JOURNAL OF NUTRITION FASTING AND HEALTH

Effect of *Bunium Persicum* Boiss. Essential Oil on the Production of Biogenic Amines (Tyramine and Histamine) and Microbiological Profile in Cheddar Cheese

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 19 Nov 2022
Accepted: 20 Feb 2023
Published: 07 Mar 2023

Keywords:
Biogenic amines
Bunium persicum Boiss
Essential oils,
Cheddar cheese
Microbiological profile
Natural antimicrobial agents

ABSTRACT

Introduction: Tyramine and Histamine, are two major biogenic amines that could be formed in different types of cheese.

Methods*: Bunium persicum* essential oil (BPEO) in different concentrations (0.05, 0.1, 0.2 and 0.4% (v/v)) was added to milk. The amount of biogenic amines (tyramine and histamine) was specified by reverse phase HPLC (RP-HPLC) after extraction from the cheese. Different microbiological analyses (mesophilic lactobacilli, aerobic mesophilic bacteria, *Enterobacteriaceae*, *lactococci*, *enterococci*, and yeasts) were performed during the ripening cheese.

Results: The amount of biogenic amines were lower in cheese samples containing 0.1, 0.2, and 0.4% BPEO in comparison with control samples. Tyramine quantity increased from 8.90 mg/kg on start day to 172.40 mg/kg on the last day of ageing in the control group also the mean concentration of histamine was 6.31 mg/kg that reached to 41.16 mg/kg in the control samples at the end of study period. The most microbiological decrease was detected in yeasts, and the minimum microbiological decrease was observed in *Enterobacteriaceae* population. All the cheese samples were acceptable to the panelists. The increase in BPEO concentrations in cheese samples caused a further reduction in biogenic amines quantity and microbial population. Cheddar cheese sample with 0.2% BPEO demonstrated the highest acceptability and quality among all the cheese samples.

Conclusion: The results of this study revealed *Bunium persicum* EO could be utilized as a reducing agent for biogenic amines, and a natural antimicrobial and flavoring agent in Cheddar cheese, for improving consumers' health.

► Please cite this paper as:
Raoofi M, Noori N, Khanjari A. Effect of Bunium Persicum Boiss. Essential Oil on the Production of Biogenic Amines (Tyramine and Histamine) and Microbiological Profile in Cheddar Cheese. J Nutr Fast Health. 2023; 11(1): 32-40. DOI: 10.22038/JNFH.2023.69084.1411.

Introduction

Biogenic amines are mostly formed by way of the decarboxylation of some free amino acids. The presence of biogenic amines in food is undesirable. Biogenic amine is formed during natural metabolism in microorganisms, plants, and animals. If the concentrations of these compounds are increased in food, it can be harmful to susceptible individuals (1). Extreme oral intake of biogenic amines such as tyramine and β- phenylethylamine may cause implications like nausea, headaches, and consecutive fluctuations in blood pressure (2). Consumption of certain fish and cheese containing high levels of histamine could result in histamine poisoning (3-4). Interactions between biogenic amines with other compounds should be considered; for example, nitrosamines that are potential carcinogens can be formed due to interactions between nitrites and biogenic amines (5). The presence of some biogenic amines, such as putrescine, cadaverine, and tryptamine, results in risen toxicity of histamine, because they can have an inhibitory effect on histamine detoxifying enzymes such as Diamine oxidase, Histone methyltransferases, and Monoamine oxidase (6). Consumption of greater than 40 mg/meal of biogenic amines is considered a potentially toxic level. Cheese is a foodstuff that contains a high amount of protein which is prone to the production of biogenic amines. It is documented that the sum levels of histamine, tyramine, cadaverine, and putrescine higher than 900 mg/kg of cheese is not safe for consumers

