Quantitative analysis of biogenic amines and chemical properties of local pasta (Aushary) cheese in Sulaymaniyah governorate in the final stage of ripening

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Abstract

This study examined 15 traditional cheese samples produced in rural villages of the Qaladiza district in Sulaymaniyah province, along with one control sample produced under strict hygienic standards in a controlled dairy facility. All samples underwent detailed physico-chemical analysis, with a particular focus on biogenic amine content and a direct comparison between the village-produced cheeses and the laboratory control. Tyramine, 2-Phenylethylamine, Cadaverine, Histamine, and Spermidine were found in the cheese. The results showed that there are significant differences (0.05*p*) in the chemical characteristics of the laboratory and the traditional Pesta cheese within the different ripening periods (0, 90, and 180) days, the cheese was hard cheese due to their contents of moisture (21.23-36.20) % and fat (29.25-43.06) % at six months of ripening. The study showed very low BAs levels less than the permissible limits at treatments T2, T4, T7, T8, T9 and T15 and read to (183.15, 138.87,22.84,253.94, 233.12 and 191.6) mg/kg respectively while no BAs detection in the T5, T6 and T10 and although the T1, T3, T11, T12, and T14 contain high level of some BAs and were (925.82, 837.97, 585.56, 379.04, and 420.5)mg/kg respectively but still under than the permissible limits, Spermidine was the highest and Tyramine was the lowest (1374.32-0.35)mg/kg respectively so concluded this type of cheese needs more interest manufactured and ripened under high hygiene conditions. Several parameters, including pH, Acidity, and sodium chloride and fat concentration, have also been linked and have role to the formation of BA in cheese.

Keywords: Traditional ripened cheese, Biogenic amines, Protected Denomination of Origin.

Introduction

Pesta cheese is one of most popular cheeses, hard or very hard Kurdish cheese in the Kurdistan region of Iraq, found in other Kurdish regions such as Turkey, Iran, and Syria but in different names, is a unique ripened cheese has a special sharp flavour, produced from cow, ovine, and goat milk by the nomadic and villagers' people. It is consumed

widely in northern Iraq[1] and therefore should be registered as Protected Geographical Indication (PGI) and Protected Denomination of Origin (PDO) and that will help to giving the specifications for local Pesta cheese in Kurdistan region, there have many studies on the chemical composition, physicochemical, and microbial properties, sensory evaluation of this cheese due to its importance, However, studies on Biogenic Amines (BAs) are few and limited, in generally cheese is a type of food characterized by its organoleptic properties, especially aroma and flavour as a result of these properties some sensory active substances are formed results of these biochemical reactions [2] .

BAs play various roles in the functioning of the human body. They result by oxidation processes in food. BAs are mostly found in fermented products like fish, meat, wine, and cheeses. Being in small amounts does not affect people's health, but in high levels can cause poisoning[3]. The most important BA present in cheeses are the histamine, then tyramine, cadaverine, and putrescine [4, 5]. Add this Biogenic amines (BAs) are natural toxicants produced during the metabolism of their precursor amino acids or due to the proteolytic activities of some microorganisms, histamine is State the internationally permissible limits for cheese. all the spry biogenic amine and is most involved in food poisoning. BAs are organic compounds with biological activity, formed by the decarboxylation of amino acids. Even if they are produced naturally by, animals, microorganisms, and plants, the consumption of foods containing large amounts of these BAs can have toxicological effects [6] or BAs formed by transamination of ketones and aldehydes by amino acid transaminases, as these Biogenic Amines are act as a spoilage indicator, their presence determines the quality of food [7]. Biogenic amine formed by the action of microorganisms, which are added during the preparation process or by contamination, also lowers the amino acid index in cheese [8] and counted as a potential microbiological toxicological hazard in old cheese [9] an excessive content of BAs proves to be toxic (diarrhea, food poisoning, vomiting, sweating, or tachycardia). Moreover, they can accelerate carcinogenesis [10] BAs can accumulate in high amounts and in some types of cheeses; more than 1000 mg of them have been detected per kilogram of cheese [11].

