رقم البحث (5)

ENTEROBACTERIACEAE COUNTS AND EVALUATION OF HISTAMINE CONCENTRATION OF EGYPTIAN SOFT DAMIETTA CHEESE MADE FROM RAW AND PASTEURIZED MILK

BY

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ABSTRACT

A Three hundred random samples Damietta white soft cheese were collected randomly from different marked dairy shops and supermarkets in Dakahlia Governorate, Egypt in sterile water proof plastic bags. The collected samples were transferred to the laboratory in an ice box with a minimum of delay to be examined bacteriologically and chemically . Each sample was divided into two parts the first for bacteriological examination while second part used for detection histamine . Total Enterobacteriaceae count of examined cheese samples in Small Glossaries ranged from 100 to 5×10^7 with a mean value of $3\times10^6 \pm 0.77\times10^6$ while in Modern manufactures ranged from 100 to 4×10^7 with a mean value of $8\times10^5 \pm 1.3\times10^5$. Histamine concentration mg/100g of examined cheese samples in Small Glossaries ranged from 0.4 to 31 with a mean value of 9.2 ± 8.1 while in Modern manufactures ranged from 0.2 to 32 with a mean value of 6.6 ± 5.3. Positive High Significally Correlation coefficient between Histamine concentration mg/100g and Enterobacteriaceae count/g of cheese samples.

INTRODUCTION

White soft cheese is one of the common delicious cheeses consumed in Egypt. There are many varieties of white soft cheese depend on the technique of manufacture, salt percentage and many other factors. Domiati cheese is pickled cheese manufacture by the traditional methods using 5-7% salted milk, **(Abd El Salam et. al., 1976 and Abou- Donia, 1986).** Biogenic amines are organic bases and biologically active compounds naturally occurring in animals, plants and microorganisms. Cheese, like other fermented foods, usually

contains high levels of biogenic amines, Which result from the breakdown of amino acids by the action of decarboxylase producing microorganisms **(Leuschner and Hammes,1998).** The most important biogenic amines from the food hygiene point of view are primary amines which include tyramine, histamine and cadaverine **(Marino et. al., 2000).** Such amines were recorded as the most abundant biogenic amines in cheese **(Bonetta et. al., 2008).**

Cheese is a histamine-rich food and may contain up to 500 mg/kg **(Roig-Sague´s et al., 2002).** It is one of the most commonly implicated food products associated with histamine poisoning **(Taylor et. al.,1982; Sumner et. al., 1985)**. Increases in the amine content of cheese may be attributable to various micro-organisms. These micro-organisms may make up the flora associated with the milk used to make the cheese, by contamination during cheese making and/or storage, or may be added to the cheese deliberately in starter cultures **(Joosten, 1987; Sumner et. al., 1990; Joosten and Nunez, 1996).**

The factors which are related to the formation of biogenic amines in dairy products, are the microflora that can produce decarboxylases, microbiological quality of the milk, cheese production technology, cheese ripening conditions and hygienic conditions during production processes **(Elsanhoty et. al., 2009;Rak., 2010).** A high concentration of these amines could be used as an indicator of poor hygienic quality of cheese **(Novella-Rodriquez et. al., 2002).**

Monitoring of some bacterial species; Enterobacteriaceae, Enterococci and coliforms are often responsible for production of biogenic amines in cheese especially when their counts exceed 10^6 cfu/g **(Sharaf et. al., 1997).**

The aim of this study was to establish whether there was a correlation between the concentration of histamine of Egyptian soft Damietta cheese made from raw and pasteurized milk and the presence of counts of Enterobacteriaceae.

MATERIALS AND METHODS

A Three hundred random samples white soft cheeses (200 samples from small glossaries and 100 samples from modern factories) were collected randomly from different marked dairy shops and supermarkets in Dakahlia Governorate, Egypt in sterile water proof plastic bags. The collected samples were transferred to the laboratory in an ice box with a minimum of delay to be examined bacteriologically and chemically . Each sample was divided into two parts the first for bacteriological examination while second part used for detection histamine .

