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Occurrence, Concentration and Acceptability of Biogenic Amines in Some Chicken Meat Cuts

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ABSTRACT

THE CURRENT research article aims to find a safe and quick way to reduce the amount of biogenic amines (BAs) in meat and meat products because, regrettably, high free amino acid levels combined with unsuitable storage and transportation conditions that lead to the formation of serious BAs through bacteriological decarboxylation, which poses serious health risks, particularly allergic reactions brought on by histamine poisoning. In Menofiya Governorate markets in Egypt, 90 samples of raw, chilled chicken breast, thigh, and wing (30 of each) were randomly selected from local poultry selling points in order to determine the presence of several BAs, such as putrescine, tyramine, cadaverine, and histamine, using HPLC. Additionally, a research was carried out to see the ability of *Bacillus polymyxa* (10^7 CFU/ml) if it could biodegrade experimentally implanted histamine and tyramine (50 mg/100g) under chilling storage (4^{O}C). Results showed that the greatest BAs levels were found in the wings, followed by the thigh and breast samples, respectively; where, substantial differences ($P \leq 0.05$) between all of the samples that were analyzed. The results of the *B. polymyxa* degrading impact showed a considerable decrease in the amounts of histamine and tyramine in chicken fillet samples, with reduction percentages of 71.6% and 76.2% following a 24-hour period of refrigeration, respectively. Based on the results obtained, raw chicken meat parts may be a substantial source of health risks depending on the concentrations of BAs; moreover, the probiotic biodegradation demonstrated a viable and safe method of biocontrolling BAs to prevent BAs health risks.

Keywords: Biogenic amines, Probiotic Biodegradation, Chicken, Egypt, HPLC.

Introduction

Poultry meat production and consumption have rapidly increased globally, and this trend is expected to continue in many areas of the world. Factors such as affordability, lack of barriers based on culture or religion, and nutritional and dietary value are the main reasons why customers are drawn to chicken meat [1-3].

A common chemical compounds produced from free amino acids decarboxylation are biogenic amines. They are found in foods naturally in low concentrations, but they are also produced in larger quantities under certain circumstances. The primary factors affecting the formation of biogenic amines are the quality of fresh food, the temperature during storage, the presence of microorganisms that have the active ability of decarboxylase enzyme, and the availability of circumstances that promote the growth of these microorganisms and the synthesis of their decarboxylase enzyme [4-7].

Histamine, tyramine, tryptamine, and putrescine—which are produced by the enzymatic decarboxylation of histidine, tyrosine, tryptophan, and ornithine, respectively—are the most significant biogenic amines found in chicken meat and meat products [8]. When the activity of aminooxidases, the enzymes responsible for the detoxification of these compounds, is inhibited, biogenic amine concentrations in food might occasionally reach levels that are harmful to consumers with increased sensitivity to these toxins [9].

One well-known chemical intoxication with a brief incubation period is histamine poisoning, which can occur in half to one hour. Many symptoms,

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including urticaria, edema, localized inflammation, and rash, are frequently present [10]. Conversely, there are few reports of cadaverine and putrescine poisoning. Putrescine and cadaverine have been linked to acute adverse effects, including increased cardiac output, lockjaw and paresis of the extremities, dilatation of the vascular system, hypotension, and bradycardia (which may result in heart failure and cerebral hemorrhage) [11, 12]. Additionally, both have indirect toxic effects by amplifying the toxicity of other BA, such as histamine.

While numerous studies were carried out with the goal of lowering the amounts of BAs in various food samples, the use of probiotics or degrading bacteria has emerged as a potentially effective method in recent times, particularly for lowering the levels of BAs in fermented foods [13].

Thus, the goal of the current investigation was to ascertain the formation of BAs in samples of raw chicken flesh while also carrying out a biocontrol treatment experiment.

Material and Methods

Collection of samples

From Shebin Elkom city, Menofiya governorate, thirty randomly selected samples of chicken meat products, comprising 30 pieces of each of the breast, thigh, and wings, were collected from various supermarkets. Biogenic amines (histamine, tyramine, putrescine, and cadaverine) were examined in the collected samples. It was also investigated how certain trials might be used to control the presence of such harmful substances in chicken meat products.

