.3	3.00±0.3	2.75±0.3	2.70±0.3	2.44±0.3
Glycine	0.92±0.1	0.79±0.11	0.77±0.11	0.80±0.1	0.86±0.09	0.92±0.11	0.80±0.1	0.72±0.08	0.71±0.08	0.85±0.1	0.82±0.1	0.74±0.08
Alanine	1.56±0.1	1.55±0.19	1.54±0.19	1.34±0.13	1.40±0.1	1.14±0.13	1.44±0.1	1.38±0.1	1.31±0.1	1.42±0.1	1.40±0.1	1.38±0.1
Cysteine	0.18±0.03	0.18±0.03	0.18±0.03	0.16±0.01	0.14±0.01	0.16±0.01	0.17±0.01	0.16±0.01	0.16±0.01	0.26±0.03	0.21±0.03	0.15±0.02
Valine	1.72±0.2	1.61±0.22	1.61±0.22	1.75±0.17	1.45±0.12	1.31±0.12	1.64±0.15	1.09±0.1	1.04±0.1	1.59±0.1	1.55±0.1	1.32±0.11
Methionine	0.98±0.1	0.95±0.14	0.95±0.14	0.97±0.11	0.81±0.07	0.95±0.1	0.77±0.1	0.85±0.07	0.84±0.07	1.11±0.15	1.01±0.15	0.85±0.1
Isoleucine	2.48±0.22	2.35±0.31	2.35±0.31	2.20±0.15	2.08±0.2	2.07±0.21	2.19±0.2	2.02±0.17	1.95±0.17	2.41±0.3	2.33±0.3	2.20±0.19
Leucine	3.02±0.3	2.45±0.3	2.45±0.3	2.63±0.23	2.40±0.16	2.22±0.23	2.48±0.22	2.18±0.15	2.11±0.15	2.73±0.3	2.66±0.3	2.35±0.18
Tyrosine	2.06±0.2	1.87±0.2	1.85±0.2	1.42±0.14	1.67±0.1	1.52±0.13	1.74±0.12	1.55±0.1	1.53±0.1	1.98±0.2	1.94±0.2	1.74±0.1
Phenylalanine	1.72±0.2	1.88±0.22	1.86±0.22	1.17±0.11	1.48±0.1	1.31±0.12	1.53±0.1	1.39±0.11	1.36±0.11	1.73±0.2	1.66±0.2	1.54±0.1
Histidine	1.80±0.2	1.94±0.23	1.91±0.23	1.55±0.25	1.83±0.2	1.47±0.11	1.68±0.12	1.57±0.11	1.56±0.11	1.82±0.2	1.79±0.2	1.60±0.1
Lysine	1.12±0.1	0.99±0.17	0.95±0.17	0.77±0.09	0.85±0.1	0.66±0.02	0.78±0.06	0.80±0.08	0.78±0.08	0.92±0.1	0.87±0.1	0.81±0.1
Tryptophan	0.94±0.1	0.98±0.16	0.95±0.16	0.83±0.1	0.79±0.08	0.73±0.11	0.79±0.07	0.85±0.1	0.82±0.1	0.99±0.11	0.95±0.11	0.91±0.1
Arginine	1.10±0.1	1.11±0.16	1.01±0.16	1.15±0.13	0.78±0.09	0.82±0.12	0.75±0.1	0.78±0.1	0.75±0.1	0.83±0.09	0.79±0.09	0.69±0.05
Total	35.10±3.3	33.93±2.9	33.61±2.9	29.26±2.7	27.98±2.6	30.08±1.9	29.19±2.5	27.18±2.9	25.74±3.0	33.12±3.1	32.3±3.1	26.62±2.8

Averaged data of analyzed Cheese samples in triplicates.

during storage period, while the number of *Enterobacteriaceae* remained high during the ripening/storage period, despite a slight decrease at the end of ripening period. All bacterial groups except for coliforms were maximum in young cheeses. Numbers of *Lactococcus* sp. were slightly higher than those of *Lactobacilli* and total mesophilic bacteria. The difference of *Lactococci* counts from the other groups was maximum three log units. The predominance of *Lactococcus* sp. during the early stages of raw milk cheeses ripening was reported (Manolopoulou et al., 2003). Lactic acid bacteria (*Lactococci, Lactobacilli* and *Enterococci*) were quantitatively the dominant groups, and change of their viable numbers was significant (P≤0:01) throughout the ripening period. Numbers of *Enterococcus* sp. in all samples of Ras cheese were almost the same in Blue cheese. The presence of *Enterococci* sp. in high numbers could be due to their tolerance to a wide range of environmental conditions such as low temperature, high salt content and acidity (Lorencová et al., 2012; Buňková et al., 2010). Because of these properties, although all microorganisms were effected from salt significantly (P≤0:05), *Enterococcus* sp. were not. *Enterococci* are a group of microorganisms that may influence the ripening process due to their proteolytic and lipolytic activities and their ability to stimulate acid production by some *Lactococci* (Sarantinopoulos et al., 2001). Total mesophilic aerobic bacteria increased reaching their highest numbers during a 45 day ripening period at cold storage, and then rapidly declined. Numbers of microorganisms indicative of the hygienic quality, such as coliforms, *Enterococcus* sp. and *Lactococcus* sp. were present in cheese at relatively high levels. These counts suggest that contamination was very high in raw milk. Numbers of coliforms and

Table 4. Means counts of microorganisms in Egyptian market cheeses.