Microbiological analysis of cheese

A sample of about 10 g was taken aseptically. It was added to 90 ml of 2% (w/v) trisodium citrate solution (45 ° C) and homogenized with a Stomacher for 2 min. Portions of 0.1ml of the cheese samples were plated in duplicate on violet red bile glucose agar (Oxoid) for Enterobacteriaceae (incubation for 18±24 h at 30°C), Further identification to the species level was performed using API 20 E(BioMeÂrieux, France).

Quantitative analysis of Histamine using RIDASCREEN® Histamin

RIDASCREEN® Histamin (Art. No.: R1601, 96 wells ,R-Biopharm AG, Darmstadt, Germany) is a competitive enzyme immunoassay for the quantitative analysis of histamine in food. All reagents required for the enzyme immunoassay - including standards – are contained in the test kit. The test kit is sufficient for 96 (including standards).A microtiter plate spectrophotometer is required for quantification. The procedures were adopted according to instruction of manufacturer's.

RESULTS

Table (1): Prevalence and Statistical analytical results of Enterobacteriaceae counts of examined cheese samples.

Source	No. of examined samples	positive samples		Min	Max	Mean	S.E.M. ±
		No	%				
Small Glossaries	200	105	52.5	100	5×10^7	3×10^6	0.77×10^6
Modern manufactures	100	30	30	100	4×10^7	8×10^5	1.3×10^5
Total	300	135	48	100	5×10^7	2.8×10^6	0.98×10^6

Fig (1):prevalence of Enterobacteriaceae of examined cheese samples.

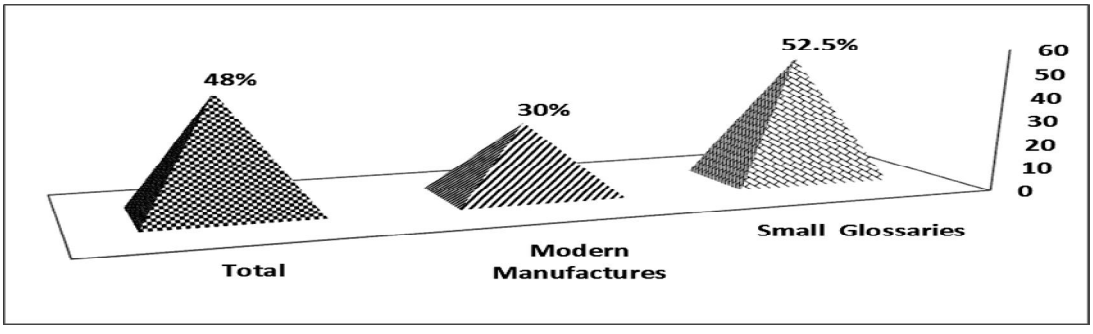
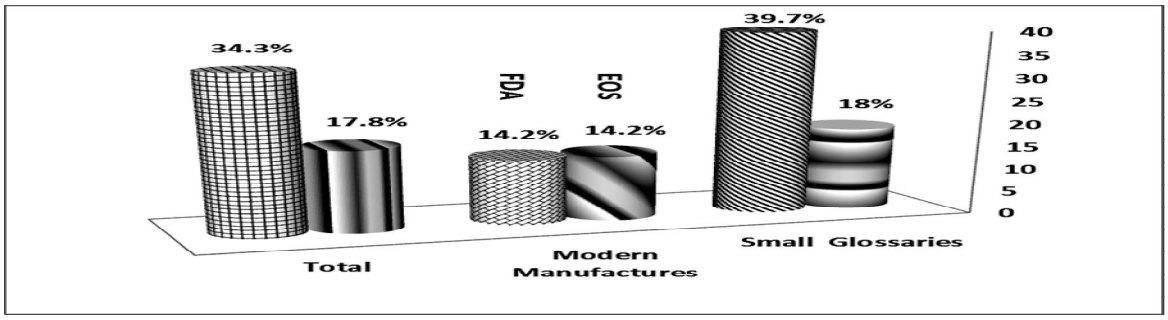


