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STUDIES ON HISTAMINE IN SOME CHEESE

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Abstract

The present study was carried out to throw light on the level of biogenic amines (histamine) in some cheese and to evaluate the effects of *L. acidophuls* and *B. bifidum* "probiotic" on the formation of histamine during the manufacture and storage of domiati cheese for 4 months at 4°C and 25°C. A total of 100 cheese samples (25 each of Mish, Domiati, Cheedar and Rommano cheeses) were collected from super markets and dairy shops in Beni-Suef city, Egypt. The mean concentration of Histamine (Hi) in Mish, Domiati, Cheddar and Rommano cheeses were 114.16, 38.24, 9.96 and 126.77mg/kg respectively. Histamine levels were detected with higher concentration above the permissible limits (200mg/kg) in 12%, 0%, 0% and 20% of the examined Mish, Domiati, Cheedar and Rommano cheese samples respectively. Also the objective of this study assess the histamine formation in manufactured Egyptian Domiati cheese stored at 4°C and 25°C for four month and evaluate the capacity of *L.hilgardii* to production of histamine and evaluate the capacity of *Lactobacillus acidophilus* and Bifido bacterium as probiotic on the growth of *L.hilgardii* and histamine formation. The public health significance and suggested precautions for minimizing the level of such biogenic amine were discussed.

Introduction

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 contamination. Biogenic amines (BAs) are low molecular weight nitrogenous bases, they were found in a variety of foods, such as fish, meat, cheese, vegetables and wines (Mohamed *et al.,* 2013). Biogenic amines content can vary widely among and within cheese varieties and even between different sections of the same cheese (Novella Rodríguez *et al.,* 2003).

The most abundant BAs in cheeses are histamine (HIS), tyramine (TYR), putrescine (PUT) and cadaverine (CAD) (Linares *et al.,* 2011). Higher levels of BAs may be formed before the foods appear spoiled or organoleptically unacceptable. Both microbial ecology and BAs levels of cheeses during ripening are significantly influenced by the type of milk used and the technological treatment applied to the raw materials, as well as the storage conditions of the product (Lanciotti *et al.,* 2007).

Biogenic amines formation through the microbial decarboxylation of amino acids is dependent on the specific bacterial strains present, the level of decarboxylase activity and the availability of the amino acid substrate (*Suzzi and Gardini,* 2003). Also in most industrial dairy fermentations, starter cultures are used to ensure the standard quality of the final products. Some LAB generally used as starter cultures, may have specific amino acid decarboxylase activities and thus, the potential to synthesize BAs that could be accumulated in the dairy products. Belonging to this group are lactococci, lactobacilli and streptococci.

The consumption of food containing BAs is responsible for many pharmacological effects that lead to several types of foodborne disease such as histamine poisoning (scombroid poisoning) (CDC, 2000) and tyramine toxicity (cheese reaction); such toxicity of both histamine and tyramine appears to be enhanced by the presence of other amines, that is CAD, PUT and others. Moreover, BAs are considered as carcinogens because of their ability to react with nitrites and form potentially carcinogenic nitrosamines (Shalaby *et al.,* 2016).

Histamine poisoning is the most common food borne problem caused by BA. At nontoxic doses, food borne histamine can cause intolerance symptoms such as diarrhea, hypotension, headache, pruritus and flushes. Just

75 mg of histamine, a quantity commonly present in normal meals, can induce symptoms in the majority of healthy persons with no history of histamine intolerance (Wohrl *et al.,* 2004, Coleman *et al.,* 2004, Jansch, 2004). The symptoms appeared due to ingestion of biogenic amines – rich food or conjugation with potentiating factors including consumption of amine oxidase- inhibiting drugs or alcohols or persons suffering from gastrointestinal diseases which may finally lead to heart failure or even brain hemorrhage (Premont *et al.,* 2001).

The first reported case of histamine poisoning from cheese was in 1967 and caused by Gouda cheese. The United States and the EC didn't set a limits for histamine in cheese, but some studies had suggested 900 mg/1kg cheese for the sum of histamine, tyramine, putrescine and cadaverine, but the legal limit for histamine, stipulated by (FDA, 2001) was (100 mg/kg) in fish, while (*Egyptian standard,* 2008) recorded the acceptable limit of histamine cheese samples by (200 mg/kg) in mish cheese.