The ripening stage is the most important stage during cheesemaking, where the most intensive physical and chemical changes occur. During ripening, casein decomposes, leading to the accumulation of free amino acids (FAAs), which can be converted into a number of sensory active substances by the effect of the microflora, and they might also be decarboxylated into biogenic amines (Bas)[12]. It is very important to detect biogenic amines due to the defects associated with it, such as off-Flavors, Odors, Headaches during consumption, and about cheese effect are stinging or burning sensation in the mouth with unwanted gas formation (CO_2) by non-starter bacteria. The aims of study, produce Pesta cheese (control sample) in the laboratory in same local and traditional method with same conditions of original Pesta cheese production for comparative with fifteen Pesta cheese samples produced locally and studying chemical composition of cheese samples, evaluate hazard and safety assessment of cheese consumption in the

Sulaymaniyah governorate, and analysed for their biogenic amine contents after six months of maturing, and determination of the biogenic amine profiles and theirs quantities of eight amines (Tyramine(Tym), Tryptamine(Try), Putrescine(Put), phenylethylamine(Phe), Cadaverine(Cad), Histamine(Him), Spermidine(Spd), and Spermine(Spm)) in Pesta cheese by using high-performance liquid chromatography HPLC. Determination of the level of toxic amines as an alarm for the risk in consumption.

Materials and Methods

Determination of Chemical and Physical Composition to Pesta Cheese

A total of fifteen samples of Pesta cheese, produced locally by different producers, were studied over six months of maturation and storage, along with a control sample produced in the laboratory under aseptic conditions according to [1]. The study aimed to determine the content of biogenic amines (tyramine, tryptamine, putrescine, 2-phenylethylamine, cadaverine, histamine, spermidine, and spermine), in addition to evaluating the physicochemical properties, including fat, protein, moisture, ash, NaCl, acidity, and pH. Each cheese sample was analysed in triplicate.

Determined fat % in cheese using a hexane solvent extraction method by [13] the extraction by using hexane was conducted using a Soxhlet apparatus (Buchi/Switzerland) equipped with a heating plate (40-60℃). 2 g of dried cheese (dried at 103℃ for 4 hours) was subjected to fat extraction for approximately eight hours, and a syphoning rate of 4/hr. Moisture% was determined following the [13] method, a drying oven set at 102℃/ 4 hours, pH Values and titratable acidity (TA) values of cheese were measured according to [13] by adding D.W at 40℃ to 10g of sample until the total volume reached 105 ml. The mixture was shaken and filtered, approximately 25ml (about 2.5 g of cheese) of filtered solution was titrated with 0.1N NaOH, and phenolphthalein was used as an indicator. The following equation was then applied: TA% = ((0.1 NaOH volume) x 0.009 x 100)/ (weight of the sample). pH value was measured by immersing the electrode of the pH meter into the filtered solution. A muffle furnace (Dahlan Labtech Co., Ltd./Korea) was used to determine ash% at 550 ℃ till all organic matter was burnt off. Meanwhile, The general nitrogen content was determined using the micro Kjeldahl method according to the International Dairy Federation [14], 1 gram cheese separated and used for Kjeldal analysis by using 15 ml sulfuric acid added to 5 gr catalyser (0.5 gr cuSO4+4.5 gr KSO4), digested in 300 °C for 3 h, then 75 ml dH2O added and read in the Kjeldal (Kjeltec Auto 1030 analyser), the protein percentage was evaluated by multiplying total nitrogen by a factor of 6.38.

Sodium chloride (NaCl) was determined by using Fourier Transform Infrared (FTIR) spectroscopy (Bruker MPA II Co., Germany) according to [15].

All chemical standards of BGa (β-phenylethylamine (C8H11N), histamine (C5H9 N3), putrescine(C4 H12N2), cadaverine(C5 H14 N2), tryptamine (C10H12N2), spermine (C10H26N4), tyramine (C8H11NO), spermidine(C14H47N6 O12P3) were supplying (Sigma Chemical Co., St. Louis, MO, USA).

Extraction of samples for HPLC analysis: Analysis of biogenic amines in the samples of cheese matrices was based on the procedure of [16]. Briefly, 12.5 mL of 0.4 M perchloric acid was added to 5 g of the cheese samples, and the mixture was homogenized for 15 minutes using a vortex mixer, put in a refrigerator for 30 minutes, and centrifuged at 5000 ×g for 15 min. The supernatant was collected for use. The biogenic amines were derived according to the procedures offered [16] then was kept at −25 °C until injected by HPLC.

