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Chemical, Microbial Counts and Evaluation of Biogenic Amines During the Ripening of Egyptian Soft Domiati Cheese Made from Raw and Pasteurized Buffaloes Milk

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Abstract: This study was conducted to compare the usage of raw, pasteurized and pasteurized buffalo milk with starter culture in the manufacture of Egyptian soft Domiati cheese. In addition, evaluate the chemical, microbial characterization and evaluation of biogenic amines throughout the ripening. The physical and chemical properties of the manufactured cheese were different. Soluble nitrogen, salt as well as pH values were higher in raw milk cheese in comparison with pasteurized milk cheese and pasteurized milk cheese with starter culture. Considerable changes had occurred in raw milk cheese during the storage period more than these with pasteurized milk cheese and pasteurized milk cheese with starter culture. Cheese made from raw milk showed higher microbial counts during ripening than those made from pasteurized milk. Raw milk cheese showed remarkably higher biogenic amines compared with pasteurized milk cheeses. Therefore, pasteurization of milk led to a decrease in final biogenic amine content of cheese as a result of the reduction of its microbial population. The obtained results suggest that, pasteurization greatly improves the keeping quality of soft Domiati cheese and increase its shelf life and decrease the formation of biogenic amines.

Key words: Buffaloes milk, domiati cheese, pasteurized milk, starter, biogenic amines

INTRODUCTION

Biogenic amines are organic bases of low molecular weight that exhibit biological activity and are usually produced by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones and the requirements for the formation of biogenic amines in foods and beverages includes: Availability of free amino acids, the presence of decarboxylase-positive microorganisms and conditions that enable bacterial growth (Geornaras *et al*., 1995). A high protein containing ripening foodstuff, it belongs to the products where the degradation of proteins during ripening leads to the accumulation of free amino acids, which can be converted (due to the activity of bacterial decarboxylases) into biogenic amines (Innocente and Dagostin 2002). Ripening cheeses are the next (after fish) most commonly implicated food item associated with biogenic amine poisoning, quantitatively and toxicologically the most important biogenic amines (histamine, tyramine, tryptamine, phenylethylamine, cadaverine) in ripening cheeses are tyramine and histamine. Tyramine is a potent vasoconstrictor; its higher levels in an organism can lead to hypertension and migraine and can induce brain haemorrhage and heart failure (Til *et al*., 1997). Histamine (also vasoactive substance) can cause urticaria, hypotension, headache, flushing and abdominal cramps (Coleman *et al*., 2004). Tyramine and