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(7). However, there are currently no legal limits for biogenic amines in most foods, including cheese. (8). In semi-hard and hard type of cheeses, the ripening period is more extended than in other cheeses, and many microbiological and biochemical evolution are made during this period, so these kinds of cheeses are an ideal food for biogenic amines formation (9). Many factors, including milk hygiene, milk pasteurization, microbial load and interaction, pH, proteolysis level, ripening temperature and time, storage temperature, and salt level, affect the formation of biogenic amines in cheese (10). It seems that biogenic amines are mainly produced due to the existence of microorganisms with decarboxylase activity in cheese, and this feature (enzymatic decarboxylation of amino acids) can be recognized in the starter culture opportunistic microorganisms and non-starter lactic acid bacteria (9). Also, among some decarboxylase-positive micro-organisms, especially many *Enterobacteriaceae* strains found in milk, can produce histamine, cadaverine, and putrescine (11). The production of biogenic amines in cheese is inevitable, therefore adding of natural or synthetic food additives to reduce BA levels can be useful. Essential oils are natural preservatives that have acceptable antibacterial and antioxidant properties. *Bunium persicum* with vernacular name of Zireh siah, is a plant belong to Umbelliferae family that grows spontaneously in some parts of the world, such as Iran (12). Cheddar cheese is a pale yellow to off-white semi-hard British cheese that is very popular globally, and has a relatively long ripening period.

In this study, the effect of *Bunium persicum* Boiss. EO on the reduction of biogenic amines formation in cheddar cheese was investigated.

Materials and Methods

Preparation and analysis of BPEO

The seeds of *Bunium persicum* were prepared from the Kerman province, Iran. Then, 200 g of the sample was transferred to a Clevenger-type apparatus system for hydrodistillation. Next, 1 ml of obtained EO was injected into the gas chromatograph-mass spectrometry to identify the EO components. The chromatograph equipped with FID detector and DB5 capillary column (30 m × 250 µm inner diameter × 0.25 µm inner layer thicknesses), the temperature plan was as follows: the internal temperature program was 50 to 265°C; program rate was 2.5°C, and injector temperature was 250°C. The column was kept at 265 ° C for 30 minutes. Helium injection speed was 1.5 mm/min. The MS was run at 70 eV ionization energy and ionization temperature of 250 °C.

Cheddar cheese manufacturing and sampling

The cheddar cheese was prepared in a commercial dairy factory as follows: pasteurized cow's milk (65°C, 30 minutes) was poured into a stainless steel two boiler vat and then cooled at 33 ° C. Afterward; the milk was inoculated with 2% (v/v) of mesophilic starter culture (*Lactococcus lactis* and *Lactococcus cremoris*, manufactured by CHR Hansen/Horsholm, Denmark) (13). Thirty minutes after adding the starter, filter-sterilized rennet (31 ml, diluted in 500 ml of sterile distilled water) was added to the milk. Then, different concentrations of *Bunium persicum* essential oil were integrated into milk (0, 0.05, 0.1, 0.2, and 0.4%). For each concentration of BPEO, two batches of Cheddar cheese were prepared. The coagulum was cut into 0.5–1.25 cm cubes after approximately forty minutes. After that, the curd was transferred to molds to drain the whey. Then, the curd was cooked at 33°C for 15 minutes. After that, the cheese was kept warm and led to acid production at final pH of 5.52 to 5.54. Salting the cheese was accomplished at the rate of 2.5% (w/w), and the remaining whey was removed from the cheese by pressing them with a cheese presser. The cheese was stored and ripened in waxed molds for 90 days at 10 ° C. Biogenic amines level and microbiological evolution were assessed for each treatment at 0, 15, 30, 45, 60, and 90 days of the ripening period.

Microbiological Analyses

10 g of the cheese sample was taken under aseptic conditions and homogenized with 90 ml of sodium citrate (2% weight/volume) solution in the Stomacher, Lab-Blender 400 (Steward Medical, London, UK). Then, serial decimal dilutions were prepared using sterile peptone water (0.1% w/v). The microbial properties of cheddar cheese containing different concentrations of BPEO, were determined as follows: Aerobic mesophilic bacteria were counted on Plate Count Agar after incubation for 2 days at 30 °C; Mesophilic lactobacilli were determined on MRS agar (Biolife) with adjusting pH to 5.5 after incubation for 2 days at 30 °C under anaerobic conditions; *Enterobacteriaceae*

was enumerated on Violet Red Bile Glucose Agar (VRBGA) after incubation for one day at 37 ° C. Coliforms were estimated on Violet Red Bile Agar (VRBA) after incubation for one day at 37 ° C. Yeasts were counted on Peptone Dextrose Agar (PDA) after incubation for 5 days at 20°C. Enterococci were determined in Kenner Fecal Agar (Biolife) after incubation for two days at 37 ° C.