HPLC Method for Biogenic Amine Analysis

An aliquot of 20 µL of each sample was injected into the HPLC system (SYKAM Co., Germany), equipped with a C18 reverse-phase column and interfaced with a UV detector set at 254–280 nm. The analysis was performed at a flow rate of 1.0 mL/min with the column temperature maintained at 40°C.

Statistical Analysis

The results obtained from fat, protein, moisture, ash, NaCl, acidity, and pH determination, ripening index, and biogenic amines analyses were subjected to an independent sample T-test using IBM SPSS (version 20). Significant difference between cheese samples was tested at $p<0.05$ [17].

Results and Discussion

Chemical composition of Pesta cheese

The chemical structure of the cheese or any other food considered as indicator of the nutritional value or the source of the energy from another hand, so chemical structure of the Pesta cheese was studied through in the three stage of ripening periods (first, medium, last) represented by (1,90,180) days as illustrated in the table 1, significant changes in the chemical composition of cheese samples were found during the ripening process when samples were analyzed over three time points (1, 90, and 180 days) and across 16 different treatments (T1-T15) and a control treatment.

Table 1 presents the physicochemical analysis results of Pesta cheese, demonstrating a progressive decrease in moisture content during cheese maturation. Initially (day 1), treatment T9 contained the highest moisture level at 49.93%, while T15 showed the lowest initial moisture content at 30.68%. By day 90, T9 maintained the highest moisture retention at 44.18%, whereas T8 exhibited the most significant moisture loss, reaching 24.71%. At the final sampling point (day 180), T9 continued to retain the highest moisture content at 36.20%, while T15 recorded the lowest final moisture level at 21.23%. Moisture loss during cheese ripening is mainly caused by microbial activity, lactic acid production, and water evaporation [18].

Fat and protein

Concentrations increased progressively throughout the ripening period, inversely correlating with moisture content. On day 1, treatment T12 recorded the highest initial fat concentration at 33.95%, while T9 showed the lowest at 19.68%. By day 90, T9

maintained the lowest fat content at 22.57%, whereas T6 demonstrated the highest concentration at 40.49%. At the final sampling point (day 180), T6 achieved the maximum fat content of 43.06%, while T9 remained at the lowest level with 29.25%. These results indicate that T6 consistently developed higher fat concentrations during ripening, while T9 maintained relatively lower fat levels throughout the entire maturation process. This inverse relationship between moisture loss and fat concentration reflects the natural dehydration process during cheese aging, where the relative proportions of solid components increase as water content decreases [19], [20], Initially (day 1), treatment T15 exhibited the highest protein content at 31.05%, while T11 showed the lowest at 17.91%. By day 90, T15 maintained its position with the highest protein concentration at 31.45%, whereas T12 recorded the minimum level at 20.63%. At the final assessment (day 180), T15 continued to demonstrate superior protein content, reaching 31.85%, while the control treatment showed the lowest protein concentration at 22.64%. consistent with the concentration effect observed as moisture is lost during the cheese aging process [20]. Protein percentage of cheese did not high change by change of ripening periods (1, 90, 180) day in the same treatment compared to the fat percentage which was more chang may be returned to slowing of proteolysis due to decrease of water activity and increase of pH values and salts content.

pH values

It was demonstrated a general declining trend throughout the maturation period, reflecting the acidification process during cheese ripening. Initially (day 1), treatment T12 exhibited the highest pH at 7.08, indicating the most alkaline conditions, while the control treatment showed the lowest initial pH at 6.04. By day 90, T12 maintained relatively high pH levels at 6.70, whereas T11 demonstrated significant acidification, reaching 5.62. At the final sampling point (day 180), T10 achieved the highest final pH of 6.71, while T14 recorded the most acidic conditions at 5.36, caused by the decomposition of remaining lactose through lactic acid bacterial activity [21].

Titratable acidity

It exhibited progressive increases throughout the maturation period, with significant variation among treatments. Initially (day 1), multiple treatments (CONTROL, T7,T8, and T13) shared the lowest acidity level at 0.10%, while T15 demonstrated the highest initial acidity at 0.20%. By day 90, T15 maintained its position with the peak acidity at 0.26%, whereas T7 showed the minimal increase to 0.11%. At the final assessment (day 180), T15 achieved the maximum titratable acidity of 0.35%, while T8 recorded the lowest final level at 0.14%. Titratable acidity continuously rises due to two main factors. First, lactic acid bacteria convert lactose into lactic acid. Second, the decomposition of certain proteins and mineral salts also contributes to this increase in acidity [22].