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histamine are broken down in the mammalian organism by oxidative deamination catalysed by monoamine oxidase (Tomas *et al*., 2008). Toxicological importance of polyamines is based on their ability to form stable carcinogenic N-nitroso compounds and to enhance the growth of chemically induced aberrant crypt foci in the intestine (Paulsen *et al*., 1997). Polyamines are required for normal cell growth and proliferation, but are readily taken up by tumor cells; a strict control of the polyamine content in the diet of the cancer patients is therefore a matter has an extremely importance issue (Kalac and Krausov, 2005). Putrescine stimulates tyrosine kinases and the expression of particular nuclear protooncogenes and is in this sense involved in cancer pathogenesis (Ulrich *et al*., 2004). The concentration of biogenic amines in cheeses depends on variety, age and type of microflora (Innocente and Dagostin, 2002). Some biogenic amines in cheese may arise from decarboxylation of amino acids by microorganisms (Joosten and Olieman, 1986) but others can be natural (Bardocz, 1995). Biogenic amines in cheese could be a result of the decarboxylase activity of the fermentative microflora. However, these amines may also arise from the microbial activity of raw milk microbial, contaminants during cheese making (Hernandez-Jover *et al*., 1996). A high concentration of these amines could be used as an indicator of the hygienic quality of cheese (Scheneller *et al*., 1997). Domiati cheese is considered to be the most popular soft white cheese in Egypt and in other Middle Eastern countries. Domiati cheese is usually made from buffalo milk and cow milk, or a mixture but is also made from sheep or goat milk (Abou-Donia, 1986). This soft white cheese has been made from pasteurized milks containing 1 to 6% fat and by addition of 2 to 15% salt. Domiati cheese also has been made with or without the addition of starter cultures to cheese milk (Abou-Donia, 1986). To avoid the use of excessive salt and to retain the typical flavor and body characteristics of Domiati cheese, various heat treatments (50 to 95°C for 15 to 30 min) of the milk and the addition of lactic cultures to the milk prior to manufacture have been studied (Abou-Donia, 1986). Single-or mixed-strain cultures of streptococci and lactobacilli in different combinations have been used by several investigators (Abou-Donia, 1986). Generally, starter cultures govern the flavor, body and texture of the cheese and help suppress the growth of pathogenic and spoilage bacteria. Several studies have addressed the effects of the treatment of milk on the accumulation of biogenic amines in cheese made from cow and ewe milk (Ordonez *et al*., 1997; Schneller *et al*., 1997). In general, there is a greater consumption of cheese made from cow milk; however, in the Mediterranean, homemade style cheese made from goat milk is common. Nevertheless, there are few data on the occurrence of biogenic amines in Domitei buffalos cheese, or the factors affecting their formation, with the exception of data reported by Novella-Rodríguez *et al*. (2003). This investigation has been carried out to study the effect of pasteurization and starter culture on the organoleptic, chemical and microbiological quality of Domitei cheese during manufacturing and ripening. In addition to, the above aims, to study effect of milk treatment on the biogenic amine profile in Domatei cheese made from raw and pasteurized buffalos milk with and without starter culture.

MATERIALS AND METHODS

The work was conducted in Food Technology Research institute, Agriculture Research center, Giza, Egypt during (2007-2008). Fresh buffalo's milk obtained from Animal production Research Institute, dairy farms Mahalet Mousa, Kafrah El-Sheik, Egypt. Milk was immediately cooled to 5°C transported to the pilot plant and maintained cold until use, then standardized to 6 and 8.5% fat and solid not fat, respectively. Rennet powder, calcium chloride, yogurt B-6 starter (a mixed strain of *Streptococcus salivanus* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*), were obtained from Chr. Hansen's Lab. A/S (Copenhagen, Denmark). Salt was obtained from a local market. Domiati cheese was manufactured with some modifications according to Abou-Donia (1986) as follow: The standardized raw milk was used in three experimental trials. The first trial used raw milk warmed at 40°C, at which rennet, calcium chloride (0.03% w/w) were added (treatment A). The second trial used heat treated milk. The milk was heated at 75°C for 15 sec then warmed to 40°C at rennet, calcium chloride (0.03% w/w) were added (treatment B). The third trial used pasteurized milk (C), the milk was heated at 72°C for 15 sec, 2% starter cultured, calcium chloride (0.03% w/w) and rennet were added. All treatments (A, B and C) were left to coagulate in 2-3 h at 40°C. The curd was scooped and whey into molds, lined with coarse cloth (netting), to drain. The manufactured cheese was stored at 10°C in soldered tins, filled with boiled salted whey (7%) and analyzed when fresh and after 15, 30, 45, 60, 90 and 120 days of storage.

Organoleptic Examination

The cheese samples were organoleptically scored using score card for flavor (50 points), body and texture (35 points) and appearance and color 15 points). The scores were averaged by five panelists according to Nelson and Trout (1981).

Chemical Analysis

All cheese samples were chemically examined for pH using pH meter. pH was measured with an Orion pH meter (Orion Research Inc., Cambridge, MA), titratable acidity according to AOAC (2000). Moisture; salt content; fat, Total Nitrogen (TN) and soluble nitrogen content (SN) were curried out according to the method described by Kuchroo and Fox (1982) and Guinee and Fox (1993). All analysis of cheese samples were performed in triplicate.