Determination of Biogenic Amines

The biogenic amines were determined according to the method that previously described by Eerola et al. (1993) as follow: 1 gram of cheddar cheese was taken and then homogenized with Stomacher (Steward Medical, London), afterward, 2 ml of 0.1% hydrochloric acid was added to it and sonicated (Soniprep 150, MSE , ltd., UK) for 30 seconds and vortexed for 3 minutes. Next, it was centrifuged (Hettich universal 32 R, Hettich, Germany) at 3000 rpm for 5 minutes. The supernatant solution was filtered by 0.45-micron filters and injected into the HPLC (Knaver, Germany) on wavelengths of 210 nm for histamine and 225 nm for tyramine (14,15).

Sensory Evaluation

The organoleptic characteristics of cheddar cheese containing different concentrations of BPEO were assessed according to the ISO standards as follows: seven panelists were chosen from the Department of Food Hygiene and Quality control, University of Tehran (ISO 8586-1:1993). All samples were assessed using a 9-point scale as 9 indicated like extremely, 1 indicated dislike extremely, and 5 for neither like nor dislike as an acceptability point (16).

Statistical Analysis

The obtained data were analyzed using SPSS software (version 19.0 for Windows), and statistical significance (significance level was considered $p < 0.05$) among parameters in each sampling day was assessed by one-way analysis of variance (ANOVA) and followed by Tukey test. Trends of changes in microbial load and biogenic amines level in each treatment in different days were evaluated using repeated measure test.

Table 1. Composition of *Bunium persicum* Boiss. Essential oil identified by gas chromatography-mass spectrometry (GC–MS)

NO	Component	Retention index*	Quantity
1-	phelanderen	805	0.27
2-	α- Pinene	838	0.74
3-	Sabinene	1006	0.75
4-	β -Pinene	1003	1.32
5-	β -Myrcene	1076	0.56
6-	σ -Cymene	1249	6.11
7-	Limonene	1269	3.53
8-	1, 8 cineole	1282	0.1
9-	α-Terpinene	1407	11.00
10-	γ- Terpinene	1416	11.02
11-	α- Terpineolene	1534	0.38
12-	Trans Sabinene Hydrate	1618	0.14
13-	Linalool	1608	0.1
14-	Terpinene-4-ol	2035	0.86
15-	Thymol	2081	0.1
16-	3- Cyclopentyl	2116	2.2
17-	25- Cyclopentane- 1- N		
18-	Cuminaldehyde	2251	22.03
19-	cuminyl acetate	1408	5.11
20-	phelanderal	2513	0.17
21-	α- Thujene	2565	11.66
22-	1- Phenyl 1- Butanol	2592	20.72
23-	1, 4- Cyclohexadine 1-	1272	0.1
24-	26- Methanol		
	Total		
			98.97

*Retention index on DB5 column.

Results

Composition of *Bunium persicum* Boiss. EO

The compositions of different components of EO are displayed in Table 1. The results of Gas chromatography-mass spectrometry (GC–MS) analysis revealed 21 specified components comprising 98.97% of the EO. Based on the results, Cuminaldehyde (22.03%), α-terpinene (11. 13%), γ- terpinene (11.37%) and 1- Phenyl

1- Butanol (20.72%) were the main components in the EO, and other important compounds were α- Thujene (11.66%), σ -cymene (6.11%), cuminyl acetate (5.11%) and limonene (3.53%).

Table 2. Effect of *Bunium persicum* Boiss essential oils (EO) on histamine and tyramine contents (Mean ± SD) in Cheddar cheese during ripening