Domiati cheese is the most popular soft white pickled cheese in Egypt and in other Middle Eastern countries.

Lacto bacillus hilgardii one of histamine producing microorganism it is a species of bacterium found in wine, dairy products and it is one member of family lacto bacillaceae Genus lacto bacillus sub genus Betabacterin (Enologyaccess org 2014 & Le Jeune and Lonvaud 1994).

Probiotics are define as a live microorganisms that contain mutually effect to antibiotics in curing of food born illness as produce a health welfare to the consumer when ingested or administered in suffusion amounts. (FAO/WHO, 2001 and Sanders, 2003). Bacteria belonging to the genera Lactobacillus and Bifidobacterium are the most often used as probiotic supplements for food (Kasimoglu *et al.,* 2004).

There is a lack of information on the histamine content in Egyptian cheese, therefore, our study was planned to evaluate presence of histamine in some Egyptian cheese, developed histamine content during the ripening and storage period in relation to the presence of decarboxylase positive strain of lactobacillus hilgardii and the impact of Lactobacillus acidophilus and Bifidobacterium on the formation of histamine during the manufacture and storage of domiati cheese for 4 months at 4°C and 25°C.

Materials and methods

Part I: Determination of Histamine content in cheese

Collection and handling of the samples (AOAC, 2016)

A total of 100 samples; 25 each of Mish, Domiati, Rommano and Chedder cheese were randomly collected from super markets and dairy shops in Beni-Suef city, Egypt. The collected samples (250 g) were stored at 4ºC tell send to the laboratory for examination.

Quantitative analysis of histamine in collected cheeses samples by RIDASCREEN Histamine kits using ELISA Reader (Malvano, 1980).

To measure the histamine in cheese samples, a competitive ELISA was employed using RIDASCREEN® histamine kit (R-Bipharm AG, Germany). The assay was performed according to the manufacturer's recommendation.

Sample preparation

The hard cheese samples were cut into strips and passed 3 times through food chopper to grind plugs, while 250 gm of the soft cheese samples were placed in 1L cup of high-speed blender and blended for a minimum time of 2-5 min, to obtain homogeneous mixture. In plastic vials 1g of cheese sample was homogenized and 9 ml of distilled water were added and mixed well, then centrifuged for 5 min at 2500 rpm at room temperature and lipid layer was removed.

1 ml of the supernatant was mixed with 9 ml of distilled water, then 200 µl of this solution were diluted with 9.8 ml of distilled water and applied 100 µl per acylation tube.

Test procedure for ELISA

1- A sufficient number of wells into the micro well holder were inserted for all standards, controls and samples to be run in duplicate. The standard and sample positions were recorded.

2- 25 µl of acylated standard solution, control or prepared sample were added to separate wells.

3- 100 µl of the anti-histamine antibody solution to each well were added and mixed gently by shaking the plate manually and incubated for 40 min at room temperature (20 – 25 0C / 68 - 77°F). Alternatively, 30 min with a MTP-shaker (approx. 600 rpm).

4- The liquid was poured out of the wells and taped the micro well holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of the liquid from the wells and all the wells were filled with 250 µl of washing buffer and poured out the liquid again. This step was repeated two times more.

5- 100 µl of the conjugate solution to each well were added and mixed gently by shaking the plate manually and incubated for 20 min at room temperature. Alternatively, 10 min with a MTP-shaker (approx. 600 rpm).

6- The liquid was poured out of the wells and taped the micro well holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of the liquid from the wells and all the wells were filled with 250 µl of washing buffer and poured out the liquid again. This step was repeated two times more.

7- 100 µl of the substrate-/chromogen solution were added to each well and mixed gently by shaking the plate manually and incubated for 15 min at room temperature in the dark place. Alternatively, 15 min with a MTP-shaker approx. 600 rpm.