Microbiological Analysis

The cheese samples were prepared for microbiological examination according to ICMSF (1968). The treated cheese samples were examined for Total Colony Count (TCC); aerobic spore former count; total proteolytic count; *Coliform* and total mold and yeast count/g, according to American Public Health Association (APHA, 1992). All experiments were repeated in triplicate and each analysis in duplicate.

Biogenic Amines Contents (%)

Extraction Method

Biogenic amines were determined by High Performance Liquid Chromatography (HPLC) in National Research Center, Cairo, Egypt according to Bütikofer and Bosset (1994) at Mycotoxins Central and Food safety as the following; sample preparation was done by cutting the cheese by a sharp tool to small pieces s. One hundred and twenty five milliliter of Trichloroacetic acid (TCA) 5% was added to 25 g of cheese sample to percipetate the protein after that sample was blended for 3 min then filtrated throw filter paper Watt man No. 1. 10 m: from the filtrate was transferred into a test tube (20 mL) and 4 g sodium chloride (NaCl) was added in the same tube to avoid turbidity during the extraction. One milliliter NaOH (50%) was added to calibrate acidity. All results were expressed in mg kg^{-1} dry matter.

HPLC Methods

High performance liquid chromatography system (Water) applied with model 600 delivery system, model Water (486). UV detector set at 254 nm and the data were integrated and recorded by Millennium chromatography Manger software 2010 (waters Milford MA 0157). No Va PaK C18 colum 30 g × 150 mm, 5 µm. Mobile phase solvents consists of solvent A: Acetonitrile: 0.02 N acetic acid (1:9) and solvent (B) 0.02 N acetic acid: acetonitrile methanol (1:9:9) were applied in linear gradient program at rate 1 mL min.

RESULTS AND DISCUSSION

Organoleptic Properties

Data shown in Table 1 showed the organoleptic total score of fresh and refrigerated stored cheese made from raw, pasteurized milk and pasteurized milk with starter culture. The flavor in all types of cheese was improved during storage period. The flavor of raw milk cheese had the highest total score

Table 1: Organoleptic examination of Domiati cheese samples

Storage periods (days)	Treatments	Organoleptic score			
		Flavor (50)	Body and texture (35)	Appearance and color (15)	Total score (100)
Fresh zero	A	47	35	13	95
	B	44	33	14	91
	C	44	32	15	91
15	A	47	35	13	95
	B	44	33	14	91
	C	44	32	15	91
30	A	45	33	12	90
	B	42	32	13	87
	C	42	30	14	86
45	A	46	33	12	91
	B	43	32	13	88
	C	43	30	14	87
60	A	47	34	11	93
	B	44	32	12	88
	C	43	31	14	88
90	A	47	34	11	92
	B	44	32	12	88
	C	43	31	14	88
120	A	47	35	11	93
	B	43	33	12	88
	C	42	32	13	87

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture

compared to pasteurized cheese and pasteurized cheese milk with starter culture, respectively. This may be due to the natural flora initially present in raw milk which participate in flavor production (Salwa and Gala, 2002).

Chemical Analysis

Pasteurized milk cheese and this with starter culture and without revealed higher moisture content than raw milk cheese. The moisture content also decreased in all cheese types throughout the storage period and this may be due to salt concentration of the filling solution (Table 2). The fat % was slightly lower in pasteurized milk cheese without starter culture (B) and pasteurized milk cheese with starter culture (C) than in raw milk cheese, while it increased during storage period as a result of the decrease in moisture content. Concerning the salt/water %, the higher salt water content was detected in raw milk cheese than the other types of cheese either fresh or during storage. Cheese yield also affected by heat treatment. It was noticed that the highest cheese yield was obtained in pasteurized milk cheese either fresh or during the storage period. This may be attributed to the effect of pasteurization on kappa casein forming complex with B lactoglobulin which increase clotting time and subsequent cheese yield (Salwa and Gala, 2002). As shown in (Table 2) cheese made from pasteurized milk without starter culture had pH values higher than other treatments. This may be due to the effect of heat treatment on microorganisms. On the other hand, pasteurized milk cheese had the highest pH value. This trend was observed till reach the minimum pH at the end of storage period. This may be due to attribute to the high microbial content of raw milk cheese and starter culture and the greater utilization of lactic acid leading to low pH value, while pasteurized milk cheese contained the lowest bacterial count owing to the effect of pasteurization (Ghosh *et al*., 1999). Nearly similar finding were reported by Abd El-Salam *et al*. (1992) and Salwa and Gala (2002). The data presented in Table 2 show the pasteurized milk cheese had lowest Titratable Acidity (TA) than those made from raw and pasteurized milk cheese with starter culture. During cheese ripening, the T.A. increased in all types of cheese. Nearly similar finding were obtained by Abd El-Salam *et al*. (1992) and Marth and Steele (2001). The data shown in Table 2 showed the effect of pretreatment of milk on Total Nitrogen (TN) and Soluble