Components (mg/ kg)	EO concentrations (%(v/v))	Days of analysis 0	15	30	45	60	90
Histamine (mg/ kg)	0	6.31 ±0.94[a]	19.13 ± 2.27[a]	28.26 ± 2.19[a]	33.30 ± 1.63[a]	37.86±1.62[a]	43.18±2.74[a]
	0.05	5.93 ±0.68[a]	19.20 ± 1.45[a]	27.53 ± 2.40[a]	32.60 ± 0.78[a]	37.00±1.60[a]	41.00±1.10[a]
	0.1	4.66 ±1.45[a]	15.90 ± 1.21[b]	22.76 ± 0.70[b]	26.20 ± 1.55[b]	31.50±1.24[b]	35.75±0.93[b]
	0.2	4.40 ±0.75[a]	14.63 ± 1.41[b]	19.96 ± 1.55[b]	23.66 ± 1.60[bc]	28.46±1.20[c]	29.26±1.75[c]
	0.4	4.80 ±0.60[a]	11.90 ± 1.30[c]	17.60 ± 2.60[bc]	18.60 ± 2.02[d]	23.86±1.15[d]	22.20±1.11[d]
	P value	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Tyramine (mg/ kg)	0	8.90 ±0.40[a]	84.90 ± 1.87[a]	144.03 ± 2.57[a]	160.03±1.80[a]	162.16±1.77[a]	88.20±2.51[a]
	0.05	9.00 ±0.62[a]	83.43 ± 1.01[a]	140.73 ± 3.36[a]	159.30±2.78[a]	160.96±3.59[a]	171.60±2.33[b]
	0.1	8.33 ±0.50[a]	76.43 ± 1.33[b]	137.96 ± 2.07[a]	150.23±2.70[b]	151.63±2.17[b]	168.86±1.30[b]
	0.2	8.03 ±0.87[a]	68.33 ± 1.90[c]	121.16 ± 1.82[b]	128.20±2.62[c]	132.66±0.83[c]	133.88±1.51[c]
	0.4	7.46 ±0.51[a]	53.96 ± 1.32[d]	118.66 ± 1.98[b]	117.93±1.30[d]	122.30±1.80[d]	110.44±1.21[d]
	P value	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01

*P value, achieved from ANOVA. Means in columns with Non-common letters are significantly different (P <0.05).

Biogenic Amines

The amount of tyramine and histamine in cheddar cheese in untreated and EO treated samples are shown in Table 2. Tyramine contents in all groups have increased during the ripening period, especially during the first 30 days of this period. Tyramine contents increase less severely in the untreated samples than in cheese groups with 0.05% and 0.1% of EO. Tyramine quantity increased from 8.90 mg/kg on started day to 172.40 mg/kg on last day of ripening in the control group. Histamine amounts formation such as tyramine followed an identical model with a slightly lower slope at the primary aging period. In the last phase of maturity, the mean concentration of histamine was 6.31 mg/kg then reached to 41.16 mg/kg. At the end of the ripening time, the quantity of tyramine decrease at 0.1, 0.2, and 0.4% of BPEO concentrations to 10, 28, and 41%, respectively, compared with the untreated samples. Moreover, histamine decrease in the alike concentrations were 17, 32, and 48%. In 0.05% of BPEO samples, a significant reduction was not observed in tyramine and histamine amounts compared to the control samples.

Microbiological Analyses

Enterococci

The quantity of various microbial categories in Cheddar cheese in BPEO-treated and control group during ripening period is displayed in Table 3. In the untreated sample, the quantity of *enterococci* bacteria reached 8.36 log CFU/g at the end of ripening period, while on the first day, the amount of this bacteria was 3.44 log CFU/g. The maximum growth of *enterococci* was recognized in the first 30 days of the maturation period. This considerable growth could be because of the existence of lactose at the primary period of ripening, which reaches more than 3 log CFU/g. Then, *enterococci* loads are reduced compared to the first month of maturing phase; This result is perhaps relevant to reducing lactose quantity. The possible interpretation of inefficiency at a concentration of 0.05% *Bunium persicum* EO may be considered due to the high amount of fat and proteins in cheese samples since it is generally supposed that the great content of proteins or fat in foodstuffs have a protective function for microorganisms against antimicrobial agent like EOs (17). *Enterococci* had the highest correlation coefficient with BA manufacture because an almost similar increment pattern was observed among *enterococci* quantity and histamine and tyramine contents during the ripening period (correlation coefficient= 0.98 and 0.96 for histamine and tyramine, respectively).

Mesophilic lactobacilli

As displayed in Table 3, this kind of bacteria is the predominant group in the primary ripening period because of its existence in the starter culture. A sharp increase in mesophilic lactobacilli counts is diagnosed within the first 15 days. After that, the quantity of them reduced slowly and reach 7.20 log CFU/g on the final day of ripening. On the initial day of maturing, loads of mesophilic lactobacilli in 0.4% BPEO concentration were approximately 0.05 log CFU/g, lower than untreated samples but not significantly. In 0.1, 0.2, and 0.4% BPEO-treated groups compared to the untreated samples, on the 15th day, almost 05, 1.2, and 1.5 log CFU/g decrease of this bacteria were observed, respectively, (*P*<0.05). According to Wang *et al*. (2022), a certain correlation between the release of free amino acids and the action of microbiological peptidase (18). The quantity of mesophilic lactobacilli with 0.1% BPEO concentration during 15 days of ripening significantly decreased, while Tyramine and histamine contents did not reduce significantly (*P*<0.05).