8- 100 µl of the stop solution were added to each well and mixed gently by shaking the plate manually and the absorbance at 450 nm was measured against an air blank within 10 min after addition of the stop solution.

A special software, the RIDA®SOFT Win, was available for evaluation of the RIDSCREEN® enzyme immunoassays. The histamine concentration in mg/100g corresponding to the absorbance of each sample was read from the calibration curve and multiplied by corresponding dilution factor (5000).

Part II: Impact of probiotic bacteria on the production of histamine during production and storage of domiati cheese

Bacterial strains

Active bacteria strains {*Lactobacillus hilgardii*(8290), *Lactobacillusacidophilus* (20079), *Bifidobacteriumbifidum*(20082)} were obtained from Cairo MIRCEN, Egypt. Probiotic strains were propagated in de Man Rogosa and Sharpe (MRS-OXOID) broth supplemented with 0.05% L-cysteine hydrochloride (Sigma, Buchs, Switzerland) at 37°C for 24 h under an atmosphere of 5% CO_2 for *Lb. acidophilus* and anaerobically for *B. bifidumwhile Lb. hilgardii* strain was propagated at 37°C for 24.

Domiati cheese manufacturing

Domiati cheese was manufactured according to the procedure described by (Abou-Donia, 1986). Itcan be summarized as follows: Fresh raw buffalo's milk was obtained from the Milk Collection Unit, Faculty of Agriculture, Ain Shams University and transferred to the laboratory under Cooling condition. The milk was heated in water bath at 80°C with agitation for 10 min. After that the milk was cooled to 40°C, calcium chloride and sodium chloride were added at levels of (0.02) and (8%) respectively. Following the addition of rennet (CHr.Hansen NATUREN) 0.5g/100 kg milk as coagulating agent. The milk was divided into four batches:

1. The first batch (T1) was maintained without any further inoculation (negativecontrol)

2. The second batch (T2) of milk was separately inoculated with *L. hilgardii* to obtain a concentration of 10^8cfu/ml of cheese making milk.

3. The third batch (T3) of milk was separately inoculated with *L. hilgardii* and *Lb. acidophilus* to obtain a concentration of 10^8 and 10^9cfu/ml respectively.

4. The fourth batch (T4) of milk was separately inoculated with *L. hilgardii* and *B. bifidum* to obtain a concentration of 10^8 and 10^9cfu/ml respectively.

After 40–50 min, cheese milk was renneted at 31–32°C and coagulation took place within 40–50 min. The curds were cut and left for 10–20 min for partial removal of the whey, and the curds were transferred into perforated molds lined with muslin cloth for further removal of whey and texturization of cheese curd without pressure. The whey was collected and stored at 5°C for making brine. Molds were turned every 2–4 h to form a firm curd. After four-five turns, the molds were left undisturbed overnight. Subsequently, cheese blocks were cut into uniform size and transferred to the whey brine solution for about 22–24 h.

The resultant cheeses were packed in plastic containers with whey and stored in refrigerator at 4°C and at 25°C for 4 months. The samples were taken from cheese samples at Zero time, one, two, three, and four months of cold storage (4°C) and room temperature (25°C) for chemical and bacteriological evaluation.

In general, 10 g of cheese samples were homogenized with 90 mL of 2% sterilized sodium citrate solution in a Colworth stomacher at 45°C for 2 min. Additional 10-fold serial dilutions were made using sterile 0.1% peptone water ((APHA, 2001)).

To enumerate *L. acidophilus, L. hilgardii* and *B. bifidum*, 0.1 mL of the serially diluted samples were pour-plated on de Man Rogosa and Sharpe (MRS) agar (CM0361B, Oxoid, UK) supplemented with 0.05 % L-cysteine hydrochloride and evenly spread by sterile bent glass rod. Colonies on the plates were counted after 48 h of incubation at 37ºC under appropriated conditions (Souza and Saad 2009).

Quantitative detection of BAs (histamine) in Domiati cheese: Quantitative detection of BAs (histamine) in stored batches at zero time, one, two, three, and four months respectively at 4°C and 25°C as previously.

