Food & Health 2023, 6(2): 37-42

Food & Health Journal

Journal homepage: fh.srbiau.ac.ir

A primary comparison for major biogenic amines variation during preserving red meat cuts in room temperature, refrigerator, and frozen condition

Afshin Motaghifar [1], Behrouz Akbari-Adergani [2*], Noordahr Rokney [1], Abbasali Motallebi Moghanjoughi [1]

[1] Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Water Safety Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

ARTICLE INFO

Original Article

Article history:
Received 17 March 2023
Revised 28 May 2023
Accepted 03 June 2023
Available online 20 June 2023

Keywords:
Biogenic amines
Cadaverine
Putrescine
Meat putrefaction
Storage temperature

ABSTRACT

Unlike bacterial and viral pathogens, certain naturally occurring substances like biogenic amines (BAs) can also cause food poisoning. BAs are associated with various health problems, including allergic reactions and even cancer development. This study aimed to quantify the levels of putrescine and cadaverine, two major BAs, in red meat using high-performance liquid chromatography. The method can monitor changes in the concentrations of these dominant BAs. The results showed that the increase in cadaverine and putrescine levels under three temperature conditions [room temperature (25 °C) for 4 days, refrigeration (2-4 °C) for 6 days, and freezing at -18 °C for one year] was significantly different. While putrescine levels rose at room temperature, cadaverine amounts increased more noticeably under refrigeration and freezing. BA formation also varied with different meat cuts used in processed meats. Statistical analysis revealed that meat storage methods, even under proper cooling, can lead to BA production depending on temperature and the presence or absence of microorganisms on the meat. In conclusion, controlling storage temperature is essential to minimize potential BA accumulation in red meats.

1. Introduction

Meat is an essential source of protein that plays a crucial role in cell structure. Even though plant-based foods provide some protein, meat helps compensate for the lack of essential amino acids in plant-based diets. Many researchers have focused on microbial changes and cross-contamination in the past decade. One of the challenging and influential factors in food safety has been the presence of harmful residues in nutrients, plant pesticides, and allergens, which many studies have reported. The prospect of this topic is food preparation without potentially dangerous agents that make it appropriate for consumers to eat, from farm to fork or table, during processing. Despite this fact and the noticed increase in food safety, one out of every 10 people in the community suffers from food poisoning due to poisoning with biogenic amines (BAs) (1). The formation of BAs such as histamine, tyramine, cadaverine, putrescine, spermidine, and spermine at doses beyond the threshold can cause numerous diseases, including headaches, palpitations, nausea, diarrhea, allergies, and hypersensitization reactions (2). BAs are generally divided into two categories based on the origin of endogenous and exogenous amino acids. The first category consists of tissue-made neurotransmitters, including catechol-amines such as dopamine, epinephrine, and norepinephrine, indol-amines such as serotonin, melatonin, and 5-hydroxytryptamine and ultimately histamine. These factors are present in fish and fruits and also play a role as neurotransmitters (3, 4). The second category of BAs is formed from bacterial decarboxylase enzymes, created and named after their initial amino acids, formed in raw and processed foods (4). These exogenous amino acids include histamine, tryptamine, putrescine, cadaverine, 2- phenylethyl amine, tyramine, spermine, and spermidine (5). Despite the occurrence of food poisoning throughout a person's life, estimating the number of diseases and deaths caused by food poisoning is complex and unclear. Therefore, in the formation of BAs, three factors are mainly effective. These

*Corresponding author: Water Safety Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran.