Ash content

A gradual declining trend in ash content was observed across all treatments during the maturation period. Initially (day 1), treatment T5 recorded the highest ash concentration at 7.63%, while the control treatment showed the lowest at 3.86%. By day 90, T5 still demonstrated the highest ash content at 7.29%, whereas the control maintained the lowest level at 3.69%. At the final assessment (day 180), T2 recorded the highest ash content at 7.17%, while T12 exhibited the lowest final concentration at 3.61%.

This decline is attributed to the formation of acid, which dissolves micellar calcium phosphate and facilitates the removal of minerals from whey [23]. Calcium levels decrease as the milling pH decreases, while no clear pattern is observed for potassium and sodium. This is because lower pH during milling promotes the loss of non-micellar calcium into the whey. Therefore, milling pH plays an important role in determining the extent of calcium loss [24].

Sodium chloride (NaCl)

The content showed a progressive increase throughout the maturation period, with notable variation among treatments. Primarily (day 1), treatment T2 contained the highest salt concentration at 3.28%, while the control treatment exhibited the lowest at 0.40%. By day 90, T2 maintained its leading position with a slight increase to 3.30%, whereas the control treatment remained unchanged at 0.40%. At the final sampling point (day 180), T2 achieved the maximum salt content of 3.49%, demonstrating continued salt accumulation, while T10 recorded the lowest final concentration at 0.49%. Salt content increased over the ripening period mainly due to moisture loss, which concentrated the salt in the cheese matrix [25].

Table (1): Mean of physicochemical composition of Pesta cheese during storage

The treatments	Ripening periods (day)	Fat %	Protein %	Moisture %	Ash %	NaCl %	Acidity %	pH
Control	0	25.89^{b} ^{cde}	21.06^{g}	48.29^{ab}	3.86^{g}	0.40^{m}	0.10^{d}	6.04 ^{m}
	90	31.78^{d} ^{e}	21.84^{n}	42.12^{a}	3.69^{g}	0.40^{m}	0.13^{fgh}	5.70^{j}
	180	38.17^{b} ^{cd}	22.64^{m}	35.85^{a}	3.62^{i}	0.63^{l}	0.17^{hi}	5.63 ^{h}
T1	0	22.84^{c} ^{de}	26.36^{d}	43.71^{bcd}	5.82^{c}	1.27^{i}	0.14^{b}	6.62 ^{h}
	90	30.47^{es}	26.72^{h}	36.28^{bcd}	5.24^{de}	1.39^{g}	0.16^{cde}	6.24 ^{e}
	180	38.77^{a} ^{bcd}	27.05^{h}	27.88^{bcde}	5.14^{cd} ^{e}	1.39^{h}	0.20^{hi}	6.18 ^{cd}

