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## Biogenic amines' residues in meat products with a reference to their microbial status

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### Abstract

**Background:** Meat products are widely recognized as substantial sources of protein derived from animals. Biogenic amines (BAs), naturally occurring toxins, are generated via the metabolism of specific amino acids by a vast array of microorganisms, including pathogenic and nonpathogenic strains.

**Aim:** The aim of this study was to ascertain the quantity of BAs produced in five meat products that are commercially available in Egypt. Additionally, the estimated daily BA intakes of the Egyptian populace as a result of consuming these animal products were computed. Additionally, a study was undertaken to investigate the relationship between total BAs (TBAs) and microbial counts, specifically total bacterial counts (TBCs), total psychrophilic counts (TPsC), total *Staphylococcus aureus* (TSC), and total Enterobacteriaceae count (TEC) as they pertained to the meat products under investigation.

**Methods:** One hundred samples of meat products (n = 20 for each) were selected at random from Egyptian markets. The collected samples included minced meat, luncheon, sausage, pasterma, and canned meat. The microbiological status and BA content of these samples were evaluated.

**Results:** Total BAs were calculated for the examined samples beef mince had the highest TBA content at  $918.22 \pm 21.3$  mg/Kg followed by sausage at  $575.1 \pm 12.8$  mg/Kg, luncheon at  $567.1 \pm 17.8$  mg/Kg, pasterma at  $417.0 \pm 31.8$  mg/Kg, and canned meat at  $242.8 \pm 21.8$  mg/Kg. The calculated estimated human daily intake (EDI) values for TBA ranged between 21.24 in canned meat to 80.34 in beef mince. It was determined that beef mince had the highest microbial contamination rates as indicated by the high TBC, TPsC, TSC, and TEC at  $5.69 \pm 0.4$ ,  $4.2 \pm 0.5$ ,  $2.4 \pm 0.2$ , and  $4.69 \pm 0.1$  log 10 cfu/g. Such counts were  $3.6 \pm 0.2$ ,  $2.4 \pm 0.2$ ,  $1.2 \pm 0.1$ , and  $4.3 \pm 0.2$  log 10 cfu/g in sausage,  $3.4 \pm 0.3$ ,  $2.2 \pm 0.1$ ,  $1.1 \pm 0.1$ , and  $4.0 \pm 0.1$  log 10 cfu/g in luncheon,  $2.5 \pm 0.1$ ,  $1.0 \pm 0.1$ ,  $1.4 \pm 0.08$ , and  $2.69 \pm 0.2$  log 10 cfu/g in pasterma; while none of the examined canned meat harbored microbial contamination.

**Conclusion:** This study indicated the presence of several BAs in meat products sold in Egypt. According to the EDI values of the examined BAs, the consumption of meat products by the Egyptian populace did not pose a risk. However, it is imperative that the handling, storage, distribution, and promotion of meat products conform to sanitary protocols.

**Keywords:** Meat products, Biogenic amines, Health risk assessment, Microbiological status.

### Introduction

Meat products, including mince, luncheon, sausage, pasterma, and canned meat are considered to be highly significant sources of animal-derived protein. Additionally, they contain substantial amounts of energy, bioactive peptides, and vitamins (Morshdy *et al.*, 2023). These products serve as significant sources of the trace elements zinc (Zn) and copper (Cu), both of which are critical for the proper functioning of bodily systems and physiological processes (Darwish *et al.*,

2014). In addition, compared to red meat, meat products have a distinct aroma and flavor, which contributes to their popularity among children (Elhelaly *et al.*, 2022; Morshdy *et al.*, 2022). On the other hand, throughout the production of these items, non-meat components and food additives, including olives, seasonings, and so forth, are introduced. Such additions may facilitate the microbial contamination of the finished products and could lead to the production of several toxic metabolites, such as biogenic amines (BAs).