Determination of BAs in chicken meat samples using HPLC

Histamine, cadaverine, tyramine and putrescine were determined in all examined samples according to Krause *et al.* [14] for samples' extraction, and Pinho *et al.* [15] for the next step of dansyl-amine formation that was dissolved in 1ml methanol and 10μl were injected in HPLC.

An Agilent 1100 HPLC system (Agilent Technologies, Germany) with a UV detector (Model G 1314A) set at a wavelength of 254 nm was utilized to determine the amount of dansylamines using high performance liquid chromatography (HPLC). HPLC gradient solvent program for separation of biogenic amines as follow: time (min.): 0, 10, 15, 20 and 25; flow rate (ml/min): 1 for all; solvent A% (0.02N acetic acid): 60, 20, 15, 60 and 60; solvent B% (methanol): 20, 40, 35, 20 and 20; solvent C% (acetonitrile): 20, 40, 50, 20 and 20, respectively (Fig. 1A, B).

Impact of probiotics on BAs concentrations in chicken fillets after experimental inoculation

Preparation of bacterial suspension was performed according to Eom et al. [16]

An overnight culture of the *Bacillus polymyxa* strain was prepared by cultivating it for 24 hours at 37ºC in Brain Heart Infusion (BHI) Broth. A milliliter of the grown bacterial suspension was decimally diluted with 0.1% w/v sterile peptone water (Merck, Darmstadt, Germany). Thus, a volume of the culture broth equivalent to roughly 10^7 bacteria was centrifuged, and the bacterial pellets were twice washed with deionized water.

Binding assay was performed according to Halttunen *et al.* [17]; where, chicken fillet was allowed to be incubated with *B. polymyxa* pellet and the experimentally inoculated BAs for 24h with fine shaking.

Experimental grouping

Histamine- and tyramine-contaminated chicken fillets were used as the control assay (G1). The test group, on the other hand, consisted of chicken fillets that were contaminated with histamine (50 mg/kg) and treated with *B. polymyxa* (G2), and chicken fillets that were contaminated with tyramine (50 mg/kg) and treated with *B. polymyxa* (G3). Histamine and tyramine levels in the samples were measured using HPLC after they were acidified with ultrapure HNO_3 and analyzed at zero, eight, sixteen, and twenty-four hour intervals.

Statistical analysis

One-way analysis of variance (ANOVA) with Duncan post-hoc analysis was performed on the collected data using SPSS® version 16.0. It was deemed statistically significant when the statistical probability (p value) was less than 0.05.

Results

Referring to the recorded occurrence, concentration and acceptability levels of the investigated BAs in Table (1); histamine, tyramine, putrescine and cadaverine were detected in 48.8%, 38.8%, 62.2% and 52.2% of the total examined samples, respectively. The recorded results showed that wing samples had significantly higher BAs concentrations ($P \leq 0.05$) than thigh and breast samples, respectively; and consequently lower acceptability level. Moreover, 75.5%, 81.1%, 62.2% and 72.2% were the acceptability ratio (%) of the examined samples in relation to their BAs concentration in accordance with the Egyptian standards guidelines.

The results recorded in Fig. 2 shows the reduction rates achieved in an experimental study that was conducted to determine the inhibitory effect of *B. polymyxa* on the experimentally inoculated histamine and tyramine in chicken fillet samples. Results revealed a significant ($P \leq 0.05$) reduction in the histamine and tyramine levels, with reduction rates of 71.6 % and 76.2%, respectively after 24h of chilling storage (4±1^{O}C) (Fig. 2).

Discussion

Poultry meats are highly perishable foods, and the deterioration time varies from 4 to 10 days after slaughtering depending on the hygienic quality, microbial load and storage conditions [18-21].

Food safety and quality have been closely linked to biogenic amines (BAs). Despite the fact that they are found in humans and animals in nature, the primary mechanism for their availability in food is bacterial decarboxylation of free amino acids [22].

The release of free amino acids from tissue proteins—which provide a substrate for the decarboxylase processes that generate the biogenic amines—may be significantly influenced by proteolysis, either bacterial or autolytic [23].

