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EVALUATION OF FORMATION 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 buffalo milk either raw, pasteurized or pasteurized with starter culture in the manufacture of Egyptian soft Domiati cheese. In addition, studing the chemical, microbial characterization and evaluation of biogenic amines throughout the ripening. The results revealed that organoleptic 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 increases its shelf life and decreases the formation of biogenic amines.

Keywords: Buffaloes milk, Domiati cheese, pasteurized milk, starter and 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. 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 & 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 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 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 & 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 & 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 ºC to 95 ºC 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-Rodrı´guez *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 the 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

This 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* ºC transported to the pilot plant, and maintained cold until use, then standardized to 6% & 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 follows: The standardized raw milk was used in three experimental trials. The first trial used raw milk warmed at 40ºC, 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 75ºC for 15 sec then cooled to 4 ºC and warmed to 40 ºC at rennet, calcium chloride (0.03% w/w) were added (treatment B).

The third trial used pasteurized milk (C), the milk was heated at 75ºC for 15 sec then cooled to 4 ºC and warmed to 40 ºC at 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 ºC. The curd was scooped and whey into molds, lined with coarse cloth (netting), to drain. The manufactured cheese was stored at 10 ºC 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.

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, cheese yield; total nitrogen (T.N.) and soluble nitrogen content (S.N.) were curried out according to the method described by Kuchroo and Fox (1982) and Guinee and Fox (1993). All analyses 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.

Organoleptic examination

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

Biogenic amines contents %

Extraction method

Biogenic amines were deterimined by High performance Liquied Chromatography (HPLC) in National Research Center, Cairo, Egypt according to Bütikofer & Bosset (1994) at Mycotoxins Central & Food safety as the following; sample prepration was done by cutting the cheese by a sharp tool to small pieces s. 125 ml of Trichloroacetic acid (TCA) 5% was added to 25gm 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 ml from the filtrate was transferred into a test tube (20 ml) and 4g sodium chloride (NaCl) was added in the same tube to avoid turbidity during the extraction. 1 ml NaOH (50%) was added to calibrate acidity. All results were expressed in mg/kg dry matter.

HPLC conditions:

HPLC system (Waters) applied with model 600 delivery system, model Waters (486). UV detector set at 254nm and the data were integrated and recorded by Millennium chromatography Manger software 2010 (waters Milford MA 0157). No Va PaK C18colum 30 gx150mm, 5 µm. Mobile phase solvents consists of solvent A: Acetonitrile: 0.02 N acetic acid (1:9) and solvent (B) 0.02N acetic acid: acetonitrile : methanol (1:9:9) were applied in linear gradient program at rate 1 ml min.

RESULTS AND DISCUSSION

Chemical analysis

The effect of pretreatment of milk on the most important parameters of the manufactured Domiati cheese as moisture content, fat %, salt/water % and cheese yield were recorded in Table (1). Heat treatment 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. 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 heat treatment on kappa casein forming complex with B lactoglobulin which increase clotting time and subsequent cheese yield (Salwa and Galal. 2002). As shown in Table (1) cheese made from heat treatment milk without starter culture had pH values higher than other treatments.

Table 1: 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	9.7
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

*** S.N. / T.N. = soluble nitrogen/total nitrogen% *F/D = fat / dry matter%**

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 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 heat treatment milk cheese contained the lowest bacterial count owing to the effect of heat treatment (Ghosh *et al*., 1999). Nearly similar findings were reported by Abd El-Salam *et al*. (1992); Salwa and Galal, (2002). The data presented in Table 2 show the pasteurized milk cheese had lower titratable acidity (T.A.) 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 findings were obtained by Abd El-Salam *et al*. (1992); Marth & Steele,(2001). The data illustrated in Table 2 showed the effect of pretreatment of milk on total nitrogen (T.N.) and soluble nitrogen (S.N.) content of the manufactured cheese. Heat treatment milk(75ºC for 15 sec) milk cheese showed the lowest total nitrogen (T.N.%). During storage period, T.N.% increased in all types of cheese. The highest values of S.N./T.N. % were recorded with the raw milk cheese either fresh or during storage followed by heat treatment milk (75ºC for 15 sec)milk cheese and heat treatment milk (75ºC for 15 sec) milk cheese with starter culture respectively.

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The lower rate of ripening in heat treatment milk(75ºC for 15 sec) 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 Galal, 2002).

Microbiological analysis

Total colony count (T.C.C.)

Data presented in Table 3 shows quite clearly that there was an increasing in total colony count (T.C.C.) in the cheese of the three manufacture trials at refrigerated storage. The T.C.C. 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 & Galal. 2002). It could be noticed that T.C.C. of pasteurized milk cheese was less than other trials. This was probably due to the destruction of bacteria by milk heat treatment milk(75ºC for 15 sec) process and rapid cooling of milk at 5ºC 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).

