

DETERMINATION OF HISTAMINE IN PESTA (AUSHARY) CHEESE

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ABSTRACT

The present study aimed to set the level of histamine in pesta cheese. Fifteen samples of Pesta cheese were collected from Sulaimani local markets, Iraq. The level of histamine was assayed using High Performance Liquid Chromatography (HPLC). In comparison with the recommended standard by Food and Drug Administration, high levels of histamine in 33.33% of cheese samples exceeded the permissible limits (100 mg/kg) and that pose a risk to public health.

INTRODUCTION

Pesta cheese (or called Aushary, Jahjy or Peza) is one of the most popular traditional Kurdish semi-hard ripened cheese, it is produced in villages by coagulating raw milk obtained from goats, sheeps or cows which is then put into an animal leather bag with the addition of dried cheese whey, some herbs (garlic, celery, shallots , peze and jahjy) and salt and then pressed and squeeze manually to be stored into caves for a period of ripening between 4- 6 months. Presently some villagers use polyethylene bags instead of the animal leather bag (18).

Histamine, is a type of diamines, accumulate in cheese during ripening (1, 21 and 22). The main factors that control the formation, accumulation and the type of amines are probably the availability of amino acids (and hence proteolysis of cheese) and the presence of bacteria which decarboxylate amino acids (26). Other factors may also contribute to histamine accumulation, such as pH, salt concentration, water availability, temperature, duration of ripening, storage, bacterial density, presence of cofactor and amine catabolism (20 , 10 , 9 and 15). The main symptoms of histamine –rich food is facial flushing, urticaria, edema, diarrhea, stomachache, cramps, hypotension, anaphylaxis, headache with respiratory disorders (6). Several outbreaks of histamine poisoning have occurred following the consumption of cheese, particularly Swiss and Cheddar, containing high levels of histamine (22, 23, 25). According to previous investing level of 100mg of histamine in kg of sample can cause histamine poisoning (9, 13). The formation and accumulation of biogenic amines is related to the hygienic conditions of cheese production and storage. For this reason biogenic amines are considered a potential biomarker of hygienic conditions during cheese production (13, 17).

The aim of this work was to verify the presence of histamine in Pesta cheese. The knowledge of histamine levels in Pesta cheese is necessary to make an assessment of the health hazard arising from the consumption of these products especially by susceptible individuals. Furthermore, the results of the present study could provide basic information to improve cheese quality with respect to biogenic amines content.

MATERIALS AND METHODS

A total of 15 samples of pesta cheese were collected from Sulaimani local markets, Iraq, to determine their content of histamine, compositional analysis

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(moisture, pH, and NaCl concentration) as well as Trichloroacetic Acid-soluble nitrogen (TCA-SN) of total nitrogen(TN).Moisture content was determined according to (12). Total nitrogen content was determined using the Kjeldahl method (2). The pH was measured by pH-meter after calibration with fresh standard buffers at pH 4.0 and pH 7.0.Salt content was determined using Volhard method (2). proteolysis was assessed by determination of TCA-SN as percentage of TN and determined by the Kjeldahl method according to (8).

Histamine was determined by taking cheese sample (20g) and then homogenized for 2 minutes with 100 ml of Trichloroacetic Acid (5% wt/ vol). After centrifugation at 2000 rpm for 15 minutes at 4°C, the fat was removed and its traces were eliminated from supernatant by extraction with diethyl ether (3×15ml). Before HPLC analysis, the residual ether was removed using a rotary evaporator and the volume of the aqueous solution was adjusted to 50ml with HPLC water and filtered through a 0.2 µm syringe filter. Histamine was determined using a previously optimized ion-pair HPLC method (4).

The total aerobic bacterial count and coliform bacteria in cheese were estimated by plate count agar and MacConkey agar, respectively (3).

Statistical analysis

Correlation coefficients among biogenic amines levels and quality parameters ($p \leq 0.05$) were determined by linear regression analysis of individual values.

RESULTS AND DISCUSSION

The relationship between histamine content and other parameters related with ripening and proteolysis was studied. Table 1 shows that the pH value of all samples ranged between (5.6-5.8) which is optimum for enzyme decarboxylating activity (11 , 17) and eight samples of pesta cheese contains less than 3% NaCl ,these samples presented favorable conditions for histamine accumulation (7 , 16). The presence of histamine in samples with NaCl higher than 3% was attributed mainly to the presence of bacteria belong to *Staphylococcus* (14) especially *S. aureus* which is strong histamine producer. Table 2 represent the degree of proteolysis of cheese samples and was confirmed by a very high percentage TCA-SN of TN (up to 60.97 %). Three samples showed that TCA-SN of TN was higher than 50% (59.5, 55.79, and 54.1) % and eight samples showed that TCA-SN of TN was higher than 40% (48.98, 47.81, 43.44, 43.2, 42.53, 42.43, 40.96, and 40.7) %. However, lowest TCA-SN of TN values recorded were (34.58, 28.30, and 28.2) %. Regression analysis of the histamine showed no significant correlation ($p \leq 0.05$) with pH, TCA-SN of TN and moisture while NaCl correlated significantly ($p \leq 0.05$) with formation and accumulation of histamine

Table 3 showed high histamine levels more than the permissible limits at samples 1,2,5,8,and 15 and reached to (145.74, 495.73,290.45,600.55, 203.67) mg/kg respectively and this will lead to unhealthy and risky conditions, it can be concluded this type of cheese is manufactured and ripened under poor hygiene conditions.