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BAs, which are naturally occurring toxins, are produced by an extensive variety of microbes, including pathogenic and nonpathogenic strains, and through the metabolism of particular amino acids. In Marcobal *et al.* (2012) BAs were classified into 1) Monoamines, such as tyramine (TYM) and histamine (HIS), are produced through a unicomponent decarboxylation process involving their respective amino acid precursors, histidine and tyrosine. 2) Diamines, including cadaverine (CAD) that is generated through the decarboxylation of lysine. Putrescine (PUT) is another diamine that can be synthesized either directly or indirectly via a single-step decarboxylation of ornithine and agmatine (Wunderlichová *et al.*, 2014) after arginine hydrolysis. 3) Polyamines such as spermine (SPM) and spermidine (SPD), which are formed via the hydrolysis of arginine into ornithine and agmatine (Shah and Swiatlo, 2008). BAs are essential for numerous cellular processes, including cell division, and tissue repair, when present in physiological amounts (Galvano *et al.* 2012; Benkerroum 2016, Ma *et al.* 2020). Prolonged ingestion of these BAs is improbable to result in severe adverse effects, including hypertension, allergic reactions, neurological complications, or fatality (Medina *et al.*, 2003). In addition, specific BAs, including PUT and CAD, have been associated with the progression of gastric cancer due to the fact that they can be converted into N-nitrosoamines by microorganisms in the gastrointestinal tract, and such compounds are known for their carcinogenic potentials (Koutsoumanis *et al.*, 2010). As per the findings of the EFSA Panel on Biological Hazards (BIOHAZ) report (EFSA, 2011), intoxication incidents in Europe have been associated with the consumption of meat and fish containing elevated concentrations of BAs. The assessment of meat products for the presence of BAs is considered a reliable indicator of the quality and hygiene of such products (Koutsoumanis *et al.* 2010; Benkerroum, 2016). Information regarding the quantities of BAs present in the different varieties of meat products sold in Egypt and the proportion of these products that contribute to the estimated daily intake of BAs by the population is noticeably deficient. The formation of BAs in the food substrate indicates the progression of meat spoilage. Therefore, investigation of the microbial load of the food subject might reflect the formation scenario of BAs. However, the association between microbial counts and BA production has been less informed. Therefore, the main aim of the present research was to determine the amount of BAs generated in five meat product varieties that are widely sold in Egypt: beef mince, luncheon, sausage, pasterma, and canned meat. The daily estimated intakes of BAs by the Egyptian population due to the consumption of these meat products were also calculated. Furthermore, an investigation was conducted on the relationships between total BAs (TBAs) and microbial counts, including total bacterial counts (TBCs), total

psychrophilic counts (TPCs), total *Staphylococcus aureus* (TSC), and total enterobacteriaceae count (TEC) on the examined meat products.

## Materials and Methods

### *Samples collection*

A hundred meat product samples were selected at random ( $n = 20$  for each type of meat products) from local markets in Egypt. Minced meat, luncheon, sausage, pasterma, and canned meat were the meat product types collected. Following chilling, the collected samples were transported directly to the laboratory of the Meat Hygiene, Faculty of Veterinary Medicine, Zagazig University for microbiological analysis and determination of their BA contents.

### *Determination of BA concentrations*

Before analysis, 10 g and 100 ml of 10% trichloroacetic acid were used to homogenize each sample. The homogenates were subsequently extracted for an hour at 5,000 rpm and 4°C before being centrifuged for twenty minutes. The supernatants underwent filtration through Whatman filter No. 1. The filtrates were subsequently stored at 4°C until analysis. The quantitative estimation of BAs was conducted using an amino acid analyzer (HITACHI, Japan), as stated by Kononiuk and Karwowska (2019). Utilizing amine standards (Merck KGaA, Darmstadt, Germany), the concentrations of the tested BAs were determined. The BA concentrations were denoted in milligrams per kilogram of meat products.

### *Estimated daily intakes of BA*

To calculate Egypt's estimated human daily intake (EDI) of total BAs from meat product consumption, the subsequent formula was applied:

EDI is equal to  $C_i$  multiplied by FIR.

In Egypt, the meat products ingestion rate is denoted as FIR, while the concentration of BAs in meat products is denoted as  $C_i$ . FAO (2003) reported that the daily meat products ingestion rate (FIR) in Egypt is 87.5 g.

### *Total microbial counts in meat products*

TBC, TPSC, and total TSC counts were conducted in accordance with the APHA (2001) recommendations. In brief, 25 g of each meat product sample was mixed with 225 ml of sterile buffered peptone water 0.1% while the mixture was spun at 2,500 rpm for 2 minutes. Following the  $10^{-1}$  dilution of these homogenates, decimal dilutions were performed. In two sterile Petri dishes, one milliliter (ml) of each dilution was combined with fifteen milliliters of nutrient agar (Oxoid) for TBC and TPSC, Baird Parker media for TSC, and violet red bile glucose agar (VRBG) for TEC. The inoculation plates were meticulously combined and left to solidify before incubation at 37°C for 24 h with TBC, TSC, and TEC, or at 7°C for 10 days with TPSC. The agar plates containing 30–300 pinpoint colonies were identified by the following calculations and records:

TBC, TPSC, TSC, and TEC/g = average number of colonies  $\times$  reciprocal dilution.