E-mail address: analystchemist@yahoo.com (Behrouz Akbari-adergani).

factors are firstly associated with raw materials, including pH, chemical composition, especially access of microbial agents to protein, especially amino acids; secondly, associated with storage and processing including the storage conditions of raw, dry, cooked or fermented materials, hygienic conditions; and finally it depends on conditions and methods of packaging, temperature and period of storage and cross-contamination with microbial agents, including gram-positive or gram-negative bacteria possessing amino-oxidase enzymes (5- 7). BAs are produced by the decarboxylation of their precursor amino acids, which are found in foods and are influenced by amino acid decarboxylase enzymes of microbial agents by removing the carboxyl group and releasing carbon dioxide (8). The poisoning effects of these BAs mainly depend on a variety of factors, including individual sensitivity, the dominant BAs, the use of monoamine oxidase inhibitors (MAOIs), alcohol consumption, and interactions with the amino oxidase enzyme system that produces these BAs (9). Among the factors contributing to the formation of BAs is the processing and preparation of food products from these materials, including the preparation of sausages and minced products and using spices and flavors, which can increase the speed of BA formation. It has been reported that even the type of spice was effective in BA formation (10). Different methods have been designed to control BA production, including herbal extracts, particular starter cultures, modified atmosphere packaging (MAP), or even consumption as fresh food (11, 12). The only BAs naturally found in food with meat origin are spermine and spermidine, and the effect of microbial agents mainly causes the other BAs. The amount of BAs is considered a quality index that was first calculated and reported for seafood and is now presented for all food groups, as well as the biogenic amine index (13). In red meat, BAs should not exceed 5 mg/kg, and the harmful amount of this index for the body is 5 to 20 mg/kg. The amount of meat BAs in poor hygienic conditions and inadequate quality is 20 to 50 mg/kg, and in rotten meat is more than 50 mg/kg (14). It remains unclear how much of these two BAs can be toxic or lead to allergic and carcinogenic complications. Still, one study has shown that the two BAs indirectly induce allergies, and continuous consumption amounts of BAs, especially with nitrite salts, induce cancer (15). The main purpose of this study was to determine the changes in these BA indexes, especially putrescine and cadaverine, and the initial comparison of the main BA changes during the storage of meat cuts at room temperature, in the refrigerator and when frozen using red meat prepared from the cow.

2. Materials and methods

2.1. Sample preparation

Meat samples were obtained from an industrial slaughterhouse located in western Tehran, Iran. Samples were collected from four cuts: neck, sirloin, rib set, and flank. These cuts are predominantly used for meat processing and the production of meat by-products. After slaughter and cooling at the slaughterhouse, the samples were transported to the laboratory for analysis.

2.2. Extraction of meat samples

Initially, a sample of the meat was tested for the determination of BAs content, which first minced the meat samples and combined 5 grams with 20 ml of 0.4 molar $HClO_4$ and homogenized for 5 minutes at 2500 rpm. The sedimentary solid part was re-extracted twice. After combining both ready-made solutions with a volume of 40 ml, it was re-dissolved with 50 ml of 0.4 molar $HClO_4$ and reached 90 ml. One ml of acidic extract prepared in this method was used as a standard solution and measured by high-performance liquid chromatography. The rest of the meat was divided into three parts and packaged and kept in 50 grams packages and put in three temperature conditions including room temperature (25 °C) for 4 days, refrigerator conditions (2-4 degrees Celsius) for 6 days, and freezing (-18 °C) for one year) to maintain the above method at specified periodic times and based on the desired numbers on specific days for testing and preparing the extract (Table 1).

Table1. Storage conditions for different types of meat cuts.

Temperature (°C)	Time (Day)											
Ambient 25°C	1	2	3									
Fridge 4°C	1	2	3	4	5	6						
Freeze -18°C	30	60	90	120	150	180	210	240	270	300	330	360