Table 3. Effect of *Bunium persicum* Boiss. Essential oil (EO) on microbiological counts (Mean _ SD) in Cheddar cheese during the ripening period

Microbiological Viability (Log CFU/g cheese)	EO concentrations (% (v/v))	Days of analysis					
		0	15	30	45	60	90
Enterococci	0	3.44 ± 0.19^{a*}	5.07 ± 0.25^{a}	6.63 ± 0.18^{a}	7.24 ± 0.12^{a}	8.20 ± 0.14 ^{a}	8.36 ± 0.16 ^{a}
	0.05	3.40 ± 0.15^{a}	5.11 ± 0.11 ^{a}	6.57 ± 0.12^{a}	7.30 ± 0.14^{a}	8.18 ± 0.23^{a}	8.16 ± 0.13^{a}
	0.1	3.41 ± 0.25^{a}	4.87 ± 0.15^{a}	6.45 ± 0.21^{ab}	7.10 ± 0.21^{a}	8.08 ±0.18 ^{ab}	7.93 ±0.24 ^{ab}
	0.2	3.31 ± 0.11^{a}	4.77 ±0.16^{ab}	6.04 ± 0.29^{b}	6.73 ± 0.14^{b}	7.48 ± 0.34^{bc}	7.54 ± 0.14^{b}
	0.4	3.18 ± 0.15^{a}	4.42 ± 0.13^{b}	5.23 ± 0.17^{c}	6.20 ± 0.06^{c}	7.00 ± 0.35^{c}	7.10 ± 0.11^{c}
	P value	>0.05	<0.01	<0.001	<0.001	<0.01	<0.01
Mesophilic lactobacilli	0	5.48 ± 0.32^{a*}	8.54 ± 0.11^{a}	8.16 ± 0.21^{a}	7.83 ± 0.16^{a}	7.64 ± 0.07 ^{a}	7.20 ± 0.38 ^{a}
	0.05	5.34 ± 0.20^{a}	8.39 ± 0.24^{ab}	8.17 ± 0.09^{a}	7.93 ± 0.28^{a}	7.58 ± 0.26^{a}	7.37 ± 0.18^{ab}
	0.1	5.41 ± 0.33^{a}	7.98 ± 0.11^{bc}	7.95 ± 0.14^{ab}	7.57 ± 0.08^{a}	7.31 ±0.09 ^{ab}	6.94 ±0.05 ^{ab}
	0.2	5.22 ± 0.12^{a}	7.83 ± 0.23^{c}	7.59 ± 0.17^{b}	7.07 ± 0.22^{b}	6.83 ± 0.19^{bc}	6.63 ± 0.27^{b}
	0.4	5.02 ± 0.36^{a}	7.02 ± 0.22^{d}	7.08 ± 0.17^{c}	6.40 ± 0.18^{c}	6.43 ± 0.38^{d}	5.75 ± 0.20^{c}
	P value	>0.05	<0.001	<0.001	<0.001	<0.05	<0.001
Lactococci	0	3.48 ± 0.19^{a*}	4.55 ± 0.18^{a}	5.13 ± 0.21^{a}	6.21 ± 0.24^{a}	6.55 ± 0.16 ^{a}	7.35 ± 0.22 ^{a}
	0.05	3.36 ± 0.11^{a}	4.48 ± 0.12^{a}	5.08 ± 0.18^{a}	6.43 ± 0.45^{a}	6.35 ± 0.12^{a}	7.28 ± 0.21^{a}
	0.1	3.41 ± 0.15^{a}	4.30 ± 0.19^{a}	4.73 ± 0.17^{ab}	5.92 ± 0.18^{ab}	6.19 ±0.18 ^{ab}	7.05 ±0.04 ^{ab}
	0.2	3.44 ± 0.08^{a}	4.19 ±0.16^{ab}	4.55 ± 0.19^{bc}	5.56 ± 0.13^{bc}	5.92 ± 0.29^{bc}	6.84 ± 0.14^{b}
	0.4	3.20 ± 0.14^{a}	3.89 ± 0.14^{b}	4.20 ± 0.20^{c}	5.25 ± 0.16^{c}	5.72 ± 0.19^{c}	6.27 ± 0.15^{c}