The number of colonies is expressed as log 10 cfu/g.

### Statistical analysis

Every value was presented as the mean  $\pm$  standard error, and all measurements were conducted twice. The statistical analysis was evaluated utilizing the Tukey-Kramer HSD test. The JMP statistical software from SAS Institute Inc., Cary, NC was utilized to conduct a Pearson correlation analysis. Statistical significance was established for all analyses using the JMP statistical program, developed by SAS Institute Inc. in Cary, NC. A significance level of  $p < 0.05$  was utilized for this purpose.

### Results

The results indicated that HIS was detected in all examined meat products. The mean concentrations of HIS were the highest in minced meat ( $225.3 \pm 5.2$  mg/Kg), followed by that sausage, and luncheon at  $168.6 \pm 5.9$  and  $164.0 \pm 3.4$  mg/Kg, pasterma ( $107.2 \pm 4.4$  mg/Kg), and canned meat ( $66.2 \pm 4.1$ ), ( $p < 0.05$ ), respectively (Fig. 1A). The EDI values of HIS resulting

from the consumption of different meat products retailed in Egypt ranged from 5.79 mg/day for canned meat to 19.71 mg/day for beef mince, as shown in Table 1.

TYR was also detected in all examined meat product samples. The meat products with the highest concentrations of TYR residues were beef mince ( $265.8 \pm 6.9$  mg/Kg), followed by luncheon ( $173.2 \pm 7.1$  mg/Kg), sausage ( $149.4 \pm 8.9$  mg/Kg), pasterma ( $112.6 \pm 12.6$  mg/Kg), and canned meat ( $78.2 \pm 6.5$  mg/Kg) (Fig. 1B). The estimated EDI values for TYR varied from 6.84 mg/day in canned meat to 23.25 mg/day in beef mince, as shown in Table 1.

Minced meat had significantly ( $p < 0.05$ ) higher CAD levels ( $240.2 \pm 7.2$  mg/Kg) > sausage ( $150.4 \pm 7.1$  mg/Kg) > luncheon ( $122.3 \pm 6.4$  mg/Kg) > pasterma ( $102.6 \pm 5.9$  mg/Kg) > canned meat ( $55.4 \pm 2.2$  mg/Kg), as shown in Figure 2A. The EDI values of CAD (mg/day) varied from 4.84 in canned meat to 21.01 in beef mince, as shown in Table 1.