Statistical Analysis

Graf pad program was used for statistical analysis. Values of different parameters were expressed as the

mean ± standard error. One-way analysis of variance (ANOVA) and (HSD) tests were used to determine significant differences in the measured attributes at P value<0.05.

Results and Discussion

Biogenic amines are valuable to judge the hygienic quality of cheese, therefore they can be used as indicator of food spoilage and can cause toxicological effects to consumers at high levels. In this study 100 cheese samples (Mish, Domiati, Cheddar and Rommano) 25 of each were analyzed to evaluate the concentration of histamine.

The achieved Results declared that the occurrence of histamine in the all examined samples of Mish, Domiati, Cheedar and Rommano cheeses were 100, 64, 56 and 100% respectively. Table 1 Furthermore, the mean values of histamine (mg/kg) in the examined samples of Mish, Domiati, Cheedar and Rommano cheeses were 114.16 ± 2.95, 38.24 ± 1.85, 9.96 ± 0.46 and 126.77 ± 4.69 respectively table 2. The reported data pointed out that the highest histamine mg/kg levels was detected in the examined Rommano cheese followed by Mish cheese, while the lowest concentration was obtained in Domiati and Cheedar cheese samples table 2. The higher results of histamine were obtained by *El-Kosi et al.,* (2009) in the examined manufactured Rommano Egyptian cheese (1753 mg/kg) , *Ibrahim and Amer* (2010) in the examined Cheddar and Rommano Egyption cheese (129.8 and 174.3 mg/kg) respectively at a percentage of 73.3% and 86.6% respectively*, Rabie et al., (2010)* in the examined Mish cheese (2150 mg/kg), *El-Zahar (2014)* in Mish cheese (310mg/kg), *Abo-El-Makarem and Amer (2016)* in the manufactured Egyptian hard Rommano cheese (645 ± 0.10 mg/kg), *Swelam and Mehanna (2017)* in the examined Rommano cheese (1149 ± 9.1 mg/kg) and *El-Leboudy et al., (2019)* in the examined Mish, Rommano, Damietta and Cheddar as the histamine level were 170.2±3.34, 169.3±4.01, 132.8±3.5 and 130.4±3.91 mg/kg respectively. From other side, lower results were reported by *Amine et al., (2007)* in the examined Mish , Damietta, Rommano and Cheddar whereas all results were lower than oral critical dose, *Linares et al., (2012)* in the examined Cheddar cheese (25.4mg/kg), *Al-Dawlatly (2017)* in the examined Damietta and Rommano cheese (5.6 and 79 mg/kg) respectively. Also, *Nazem et al., (2019)* reported that the mean of histamine in the examined Rommano cheese was 84.9±2.59 mg/kg.

Table 1: Incidence of histamine in the examined cheese samples.

Type of cheese samples	No. of samples	Positive samples	
		No.	%
Mish	25	25	100
Domiati	25	16	64
Cheddar	25	14	56
Rommano	25	25	100

Table 2: Statistical analytical results of histamine content among the positive examined samples (mg/kg).

Type of cheese	Minimum	Maximum	Mean ± S.E
Mish	7.32	250	114.16 ± 2.95
Domiati	0.00	180	38.24 ± 1.85
Cheddar	0.00	37.8	9.96 ± 0.46
Rommano	16	417.5	126.77 ± 4.69

Table 3: Comparison of histamine levels in different cheese samples with Egyptian standard (2008) (n=25).

Samples	Maximum Permissible limit (mg/kg)	samples within permissible limit		samples above permissible limit	
		No.	%	No.	%
Mish	200[b]	22	88	3	12
Damiati	200[b]	16	64	0	00
Cheddar	200[b]	14	56	0	00
Rommano	200[b]	20	80	5	20

b = Maximum permissible limits of histamine according to Egyptian standard (2008) (200mg/kg).

Inspection of table 3 revealed that 3 (12%) and 5 (20%) of the examined Mish and Rommano cheese samples exceeded the maximum permissible limit of histamine (200 mg/kg) which recommended by *Egyption standard (2008)* respectively.