2.3. HPLC analysis

After standard preparation, six standard solutions with concentrations ranging from 5 to 250 micrograms were prepared by mixing the two BAs (cadaverine and putrescine). Each standard solution (1 mL) was used to create a calibration curve. The standards were mixed with saturated sodium chloride and sodium bicarbonate solution at 70°C for 10 minutes. The mixtures were then combined with acetonitrile and distilled. Separate calibration curves were generated for each BA by measuring the standards and plotting concentration versus peak area (Fig. 1). Next, for preparation of meat extracts, minced meat (1 mg) was mixed with 200 µL of 2 M sodium hydroxide and buffered with 300 µM saturated sodium bicarbonate. Dansyl chloride solution (2 mL) was added to react with the BAs and heated in a water bath at 60°C for 10 minutes. Ammonium hydroxide (100 µL, 28%) was added and mixed for 15 minutes to stop the dansylation reaction and remove excess dansyl chloride. The distilled solution was diluted and stabilized by adding 5 mL acetonitrile before filtration and injection into the HPLC for analysis. The prepared samples were coded and analyzed in two blinded tests. Chromatography was performed using an HPLC system with UV detection at 254 nm. A C18 reverse phase column (ODS2-C18, 5 µm, 4.6 x 250 mm) was used at 40°C. A gradient elution method was employed using two

mobile phases: A) 5% water, 95% acetonitrile, and B) 25% water, 75% acetonitrile. The gradient composition over time was 45% A at 5 minutes, 45% A at 30 minutes, 60% A at 50 minutes, 80% A at 55 minutes, and 80% A at 60 minutes. The flow rate was 0.1 mL/min, and the injection volume was 5 µL.

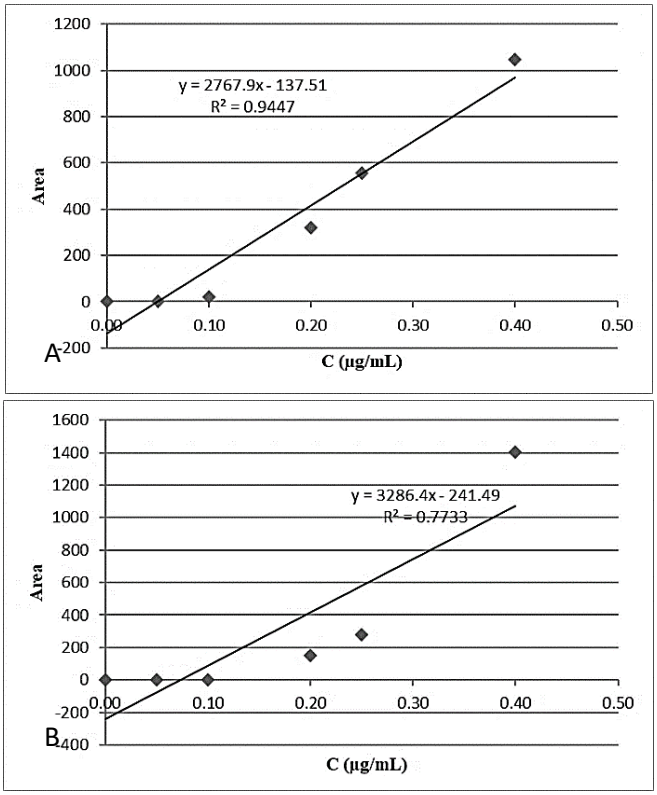

Fig. 1. Standards of putrescine (A) and cadaverine (B).

2.4. Microbial analysis

Microbial analysis was performed on meat samples stored at room temperature (25°C) and refrigerator temperature (2-4°C). For each temperature, 10 grams of meat was homogenized in a sterile blender (Homogenizer IKA-WERKG, RET basic C) with 90 mL of normal saline for 10 minutes at 3000 rpm. Serial dilutions from 10-1 to 10-10 were then prepared by transferring 1 mL of the homogenized sample to 9 mL of sterile distilled water. For total viable count analysis, aliquots from appropriate dilutions were plated onto nutrient agar and incubated at 37°C for 24 hours. Selective and differential media were also used. MacConkey agar was used to isolate coliforms, such as *Escherichia coli*, with incubation at 37°C for 24 hours. In addition, Mannitol Salt agar was used to isolate *Staphylococcus aureus* based on mannitol fermentation, followed by incubation at 37°C for 24 hours.