T2	0	29.05^{a} bc	26.53^{d}	35.07^{fgh}	7.36^{a}	3.28^{a}	0.15^{b}	6.86 d
	90	32.39^{d} e	27.05^{f}	30.80^{efg}	7.22^{ab}	3.30^{a}	0.17^{bcd}	6.58 c
	180	37.05^{c} d	27.56^{g}	24.27^{def}	7.17^{a}	3.49^{a}	0.23^{cdeh}	6.15 d
T3	0	25.73^{b} cde	26.66^{d}	41.83^{cde}	4.97^{de}	1.08^{k}	0.14^{b}	6.90 c
	90	34.72^{c} d	26.89^{g}	33.50^{def}	4.81^{de} f	1.19^{i}	0.15^{def}	6.58 c
	180	40.82^{a} bc	27.12^{h}	26.97^{bcde}	4.77^{cd} ef	1.25^{i}	0.16^{hi}	6.32 b
T4	0	33.27^{a}	24.854^{h}	36.65^{efgh}	5.02^{de}	1.30^{i}	0.12^{cd}	6.66 g
	90	39.37^{a} b	25.81^{i}	30.71^{efg}	4.81^{de} f	1.43^{g}	0.13^{efg}	6.21 e
	180	42.28^{a} b	26.78^{j}	25.79^{cdef}	4.67^{de} fg	1.50f^{g}	0.17^{gh}	6.19 cd
T5	0	23.95^{c} de	25.26^{e}	41.84^{cde}	7.63^{a}	2.51^{b}	0.11^{cd}	6.15^{l}
	90	31.55^{d} e	25.52^{j}	32.31 ^{defg}	7.29^{a}	2.62^{b}	0.12^{gh}	5.75^{i}
	180	39.21^{a} bcd	25.76^{l}	27.79 ^{bcde}	5.96^{b}	2.74^{b}	0.25^{bcd}	5.43^{j}
T6	0	31.66^{a} b	26.35^{d}	36.08^{efgh}	4.85^{ef}	1.42^{f}	0.14^{b}	6.70 f
	90	40.49^{a}	26.63^{h}	27.67^{gh}	4.52^{ef}	1.43^{g}	0.15^{def}	6.24 e
	180	43.06^{a}	26.92^{hi}	23.10^{ef}	3.99^{hi}	1.48^{g}	0.17^{hi}	6.21 c
T7	0	30.49^{a} b	24.30^{f}	35.90^{efgh}	6.73^{b}	1.71^{d}	0.10^{d}	6.25 k
	90	34.82^{c} d	24.66^{k}	32.61^{defg}	6.54^{bc}	1.86^{d}	0.11^{h}	5.84 h
	180	39.85^{a} bc	25.04^{k}	28.79^{bcd}	6.24^{cd}	1.90^{d}	0.20^{eh}	5.53^{i}
T8	0	28.91^{a} bc	27.78^{c}	34.65^{gh}	6.68^{b}	1.87^{c}	0.10^{d}	6.48^{i}
	90	37.66^{a} b	28.01^{e}	24.71^{h}	6.38^{c}	2.08^{c}	0.12^{gh}	6.12 f
	180	39.66^{c} d	28.23^{e}	24.16^{def}	6.24^{b}	2.17^{c}	0.14^{i}	6.09 e

T9	0	19.68^{e}	27.92^{c}	49.93^{a}	4.82^{ef}	0.79^{l}	0.15^{b}	6.13^{l}
	90	22.57^{g}	28.34^{d}	44.18^{a}	4.62^{def}	0.86^{k}	0.17^{bcd}	5.68^{j}
	180	29.25^{e}	28.78^{d}	36.20^{a}	4.09^{ghi}	0.87^{k}	0.27^{b}	5.63^{h}
T10	0	27.62^{abcd}	18.76^{h}	47.68 ^{abcd}	4.84^{ef}	0.40^{m}	0.14^{b}	6.97^{b}
	90	30.72^{e}	23.55^{l}	40.56^{ab}	4.72^{def}	0.46^{l}	0.17^{bc}	6.66^{b}
	180	35.22^{d}	28.37^{e}	31.80^{ab}	4.41^{fgh}	0.49^{m}	0.22^{deh}	6.51^{a}
T11	0	33.28^{a}	17.91^{i}	40.82^{def}	5.56^{cd}	1.58^{e}	0.15^{b}	6.06^{m}
	90	36.77^{bc}	22.92^{m}	32.61^{defg}	5.35^{d}	1.69^{e}	0.19^{b}	5.62^{k}
	180	37.82^{bcd}	27.99^{f}	28.59^{bcde}	5.33^{c}	1.78^{e}	0.25^{bcd}	5.44^{j}
T12	0	33.95^{a}	17.93^{i}	42.81^{bcd}	4.30^{fg}	1.08^{k}	0.12^{c}	7.08^{a}
	90	38.98^{ab}	20.63^{o}	35.24^{cde}	4.09^{fg}	1.20^{i}	0.14^{efg}	6.70^{a}
	180	40.71^{abc}	26.38^{k}	30.71^{bc}	3.61^{i}	1.23^{i}	0.26^{bc}	6.31^{b}
T13	0	25.72^{bcde}	29.88^{b}	39.91^{defg}	5.22^{cde}	1.19^{j}	0.10^{d}	6.79^{e}
	90	30.97^{e}	30.07^{b}	33.26^{def}	5.11^{de}	1.31^{h}	0.12^{gh}	6.35^{d}
	180	38.04^{bcd}	30.61^{b}	25.10^{cdef}	4.55^{efgh}	1.52^{f}	0.15^{i}	5.87^{f}
T14	0	21.74^{de}	27.93^{c}	44.71^{abcd}	5.11^{de}	0.98^{k}	0.14^{b}	6.31^{j}
	90	25.91^{f}	28.95^{c}	39.52^{abc}	4.89^{def}	1.07^{j}	0.17^{bcd}	5.89^{g}
	180	37.22^{cd}	29.99^{c}	27.07^{bcde}	4..80^{cdef}	1.15^{j}	0.23^{bcde}	5.36^{k}
T15	0	32.70^{a}	31.05^{a}	30.68^{h}	5.02^{de}	1.38^{g}	0.20^{a}	6.26^{k}
	90	38.04^{abc}	31.45^{a}	28.55^{fgh}	4.87^{def}	1.50^{f}	0.26^{a}	5.87^{gh}
	180	40.03^{abc}	31.85^{a}	21.23^{f}	4.54^{efgh}	1.51f^{g}	0.35^{a}	5.77^{g}