Referring to the recorded results in Table (1), it is obvious that wing samples revealed significantly higher BAs contents than the other examined samples, which came in contrary with the recorded results of Abd El Zahir [24] who found that thigh samples had higher histamine levels than wing samples; while agreed with the recorded results of Ibrahim *et al.* [25] who found that the BAs levels were higher in thigh samples than the breast samples; which may be attributed to the microbial content of each samples, amount of free amino acids, and the availability of the favorable conditions for amino acids decarboxylation [26].

Histamine is categorized as a heterocyclic diamine due to its chemical makeup and quantity of amine groups. Histamine is involved in several critical physiological processes in humans, such as blood pressure regulation, cellular growth control, allergy response, and synaptic transmission [27].

The current obtained results of histamine came higher than those recorded by Abd El Zahir [24] (8.41 and 10.75 mg/kg for wing and thigh with 95% acceptability, respectively); where the acceptability rate was 95% for both wing and thigh as well), Ibrahim *et al.* [25] and Hassan et al. [28] (10.88 and 8.17 mg/kg with the incidence of 80% for thigh and breast samples, with acceptability ratio of 90 and 100%, respectively).While, it does not agree with the recorded results of Balamatsia *et al.* [29], Ntzimani *et al.* [30], and Gallas *et al.* [31] who did not find histamine in the examined chicken breast samples.

Symptoms of histamine intoxication include headaches, palpitations, hypotension, urticarial and other rashes, edema, mouth burning, vomiting, diarrhea, cramps, and swelling. These symptoms can also be respiratory, hematological, gastrointestinal, and neurological in nature [32]. On the other hand, the formula for cadaverine is (CH2)5(NH2)2. Classified as a diamine, which is typically found in trace amounts in living things, but is frequently linked to the putrefaction of animal tissue following bacterial lysine decarboxylation via protein hydrolysis [33].

The current obtained results of cadaverine were higher than those of Abd El Zahir [24] (4.18 and 2.97 mg/kg for thigh and wing, respectively; where all of the examined samples were within MRLs of cadaverine). While came in agreement but in lower incidence (%) with the recorded results by Ibrahim *et al.* [25] (12.65 and 9.86 mg/kg for thigh and breast samples, with acceptability rate of 80% and 100%, respectively; with the incidence of 100%); but, lower results were recorded by Balamatsia *et al.* [29] (19.8 mg/kg in breast samples). While did not agree with the obtained results of Ntzimani *et al.* [30] who did not find cadaverine in their examined samples.

Despite the fact that histamine and tyramine appear to have more potent pharmacological effects than putrescine and cadaverine [12]. Acute adverse effects, including elevated cardiac output, lockjaw and paresis of the limbs, vascular system dilatation, hypotension, and bradycardia (which may result in heart failure and brain hemorrhage), have been linked to the ingestion of these vasoactive BAs [34]. Furthermore, by increasing the toxicity of other BA, like histamine, both have indirect harmful effects [35].

The current obtained results of putrescine were higher than those of Ibrahim *et al.* [25] (4.54 and 4.15mg/kg for thigh and breast samples, with acceptability rate of 100%; with the incidence of 70% and 60%, respectively). While not agreed with the obtained results of Ntzimani *et al.* [30] who did not find putrescine in their examined samples.

With the ability to release catecholamines indirectly, tyramine is a trace monoamine [36]. Tyramine use primarily affects the peripheral cardiovascular system. A hypertensive crisis may be triggered by tyramine overconsumption, particularly when paired with monoamine oxidase inhibitors (MAOIs) [37].The current obtained results of tyramine were higher than those of Abd El Zahir [24] (4.98 and 2.97 mg/kg for wing and thigh, respectively; where all of the examined samples were within MRLs of cadaverine. While lower results were recorded by Balamatsia *et al.* [29] (0.2 mg/kg in breast samples). While did not agree with the obtained results of Ntzimani *et al.* [30] who did not find tyramine in their examined samples.