2.5. Statistical analysis

Microbial load (total viable count, TVC) and total volatile basic nitrogen (TVB-N) were evaluated along with putrescine and cadaverine levels using HPLC measurements. Putrescine and cadaverine concentrations in meat cuts stored under different temperature conditions (room temperature, refrigeration, freezing) were statistically compared using Kruskal-Wallis and one-way ANOVA tests in SPSS. The results showed that increases in putrescine levels based on these tests were not statistically significant ($p>0.05$), whereas increases in cadaverine levels between storage conditions were significant ($p<0.05$). Mean putrescine and cadaverine levels were calculated for each storage temperature. Graphs were also generated to visualize differences over time and storage methods. TVC and TVB-N analysis validated trends observed for the bacterial amines measured by HPLC. This corroborated quality evaluations, especially for frozen meat samples.

3. Results

3.1. Change of BAs by temperature

The data from this study demonstrated that temperature changes influenced the amounts of the two biogenic amines (BAs) during their formation. Specifically, the amounts showed distinct patterns at room, refrigerator, and freezing temperatures. At room temperature (25°C), the average amounts of the putrescine and cadaverine in the rib, neck, sirloin, and flank samples increased over 3-4 days. For example, in the rib samples, the amounts of putrescine increased from 0.371974 to 0.324273 mg/kg over 3 days. In the refrigerator (2-4°C), the amounts in all cuts increased over 6 days. For instance, in the neck samples, the amounts of putrescine increased from 0.72657 to 1.293275 mg/kg. However, in frozen samples, the amounts did not show a consistent increasing or decreasing pattern over time. In the flank samples, the amounts of putrescine changed from 0.004235 to 0.229875 mg/kg. The HPLC results indicated that putrescine and cadaverine amounts consistently increased with storage time at room and refrigerator temperatures. But at freezing temperatures, the changes were more variable. This suggests that microbial activity and BA production continue at higher temperatures but are inhibited at freezing temperatures. Overall, the data demonstrate that temperature is a crucial factor influencing biogenic amine formation in beef samples (Tables 2 and 3).

3.2. Total bacterial count of red meat at 25 °C

The results obtained from bacterial count showed that the levels of both BAs in neck cuts stored at room temperature increased over three days, with the total count rising from 4.64 ± 0.21 to 8.64 ± 0.32 log CFU/g. Similarly, the levels of both BAs increased simultaneously in neck cuts, causing the total microbial load to rise from 4.78 ± 0.18 to 8.78 ± 0.25 log CFU/g. For sirloin cuts stored at room temperature, the total microbial load increased by the third day from 4.23 ± 0.16 to 8.52 ± 0.28 log CFU/g. Finally, in flank cuts stored at room temperature, the total microbial load rose from 4.18 ± 0.19 to 8.14 ± 0.24 log CFU/g by the third day.

3.3. Total bacterial count of red meat in the refrigerator

The total bacterial count of rib cuts stored at refrigerated temperature increased from 4.74 ± 0.21 to 6.36 ± 0.28 log CFU/g due to bacterial growth. Neck samples stored at refrigeration temperature showed an increase in total bacterial counts over six days. On the first day, the count was 4.73 ± 0.19 log CFU/g, reaching 4.96 ± 0.16 log CFU/g on the second day. On the third, fourth, and fifth days, the bacterial count increased to 5.55 ± 0.17, 6.08 ± 0.15, and 6.41 ± 0.13 log CFU/g, respectively. By the sixth day, the total count had risen to 6.64 ± 0.11 log CFU/g. For sirloin cuts stored at refrigerated temperature, the microbial count increased from 5.05 ± 0.14 to 7.08 ± 0.26 log CFU/g by the sixth day. Similarly, in flank cuts refrigerated, the total microbial count rose from 4.52 ± 0.18 log CFU/g on the first day to 6.28 ± 0.24 log CFU/g by the third day. By the sixth day, the count reached 6.33 ± 0.22 log CFU/g.

Table 2. Putrescine levels (mg/kg fresh weight) in various meat cuts stored at different temperatures and time periods.