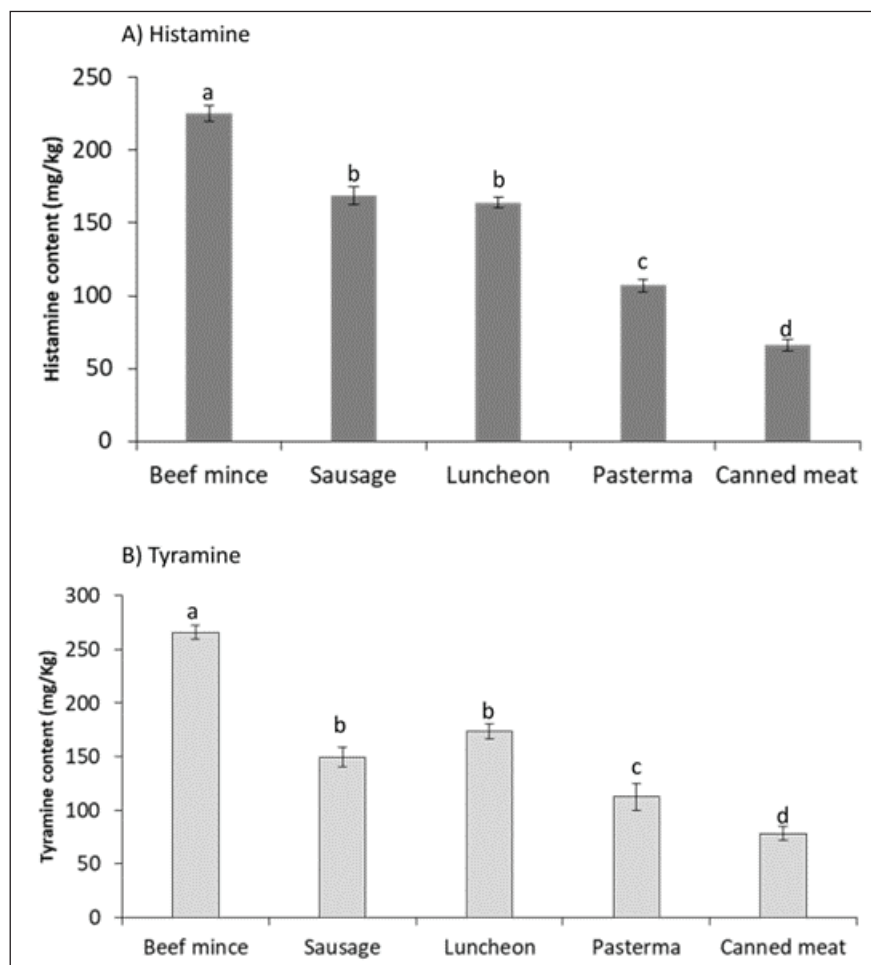


Fig. 1. Total contents of (A) HIS and (B) TYM (mg/kg) in the examined meat products. Columns carrying different letter are significantly different.

**Table 1.** Estimated daily intakes (mg/day) of different biogenic amines detected in the examined meat products.

|                       | Beef mince | Sausage | Luncheon | Pasterma | Canned meat |
|-----------------------|------------|---------|----------|----------|-------------|
| Histamine             | 19.71      | 14.75   | 14.35    | 9.38     | 5.79        |
| TYM                   | 23.25      | 13.07   | 15.15    | 9.85     | 6.84        |
| CAD                   | 21.01      | 13.16   | 10.70    | 8.97     | 4.84        |
| PUT                   | 14.18      | 7.89    | 7.82     | 6.96     | 3.15        |
| SPM                   | 0.81       | 0.42    | 0.50     | 0.54     | 0.19        |
| SPD                   | 1.36       | 1.01    | 1.08     | 0.77     | 0.42        |
| Total biogenic amines | 80.34      | 50.31   | 49.61    | 36.48    | 21.24       |

Regarding PUT, the acquired data indicated the formation of PUT in all analyzed meat products. Like CAD, minced meat had the highest content of PUT residues by a significant margin. The examined meat products exhibited PUT concentrations ranging from 36.0 to 162.1 mg/kg (Fig. 2B). Table 1 presents the EDI values of PUT (mg/day) in the tested meat products, which exhibited the highest EDI value for beef mince at 14.18.

Meat product samples that were analyzed underwent SPM concentration estimations. The results of the study revealed that canned meat contained the least amount of SPM ( $2.2 \pm 0.1$  mg/Kg), while beef mince had the highest SPM content ( $9.2 \pm 0.5$  mg/Kg) (Fig. 3A).

SPD is the second polyamine that is being examined in this study. The mean SPD concentrations in the tested meat products were presented in Figure 3B. Beef mince had the highest SPD content ( $15.62 \pm 0.8$  mg/Kg) while canned meat had the least concentrations ( $4.8 \pm 1.1$  mg/Kg). For SPD, EDI values were calculated for each meat product analyzed (Table 1), which ranged between 0.42 in canned meat to 1.36 in beef mince.

Total BAs were calculated for the examined samples, beef mince had the highest TBA content at  $918.22 \pm 21.3$  mg/Kg followed by sausage at  $575.1 \pm 12.8$  mg/Kg, luncheon at  $567.1 \pm 17.8$  mg/Kg, pasterma at  $417.0 \pm 31.8$  mg/Kg, and canned meat at  $242.8 \pm 21.8$  mg/Kg (Fig. 4). The computed EDI for TBA ranged between 21.24 in canned meat to 80.34 in beef mince (Table 1). Consequently, we broadened the scope of this study to include an examination of the microbiological status of the meat products being studied. It was determined that beef mince had the highest microbial contamination rates as indicated by the high TBC, TPSC, TSC, and TEC at  $5.69 \pm 0.4$ ,  $4.2 \pm 0.5$ ,  $2.4 \pm 0.2$ , and  $4.69 \pm 0.1$  log 10 cfu/g. Such counts were  $3.6 \pm 0.2$ ,  $2.4 \pm 0.2$ ,  $1.2 \pm 0.1$ , and  $4.3 \pm 0.2$  log 10 cfu/g in sausage,  $3.4 \pm 0.3$ ,  $2.2 \pm 0.1$ ,  $1.1 \pm 0.1$ , and  $4.0 \pm 0.1$  log 10 cfu/g in luncheon,  $2.5 \pm 0.1$ ,  $1.0 \pm 0.1$ ,  $1.4 \pm 0.08$ , and  $2.69 \pm 0.2$  log 10 cfu/g in pasterma; while none of the examined canned meat harbored microbial contamination (Fig. 5).