Table 2: Chemical composition of Domaitei cheese

Storage period\days	Cheese trails	Chemical composition Moisture (%)	Fat (%)	F\D	Acidity (%)	pH	Salt (%)	SN/TN* (%)
Zero (fresh)	A	59.65	18.20	45.11	0.22	6.45	7.46	9.58
	B	60.75	17.90	45.61	0.20	6.45	7.40	8.72
	C	61.40	17.60	45.65	0.22	6.42	7.24	9.40
15	A	58.91	18.65	45.38	0.33	6.05	7.60	11.26
	B	59.48	18.55	45.78	0.28	6.15	7.65	10.29
	C	59.95	18.25	45.57	0.25	6.32	7.51	9.70
30	A	57.90	19.30	45.84	0.43	5.88	7.85	11.38
	B	59.35	19.10	45.86	0.32	6.05	7.96	10.70
	C	58.80	18.90	45.87	0.28	6.19	7.83	9.87
45	A	57.41	19.55	45.89	0.49	5.65	7.98	12.11
	B	58.09	19.28	46.00	0.38	5.90	8.01	11.13
	C	58.55	19.05	45.96	0.31	6.08	7.94	9.92
60	A	58.15	19.88	46.39	0.55	5.35	8.14	13.05
	B	57.88	19.45	46.18	0.43	5.80	8.12	11.82
	C	58.22	19.25	46.07	0.37	5.98	7.99	10.07
90	A	56.75	20.18	46.66	0.62	5.15	8.19	14.06
	B	57.25	19.85	46.43	0.48	5.75	8.30	12.75
	C	57.75	19.50	46.15	0.42	5.88	8.14	10.44
120	A	55.90	20.75	47.05	0.73	4.90	8.41	16.19
	B	57.18	20.05	46.82	0.48	5.80	8.43	13.95
	C	57.55	19.05	46.76	0.40	5.95	8.25	10.83

*SN/TN: Soluble nitrogen/total nitrogen% *F/D: Fat/dry matter%

Nitrogen (SN) content of the manufactured cheese. Pasteurized milk cheese showed the lowest Total Nitrogen (TN%). During storage period, TN% increased in all types of cheese. The highest values of SN/TN% were recorded with the raw milk cheese either fresh or during storage followed by pasteurized milk cheese and pasteurized milk cheese with starter culture respectively. The lower rate of ripening in pasteurized milk cheese may be due to the destructive effect of heat treatment on the natural flora and milk enzymes which in turn affect fat and protein degradation (Salwa and Gala, 2002).

Microbiological Analysis

Total Colony Count (TCC)

Data presented in Table 3 shows quite clearly that there was an increasing in Total Colony Count (TCC) in the cheese of the three manufacture trials at refrigerated storage. The TCC of cheese in all manufactured trials gradually increased until 60 days of refrigerated storage. This increase can be explained by the sufficient change in the environmental conditions which happen during cheese storage and allow the growth and multiplication of microorganisms (Salwa and Gala, 2002). It could be noticed that TCC of pasteurized milk cheese was less than other trials. This was probably due to the destruction of bacteria by milk pasteurization process and rapid cooling of milk at 5°C before renneting which drastically reduce the growth rate of microorganisms than raw and heat treated milk cheese (Rehman *et al*., 2000; Masud *et al*., 2007).