Rib set			Neck			Sirloin			Flank		
3 days (room)	**6 days (ref.)**	**12 months (freez.)**	**3 days (room)**	**6 days (ref.)**	**12 months (freez.)**	**3 days (room)**	**6 days (ref.)**	**12 months (freez.)**	**3 days (room)**	**6 days (ref.)**	**12 months (freez.)**
0.371974	0.189	**0.00**	0.114125	0.072657	**0.00**	0.089561	0.123756	**0.00**	0.086863	0.007201	**0.00**
0.317398	0.31908	0.027381	0.126234	0.132063	0.015623	0.083127	0.197123	0	0.082682	0.136875	0.004235
0.324273	0.337346	0.05652	0.138528	0.129653	0.028435	0.070135	0.176272	0	0.165852	0.170167	0.005234
	0.453673	0.09765		0.126948	0.029356		0.542396	0		0.216371	0.006159
	0.503711	0.08267		1.068327	0.032536		0.516759	0		0.223844	0.008235
	0.528004	0.10232		1.293275	0.125629		0.530464	0.132821		0.236287	0.005184
		0.16823			0.128345			0.138834			0.128138
		0.102158			0.129333			0.131217			0.146002
		0.10093			0.117038			0.133599			0.153405
		0.198659			0.122314			0.132077			0.190724
		0.191363			0.118232			0.110201			0.227185
		0.182165			0.125234			0.202321			0.229875

Table 3. Cadaverine levels in various meat cuts stored at different temperatures and time periods.

Rib set			Neck			Sirloin			Flank		
3 days (room)	**6 days (ref.)**	**12 months (freez.)**	**3 days (room)**	**6 days (ref.)**	**12 months (freez.)**	**3 days (room)**	**6 days (ref.)**	**12 months (freez.)**	**3 days (room)**	**6 days (ref.)**	**12 months (freez.)**
0.091036	0.00008	**0**	0.028103	0.125079	**0**	0.008543	0.113867	**0**	0.001416	0.058212	**0**
0.097582	0.001682	0.702518	0.038215	0.139922	0.802321	0.016682	0.112765	0.042215	0.004152	0.074649	0.856123
0.191334	0.024299	0.771911	0.056123	0.148799	0.806918	0.024413	0.129823	0.053208	0.006861	0.082685	0.885042
	0.480061	0.783942		0.174585	0.811123		0.131116	0.067209		0.095211	0.837234
	0.55161	0.803257		0.252338	0.824325		1.172955	0.073299		0.623359	0.884231
	0.599837	0.884165		0.981365	0.874266		1.203026	0.879231		0.64165	0.848247
		0.826165			0.882968			0.878718			0.846554
		0.764216			0.773024			0.917318			0.921175
		0.745865			0.781666			0.879213			0.931422
		0.744216			0.918545			0.846531			0.939485
		0.720167			0.788177			0.855201			0.942862
		0.700691			0.873672			0.882435			0.950959

3.4. Total bacterial count of red meat in freezing condition

The results of the total bacterial count at the freezing temperature in frozen meat samples at the cold of -18 °C, after 12 months, showed a significant difference in the content of the putrescine and cadaverine as the main BAs, and it indicated changes in chemical properties of meat. Its importance is that the values of both BAs had increased dramatically, while unlike the specimens tested at two temperatures, room and refrigerator, the production of two BAs at freezing temperature was the same as in the refrigerator. The statistical results of the two putrescine and cadaverine were obtained in terms of the statistics ratio of f-value 0.56297 and 5.62958 and the P-value 0.642592 and 0.002574, respectively.