Cheese type	Ripening/ storage period (month)	Aerobic mesophilic bacteria (10^6 cfu/g)	Coliform group (10^2 cfu/g)	Moulds and Yeasts (10^3 cfu/g)	Lactococci (10^4 cfu/g)	Enterococci (10^4 cfu/g)	Lactic acid bacteria (10^6 cfu/g)
Mish	6	7.59± 0.13^{b}	4.98± .15^{ef}	3.89 ± 0.21^{f}	7.13± 0.19^{a}	6.45± 0.22^{a}	10.24±0.09^{a}
	12	7.37±0.42b^{c}	5.07± .44de	4.05± 0.20^{ef}	6.94± 0.31^{b}	6.38± 0.87^{b}	9.66±0.12^{b}
	24	6.84± 0.06^{fg}	5.27± .59^{cde}	4.33± 0.63^{de}	6.75± 0.18^{c}	6.33± .44^{bc}	9.50±0.17^{bc}
	36	6.28± 0.04^{i}	5.34± .57^{cd}	4.74± 0.90^{c}	6.66± .06^{cde}	6.13± 0.54^{c}	9.23±0.08^{def}
	48	6.09± 0.18^{i}	5.53± .35^{bc}	4.82± 0.42^{c}	6.34± 0.37^{f}	5.88± 0.30^{d}	9.15±0.13^{ef}
Ras	6	7.13± 0.20^{de}	4.5± 0.23^{g}	4.05± 0.20^{ef}	6.67±0.20^{cd}	5.82±0.18^{de}	9.65±0.20^{b}
	9	6.95± 0.24^{ef}	4.63± 0.21^{g}	4.37± 0.43^{d}	6.58± .17de	5.78± .39de	9.55±0.13^{bc}
	12	6.65± 0.21^{gh}	4.72± .42^{gf}	4.79± 0.61^{c}	6.50± .13^{ef}	5.73± .47de	9.30±0.11^{de}
	24	6.51± 0.18^{h}	3.93± 0.23^{h}	5.01± 0.39^{bc}	6.40± 0.19^{f}	5.63± 0.38^{e}	9.13±0.07^{ef}
Blue-viened	6	7.92± 0.24^{a}	5.84± 0.25^{a}	4.82± 0.42^{c}	4.51± 0.14^{g}	5.88± 0.41^{d}	9.36±0.011^{cd}
	9	7.37± 0.21^{bc}	5.65± .26^{ab}	5.23± 0.16^{ab}	4.38± 0.21^{g}	5.78± .44de	9.30±0.06^{de}
	12	7.22± 0.58^{ed}	5.27± .19^{cde}	5.37± 0.39^{a}	4.00± 0.09^{h}	5.73± .30de	9.08±0.08^{f}

Microbiological composition of Egyptian market cheeses (means ± SD). Means log counts in triplicates.

Enterococcus sp. were not reduced significantly (P≤0:05), while numbers of *Lactobacilli* sp. were also reduced significantly depending on the ripening time (P≤0:01), but they remained alive. This can be explained by the pH levels and the quantity of lactic acid. Counts of yeasts and moulds were in Mish cheese similar to other findings in Ras cheeses. During the ripening/storage period, the numbers were not significantly decreased (P≤0:01), and they had relatively high counts in Blue cheeses. Yeasts were present at various levels among the distinct cheeses, grouped from dairy markets, the differences in numbers may be due to the distinct pH and salt concentrations found between the corresponding cheeses, although no significant correlations resulted. Occurrence of yeasts in cheeses was variable, because they have been associated with the production of flavour compounds as a result of their relatively strong proteolytic and lipolytic activities. However, scant information is available regarding the contribution of yeasts to synthesis of BAs in foods: a histidine-decarboxylase activity was found in yeasts of the genera Debaromyces and Candida isolated from fermented meat (Montel et al., 1999) and such an activity was actually above that observed in lactic acid bacteria. Macedo et al. (1995) found that the presence of yeasts was closely related to lactic acid utilization, while their contribution to the ripening process was due to their proteolytic and lipolytic activities. In this study, the number of microorganisms such as yeasts, moulds and coliforms causing spoilage of cheeses by their putrefactive effects were decreased slightly. Formation of basic compounds from proteolysis could be as a resulting of changing of pH and a decrease of acidity. As recommendation, the permissible level of biogenic amines stipulated by Egyptian Organization for Standardization and Quality Control (EOS, 1996) should be modified to meet the more safe standard adopted by Food Drug Administration (FDA, 2001) and their levels can be lowered by using good quality raw milk and maintaining hygiene standards during manufacturing and storage processes.

Conclusions and recommendations

The main feature influencing the BAs formation is the extent of growth of microorganisms, like *Enterococci* sp. characterized by decarboxylase activity. The presence of high contents of BAs in Mish and Ras cheeses could be related to the enzymatic activity of proteases derived from microorganisms, or from another factor, that is important from a qualitative point of view, that is, in relation to the type of amino acids provided to the amino acid decarboxylating microbiota, in particular tyrosine. Therefore, a large amount of BAs in cheese reflects the bad hygienic conditions under which they are produced and stored. Accordingly, the levels of biogenic amines in different cheeses should be in accordance with the safe permissible limit recommended by FDA to ensure human safety.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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