Aerobic Spore Former Count

As shown in Table 4, gradual increase in aerobic spore former count of all manufactured cheese trials was demonstrated up to 60 days of refrigerated storage. The results showed that pasteurized milk cheese contained less aerobic spore former than other trials. Nearly similar finding were reported by El-Sissi and Neamat Allah (1996). Growth of aerobic spore former in raw milk produces extra cellular lipase enzyme which absorb on milk fat globules and concentrated in the manufactured cheese. During storage, the enzyme causes bitter flavor by hydrolysis of fats into fatty acids and glycerides. The

Table 3: The mean total colony count (cfu g^{-1}) of Domiati cheese

Storage period (days)	A	B	C
0 (fresh)	19×10^7	2.8×10^4	5.0×10^7
15	30×10^7	5.3×10^4	3.1×10^7
30	51×10^7	7.4×10^4	5.2×10^7
45	60×10^7	8.0×10^4	9.7×10^7
60	73×10^7	8.9×10^4	8.0×10^7
90	15×10^7	6.6×10^4	4.4×10^7
120	41×10^8	1.3×10^4	2.8×10^3

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture

Table 4: Total aerobic spore former count (cfu g^{-1}) of Domiati cheese

Storage period (days)	A	B	C
0 (fresh)	31×10^4	2.2×10^2	3.4×10^2
15	45×10^4	35×10^2	3.8×10^2
30	51×10^4	39×10^3	4.1×10^2
45	60×10^4	46×10^3	7.0×10^2
60	72×10^4	61×10^3	8.7×10^2
90	40×10^4	13×10^3	3.3×10^2
120	16×10^4	87×10^2	1.1×10^2

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture

Table 5: Total proteolytic count of Domiati cheese

Storage period (days)	A	B	C
0 (fresh)	35×10^4	1.1×10^2	1.6×10^2
15	51×10^4	1.7×10^2	2.0×10^2
30	59×10^4	2.4×10^2	3.4×10^2
45	77×10^4	4.8×10^2	5.6×10^2
60	67×10^4	2.7×10^2	2.5×10^2
90	42×10^4	1.0×10^2	1.5×10^2
120	120×10^3	1.0×10	1.0×10

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture

enzyme could be inactivated by pasteurization, while in raw cheese milk the enzymes were still active. So, raw milk cheese may subjected to rapid spoilage than other treatments (Chen *et al*., 2003).

Total Proteolytic Count

As shown in Table 5 the total proteolytic count of cheese was increased in all manufactured trials up to 60 days and then decreased until the end of 120 days of storage. Pasteurized milk cheese demonstrated significant decrease in total proteolytic count than raw and pasteurized milk cheese. At the end of 120 days refrigerated storage, pasteurized milk cheese showed the lowest values of proteolytic organisms. Nearly similar finding was recorded by Urbach (1993) and Ordonez *et al*. (1997). Proteolysis is the most important process happens during cheese storage. It contributes to cheese off-flavor, off odor and abnormal texture through the break down of the released proteolytic products such as amino acids and peptides into amines and acids. Their growth in cheese leading to production of protease enzyme which affect on the plasmin and plasminogen of the casein micelle leading to slow cheese making and low cheese yield. The enzyme could not affected by heat treatment but may be destroyed at 70°C for 15-30 sec. This explain the relationship between the high proteolytic count and the low cheese yield in raw and heat treated milk cheese (Beuvier *et al*., 1997).