Actually, the existence of BAs in the studied chicken meat samples is very interesting for two reasons: first, since they may serve as quality indicators, and second, because of their potential toxicological implications, as excessive dietary histamine levels may be harmful to some consumers [38].

Broadly speaking, variations in the amount of biogenic amines within and between product classes were statistically significant (P≤0.05). Numerous factors, including the makeup of the microbiota, the chemico-physical parameters, the hygienic practices used during processing, and the accessibility of precursors, influence these variations [39, 40].

However, bacterial decarboxylation can be inhibited to control or prevent the formation of biogenic amines in food. A variety of methods have been reported to limit microbial growth, including the use of food additives that were deemed impractical based on fishing, hydrostatic pressures, irradiation, and controlled atmospheric packaging (CAP) [41]. As a result, additional biocontrol strategies, such as the use of amine-negative bacteria or bacterial amine oxidase, must be developed to regulate BA levels.

The second aim of the current study was to biocontrol the levels of BA in the examined samples. Therefore, the biodegrading effect of *B. polymyxa* on the artificially inoculated histamine and tyramine (50 mg/Kg) in chicken fillet samples was investigated. Its effect appeared to be potentially good and rapid, where 71.6% and 76.2% of the inoculated histamine and tyramine declined within 24h or refrigeration, respectively.

The present findings were consistent with the earlier findings [42, 43], which showed that throughout fermentation, the total biogenic amine contents in the control samples were significantly higher ($p < 0.05$) than those of the inoculated samples. Following 120 days of fermentation, the inoculated samples' histamine and total biogenic amine levels decreased by 34.0% and 30.0%, respectively; and Roselino *et al.* [44] who recorded a significant reduction in different BAs in probiotic treated salami samples. Moreover, nearly similar results were recorded by Samir *et al.* [45] who reported a significant reduction in the experimentally inoculated histamine levels after addition of *B. polymyxa,* in fish fillet samples, for 24h of refrigeration, with reduction % of 81.8%. Also, after 12 and 24 hours of storage at 5°C, Saad *et al.* [46] observed a significant decrease in the level of histamine, which dropped from 40 mg/kg at 0 h to 13.1 and 6.6 mg/kg with reduction (%) of 67.3% and 83.5%, respectively; similarly, the levels of tyramine decreased with 58.0 and 71.8% after 12 and 24 hours of cold storage, respectively.

Histamine oxidase and dehydrogenase, which can catalyze the oxidative deamination of histamine to imidazole acetaldehyde and ammonia, are two possible mechanisms by which *B. polymyxa* may regulate the synthesis of histamine. Furthermore, in the presence of water and oxygen, histamine oxidase can catalyze the conversion of histamine to imidazole acetaldehyde, ammonia, and hydrogen peroxide [47]; Lee *et al.* [43] proposed two mechanisms for the action of *B. polymyxa* on the reduction of biogenic amines: (1) *B. polymyxa* competes with the current microorganisms that can produce BAs; and (2) *B. polymyxa* has the capacity to enzymatically break down the BAs.

Conclusion

Based on the results obtained, it can be concluded that there is a considerable variance in the potential creation of BAs in various slices of chicken meat, with the highest concentration of BAs found in wing samples, followed by thigh and breast samples, in that order. In addition, *Bacillus polymyxa* showed a possible quick breakdown effect on the levels of histamine and tyramine, which makes it an effective bio-controlling technique for BAs in chicken meat slices during refrigeration storage.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study did not use any living animal nor human subjects.