The detectable level of histamine with in permissible limit in 88, 64, 56 and 80 % of Mish, Domiati, Cheedar and Rommano cheese respectively, may be attributed either to its presence in low percentage due to it may be was catabolized by microorganisms or enzymes (Chang *et al.,* 1985).

Histamine can be related to contamination of cheese with species of some proteolytic bacteria which can convent the histadine into histamine. Although the manufacturing protocol for processed cheese includes high temperature treatment, biogenic amines are not affected by this treatment and are passed from the raw materials (milk) to the final product (processed cheese) once biogenic amines are formed, it is difficult to destroy them by pasteurization or cooking. Therefore, biogenic amines formation should be controlled by strict use of good hygiene in both raw material and manufacturing environment, with corresponding inhibition of spoiling microorganisms (Santos, 1996).

Also it is of great concern to mention that the presence of biogenic amines in cheese could be attributed to lactic acid cultures with decarboxylase activity *(Bover and Holzapfel, 1999)*. Moreover, the high storage temperature may favor the growth of some undesirable bacteria and enhances their decarboxylation activity against the amino acids of processed cheeses resulting in formation of such serious biogenic amines (Bover *et al.,* 2000).

Lacto bacillus hilgardii one of histamine producing microorganism. Identification of histamine-producing (histidine decarboxylase-positive [HDC+]) bacteria is difficult since they belong to diverse species and only some strains of a given species are histamine producers. HDC+ bacteria differ from non-histamine-producing (HDC+) bacteria by the presence of HDC, the enzyme that converts histidine into histamine and CO_2. Two different kinds of HDCs were found in gram-negative and gram-positive bacteria. HDCs of gram-negative bacteria use pyridoxal phosphate as a cofactor for activity, whereas HDCs of gram-positive bacteria use a different catalytic mechanism based on a pyruvoyl group linked at the active site (Taylor, 1986).

Lactobacilli and bifidobacteria are recognized as a good quintessence for health-promoting components of the microflora. Lactobacilli has a health significance in gastrointestinal troubles as reduce constipation, infantile diarrhea, traveller's diarrhea, irritable bowel syndrome, lactose-intolerant individuals and resist infections such as Salmonella. Bifidobacteria also obtain a healthy effect as stimulate the immune system, reduce pathogen colonization, aid in restoring of normal flora after antibiotic treatment, produce B vitamins, help in reduction of blood ammonia and cholesterol concentration (Gibson, 2002).

The reported data in Graph (4) proved that the histamine level increased by increasing time of storage at 4°C in control (T1) from 0 mg/kg at zero time to reach 2.5, 14.9 and 18.2 mg/kg at 30, 60, 90 day respectively, with the exception of the last month (4th month) the histamine level decreased to 14.7 mg/Kg, Also in the (T2) which inoculated with *L. hilgardi*, we found that the histamine level increased from 0 mg/kg at zero time to reach 2.5, 18.5 and 19.4 mg/kg, respectively, with the exception of the last month the histamine level decreased to 13.9 mg/kg. Also proved that the (T3 and T4) which contained *L. acidophlus* and *B.bifidum* as probiotics to study its effect on L.hilgardii and histamine formation, we found that *L. acidophuls* had negative effect on the level of histamine more than *B. bifidum* whereas the histamine level in (T 3) increased by time from 0 mg/kg at zero time to reach 2.5, 9.5 and 18.6 mg/k g, respectively, with the exception of the last month the histamine level decreased to 15 mg/kg, In (T4) we found that the histamine level increased by time from 0 mg/kg at zero time to reach 2.5, 14.4 and 18.5 mg/kg at 4°C, respectively, with the exception of the last month the histamine level decreased to 11.6 mg/kg.