Total Coliform Count

Data in Table 6 summarized the total Coliform count in different cheeses. From the data it could be seen that Coliform counts markedly decreased with heat treatment and completely disappeared in

Table 6: The mean total Coliform count (MPN g^{-1}) of Domiati cheese

Storage period (days)	A	B	C
0 (fresh)	120×10³	ND	ND
15	300×10³	ND	ND
30	44×10⁴	ND	ND
45	61×10⁴	ND	ND
60	23×10³	ND	ND
90	15×10³	ND	ND
120	2.1×10³	ND	ND

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture; ND: Not detected

Table 7: The mean total Coliform count (MPN g^{-1}) of Domiati cheese

Storage period/days	A	B	C
0 (fresh)	6.1×10^6	4.7×10^2	5.2×10^2
15	6.5×10^6	5.9×10^2	6.1×10^2
30	7.8×10^6	6.2×10^2	6.7×10^2
45	8.3×10^6	69×10^3	7.1×10^2
60	9.3×106	8.6×10^2	9.1×10^2
90	2.1×10^6	5.1×10^2	5.0×10^2
120	1.8×10^6	2.1×10^2	1.9×10^2

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture

cheese made from pasteurized milk. The obtained results can explain the blowing defects which may appear in cheese made from raw milk due to gas production by Coliform (Moatsou, 2001; Salwa and Gala, 2002).

Total Mold and Yeast Count

The total mold and yeast count were higher in cheese made from raw milk in comparison with other treatments (Table 7). This increase may be correlated to the higher acidity of raw milk cheese which may improve their growth. Nearly similar findings were reported by Salwa and Gala (2002). Yeast and mould are considered as spoilage organisms resulting in flavor and textural deterioration including softening, discoloration and slime formation (Besancon *et al*., 1992). International microbial legislation for soft cheese should not exceed 10^2-10^3 cfu g^{-1} with their freedom from all pathogenic microorganisms, raw milk cheese is more likely to serve as a vector for food borne illness.

Biogenic Amines Contents

The data presented in Table 8 demonstrated the effect of pasteurization and starter culture in the formation of biogenic amines in Egyptian soft Domiati cheese made from buffaloes' milk during ripening period 120 days at 10°C. The contents of biogenic amines were affected differently by the tested variability factors in the present experiment pasteurization and starter culture. The data showed that there was an increased progressively in the biogenic amines (Tyramine TY, tryptamine TR, β-phenylethylamine PHE, putrescine PU, cadaverine CA, histamine HI) during ripening. The final amounts of TY and CA in treatment A were higher than in other than treatments (B and C) this could be explained the contamination of raw milk by the enterococci and also lactobacilli. According to Kebary *et al*. (1999), the content of TY and PHE and CA in cheese are associated with the number of enterococci. The accumulation of TY has also been related to non-starter lactic acid bacteria, mainly lactobacilli (Novella-Rodrıeguez *et al*., 2004). The production of TY was also related to lactococci (Durlu-Ozkaya *et al*., 2001). On the other hand, the content of TY and PHE amines in both kinds of cheese treatment B and C were similarly results indicate that the starter culture that used was unable to produce biogenic amines. The obtained results are agreement with the results obtained by Novella-Rodrıguez *et al*. (2004). The TY and PU contents observed in this work were similar or higher than

Table 8: Biogenic amines contents (mg kg^{-1} dry matter) of Domiati cheeses made from raw and pasteurized Egyptian buffalos milk during storage period (120) days