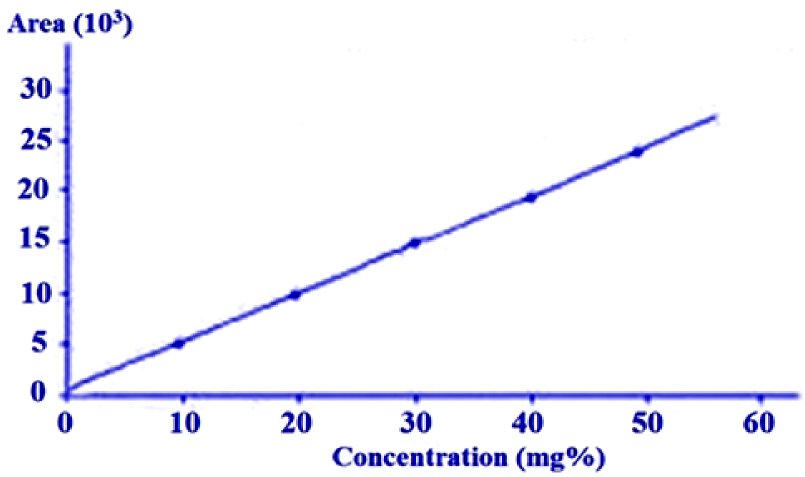

Fig. (1A). Calibration curve of biogenic amine by using HPLC

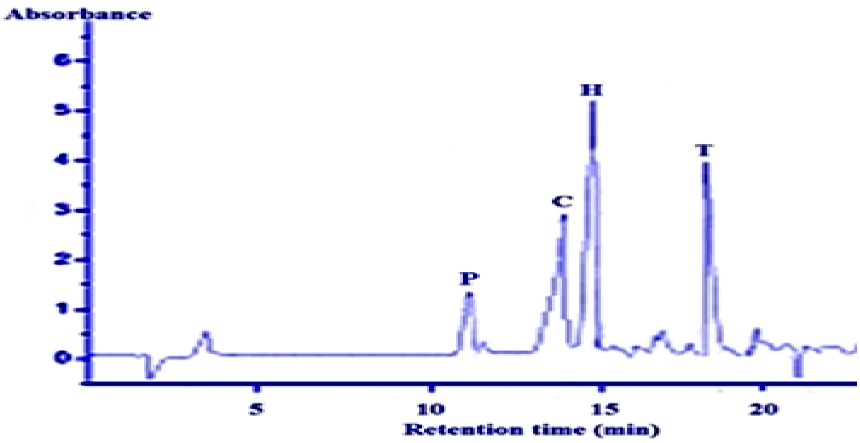

Fig. (1B). HPLC-derived chromatograms of the biogenic amine standard solution regions.
C: Cadaverine, **H:** Histamine, **P:** Putrescine, **T:** Tyramine

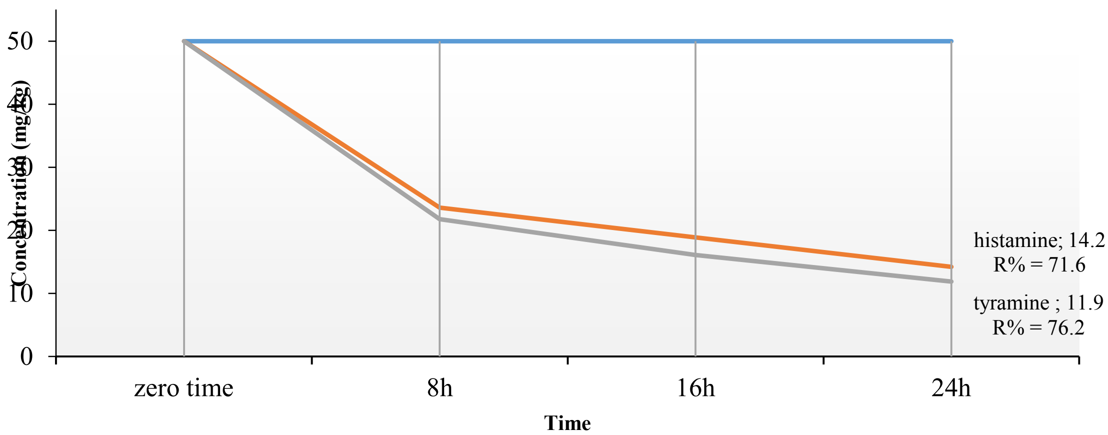

Fig. 2. Effect of *B. polymyxa* culture (10^7 CFU/g) on the levels of histamine and tyramine experimentally inoculated to chicken fillets (50 mg/Kg).

TABLE 1. Incidence, concentrations and acceptability levels of biogenic amines (BAs) (mg/Kg) in the examined samples of chicken meat products (n=30).