### Discussion

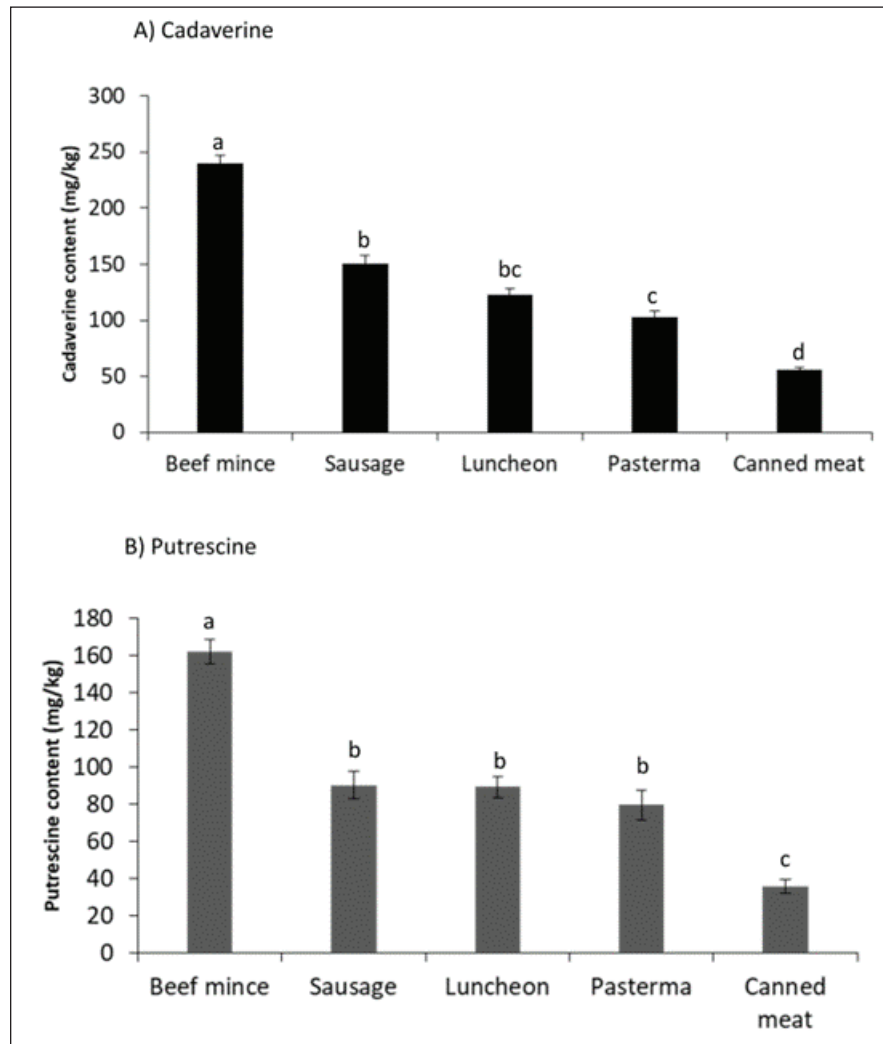
Since quite some time, it has been acknowledged that BAs are present in a vast array of foods, including

meat and meat products. Meat holds significant dietary importance in developed countries. The occurrence of these amines in food is noteworthy for two reasons: initially, from a toxicological standpoint, as specific consumers may become sick when exposed to elevated concentrations of dietary BAs; and second, because they may serve as potential indicators of product quality. The present study involved the measurement of six BAs in five distinct varieties of meat products that are available for purchase in Egypt. The variety and microbiological condition of the meat products had a significant effect on the total and individual BAs produced.

Comparable concentrations of the tested BAs were detected in several reports. In Egypt, the concentrations of HIS, TYM, PUT, CAD, SPM, and SPD were determined in a sample of Egyptian fermented foods. Blue cheese, Mesh cheese, smoked and fermented prepared sausage smoked and salted fermented fish [Feseekh], salted sardines, and anchovies comprised the foods that were inspected. The sausage fermented in Egypt exhibited the most elevated concentration of total BAs (2,482 mg/kg), with blue cheese and mesh cheese following suit at 2,014 and 2,118 mg/kg, respectively. Smoked cooked sausage contained the least amount of concentration (111 mg/kg). HIS levels in Feseekh were found to be elevated at 521 mg/kg, while blue cheese exhibited the maximum TYM content at 2,010 mg/kg. Certain traditional Egyptian foods may pose a health risk due to the concentration of BAs, particularly HIS, as suggested by these results (Rabie *et al.*, 2011). Likely, in a study conducted on fermented sausage retailed in Greece. The BA analyzed contained elevated concentrations of TYM, PUT, HIS, and CAD, with respective ranges of 0 to 510 mg/kg (median: 197.7 mg/kg), 0 to 505 mg/kg (median: 96.5 mg/kg), 0 to 515 mg/kg (median: 7.0 mg/kg), and 0 to 690 mg/kg (median: 3.6 mg/kg) (Papavergou *et al.*, 2012).