Table 3: The mean total colony count (cfu/g) of Domiati cheese

Storage period/days	A	B	C
0(fresh)	$19x10^7$	$2.8\ x10^4$	$5.0\ x10^7$
15	$30x10^7$	$5.3x10^4$	$3.1x10^7$
30	$51x10^7$	7.4x104	$5.2x10^7$
45	$60x10^7$	$8.0x10^4$	$9.7x10^7$
60	$73x10^7$	$8.9x10^4$	$8.0x10^7$
90	$15x10^7$	$6.6x10^4$	$4.4x10^7$
120	$410x10^7$	$1.3x10^4$	$0.00028x10^7$

A= cheese made from raw milk
B = cheese made from pasteurized buffaloes milk
C= cheese made from pasteurized buffaloes milk with starter culture

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 findings 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 enzyme could be inactivated by heat treatment milk (75ºC for 15 sec) 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); 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 70ºC 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) .

Table 4: Total aerobic spore former count (cfu/g) of Domiati cheese

Storage period/days	A	B	C
0(fresh)	$31x10^4$	$2.2x10^2$	$3.4x10^2$
15	$45x10^4$	$35x10^2$	$3.8x10^2$
30	$51x10^4$	$390x10^2$	$4.1x10^2$
45	$60x10^4$	$460x10^2$	$7.0x10^2$
60	$72x10^4$	$610x10^2$	$8.7x10^2$
90	$40x10^4$	$130x10^2$	$3.3x10^2$
120	$16x10^4$	$87x10^2$	$1.1x10^2$

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)	$35x10^4$	$1x10^2$	$1.6x10^2$
15	$51x10^4$	$1.7x10^2$	$2x10^2$
30	$59x10^4$	$2.4x10^2$	$3.4x10^2$
45	$77x10^4$	$4.8x10^2$	$5.6x10^2$
60	$67x10^4$	$2.7x10^2$	$2.5x10^2$
90	$42x10^4$	$1x10^2$	$1.5x10^2$
120	$12x10^4$	$0.1x10^2$	$0.1x10^2$

A= cheese made from raw milk
B = cheese made from pasteurized buffaloes milk
C= cheese made from pasteurized buffaloes milk with starter culture

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 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 Galal, 2002).

Table 6: The mean total *coliform* count (MPN/g) of Domiati cheese

Storage period/days	A	B	C
0(fresh)	$120x10^3$	ND	ND
15	$300x10^3$	ND	ND
30	$440x10^3$	ND	ND
45	$610x10^3$	ND	ND
60	$23x10^3$	ND	ND
90	$15x10^3$	ND	ND
120	$2.1x10^3$	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

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 & Galal (2002). Yeast and mold 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 with their freedom from all pathogenic microorganisms, raw milk cheese is more likely to serve as a vector for food borne illness.

Table 7: The mean total Mold and Yeast (MPN/g) of Domiati cheese.

Storage period/days	A	B	C
0 (fresh)	$6.1x10^6$	$4.7x10^2$	$5.2x10^2$
15	$6.5x10^6$	$5.9x10^2$	$6.1x10^2$
30	$7.8x10^6$	$6.2x10^2$	$6.7x10^2$
45	$8.3x10^6$	$69x10^3$	$7.1x10^2$
60	9.3x106	$8.6x10^2$	$9.1x10^2$
90	$2.1x10^6$	$5.1x10^2$	$5.0x10^2$
120	$1.8x10^6$	$2.1x10^2$	$1.9x10^2$

A= cheese made from raw milk
B = cheese made from pasteurized buffaloes milk
C= cheese made from pasteurized buffaloes milk with starter culture

Biogenic Amines contents

The data presented in (table 8 and figure 1) demonstrated the effect of pasteurized milk and starter culture in the formation of biogenic amines in Egyptian soft Domiati cheese made from buffaloes' milk during ripening period 120 days at 10ºC. The contents of biogenic amines were affected differently by the tested variability factors in the present experiment pasteurized milk and starter culture. The data showed that there was an increase progressively in the biogenic amines (Tyramine TY, tryptamine TR, *β*-phenylethylamine PHE, putrescine PU, cadaverine CA, histamine HI) during ripening.

Table 8: Organoleptic examination of Domiati cheese samples.

Storage periods (days)	Treatments	Organoleptic score			
		Flavor (50)	Body & texture(35)	Appearance & 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

The final amounts of TY and CA in treatment A were higher than in other 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 contents 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 treatments B and C have similar results were indicate that the starter culture that used was unable to produce biogenic amines. The obtained results are agree with the results obtained by Novella-Rodrıguez *et al*. (2004). The TY and PU contents observed in this work were similar or higher than those reported elsewhere in various cheeses, like Idiaza´bal cheese (Ordonez *et al*., 1997) and Manchego cheese (Fernandez-Garcı´a *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).