4. Discussion

The results showed that putrescine and cadaverine levels in the frozen rib cut samples did not change over the first 5 months. However, in other sample parts (room, refrigerators, freezing temperatures), both BAs increased based on peak area from the first month of storage. Changes were more prominent in other meat parts (neck, flank, sirloin) stored under the same conditions. After five months of freezing storage, TVBN and BA levels reached 7.23 and 36.52 mg/gr, respectively. In a separate study, cadaverine and putrescine levels increased by 1121.48 and 118.98 mg/kg in meat stored at -18°C for 12 months without any change (16). Rapid freezing after packaging likely decreased muscle metabolism and water content in meat, which is consistent with other reports (15). This explains the observed daily logarithmic decrease in microbes in frozen samples, making microbial testing insignificant. The study was limited by some variability in storage conditions like temperature maintenance

and freezing speeds, which can impact meat quality, as reported elsewhere (17). Despite freezing initially inhibiting microbial and enzymatic activity, amino acid precursors of BAs still increased after 150 days, likely due to residual enzymatic activity from cells and bacteria. Pre-freezing and residual enzymatic and autolytic activity during frozen storage can drive BA formation through bacterial/cellular enzyme and autolysis pathways observed in multiple studies despite reduced microbial activity (18). The measured putrescine levels were generally higher than cadaverine at room temperature, indicating bacterial amino-decarboxylase enzyme activity as the primary driver of production through arginine catabolism. However, some putrescine is also converted to spermine and spermidine (19), resulting in relatively higher cadaverine levels over storage time under refrigeration and freezing. Cellular enzymes and autolysis play a more significant role in BA formation at lower temperatures, reducing microbial loads more effectively. Less putrescine conversion allows cadaverine levels from lysine degradation to increase, supported by slightly higher baseline lysine levels in meat (15). Since putrescine and cadaverine are end products of arginine and lysine breakdown, putrescine production may also derive from other precursor amino acids like glutamine and ornithine (19). The amounts of putrescine and cadaverine vary in meats and products, making their quantification important for health assessment. Unlike histamine in fish, these carcinogenic BAs are the primary markers examined in meat due to linked respiratory, gastrointestinal, cardiovascular and neurological complications (20). When combined with nitrite during processing, BAs also facilitate the formation of nitrosamines that promote cancer development. Therefore, controlling BA levels in the food chain is crucial, especially for sensitive groups. Recently, techniques to prevent BA formation during meat processing and storage have been explored to ensure consumer safety. However, maintaining overall hygienic conditions through proper HACCP implementation and raw material freshness remains the most effective approach for reduction. BAs are heat-resistant and unaffected by smoking or freezing alone. Their elimination during processing is impractical, so monitoring and measurement during production are critical. Adopting multiple complementary interventions like starter cultures, enzymes, hydrostatic pressure, atmospheric modifications and additives, optimal temperature control, and shelf life management of products can help minimize BA risks. Using high-quality raw materials suitable for the method also supports BA reduction in meats and meat products.

5. Conclusion

This study examined changes in biogenic amine (BA) levels, specifically putrescine and cadaverine, under different storage temperatures. BA formation was influenced by temperature, with putrescine levels higher at room temperature and cadaverine dominating under refrigeration or freezing. BA production also varied between meat cuts used in processed products. Proper temperature control through refrigeration or freezing can help reduce BA formation. Assessing BA levels provides a valuable quality index for red meats and insight into the impacts of various storage and packaging methods. Higher microbial loads at room and refrigerator temperatures increased BAs more than freezing alone. While freezing alone may not induce organoleptic changes or affect TVN within standard limits, increasing BAs indicates degradation and safety risks even without microbial growth. Daily microbial reductions at freezing cannot dismiss BA testing for fraud detection and guaranteeing meat safety. Different preservation techniques like herb extracts, starter cultures, or MAP require continuous BA monitoring to ensure consumer health is not compromised. Putrescine and cadaverine behaved oppositely based on temperature and precursor amino acid availability. However, BA analysis serves as a critical threshold for quality evaluation and confirms meat safety, especially for vulnerable groups. Proper temperature control and hygienic processing remain essential to minimizing BA risk in meat products.

Acknowledgments

This research was conducted as part of a Doctor of Veterinary Science thesis in the Department of Veterinary Medicine at the Islamic Azad University Science and Research Branch.