HIS is responsible for severe scombroid poisoning, or HIS-intoxication, can be induced by the ingestion of elevated concentrations of HIS. Symptoms include anaphylaxis, chest pain, symptoms affecting the nervous system and cardiovascular system, gastrointestinal distress, and anaphylaxis (FAO/WHO,



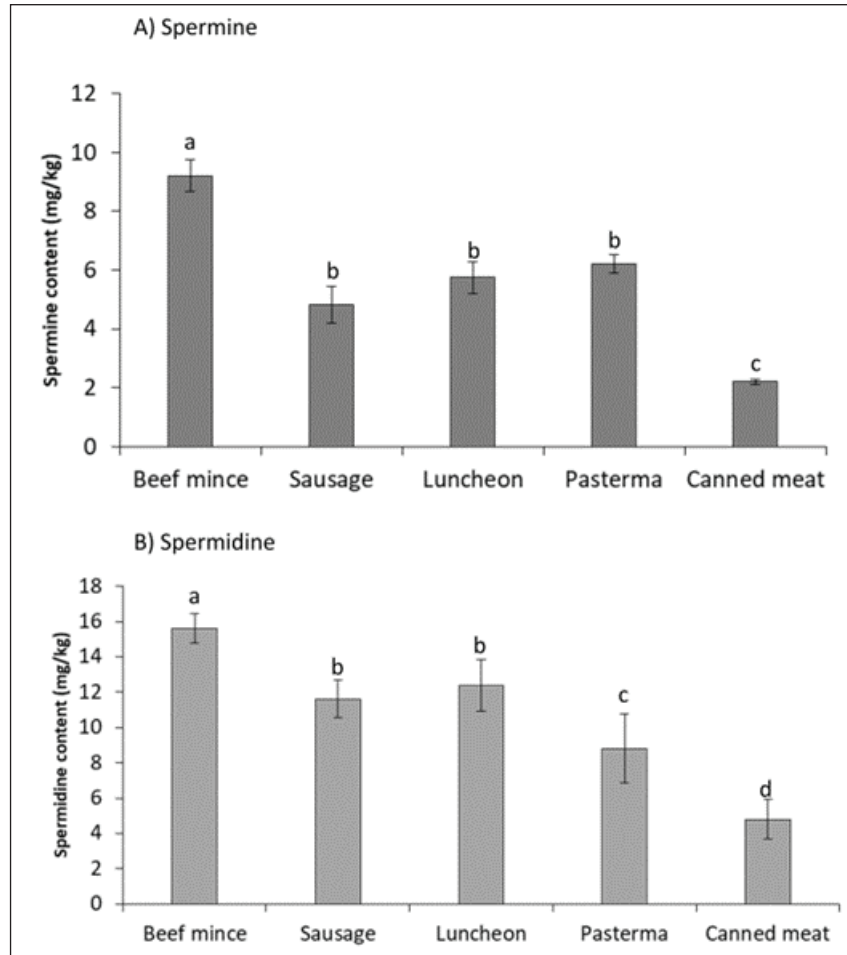


**Fig. 2.** Total contents of (A) CAD and (B) PUT (mg/kg) in the examined meat products. Columns carrying different letter are significantly different.

2012). It was established that the value of 50 mg the no known adverse effect threshold (NOAEL) for HIS by the EFSA subcommittee on biological hazards (EFSA, 2011). This may indicate that the HIS-measured EDI levels utilized in the current investigation will not have any adverse effects on the human population. Nonetheless, multiple epidemics of HIS intoxication in Taiwan have been attributed to the consumption of raw or processed fish (Chen *et al.*, 2010). Analogous instances of HIS-induced intoxication were recorded in Australia between 2001 and 2013, predominantly attributable to the consumption of tuna (Knopé *et al.*, 2014). Additionally, an outbreak of HIS intoxication involving children who had ingested canned sardines was documented in a kindergarten located in the Vojvodina province of northern Serbia (Petrovic *et al.*, 2016). This exemplifies the variability among individuals regarding their susceptibility to developing

symptoms of HIS toxicity. Potential factors contributing to this discrepancy include variations in age, dietary behaviors, and genetic predisposition (Visciano *et al.*, 2014).