The data in (Graph 5) illustrated that the histamine level increased by increasing time of storage at 25°C in (T1) from 0 mg/k g at zero time to reach 2.5, 1.8 and 19.7 mg/kg respectively, with the exception of the last month the histamine level decreased to 7 mg/kg .while in (T2) the histamine level increased from 0 mg/kg at zero time to reach 2.9, 2 and 22 mg/kg g at 25°C, respectively, with the exception of the last month the histamine level decreased to 13.1 mg/kg. while in (T3) the histamine level increased from 0 mg/kg at zero time to reach 2.5, 17.8 and 19.1 mg/k g at 25°C, respectively with the exception of the last month the histamine level decreased to 9.2 mg/kg. while in (T4) the histamine level increased from 0 mg/kg at zero time to reach 2.5, 19.3 and 20 mg/k g at 25°C, respectively, with the exception of the last month the histamine level decreased to 11.7mg/kg.

It had been reported that *L. hilgardii* one of numerous bacteria has histidine decarboxylase enzyme, which required for production of histamine in the dairy products *(*Ansorena *et al.,* 2002; Bover-Cid *et al.,* 2003; Franz *et al., 2003* and Foulquié-Moreno *et al.,* 2006), overall the results revealed that the levels of biogenic amines in the manufactured Domiati cheese increase with the increasing temperatures and ripening periods. Also, these results support the opinion that the time and the storage temperature have a great influence on biogenic amine concentrations (Valsamaki *et al.,* 2000).

A drop in BAs production at the end of storage period in the present experiment is comparable to the data of Standarova *et al.,* (2010), who recorded that histamine production in cheese reduced after 6 week of storage, Kemprda *et al.,* (2007) and Marijan *et al.,* (2017) reported that histamine level did not always increase linearly during storage of cheese. This finding may indicate that the formation of histamine in cheese depends on complex factors accelerating or retarding the production of this amine such as different numbers of decarboxylatingmicro organisms in certain parts of the cheese and internal micro environmental conditions (access to O_2 or effects caused by water).

The reported data in table 6 declared that in the control batch (T1) L.hilgardii was not detected in 4 months of ripening at 4°C, while in group (T2) we found that number of L.hilgardii decreased during the storage period

and reached levels of 7.5×10^9, 6.8×10^2 and not detected till the end of storage period at zero, one, two, three and four month respectively, but in (T3) group we found that number of L.hilgardii decreased during the storage period and reached to levels of 2×10^9, 5.5×10^2 and not detected till the end of storage period while, we found that number of *L.acidophilus* decreased during the storage period and reached to levels of 1.2×10^{10}, 5×10^3,40 and not detected till the end of storage period at zero, one, two, three and four month respectively, in (T4) group, we found that number of L.hilgardii decreased during the storage period and reached to levels of 4.2×10^9, 9×10^2 and not detected till the end of storage period, while we found that number of B.bifidum reached to levels of 1.2×10^{10}, 6×10^4, 30 and not detected till the end of storage period at zero, one, two, three and four respectively at 4°C.

The reported data in table 7 proved that in the control batch (T1) L.hilgardii was not detected in 4 months of ripening at 25°C, while in group (T2) we found that number of L.hilgardii decreased during the storage period and reached to levels of 9×10^9, 1×10^3 and not detected till the end of storage period at zero, one, two, three and four month respectively, in (T3) group we found that number of L.hilgardii decreased during the storage period and reached to levels of 1×10^9, 9×10^2cfu/g and not detected till the end of storage period, while we found that number of L.acidophilus decreased during the storage period and reached to levels of 5×10^9, 2.4×10^3cfu/g and not detected till the end of storage period at zero, one, two, three and four month respectively, in (T4) group we found that number of L.hilgardii decreased during the storage period and reached to levels of 6×10^{10}, 8.7×10^2cfu/g and not detected, while we found that number of B.bifidum reached to levels of 1×10^{10}, 4×10^3cfu/g and not detected till the end of storage period at zero, one, two, three and four month respectively at 25°C.

It is evident that there is no significant effect of probiotics on histamine production and control because P value > 0.05, while it had effect on L.hilgardii.

During ripening of cheese, marked changes in body and flavor occurred which associated largely with enzymatic degradation of casein and resulting in steady increase of free amino acids content. Some of the amino acids are subjected to further breakdown reaction as decarboxylation catalyzed by specific bacterial decarboxylases that give rise to the formation of carbon dioxide and amines. Thus, cheese could be considered an ideal environment for the amine production through bacterial decarboxylation of some amino acids *(Sattler et al., 1988)*.