	P value	>0.05	<0.01	<0.01	<0.01	<0.01	<0.001
Enterobacteriaceae	0	1.23 ± 0.09^{a}	1.78 ± 0.51^{a}	2.30 ± 0.17^{a}	2.73 ± 0.24^{a}	2.78 ± 0.15 ^{a}	2.96 ± 0.11 ^{a}
	0.05	1.15 ± 0.16^{a}	1.65 ± 0.16^{ab}	2.31 ± 0.06^{a}	2.66 ± 0.15^{ab}	2.84 ± 0.09^{ab}	2.76 ± 0.18^{a}
	0.1	1.28 ± 0.11^{a}	1.61 ± 0.17^{ab}	2.21 ± 0.16^{a}	2.56 ± 0.17^{ab}	2.76 ±0.14 ^{ab}	2.70 ±0.20 ^{a}
	0.2	1.19 ± 0.12^{a}	1.58 ±0.16^{ab}	1.91 ± 0.19^{ab}	2.26 ± 0.09^{bc}	2.48 ± 0.14^{b}	2.28 ± 0.12^{ab}
	0.4	1.15 ± 0.18^{a}	1.39 ± 0.08^{b}	1.73 ± 0.25^{b}	1.90 ± 0.13^{c}	2.11 ± 0.08^{c}	2.21 ± 0.09^{ab}
	P value	>0.05	<0.05	<0.01	<0.01	<0.001	<0.001
Aerobic mesophilic Bacteria	0	4.57 ± 0.21^{a}	5.33 ± 0.35^{a}	5.25 ± 0.28^{a}	6.96 ± 0.18^{a}	8.08 ± 0.14 ^{a}	7.64 ± 0.29 ^{a}
	0.05	4.51 ± 0.17^{a}	5.22 ± 0.13^{a}	5.12 ± 0.10^{ab}	6.77 ± 0.37^{a}	8.20 ± 0.28^{ab}	7.49 ± 0.35^{a}
	0.1	4.56 ± 0.40^{a}	5.13 ± 0.38^{ab}	5.08 ± 0.31^{ab}	6.56 ± 0.15^{ab}	8.04 ±0.07 ^{ab}	7.24 ±0.22 ^{ab}
	0.2	4.54 ± 0.11^{a}	4.58 ±0.17^{bc}	4.91 ± 0.09^{ab}	6.04 ± 0.19^{bc}	7.68 ± 0.07^{bc}	7.00 ± 0.07^{ab}
	0.4	4.34 ± 0.18^{a}	4.37 ± 0.12^{cd}	4.60 ± 0.15^{b}	5.54 ± 0.20^{cd}	7.47 ± 0.18^{c}	6.37 ± 0.16^{c}
	P value	>0.05	<0.05	<0.05	<0.001	<0.01	<0.01
Yeast	0	2.97 ± 0.18^{a}	3.31 ± 0.14^{a}	3.52 ± 0.17^{a}	4.85 ± 0.14^{a}	5.26 ± 0.18 ^{a}	6.29 ± 0.39 ^{a}
	0.05	2.96 ± 0.27^{a}	3.19 ± 0.11^{ab}	3.36 ± 0.06^{ab}	4.72 ± 0.11^{ab}	5.17 ± 0.12^{a}	6.07 ± 0.19^{a}
	0.1	2.81 ± 0.16^{a}	3.07 ± 0.11^{ab}	3.27 ± 0.23^{ab}	4.62 ± 0.16^{a}	5.09 ±0.15 ^{a}	5.91 ±0.19 ^{ab}
	0.2	2.85 ± 0.18^{a}	2.92 ±0.20^{b}	3.20 ± 0.28^{ab}	3.86 ± 0.28^{b}	4.46 ± 0.21^{b}	5.47 ± 0.18^{b}
	0.4	2.89 ± 0.10^{a}	2.59 ± 0.14^{c}	2.95 ± 0.13^{b}	3.15 ± 0.09^{c}	3.74 ± 0.28^{c}	4.29 ± 0.15^{c}
	P value	>0.05	<0.01	<0.05	<0.001	<0.001	<0.001

*P value obtained from ANOVA is mentioned. Means in columns without common letters are significantly different (P < 0_05).