A maximal allowable concentration of TYR in Austria was suggested by Paulsen *et al.* (2012) as 950 mg/Kg. This suggests that the populations of Egypt would not experience adverse effects due to the levels identified in the present research. However, it should be noted that TYR, an acknowledged vasoactive BA, has been associated with complications such as hypertension crises, cardiac failure, and increased heart rate (Benkerroum, 2016). Therefore, utmost caution is necessary, particularly for individuals who are exceptionally susceptible. The ingestion of greater amounts of CAD may result in an escalation of the toxicity of HIS. CAD is also associated with gastric and intestinal cancers (Benkerroum, 2016).



**Fig. 3.** Total contents of (A) SPM and (B) SPD (mg/kg) in the examined meat products. Columns carrying different letter are significantly different.

The scarcity of evidence regarding NOAEL and the risk assessment of PUT can be attributed to the fact that PUT produces the least hazardous effects (Koutsoumanis *et al.*, 2010). On the contrary, PUT could potentially intensify the deleterious impacts of TYR and HIS by impeding their oxidative inhibition mechanism (Bulushi *et al.*, 2009). Moreover, neurological disorders and gastrointestinal malignancies have been associated with PUT (Benkerroum, 2016).

NOAEL SPM in meat products is, to the best of our knowledge, poorly understood and undocumented. However, SPM has been associated with several neurodegenerative disorders and neurological conditions, such as cancer, psoriasis, and epilepsy (Benkerroum, 2016). It was reported that SPD acts as a precursor for *N*-nitrosopyrrolidine (Drabik-Markiewicz *et al.*, 2011). In addition, SPD is associated with ischemia and cystic fibrosis and promotes the development of malignancies (Benkerroum, 2016).

Their elevated concentration of total BAs is most likely attributable to the high concentration of the corresponding free amino acids in meat products, which includes them (Biji *et al.*, 2016).

Microbial counts and the formation of BAs have been found to be positively correlated in fish (Visciano *et al.*, 2014; El-Ghareeb *et al.*, 2021), camel meat (Tang *et al.*, 2020), and cheese (Ma *et al.*, 2020). Besides, it was reported that the presence of BAs in processed meat products and minced meat is one indicator of low-quality raw materials. The most prolific producer of HIS was *Escherichia coli*, which was followed by *Proteus mirabilis* and *Morganella morganii*, in that order. The strains *Citrobacter freundii* and *Enterobacter spp.* exhibited the greatest PUT production level. Subsequently, *Serratia grimesii*, *Proteus alcalifaciens*, *Escherichia fergusonii*, *M. morganii*, *P. mirabilis*, *Proteus penneri*, and *Hafnia alvei* followed in that order of importance. The primary producer of CAD was *E. coli* (Durlu-Özkaya *et al.*, 2001).

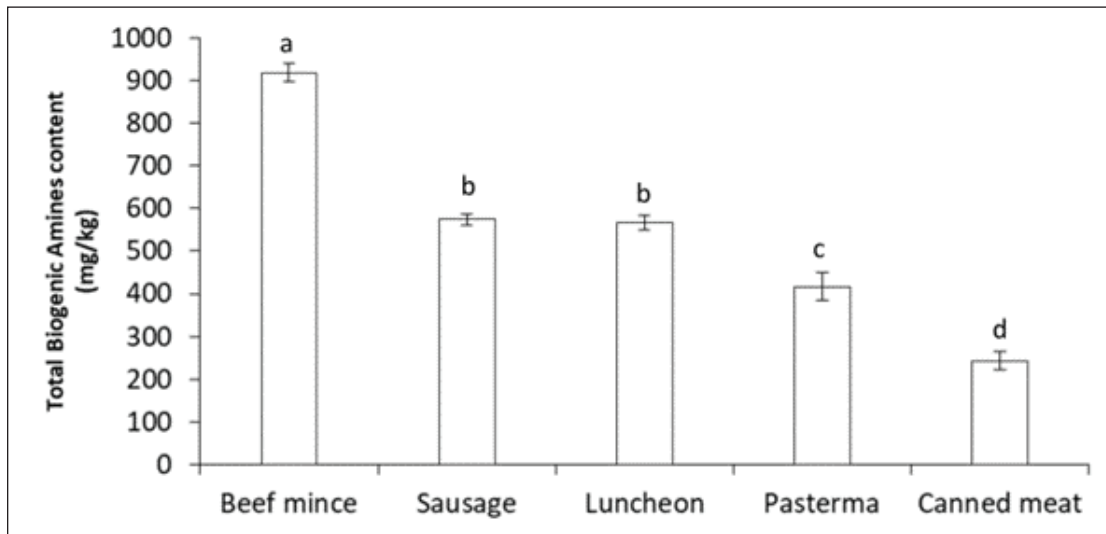


Fig. 4. Total biogenic amine contents (mg/kg) in the examined meat products. Columns with different letter are significantly different.