Table (2): Statistical analytical results of Histamine concentration mg/100g examined cheese samples and acceptability according to EOS (1996) & FDA (2001)

Source	examined samples	positive samples		Min	Max	Mean	S.E.M. ±	Unacceptability			
								According EOS*		According FDA**	
		No	%					No	%	No	%
Small Glossaries	83	81	97.5	0.4	31	9.2	8.1	15	18	33	39.7
Modern manufactures	14	12	85.7	0.2	32	6.6	5.3	2	14.2	2	14.2
Total	97	93	96.6	0.2	32	8.8	7.8	17	17.8	35	34.3

***EOS (1996) 20mg/100gm** ** ** FDA(2001) 10mg/100gm**

Fig (2): Statistical analytical results of Histamine concentration mg/100g examined cheese samples and acceptability according to EOS (1996) & FDA (2001).



Table(3): Correlation coefficient between Histamine concentration mg/100g and Enterobacteriaceae count/g of cheese samples

Bacterial count	No of examined samples	positive samples		Min	Max	Mean	S.E.M. ±	Correlation coefficient R
		No	%					
Enterobacteriaceae Count	300	135	48	100	5×10^{7}	2.8×10^{6}	0.98×10^{6}	0.743**
Histamine conc.	97	93	96.6	0.2	32	8.8	7.8	

** **Positive High significally Correlation coefficient**

DISCUSSION

Cheese is probably one of the most important histamine sources of our diet. Probably is unavoidable to elaborate aged cheese without containing a certain amount of these biogenic amines, but is necessary to prevent the formation of high amount that may suppose a risk for health of the consumers. Therefore, producers must reduce the amount of contaminating microorganisms such as enterococci or Enterobacteriaceae through pasteurization and appropriate post pasteurization hygienic measures. Biogenic amine levels are significantly lower in cheeses elaborated from pasteurized milk **(Schnellar et. al., 1997).** The use of appropriate starter cultures, which able to compete with the amine-forming microorganisms. Proper storage temperature is the most effective method to prevent histamine formation in cheese.

The results given in **Table (1)** revealed that Total Enterobacteriaceae count of examined cheese samples in Small Glossaries ranged from 100 to 5×10^7 with a mean value of $3\times10^6 \pm 0.77\times10^6$ while in Modern manufactures ranged from 100 to 4×10^7 with a mean value of $8\times10^5 \pm 1.3\times10^5$.

Inspection of **Table (1) & Fig (1)** indicate that the incidence of Enterobacteriaceae in Small Glossaries 52.5% while in Modern manufactures 30%. Owing to their microbial origin, biogenic amines can be formed by microbial contamination. In this sense, ***(Rodriguez et. al., 2002)*** reported that raw milk cheese had higher level of biogenic amines as compared with pasteurized milk cheese due to its higher microbial contamination particularly with Enterobacteriaceae and Enterococci.

Biogenic amines in food are used as an indicator of the hygienic quality of raw materials employed in food manufacture as well as the hygienic quality during food processing. **(Jover et. al., 1997).** The results given in **Table (2)** revealed that Histamine concentration mg/100g of examined cheese samples in Small Glossaries ranged from 0.4 to 31 with a mean value of 9.2 ± 8.1 while in Modern manufactures ranged from 0.2 to 32 with a mean value of 6.6 ± 5.3. The difference in histamine content in these cheese products can attribute to the type of cheese, the storage temperature of the cheese, and the hygienic condition of the environment for cheese processing and handling. As histamine content in cheese samples of different type and origin varied to a great extent, obligatory monitoring of histamine should be considered as a valuable tool to quality of cheese.

Large amounts of biogenic amines in cheese could indicate a failure, from a hygienic point of view, in the milk used for cheese products or during the cheese making **(Masson et. al., 1996).**

Comparing the obtained results with the permissible limits recommended by **EOS (1996)** and **FDA (2001)** for Histamine concentration mg/100g, Small Glossaries exceeded 18% and 39.7% while in Modern manufactures exceeded 14.2% and 14.2%. The production of biogenic amines in cheeses is an extremely complex phenomenon, dependent of several variables, such as the presence of microorganisms their proteolytic and decarboxylase activities, ripening time and ripening temperature.