Lactococci

Loads of *lactococci* during the 90-day cheese ripening period increased from 3.48 log CFU/g to 7.42 log CFU/g in the control group. The quantity of *lactococci* is constantly increasing during the ripening period of the cheese in all samples. In 0.05, 0.1, 0.2, and 0.4% of the groups treated with BPEO, the count of *lactococci* significantly decreased at the end of aging by 0.07, 0.3, 0.51, and 1.08 log CFU/g compared to the untreated group.

Enterobacteriaceae

Enterobacteriaceae in the untreated group increased from 1.23 log CFU/g to 2.98 log CFU/g during the ripening period. After 90 days of ripening, loads of *Enterobacteriaceae* decreased by 0.28, 0.71, and 0.76 log CFU/g in concentrations of 0.1, 0.2, and 0.4% of BPEO compared to the control sample.

Aerobic mesophilic Bacteria

In the untreated sample, the primary counts of this bacteria were 4.59 log CFU/g, then increased up to 8.10 log CFU/g after 2 months of the maturation period. Aerobic mesophilic Bacteria reduced to 7.71 log CFU/g on the last day of ripening. In concentrations of 0.1, 0.2, and 0.4% of BPEO, loads of aerobic mesophilic 0.47, 0.71, and 1.34 log CFU/g, respectively, were remarkably decreased.

Yeast

Primary loads of these microorganisms were nearly 2.97 log CFU/g which reached 6.29 log CFU/g on the last day of maturing. In concentrations of 0.05, 0.1, 0.2, and 0.4% of BPEO-treated samples, 0.22, 0.38, 0.82, and 2.00 log CFU/g *yeast* cell decline was reported on the last day of aging.

Sensory Evaluation

The mean acceptability grades of Cheddar cheese containing BPEO and the control samples was displayed in Table 4. The cheese groups treated with BPEO at 0.1% and 0.2% were the most favorable. In general, the addition of BPEO in these two concentrations expressed improved the sensory properties and overall acceptance of cheddar cheese. All groups scored upper than the acceptability point.

Table 4. Mean scores for the acceptability of Cheddar cheese samples with different concentrations of *Bunium persicum* Boiss essential oils (EO)

Bunium persicum EO (% (v/v))	Mean scores ± SD
0	7.08 ± 0.15[a*]
0.05	6.79 ± 0.31[a]
0.1	7.23 ± 0.26[ab]
0.2	8.14 ± 0.19[c]
0.4	7.67 ± 0.45[b]

* Means in columns with Non-common letters are significantly different ($p < 0.05$).

Discussion

Biogenic amines

Biogenic amines contents are very diverse and several agents like quality and kind of milk, kind of starter culture, kind of cheese, temperature and duration of ripening, production process, and chemical properties make it hard and complicated to compare biogenic amines contents in various studies (19). According to the findings, increasing the BPEO concentration cause decreased histamine and tyramine content. Histamine is the most prevalent agent of foodborne intoxication related to biogenic amines formation, which attracts public consideration to food hygiene researches. Although the histamine amount in the untreated samples is fewer than the suggested toxicological range (100 mg/kg) (20), these quantities may be riskier in some people such as allergic consumers, Monoamine oxidase inhibitors consuming patients, and individuals that eat more cheese. Several studies have shown that *Bunium persicum* has antimicrobial effects (21,22), which could cause a decrease in the count of microorganisms and production of biogenic amines due to their decarboxylase positivity. It has been proposed that the antimicrobial activity of most EO is related to oxygenated monoterpenes and sesquiterpenes (23). BPEO has a high quantity of Cuminaldehyde, α-Terpinene, and γ- Terpinene and possesses high antimicrobial properties. Considering that in the present study, at a concentration of 0.4% of BPEO, the content of the biogenic amine produced in cheese is reduced by approximately 45%, so it can be concluded that the production of biogenic amines has a pattern dependent on the concentration of essential oil. Tyramine decreased in BPEO-treated samples, However, it was upper than the reported result limit (100 mg/kg) by Ruiz-Capillas and Herrero, (2019) (20). On the other hand, higher concentrations of 0.4% BPEO showed adverse effects on organoleptic attributes. Hence, it is suggested that the simultaneous application of BPEO with other techniques or other essential oils to control the production of biogenic amines.