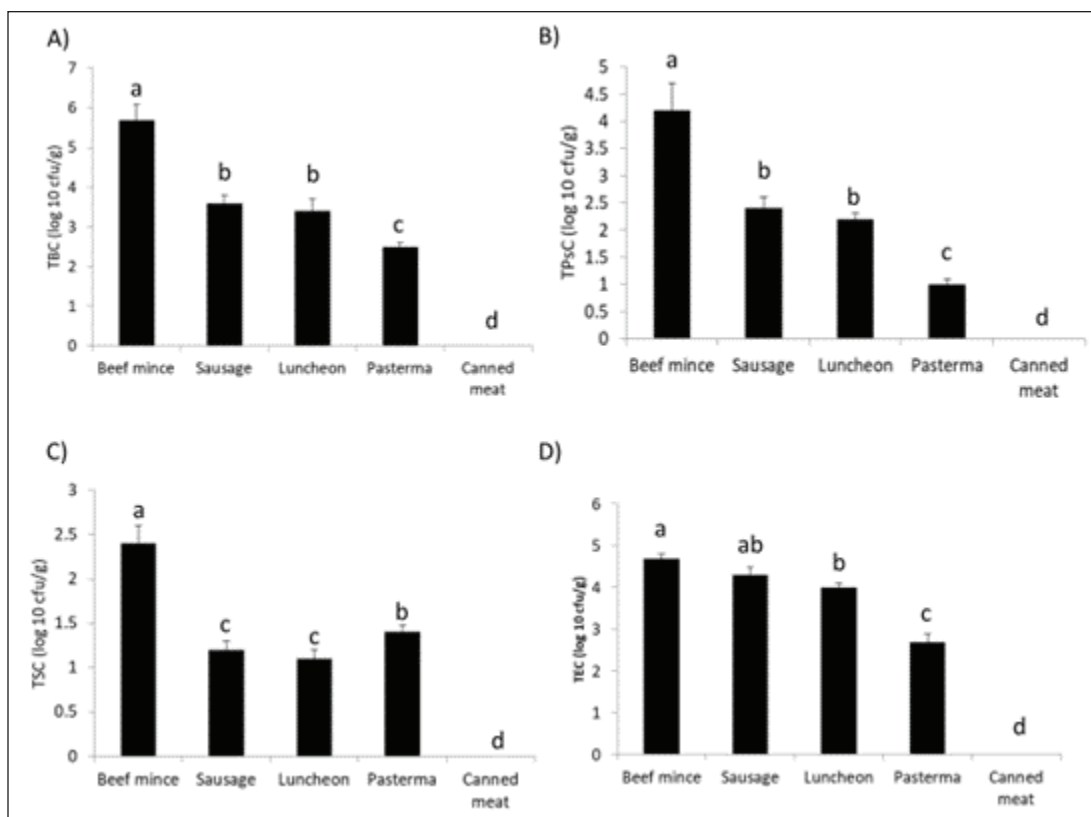


Fig. 5. (A) TBCs, (B) TPSC, (C) Total *S. aureus* counts (TSC), and (D) TEC (log 10 cfu/g) in the examined meat products. Columns with different letter are significantly different at  $p < 0.05$ .

The results of this study provide significant evidence that the amount of BA produced in meat products is contingent upon the type of the meat products, conditions of storage, and initial microbial load.

Consequently, strict adherence to industry standards during the processing and manufacturing of meat products is of utmost importance. These standards encompass the prevention of microbiological

contamination, meticulous handling of beef mince, and maintenance of a chilling temperature not exceeding 4°C (FDA, 2011).

### Conclusion

According to the results of the investigation, a number of BAs were detected in meat products sold in Egypt. Minced meat demonstrated the highest contents of total BAs and microbiological populations. The EDI values of the examined BAs indicated that the Egyptian population did not present any risk via meat product consumption. Conversely, ingesting substantial quantities of meat products that have been contaminated with BAs—more specifically, TYR and HIS—could present a significant health hazard, particularly in terms of scombroid toxicity. Processing, storage, distribution, and marketing of meat products should therefore adhere to hygienic standards.

### Conflict of interest

It is declared by the authors that they have no conflicts of interest.

### Funding

Not applicable.

### Data availability

Data will be made available from the corresponding author upon reasonable request.

### Authors' contributions

All authors equally contributed to the study.

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