Biogenic Amines Content

Histamine

Two samples (T1 and T2) tested positive for histamine presence, with average concentrations of 4.44 mg/kg and 70.32 mg/kg, respectively. Based on the [26], [27]. The acceptable range for histamine levels should fall within 50-100 mg/kg.

Cadaverine

Cadaverine was found in samples T1 and T2, showing values of 0.66 mg/kg and 0.79 mg/kg. According to standards, it was found to be 180 mg/kg body weight/day for cadaverine [28].

Phenylethylamine

2-Phenylethylamine was found in four samples, with concentration levels varying from 184.38 mg/kg to 920.59 mg/kg. Based on [26] the standard acceptable range should fall between 200 mg/kg and 1000 mg/kg; this level is considered a critical dose of this amine content in the T1 and T3, which had 920.59 and 837.97 mg/kg, respectively.

Tyramine

Tyramine was found in samples T8 and T11, with concentrations of 4.78 mg/kg and 0.35 mg/kg, respectively. The maximum permissible level for tyramine is 30 mg/kg [28].

Spermidine

This Bas was found in treatments 8, 11 and 13 in high amounts 253.59, 555.96 and 1374.32 mg/kg, respectively although it was less than the permissible limits because the toxic dose is not established in food according to [26].

The Secondary Amines

Methylamine concentrations varied significantly among the tested samples, with treatment T4 exhibiting the highest level at 138.21 mg/kg, while treatment T7 demonstrated the lowest concentration at 22.84 mg/kg. Ethylamine concentrations showed marked variation across treatments, with T9 recording the highest level at 170.21 mg/kg, while T11 exhibited the lowest concentration at 24.82 mg/kg. Di-ethylamine concentrations demonstrated considerable variation among treatments, with T12 recording the maximum level at 236.95 mg/kg, while T14 showed the minimum concentration at 53.12 mg/kg. Aniline concentrations exhibited substantial variation among treatments, with T2 recording the highest level at 112.83 mg/kg, while T15 demonstrated the lowest concentration at 7.22 mg/kg.

Table (1): Concentration (mg/kg) of biogenic amines and secondary amines detected by high-performance liquid chromatography (HPLC) in Pesta cheese samples after 180 days of ripening.

Pesta Cheese treatments	histamine	cadaverine	2-Phenylethylami	Tyramine	Putrescine, Tryntamine	Spermidine	Methylamine	Ethylamine	Di ethylamine *	Aniline *	Total
C	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
T1	4.44	0.79	920.59	n.d	n.d	n.d	n.d	n.d	n.d	n.d	925.82
T2	70.32	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	112.83	183.15
T3	n.d	n.d	837.97	n.d	n.d	n.d	n.d	n.d	n.d	n.d	837.97
T4	n.d	0.66	n.d	n.d	n.d	n.d	138.21	n.d	n.d	n.d	138.87
T5	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
T6	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
T7	n.d	n.d	n.d	n.d	n.d	n.d	22.84	n.d	n.d	n.d	22.84
T8	n.d	n.d	n.d	0.35	n.d	253.59	n.d	n.d	n.d	n.d	253.94

