QUANTITATIVE ANALYSIS OF HISTAMINE IN CHEESE COLLECTED FROM KAFR EL-SHEIKH GOVERNORATE, EGYPT

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ABSTRACT

Forty cheese samples (20 each of Ras and Edam cheese) were randomly collected from different supermarkets at Kafr El- Sheikh Governorate, Egypt.The collected samples were homogenized and packed in polyethylene bags and stored below -20 ^{o}C prior to quantitative analysis of Histamine (HIS) using Ridascreen Histamine (Enzyme Immunoassay for quantitative analysis of histamine). Histamine was found in 100 and 75% of the analyzed Ras and Edam cheese samples with a total percentage of 87.5%, and mean concentrations of 23.38± 2.04, 1.08± 0.12 and 13.82± 0.46 mg/ 100 g, respectively. Eighty% of the examined Ras cheese presented a HIS concentration over 100 mg /kg the recommended upper limit for histamine. While, the concentrations of HIS in all Edam cheese samples analyzed were lower than the maximum acceptable limit. As HIS 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 ensure quality of cheese.

INTRODUCTION

Biogenic amines are organic bases of low molecular weight, formed in foods mainly by the microbial decarboxylation of certain amino acids and it has been suggested that their concentration can be

used as an indicator for the hygienic quality of the food (***Schneller et al., 1997; Innocente and D'agostin, 2002*** *and* ***Novella-Rodriguez et al., 2002***). Histamine (HIS) is one of the most studied amines arises in foods by the growth of microorganisms that possess histidine decarboxylase and thus, foods rich in free histidine contains high level of HIS.

Histamine poisoning is a chemical intoxication resulting from the ingestion of food that contain unusually high levels of HIS (***Taylor, 1988***). Just 75 mg of histamine, a quantity commonly present in some meals, can induce symptoms in the majority of healthy persons with no history of HIS intolerance (***Wöhrl et al., 2004***).

European legislation (***Commission Regulation (EC) 2073/2005***) limits the histamine levels in fishery products to 200 mg Kg^{-1} for fresh fish and up to 400 mg Kg^{-1} for cured products. The US Food and Drug Administration consider it a danger to health if the HIS level is equal to 500 mg Kg^{-1} ***(FDA, 1995)***. Although there are no regulations governing the HIS content in most foodstuffs, some laboratories have made a recommendation to limit the presence of HIS to 100 mg Kg^{-1} in fermented food products (***Brink et al., 1990***). Although fish of the families *Scombridae* and *Scomberesocidae* are commonly implicated in incidents of HIS poisoning, non-scombroid fish, cheese and other foods have also been attributed in cases of such poisoning (***Stratton et al., 1991***).

The presence of biogenic amines especially HIS in cheese constitutes a potential public health concern because of its physiological and toxicological effects (***Önal, 2007***). HIS is a powerful biologically active chemical that can directly stimulate the heart, cause extravascular

smooth muscle to contract or relax, stimulate both sensory and motor neurons, and control gastric secretion. Therefore, a wide variety of symptoms can be attributed to this type of poisoning, rash, urticaria, eodema, nausea, vomiting, diarrhea, abdominal cramps, hypotension, headache, palpitations, tingling, flushing and burning sensations in the mouth (***Stratton et al., 1991***).

Amine contents may vary among different types of cheese; ripened cheeses usually contain higher concentrations of amines than unripened cheeses. This difference can be related to casein proteolysis during ripening (***Flick*** *and* ***Granata, 2004***). Moreover, the duration and conditions of the ripening period and starter culture types used are important factors for the production of amines. Also, bacterial quality of milk, heat treatments applied, pH, salt concentration, and temperature are the other parameters leading to differences among a variety of cheese (***Stratton et al., 1991*** *and* ***Novella-Rodriguez et al., 2003***).

Due to the impact of biogenic amines on human health and food safety, monitoring their levels in foodstuffs is still gaining importance (***Önal, 2007***).Therefore, this study was planned to determine the presence of histamine in hard and semi-hard cheese commercially available in Kafr EL-Sheikh Governorate, Egypt.

MATERIALS AND METHODS

1. Collection of samples

Forty cheese samples (20 each of Ras and Edam cheese) were randomly collected from different supermarkets at Kafr El- sheikh Governorate, Egypt. All samples were analyzed before their expiry date. The collected

samples were homogenized and packed in polyethylene bags and stored below -20 ºC prior to analysis.

2. Quantitative analysis of Histamine: using Ridascreen® Histamine (Enzyme Immunoassay for quantitative analysis of histamine), Art. No.: R1604, according to instruction of manufacturer's.