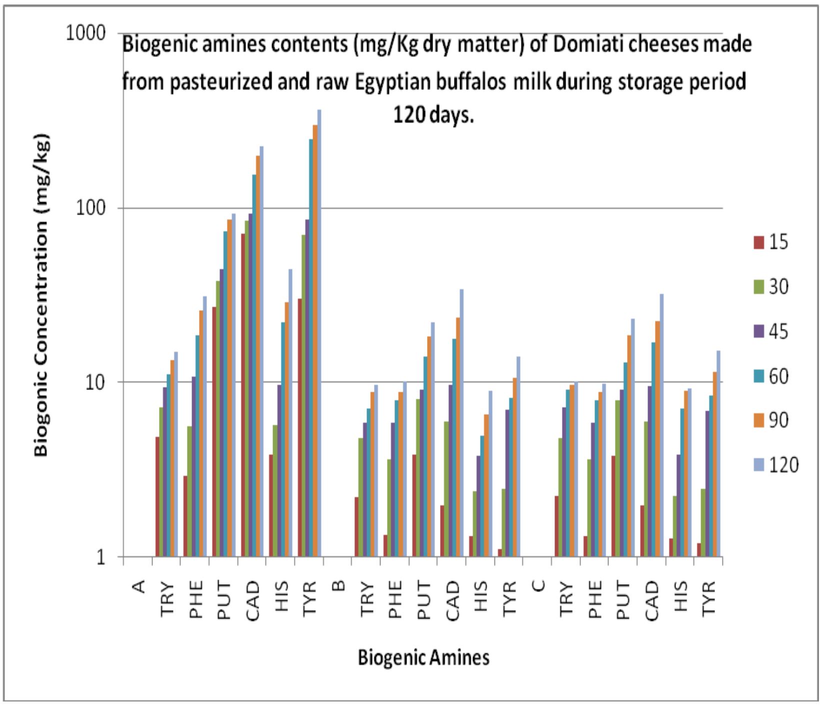

Figure 1: Biogenic amines contents (mg/Kg dry matter) of Domiati cheeses made from raw and pasteurized Egyptian buffalos milk during storage period (120) days

Likewise, the relatively high number of lactobacilli in cheeses from pasteurized milk might explain why PU, CA and HI accumulated after 15 d ripening. 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 & 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 Idiaza´bal cheese (Ordonez *et al*., 1997) and in cow cheese (Fernandez Garcı´a *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. pasteurized 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 pasteurized milk 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 to apply a Critical Control Point during the implementation of HACCP for the production of 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.

Organoleptic properties

Data illustrated in Table (1) showed the organoleptic total score of fresh and refrigerated stored cheese made from raw, heat treatment milk(75ºC for 15 sec) and heat treatment milk(75ºC for 15 sec) 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 compared to heat treatment cheese and heat treatment 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 Galal., 2002).

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تقيم تكوين الأمينات الحيوية أثناء تسوية الجبن الدمياطي المصري المصنوع من اللبن الجاموسي الخام والمبستر

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تم أجراء هذا العمل لمقارنة أستخدام اللبن الجاموسي الخام و المبستر واللبن المبستر مضاف إلية بادي وتأثير ذلك علي الخواص الكيميائية والبكتريولوجية وتقييم تكوين الأمينات الحيوية أثناء عملية التسوية. وقد أوضحت النتائج أن هناك اختلاف في الخواص الحسية والكيميائية حيث وجد أن هناك اختلاف في النيتروجين الذائب والملح ورقم الآس الهيدروجيني حيث كانوا أعلي في المعاملة المصنعة من لبن خام عن المصنعة من لبن المبستر والمصنعة من لبن المبستر ومضاف إلية بادئ. وهذه التغيرات والزيادة كانت أيضا ملحوظة خلال فترة التسوية أيضا. والجبن المصنوع من لبن خام كانت أعلي في العدد الكلي للبكتريا و الأنواع الأخرى منها بصفة عامة خلال فترة التخزين. وكذلك أيضا كانت الجبن المصنوع من لبن خام أعلي في محتواها من الأمينات الحيوية عن باقي المعاملات الأخري. من هذه النتائج المتحصل عليها يمكن أن نستنتج أن المعاملة الحرارية للبن واختيار البادي المناسب الجيد الغير قادر علي تكوين الأمينات الحيوية من العوامل الهامة التي توضع في الاعتبار لأنهما يقللان من تكوين الأمينات الحيوية و المعاملة الحرارية تؤدي الي تحسين خواص الجودة وصلاحية الجبن الدمياطي وتقلل من تكوين الأمينات الحيوية في الجبن الدمياطي أثناء التسوية.

Table 2: Biogenic amines contents (mg / Kg dry matter) of Domiati cheeses made from raw and pasteurized Egyptian buffalos milk during storage period (120) days.

Biogenic amines	Experimental Egyptian Domiati cheeses																				
	A							B							C						
	storage period 120 days																				
	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-Phenylethylamine	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	10.14	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	22.4	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	1.2	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