Biogenic amines	Experimental Egyptian domiati cheeses / Storage period 120 days / A							B							C						
	0	15	30	45	60	90	120	0	15	30	45	60	90	120	0	15	30	45	60	90	120
Tryptamine	2.29	4.68	7.19	9.43	11.16	13.34	14.87	<0.05	2.21	4.84	5.91	7.12	8.88	9.76	<0.05	2.23	4.79	7.16	9.04	9.68	10.11
β-Phenyle-thylamine	0.96	2.94	5.66	10.89	18.64	25.79	31.12	0.79	1.34	3.64	5.91	7.97	8.86	0.14	0.76	1.33	3.66	5.89	7.94	8.86	9.89
Putrescine	9.62	26.99	38.22	44.56	73.18	85.78	92.23	0.81	3.85	7.99	9.14	13.98	18.22	22.15	0.84	3.83	7.97	9.11	12.98	18.65	23.08
Cadaverine	52.47	71.19	84.35	92.47	154.12	198.11	223.32	1.25	1.97	5.97	9.65	17.87	23.43	34.44	1.34	1.99	5.94	9.61	16.88	22.40	31.98
Histamine	2.85	3.87	5.67	9.63	22.18	28.99	44.23	<0.05	1.33	2.38	3.83	4.98	6.56	8.92	<0.05	1.29	2.25	3.87	7.13	8.99	9.21
Tyramine	3.96	30.24	69.87	85.97	246.12	297.34	365.23	<0.05	1.11	2.45	6.98	8.21	10.65	14.12	<0.05	1.20	2.45	6.98	8.43	11.45	15.12

A: Cheese made from raw milk; B: Cheese made from pasteurized buffaloes milk; C: Cheese made from pasteurized buffaloes milk with starter culture

those reported elsewhere in various cheeses, like Idiazábal cheese (Ordonez *et al*., 1997) and Manchego cheese (Fernández-García *et al*., 2000). The other biogenic amines found in the different cheese samples can be ordered according to their amount as follows: PU, HI, PHE and TR, all of which showed higher contents in cheeses from raw milk (3-7 times greater) than in cheeses from pasteurized milk and pasteurized milk with starter culture. The higher content of CA, PU and HI in batch from raw milk could be explained by the higher enterobacteriaceae and lactobacilli counts in treatment A. These amines are commonly associated with enterobacteriaceae (Kebary *et al*., 1999; Komprda *et al*., 2008; Martuscelli *et al*., 2005) and they can also be produced by lactobacilli (Stratton *et al*., 1991; Novella-Rodrıguez *et al*., 2002; Lanciotti *et al*., 2007). Likewise, the relatively high number of lactobacilli in cheeses from pasteurized milk might explain why PU, CA and HI accumulated after 15 d ayripening. On other hand, the minor biogenic amines, PHE and TR, contents were also higher in cheeses from raw milk, with PHE and TR being three and two times higher than in cheeses from pasteurized milk, respectively. The production of PHE by enterococci in cheese has been related to tyrosine decarboxylase positive activity, since this enzyme can also use phenylalanine as substrate (Joosten and Nunez, 1996). In agreement with this, the cheeses with high levels of TY also exhibited high levels of PHE. However, the PHE values found in our study were lower than those reported by other researchers in ripened cheese made from cow milk (Komprda *et al*., 2008) and in Feta cheese (Valsamaki *et al*., 2000) and in goat cheese (Novella-Rodrıguez *et al*., 2004), but higher than those found in Idiazábal cheese (Ordonez *et al*., 1997) and in cow cheese (Fernández-García *et al*., 2000; Aliakbarlu *et al*., 2009). The formation of biogenic amines in Egyptian soft Domiati cheese made from pasteurized and raw buffaloes' milk with and with out starter culture is an extremely complex phenomenon, dependent of several variable factors such as the presence of microorganisms, their proteolytic and decarboxylase activities, ripening time and ripening temperature. To control the biogenic amine formation the quality of milk and hygiene during cheese manufacturing should also be optimized and standardized. Pasteurization of milk eliminates some of the bacteria that are the major cause of biogenic amine production in cheese, this being the main explanation for the lower amine contents in cheeses from pasteurized milk and pasteurized milk with selected starter culture. However, it is also clear from our results that the pasteurization and the selection of starter culture which are unable to formation of biogenic amines. The ripening time and other factors such as the degree of proteolysis can also play a principle role in amine biogenesis and should also be taken into account to avoid the formation of biogenic amines. Hence, this study recommended the Egypt government should be applied a Critical Control Point during the implementation of HACCP for the production milk and cheese manufacture and selected the suitable starter culture unable to formation of biogenic amines to obtain cheeses with low or moderate levels of biogenic amines and with high quality, safety and premium grade.

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