2.1. Preparation of Samples: Ten g. of cheese samples were homogenized; 9 ml of distilled water were added to 1g of homogenate and mixed well, then centrifuged for 5 min / 2500 g (6750 rpm) at room temperature (20 - 25 °C). Lipid layer was removed and 1 ml of the supernatant was mixed well with 9 ml of distilled water, then 200 µl of this solution were diluted with 9.8 ml of distilled water.

2.2. Test implementation

2.2.1. Test preparation

All reagents were adjusted to room temperature (20 - 25 °C) before use. The washing buffer was diluted 1:50 (1+49) with distilled water before use. The acylation reagent was reached room temperature till forms a homogeneous, crystal-free solution before use.

2.2.2. Test procedure for the acylation

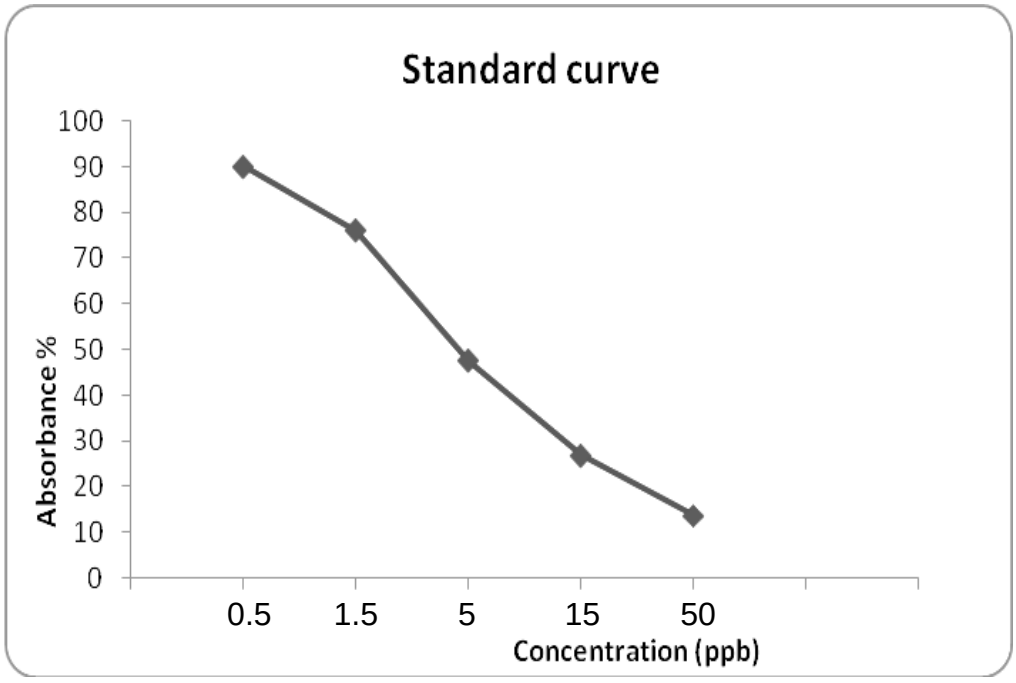
From each of the standard solution, control or prepared sample 100 µl were added to separate wells of the acylation plate, 25 µl of the acylation reagent were added to each acylation well then, 200 µl of the acylation buffer were added to each acylation well and mixed gently by shaking the plate manually. The plate was incubated for 15 min at room temperature (20 - 25 °C).

2.2.3. Test procedure for ELISA

Sufficient numbers of wells were inserted into the microwell holder for all standards, controls and samples. 25 µl of acylated standard solution, control or prepared sample were added to separate wells. 100 µl of the anti-histamine antibody solution were added to each well and mixed gently by shaking the plate manually. The plate was incubated for 40 min at room temperature (20 - 25 °C). The liquid was poured out of the wells and the microwell holder was taped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled with 250 µl of washing buffer and the liquid was poured out again. The washing process was repeated two more times. 100 µl of the conjugate solution were added to of each well and mixed gently by shaking the plate manually and incubated for 20 min at room temperature (20-25 °C). The liquid was poured out of the wells and the microwell holder was taped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled with 250 µl of washing buffer and the liquid was poured out again. The washing process was repeated two more times. 100 µl of the substrate-/chromogen solution were added to each well and mixed gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C) in the dark. 100 µl of the stop solution were added to each well, mixed gently by shaking the plate manually and the absorbance was measured at 450 nm against an air blank within 10 min after addition of stop solution.

2.2.4. Calculation

The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages. The absorption is inversely proportional to the HIS concentration in the sample. The histamine concentration in µg/kg (ppb) corresponding to the absorbance of each sample was read from the calibration curve and then further multiplied by the dilution factor (5000). According to the test preparation record, the lower detection limit is 2.5 ppm and the recovery rate is 100% for cheese.



RESULTS

Table (1): Concentration of histamine (mg/100g) in the examined cheese samples.