References

1. Franz CMA, den Besten HMW, Böhnlein C, Gareis M, Zwietering MH, Fusco V. Microbial food safety in the 21st century: Emerging challenges and foodborne pathogenic bacteria. *Trends in Food Science & Technology*. 2018;81:155-8.
2. Alvarez MA, Moreno-Arribas MV. The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends in Food Science & Technology*. 2014;39:146-55.
3. Ozcelik F, Temel MC, Ozcelik İK, Kale E. The role of biogenic amines in nutrition toxicology. *International Journal of Nutrition*. 2020;5:21-9.
4. Shiron M, Esposito L, D'Onoferio F, Visciano P, Martuscelli M, Mastrocola D, Paparella A. Biogenic amines in meat and meat products: A review of the science and future perspectives. *Foods*. 2022;11(6):788.
5. Majer-Baranyi K, Székács A, Adányi A. Application of Electrochemical Biosensors for Determination of Food Spoilage. Foods. 2023;13(4):456.
6. Ly D, Mayrhofer S, Schmidt JM, Zitz U, Domig KJ. Biogenic amine contents and microbial characteristics of Cambodian fermented foods. *Foods*. 2020;9:198.
7. Ruiz-Capillas C, Herrero AM. Impact of biogenic amines on food quality and safety. Foods. 2019;8:62-78.
8. Tolulope Omidiran A, Damilola Jenfa M. Occurrence of biogenic amines in fermented foods. Elsevier. 2023;539-48.
9. Ruiz-Capillas C, Herrero AM. Impact of biogenic amines on food quality and safety. *Foods*. 2019;8(2):62.
10. Tabanelli G. Biogenic amines and food quality: Emerging challenges and public health concerns. *Foods*. 2020;9(7):859.
11. Martuscelli M, Serio A, Capezio O, Mastrocola D. Safety, quality and analytical authentication of ḥalāl meat products, with particular emphasis on salami: A review. *Foods*. 2020;9(8):1111.
12. Wójcik W, Łukasiewicz-Mierzejewska M, Damaziak K, Bie D. Biogenic amines in poultry meat and poultry products: formation, appearance, and methods of reduction. *Journal of the Science of Food and Agriculture*. 2020;101(7):2634-40.
13. Shao X, Xu B, Chen C, Li P, Lu H. The function and mechanism of lactic acid bacteria in the reduction of toxic substances in food: a review. *Food Science & Nutrition*. 2022;62:5950-63.

14. Qu Y, Wang J, Liu Z, Wang X, Zhou H. Effect of storage temperature and time on biogenic amines in canned seafood. *Foods*. 2022;11(18):2743.
15. Nougalli Roselino M, Fonseca Maciel L, Sirocchi V, Caviglia M, Sagratini G, Vittori S,Pia Taranto M, Cardoso Umbelino Cavallini D. Presence of biogenic amines in food and their public health implications: a review. *Journal of Food Composition and Analysis*. 2021;94:103649.
16. Hernández-Jover T, Izquierdo-Pulido M, Veciana-Nogués MT, Vidal-Carou MC. Analysis of biogenic amines in probiotic and commercial salamis. *Journal of Agricultural and Food Chemistry*. 2020;44:3097-101.
17. Motaghifar A, Akbari-Adergani B, Rokney N, Mottalebi A. Evaluating red meat putrefaction in long term storage in freezing condition based on co-variation of major biogenic amines and Total Volatile Nitrogen. *Food Science and Technology*. 2020;41:123-8.
18. Dave D, Ghaly AE. Meat spoilage mechanisms and preservation techniques: a critical review. *American Journal of Agricultural and Biological Sciences*. 2011;6(4):486-510.
19. Buňková L, Buňka F, Mantlová G, Čablová A, Sedláček I, Švec P, et al. The effect of ripening and storage conditions on the distribution of tyramine, putrescine and cadaverine in Edam-cheese. *Food microbiology*. 2010;27(7):880-8.
20. Shirone M, Esposito L, D'Onofrio F, Visciano P, Martuscelli M, Mastrocola D, Paparella A. Biogenic Amines in meat and meat products: A review of the science and future prospective. *Foods*. 2022;11(6):788; 1-14.