T9	n.d	n.d	n.d	n.d	n.d	n.d	n.d	170.21	n.d	62.91	233.12
T10	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-
T11	n.d	n.d	n.d	4.78	n.d	555.96	n.d	24.82	n.d	n.d	585.56
T12	n.d	n.d	n.d	n.d	n.d	n.d	n.d	54.40	236.95	87.69	379.04
T13	n.d	n.d	n.d	n.d	n.d	1374.32	n.d	n.d	n.d	n.d	1374.3
T14	n.d	n.d	367.38	n.d	n.d	n.d	n.d	n.d	53.12	n.d	420.5
T15	n.d	n.d	184.38	n.d	n.d	n.d	n.d	n.d	n.d	7.22	191.6

C: control treatment, T: treatment, * Secondary Amines

The effect of cheese chemical composition on BAs formation

The relationship between BAs content and the other chemical parameters related to maturing and proteolysis was studied. Table (1) shows that the acidity and pH value of all samples ranged between (0.10 - 0.26) % and (5.36-7.08), respectively, which is optimum for enzyme activity to decarboxylate [29]. Based on our analytical results, pH levels significantly influenced biogenic amine formation in treatment T10, which recorded the highest pH value of 6.51. According to [30] the optimal pH range for decarboxylation reactions and biogenic amine formation is 5.0-6.5. Most samples in this study exhibited pH values within this critical range, suggesting potential conditions conducive to biogenic amine production during cheese maturation. This pH range represents a balance where decarboxylase enzymes from certain bacterial strains remain active, potentially converting amino acids to biogenic amines.

Most of Pesta cheese samples were contain less than 3% NaCl, that provides presented favourable conditions for biogenic amines accumulation excepting control treatment which produced under aseptic condition, Whereas High quantities of sodium chloride (=5%) prevent the formation of BA, most likely because they have an inhibitory effect on the rate at which bacteria that produce BA develop [31] each of T1, T3, T11, T12, T14 were contain a high level of total amins (925.82, 837.97, 585.56, 379.04, 420.5) mg/kg respectively compared to another treatments, in general sodium

chloride was have a different effective on the BAs in the cheese samples, activates tyrosine decarboxylase activity and inhibits histidine decarboxylase activity in certain sodium chloride concentrations and that reviewed by [32]. Treatment T6 exhibited the highest fat content (43.06%), which contributed to the prevention of biogenic amine formation alongside secondary amine development. The production of biogenic amines is not favoured by fat. In fact, when cheese's fat content rises, its water activity falls. Cheeses' water activity is decreasing in parallel with their increasing fat content, which inhibits proteolytic bacteria and decreases the availability of free amino acids for the synthesis of bioamines [33].

The control treatment maintained optimal processing conditions within safe parameters, effectively preventing any biogenic amine formation in the samples. This demonstrates that proper control of physicochemical factors—including pH, fat content, and processing conditions—can successfully minimize food safety risks associated with biogenic amine accumulation during cheese maturation.

Table 2 showed very low BAs levels less than the permissible limits at treatments T2, T4, T7, T8, T9 and T15 and read to (183.15, 138.87,22.84,253.94, 233.12 and 191.6) mg/kg respectively while no BAs detection in the T5, T6 and T10 and although the T1, T3, T11, T12, and T14 contain high level of some BAs and were (925.82, 837.97, 585.56, 379.04, and 420.5) mg/kg respectively but still under than the permissible limits, so concluded this type of cheese needs more interest manufactured and ripened under high hygiene conditions. Several environmental parameters, including pH, temperature, and salt concentration, have also been linked to the synthesis of BA in cheese [34].

Pesta cheese contains a good amount of protein, fat, and minerals, giving it high nutritional value and making it a good source of energy and safety. Meeting these nutritional requirements is essential in the dairy industry for ensuring public health.However, the presence of biogenic amines (BAs) in ripened cheese is a quality concern. This study revealed that the quantitative estimation of BAs in Pesta cheese treatments showed the presence of histamine, cadaverine, tyramine, and spermidine—though all were at safe levels for consumption. Nevertheless, this raises concerns regarding the quality and safety of the cheese. Therefore, it is concluded that the production of this type of cheese requires greater attention and must be carried out under strict hygienic conditions during manufacturing and ripening.

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