Types of samples	No. of examined samples	Positive samples		Concentration mg/100g		
		No.	%	Min.	Max.	Mean± SE
Ras cheese	20	20	100	1.4	33.3	23.38± 2.04
Edam cheese	20	15	75	0.25	2.3	1.08± 0.12
Total	**40**	**35**	**87.5**	**0.25**	**33.3**	**13.82±0.46**

Table (2): Frequency distribution of examined cheese samples based on their histamine concentration.

Frequency	Ras cheese		Edam cheese	
	No.	%	No.	%
0 – 5	1	5	15	100
>5 – 10	3	15	0	0
>10 – 20	1	5	0	0
>20	15	75	0	0
Total	**20**	**100**	**15**	**100**

DISCUSSION

Society is increasingly aware of the importance of diet for health, and hence, any issue relating to food safety has a considerable impact on consumer behaviour and official policy. A great effort has been made in the development of techniques to detect contaminants, such biogenic

amines, in foodstuffs. Histamine poisoning is a food-borne chemical intoxication caused by the ingestion of HIS (mainly by the intake of scombroid fish or cheeses, rich in HIS) and is the most common food-borne problem caused by biogenic amines. Different regulatory laws in the USA and the EU have established an upper limit of 100 mg/ kg of histamine in fishery products and this is the tolerance level recommended by other research institutions for fermented foods (***Brink et al., 1990***).

In this work, random samples of commercial available hard (Ras) and semi hard (Edam) cheeses made with different ripening periods were analyzed for their HIS concentrations. HIS was found in 100 and 75% of the analyzed Ras and Edam cheese samples with total percentage of 87.5%, and mean concentrations of 23.38± 2.04, 1.08± 0.12 and 13.82± 0.46 mg/ 100 g, respectively. Nearly similar result was reported by ***Antila et al (1984)***, while lower result was obtained by ***Ekbal*** *and* ***Amer (2010)***. Eighty% of the examined Ras cheese presented a HIS concentration over 100 mg /kg, the recommended upper limit for HIS as reported by ***Durlu-Özkaya et al (1999)*** *and* ***Durlu-Özkaya (2002)***.While, the concentrations of HIS in all Edam cheese samples analyzed were lower than the maximum acceptable limit.

The ageing of cheese is one of the factors that conditioned HIS formation. Cheeses with a short ageing period presented the lowest amounts of HIS than other types of cheese with long ageing period (***Artur et al., 2002***). ***Darwish (1993)*** reported that the HIS formation in Pannonia and Karavan cheeses starting from the third month of ripening and the HIS content increased in both types of cheeses while they matured, this explain the difference in HIS concentrations in Ras and

Edam cheese in our study. Also, the difference in HIS concentrations may be attributed to storage of Ras cheese in Egypt at room temperature. ***Joosten (1988)*** studied the factors influencing the amounts of biogenic amines in cheese and found that histamine formation was accelerated if the cheese stored at high temperatures (18 ºC or 21 ºC).

Levels above 500–1000 mg/kg for HIS are considered potentially dangerous to human health (***Brink et al., 1990***). Considering this, non of the examined cheese samples can be considered potentially dangerous for the consumers. However, some cheese-related cases of HIS intoxication involving individuals on drug therapy (e.g. isoniazid) have been reported after consumption of cheese containing less than 300 mg/kg (***Brink et al., 1990*** *and* ***Stratton et al., 1991***).

Cheese is probably one of the most important HIS 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 the health of the consumers. Therefore, producers must reduce the amounts 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 (***Schneller 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 HIS formation in cheese

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 ensure quality of cheese.

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التحليل الكمى للهستامين فى الجبن المجمع من محافظة كفر الشيخ - مصر

عزه مرغنى محمد ديب و حسام فاروق أحمد

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تم تجميع 40 عينة عشوائية من الجبن الراس والجبن الفلمنك (20 عينة من كل نوع) من أسواق مدينة كفرالشيخ لفحصها لتقدير تركيز الهستامين بها با ستخدام اختبار الاليزا، ولقد تبين من الدراسة وجود الهستامين بنسبة 100% فى عينات الجبن الراس ونسبة 75% فى عينات الجبن الفلمنك (بنسبة كلية 87.5%) وقد كان متوسط تركيز الهستامين 23.38± 2.04 و1.08± 0.12 (13.82± 0.46) مليجرام/100 جم علي التوالي0 وقد أظهرت الدراسة أن 80% من عينات الجبن الراس قد إحتوت على كمية هستامين أعلى من الحد المسموح به (100مجم/كجم)، بينما كان تركيز الهستامين فى جميع عينات الجبن الفلمنك أقل من الحد المسموح به هذا وقد تم مناقشة الأهمية الصحية من تواجد الهستامين فى الجبن وكيفية الحد من وجوده بها.