BAs (mg/Kg)	Meat Product	Incidence and Conc.			Acceptability		
		+ve samples		Mean ± S.E	MRL (mg/Kg)*	Accepted samples	
		No	%			No	%
Histamine (mg/Kg)	Breast	13	43.3	10.37 ± 0.59 C	20	24	80
	Thigh	14	46.7	12.95 ± 0.74 B		23	76.7
	Wings	17	56.7	16.48 ± 1.03 A		21	70
	Total	44	48.8		Total	68	75.5
Tyramine (mg/Kg)	Breast	10	33.3	8.54 ± 0.46 C	20	26	80.7
	Thigh	12	40	9.89 ± 0.53 B		25	83.3
	Wings	13	43.3	11.42 ± 0.81 A		22	73.3
	Total	35	38.8		Total	73	81.1
Putrescine (mg/Kg)	Breast	16	53.3	12.31 ± 0.65 C	20	21	70
	Thigh	19	63.3	14.06 ± 0.73 B		19	63.3
	Wings	21	70	15.92 ± 0.88 A		16	53.3
	Total	56	62.2		Total	56	62.2
Cadaverine (mg/Kg)	Breast	14	46.7	11.25 ± 0.59 C	20	23	76.7
	Thigh	15	50	12.41 ± 0.68 B		22	73.3
	Wings	18	60	14.73 ± 0.81 A		20	66.7
	Total	47	52.2		Total	65	72.2

*MRL: Maximum Residual Limit (EOS, 2010).
ABC Means with different superscript letters in the same column, within the same BAs item, are significantly different (P<0.05).

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تواجد وتركيزات ومقبولية الأمينات الحيوية في بعض قطع لحم الدجاج

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الملخص

الى جانب العديد من الفيتامينات والمعادن المهمة، يوفر لحم الدجاج مصدرًا جيدًا للبروتينات سهلة الهضم وعالية الجودة. انه من الضروري إيجاد طريقة آمنة وسريعة لتقليل كمية الأمينات الحيوية في لحوم الدواجن ومنتجاتها. تؤدي مستويات الأحماض الأمينية الحرة المرتفعة جنبًا إلى جنب مع ظروف التخزين والنقل غير المناسبة إلى تكوين أمينات حيوية خطيرة من خلال التحلل البكتيري والذي يشكل مخاطر صحية خطيرة، وخاصة الحساسية الناجمة عن التسمم بالهستامين. لذلك تم تجميع 90 عينة من صدور وأفخاذ وأجنحة الدجاج النيئة والمبردة (30 لكل منها) بشكل عشوائي من نقاط بيع الدواجن المحلية بأسواق محافظة المنوفية في مصر، من أجل قياس تركيزات الامينات الحيوية، بما في ذلك بوتريسين، تيرامين، الكادافيرين والهيستامين باستخدام جهاز الفصل الطيفي عالي الكفاءة. بالإضافة إلى ذلك، تم إجراء بحث لاستقصاء قدرة بكتريا الباسيلس بوليميكساعلى التحلل الحيوي للهيستامين والتيرامين (50 مجم/100 جم) تحت ظروف التخزين المبرد (4 درجات مئوية). أظهرت النتائج أن أعلى مستويات الأمينات الحيوية وجدت في الأجنحة، تليها عينات الفخذ والصدور، على التوالي. أظهرت النتائج تأثير الباسيلس بوليميكساعلى مستويات الأمينات الحيوية حيث احدثت انخفاضاً ملحوظاً في كميات الهستامين والتيرامين في عينات صدور الدجاج، حيث بلغت نسب الانخفاض 71.6% و76.2% بعد فترة 24 ساعة من التبريد، على التوالي. بناءً على النتائج التي تم الحصول عليها، قد تكون أجزاء لحم الدجاج النيئ مصدرًا للأمينات الحيوية ؛ بالإضافة إلى ذلك، أظهرت النتائج التجريبية قدرة الباسيلس بوليميكساعلى احداث انخفاض ملحوظ في كميات الهستامين والتيرامين.

الكلمات الدالة: الأمينات الحيوية، التحلل الحيوي ،البروبيوتيك، الدجاج، مصر