Cheese is an ideal substrate for the production of biogenic amines by microbial decarboxylation of the corresponding amino acids. Histamine content of the cheese is affected by many factors. The formation and presence of such amines depend on

a variety of factors including the presence of substrate and microbial enzymes, Temperature, pH, salt and water content, presence of enhancing substances in catabolism of amines **(Standara et.al.,2000).** The presence of biogenic amines can cause several problems for susceptible consumers, such as nausea, respiratory disorder, hot flushes, sweating, heart palpation, headache, bright red rash, oral burning **(Stratton et. al., 1991).**

The results given in **Table (3)** revealed that Positive High Significally Correlation coefficient between Histamine concentration mg/100g and Enterobacteriaceae count/g of cheese samples. The production of biogenic amines in cheese has often been linked to non-starter lactic acid bacteria and Enterobacteriaceae **(Petridis and Steinhart, 1996).**

Because the biogenic amines in food products are mainly generated by decarboxylation of the corresponding amino acids precursors, thought bacterial decarboxylase, for formation of biogenic amines are necessary these conditions **(Bodmer et. al., 1999):** 1- availability of free amino acid, but not always leading to amine formation, 2- presence of decarboxylase-positive microorganisms, 3- conditions that allow bacterial growth, decarboxylase synthesis and decarboxylase activity.

In conclusions,the results obtained indicate that the high concentration of histamine in the cheeses obtained from small groceries than samples obtained from modern factories as well as High counts of Enterobacteriaceae can be correlated with high concentrations of histamine in examined soft cheese, suggesting that histamine could be used as a quality indicator of hygienic cheese-Makin . In addition, the results presented here appear to support the need for the implementation of a well-designed good manufacture practice scheme throughout transportation, commercial distribution, purchase and storage by the consumer in order to guarantee low levels of histamine in cheese and therefore, less potential for health hazards by the time of consumption.

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الملخص العربى

العدد الكلى لميكروبات الانتريوبكتيريسى وتقييم تركيزالهستامين فى الجبن الابيض الدمياطى المصرية المصنعة من اللبن الخام والمبستر

عادل عبد الخالق- محمد الشربينى – مها العشماوى - عبدالله الشحات البيومى

فسم الرقابة الصحية على الاغذيه - كلية الطب البيطرى – جامعة المنصورة

أجريت الدراسة على عدد ثلثمائة عينة جبن ابيض طري دمياطى ممثلة فى ٢٠٠ عينة من الاجبان المصنعة بالطريقة القديمة لصغار المنتجين التى يستخدم فيها الحليب الخام بدون بسترة و ١٠٠ عينة مصنعة بالطرق الحديثة التى يستخدم فيها طريقة البسترة للحليب جمعت من أماكن متفرقة فى مدينة شربين - محافظة الدقهلية، وقد تم فحص العينات ميكروبيولوجيا لمعرفة العدد الكلى لميكروبات الانتريوبكتريسى وتركيز الهستامين و قد أوضحت النتائج أن متوسط العد الميكروبى لصغارالمنتجين $3\times10^{6} \pm 0.77\times10^{6}$ وللمصانع الحديثه $8\times10^{5} \pm 1.3\times10^{5}$. بيما كان متوسط تركيز الهستامين لصغار المنتجين ٩,٢ مليجرام /١٠٠ جم وللمصانع الحديثة كان متوسط تركيز الهستامين ٦,٦ مليجرام /١٠٠ جم .

وقد وجد أن هناك ارتباط ايجابى قوى ملحوظ بين العدد الكلى لميكروبات الانتريوبكتريسى و متوسط تركيز الهستامين. وقد تم مناقشة الأهمية الصحية من تواجد الهستامين فى الجبن وتاثيرة على صحة المستهلك وكيفية الحد من وجوده بها.