Microbiological Analyses

Enterococci

There are several sources for this bacteria; this is complicated to determine the exact ones. *Enterococci* are described as the main microorganisms in hard and semi-hard cheese because of the hygienic situation of cheese manufacture, milk contamination and their resistance to heat and salt (24). Tyramine-making microorganisms commonly existed in the *enterococci*, LAB, and *Enterobacteriaceae* groups, as expressed by Martuscelli *et al*. (2005) (25). Though *enterococci* are a subgroup of LAB, these bacteria are considered as BAs-making. Some *enterococci* play a beneficial role during the ripening period of cheese, such as lipolysis, proteolysis, citrate breakdown, probiotic function, and forming bacteriocins, while some others are relevant to cause infections in humans, and they are suspected of being a tyramine maker in cheese (26).

Mesophilic Lactobacilli

The pattern of mesophilic lactobacilli numbers changes stated by Marino *et al*. (2008) during the ripening period was different, which increased up to 7.8 log CFU/g at the end of maturing, and it was in agreement with our findings (27). The reduction of lactose at primary maturing periods may be the principal proof for lactobacilli because they are saccharolytic. The possible interpretation may be over the cellule lysis, and fewer BAs production may have happened, however increasing free amino acid and releasing aminopeptidase facilitated BAs' formation. In higher concentrations of BPEO (0.2 and 0.4%), the effects of EO intercept BAs formation. The count of mesophilic lactobacilli increased significantly in the first 15 days of the maturation period, but this kind of bacteria started to decline, unlike histamine and tyramine, after BAs production. Therefore, it is predicted that mesophilic lactobacilli are involved in the formation of BAs, especially during the first stage of maturation. Schenler *et al*. (1997) reported that *Enterococci* and *Enterobacteriaceae* are the producers of tyramine in cheese, while *lactobacilli* are not considered important in this regard (28). Starter bacteria are involved in protein breakdown, and nonstarter lactic acid bacteria (NSLAB) are effective in peptidolysis and secretion of free amino acids (29). Marino *et al*. (2000) mentioned that this bacteria must be present in large amounts (10^7 CFU/g) in cheese over a long time (at least 180 days) in order to make large amounts of tyramine and histamine (30). Therefore, it can be concluded that mesophilic lactobacilli do not have a significant effect in forming BAs but can provide the conditions for their making.

Lactococci

High levels of *lactococci* on the first days of the maturation period could be indicated by poor hygiene and heat resistance of pasteurization. Salminen and Wright, (2004) reported that several peptidases had been recognized in *lactococci* capable of degrading milk casein (31). Barbieri *et al*. (2019), reported that *lactococci* contained several strains that have been introduced as BA makers (32).

Enterobacteriaceae

Enterobacteriaceae had the most negligible reduction compared to other microorganisms studied. This result could be because of the hydrophilicity of the impermeable outer membrane to the lipophilic component, which makes gram-negative bacteria more resistant than gram-positive bacteria (29). *Enterobacteriaceae* have low heat resistance and are destroyed by pasteurization, but bad manufacturing practices could contaminate milk and cheese (33). The main and major manufactures of BA in cheese are *Enterobacteriaceae* and *Enterococci* (34). Hence, the high level of BAs in cheese could indicate poor hygienic milk handling and quality.

Yeasts

Yeasts were the most sensitive microbial population to BPEO. Our result is in accordance with the results of Khorshidian *et al*. (2018) (17). In this study, a high population of yeast was identified, which could be due to features like as high tolerance to low pH, water depletion, growth potential at low storage temperature, and high salt concentration, which causes *yeast* survival and growth in ripening cheese (29). Various yeast species like *Debaryomyces hansenii* and *Yarrowiali polytica* isolated from cheese have proved potential for BAs production like histamine and tyramine, respectively (35).

Conclusion

In general, BA-production capacity is an important feature among different microbial species. Hence, that it is better to use specific methods to detect BAs-producing micro-organisms. BPEO remarkably decreased BAs quantity and microbial population compared to the untreated sample. The use of essential oil is a practical method to reduce the BAs content that could be used in various kind of cheeses. It is also recommended that utilize BPEO alone or in combination with other natural green preservatives to reduce the formation in cheese.

Conflict of Interest

The authors declare no conflict of interest.

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