Table 1: Physicochemical properties of Pesta cheese samples

Samples number	Moisture %	pH	NaCl %
1	33.340	5.8	3.7012
2	29.297	6.5	2.0454
3	34.129	6.6	2.728
4	40.1264	6.7	2.1428
5	28.3217	5.9	3.7012
6	37.0563	6.8	2.5341
7	33.3864	6.5	5.822
8	42.7823	6.07	2.1428
9	33.0802	6.1	5.6492
10	29.3054	6.0	4.0908
11	39.8145	6.1	4.0999
12	27.5415	6.2	2.528
13	32.7906	6.0	6.0388
14	47.0287	6.2	2.1428
15	38.9359	6.2	2.4481

Table 2: Evolution of proteolysis index in Pesta cheese samples

Samples number	TCA-SN %	T.N%	TCA-SN of TN (%)
1	1.34	3.31	40.70
2	1.34	4.77	28.20
3	1.90	4.41	43.20
4	1.90	3.52	54.10
5	1.74	4.00	43.44
6	2.30	3.86	59.50
7	1.12	2.65	42.43
8	1.46	3.56	40.96
9	1.23	4.35	28.30
10	1.51	4.37	34.58
11	1.51	3.09	48.98
12	2.02	4.22	47.81
13	1.51	3.56	42.53
14	2.18	3.58	60.97
15	1.96	3.51	55.79

Table 3: Concentration (mg/kg) of histamine in Pesta cheese samples

Samples number	Histamine
1	145.74
2	495.73
3	45.54
4	76.85
5	290.45
6	18.23
7	13.32
8	600.55
9	13.62
10	45.47
11	19.99
12	26.19
13	25.93
14	62.95
15	203.67

Coliform bacteria were used as an indicator of sanitation conditions during milk processing, cheese production and storage (24). From table 4 it can be concluded that cheeses were made under poor sanitation conditions as the coliform bacterial counts were high. Numerous species of Enterobacteriaceae have been mainly related with accumulation of diamines in cheese (16,19). From table 4 it can be concluded that after a long ripening period for pesta cheese (about 6 months), and in spite of the unfavorable conditions in cheese such as low water activity, low redox potential, low pH, high salt and lack of fermentable carbohydrates (5), the total aerobic bacterial count was found to be high and this is due to contamination of raw milk, as well as, during processing and marketing.

In this work we found out that the presence of histamine in Pesta cheese is not always related to high TCA-SN of TN (%), moisture, pH and NaCl concentration, but it reflects the bad hygienic conditions during milk processing, cheese production and storage.

Table 4: Microbial population (CFU/g) in Pesta cheese samples.

Samples number	Total count	Coliform
1	8.0×10^4	2.3×10^2
2	1.4×10^5	5.0×10^2
3	1.1×10^5	2.0×10^3
4	3.9×10^4	5.0×10^2
5	1.6×10^3	4.0×10^5
6	1.0×10^4	5.0×10^1
7	2.7×10^4	3.0×10^1
8	2.7×10^4	2.5×10^2
9	7.5×10^3	2.5×10^1
10	5.1×10^4	3.0×10^2
11	5.5×10^5	3.5×10^1
12	2.7×10^5	6.0×10^2
13	4.5×10^4	5.0×10^2
14	6.4×10^5	1.5×10^5
15	3.4×10^5	3.0×10^4

Conclusions:

In conclusion, comparing the obtained results with the recommended standard by Food and Drug Administration, high levels of histamine in pesta cheese consumed in Kurdistan – Iraq exceeded the permissible value (100mg/kg) which carry a high potential risks to public health.

It is recommended to advices and subject all the locally produced cheeses to the quality control regulations and improve the cheese manufacturing process include origin of milk; good manufacture practices precisely pasteurization of milk; place of storage; and water supply. Further studies are needed in order to determine which bacteria had a role in the formation of histamine in pesta cheese and also to evaluate the effect of ripening period, ripening temperature and salt concentration on biogenic amines formation.

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تقدير مستوى الهستامين في جبن البيستا (الاشاري)

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الملخص

هدفت الدراسة الحالية الى تعيين مستوى الهستامين في جبن البيستا . تم جمع 15 عينة من جبن البيستا من الاسواق المحلية لمحافظة السليمانية في العراق وقدرت نسبة الهستامين بأستخدام الكروماتوغرافي السائل ذو الاداء العالي وبمقارنة النتائج المستحصل عليها مع المعايير الموصى بها من قبل منظمة الغذاء والدواء وجد ان 33.33% من العينات تجاوزت الحدود المسموح بها (100 ملغم/كغم) والتي تشكل مخاطر للصحة العامة .