Table 4: Concentration of histamine in four batches of manufactured Domiati cheese stored for 4 month at 4 C°(mg/kg).

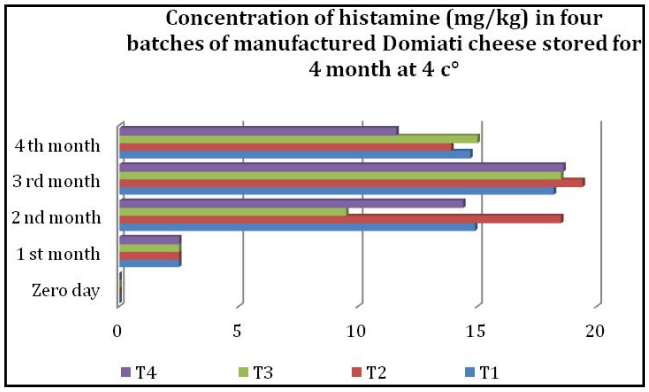

Table 5: Concentration of histamine in four batches of manufactured Domiati cheese stored for 4 month at 25 C°(mg/kg).

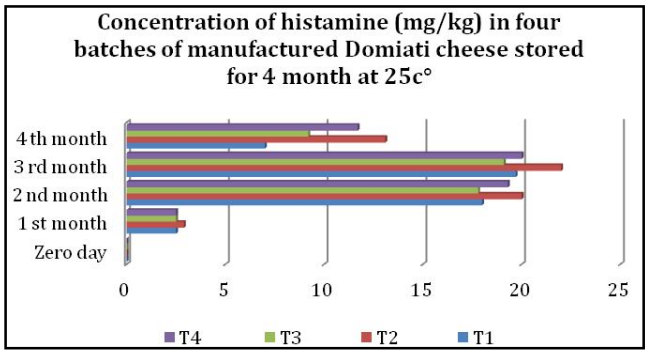

Table 6: Impact of Lb. acidophilus and B. bifidum on Lb. hilgardii organism in Domiati cheese during production and refrigerator storage.

Treat-ments	Total viable count cfu/g	Time (month)				
		Zero	one	two	Three	Four
T1	L.hilgardii	ND	ND	ND	ND	ND
T2	L.hilgardii	7.5×10^9	6.8×10^2	ND	ND	ND
T3	L.hilgardii	2×10^9	5.5×10^2	ND	ND	ND
	L.acidophilus	1.2×10^{10}	5×10^3	40	ND	ND
T4	L.hilgardii	4.2×10^9	9×10^2	ND	ND	ND
	B.bifidum	1.2×10^{10}	6×10^4	30	ND	ND

Table 7: Impact of Lb. acidophilus and B. bifidum on Lb. hilgardii organism in Domiati cheese during production and storage at room temperature.

Treat-ments	Total viable count cfu/g	Time (month)				
		Zero	one	two	Three	Four
T1	L.hilgardii	ND	ND	ND	ND	ND
T2	L.hilgardii	9×10^9	1×10^3	ND	ND	ND
T3	L.hilgardii	1×10^9	9×10^2	ND	ND	ND
	L.acidophilus	5×10^9	2.4×10^3	ND	ND	ND
T4	L.hilgardii	6×10^{10}	8.7×10^2	ND	ND	ND
	B.bifidum	1×10^{10}	4×10^3	ND	ND	ND

In conclusion, presence of high concentration of histamine in Egyptian processed cheese specially Mish and Rommano reflects the bad hygienic conditions under which they produced and stored and accordingly, the levels of biogenic amines in different cheeses should be come in accordance with the safe permissible limit that was recommended by EOS to ensure human safety. Also It is concluded that the concentrations of biogenic amines increased with the increasing temperature and ripening period and the toxic doses suggested by the concerned organizations must be taken in consideration to improve the cheese quality, protect cheese from being contaminated with amine producing bacteria and to safeguard consumers from biogenic amines poisoning, the factors that enhance biogenic amines formation should be minimized during cheese processing.

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