INHIBITORY EFFECT OF SOME SPICES ON THE FORMATION OF BIOGENIC AMINES IN MINCED MEAT STORED UNDER DIFFERENT CONDITIONS

التأثير المثبط لبعض التوابل علي تكوين الأمينات الحيوية في اللحوم المفرومة والمحفوظة عند ظروف مختلفة

Refaat M. Farghaly*, Mohammad M. Elgazzar
and Mohamed A. Hassan*****

***Dept. of Food Hygiene and Control, Fac. of Vet. Med. ,South Valley Univ.**
**** Dept. of Food Hygiene and control, fac. of Vet. Med. , Mansoura Univ., Egypt.**
*****Dept. of Food Control, Fac. of Veterinary Med. (Moshtohor), Zagazig Univ., Benha Branch, Egypt.**

Abstract

The inhibitory effect of a ground spices- mixture consisted equally of cumin, coriander, black pepper and red pepper on the formation of biogenic amines (cadaverine, histamine and putrescine) were determined by using TLC in a total of 40 samples of minced meat
stored at different temperatures for various periods. The examined samples were divided into 4 groups (n=10) according to the concentration of spices; one group without spices (0.0%) and other three groups contained 0.1%, 0.3% and 0.5% of spices- mixture and the biogenic amine contents were detected under 3 conditions; at room temperature without storage (0 day), at 4ºC for 1day and at –5ºC for 14 days.

The chromatographic analysis exhibited higher level of putrescine (21.2 mg /100 gm ±0.47), followed by histamine (11.1 mg /100 gm ±0.34) and cadaverine (9.4 mg /100 gm ±0.47) in the examined samples at room temperature before storage and without spice mixture. Slight inhibitory effect obtained when examined samples mixed with spices and stored at 4ºC for 1 day, specially with 0.5% spices; putrescine (19.2 mg /100 gm ±0.83), histamine (8.1 mg /100 gm ±0.63) and cadaverine (8.1 mg /100 gm ±0.76) .However, great inhibitory effect on the formation of putrescine (12.3 mg /100 gm ±0.62), histamine (6.2 mg /100 gm ±0.93) and cadaverine (5.8 mg /100 gm ±1.08) was achieved after mixing the minced meat samples with 0.5% spices and stored at –5ºC for 14 days.

Higher frequencies; 6(60%), 8(80%), 10(100%) recovered in the non spiced samples of minced meat without storage that contained cadaverine, histamine and putrescine levels more than that recorded by FDA (2001) regulatory limit (10 mg / 100 gm), respectively. While, lower frequencies; 0(0%), 1(10%) and 3(30%) were

found in samples mixed with spices (0.5%) conjugated with storage temperature of -5ºC for 14 days for their three amines- contents more than this limit, respectively. According to (EOS, 1996), neither of all the samples contained cadaverine 0(0%), nor most of them possessed histamine of higher concentration than the permissible limit (20 mg / 100 gm). However, higher incidence; 7 (70%) was revealed in the examined samples of minced meat without spices and stored at 4ºC for 1 day, that contained putrescine level more than the permissible limit. But, no incidence 0 (0%) of all spiced samples mixed with (0.5%) spices and stored at -5º for 14 days, contained putrescine level higher than this permissible limit.

Public health importance of biogenic amines in processed meat and suggested recommendations to inhibit their formation in food and to avoid their harmful effect in food spoilage and intoxication were discussed.

Materials and Methods

Collection and preparation of samples:

Forty minced meat samples (200 gm of each) were collected from different supermarkets in Qena province. These samples were divided into 4 groups (n= 10) according to the concentration of spice mixture (equal parts of sterilized cumin, coriander, black pepper and red pepper); one group without spices and other 3 groups mixed with 3 different concentrations of spice mixture (0.1%, 0.3 % and 0.5 %). The four groups were examined under three conditions; room temperature (32º C) without storage, 4ºC for 1 day and -5º C for 14 days, subsequently.

Estimation of biogenic amines:

Cadaverine, histamine and putrescine were identified by thin layer chromatography (TLC), as described by Shalaby (1994). Values are given as average of three replicates.

1-Reagents and solutions:

- Amines: Crystalline hydrochlorides of cadaverine, histamine and putriscine, as well as, dansyl chloride (5- dimethylaminonaphthaline- 1- sulfonyl chloride) were purchased from E. Merk, Germany.
- Stock standard solutions for the tested amines: 25 mg of each standard pure amines; histamine-2HCl, cadaverine2HCl and putriscine 2HCl were dissolved in 50 ml distilled water individually.
- Working standard solutions(10 µg/ml): 2 ml of each stock standard solution were pipetted into 100 ml volumetric flask and diluted to volume with 5% trichloroacetic acid (TCA). This solution is prepared freshly each time for testing and stored in refrigerator.
- Dansyl chloride solution: 500 mg of dansyl chloride were dissolved in 100 ml acetone.
- Thin layer chromatography (TLC) plates (20x20 cm) glass sheets (pre-coated with silica gel 6.60) were purchased from E. Merk, Germany.

2- Amine extraction (for meat samples).

- 25 gm of homogenized meat sample were blended with 125 ml of 5% TCA for 3 min using a warning blender, then filtration was achieved using filter paper Whatman No.(1), England.
- 10 ml of the extracts was transferred into a suitable culture tube with 4 gm NaCl and 1 ml of 50% NaOH, then shaked and extracted 3 times by 5 ml n-butanol chloroform (1:1 v/v) stoppered and shaked vigorously for 2 min followed by centrifugation for 5 min at 3000 rpm and the upper layer was transferred to 50 ml separating funnel using disposable pasture pipette.
- To combined organic extracts (upper layer), 15 ml of n- heptane was added and extracted 3 times with 1.0 ml portions of 0.2 NHCl, then NHCl layer was collected in a glass stopper tube. Solution was evaporated just to dryness using water bath at 95ºC with aid of air currents.

3- Derivative formation (Dansyl amines):

200 µl of each stock standard solution (or sample extract) were transferred to a culture tube and dried under vacuum. About 0.5 ml of saturated NaHCO3 solution was added to the residue of the sample extract (or the standard). The tube stoppered and curefully mixed to prevent loss due to spattering. Carefuly, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The mixture was kept in a water bath at 70ºC for 10 min, then the mixture was evaporated at reduce pressure and under gentle warming to 45º C. Then, the extraction of dansylated biogenic amines was carried out using 3 times of 5.0 ml portions of diethyl- ether, stoppered, shaked carefully for 1 min and the ether layers were collected in a culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35ºC in dry film and dissolved in 1 ml methanol, then 10µl injected in TLC plates (Paulsen and Bauer, 1996).

4- Separation of dansyl amines.

The chromatographic separation was carried out to separate the three dansyl amines by one- dimensional TLC. On 2 cm from the bottom edge of the TLC plate and at intervals of 1 cm, the following samples were applied using a microsyringe: 10, 15, 20, 30 and 50 µl of dansyl amine standard, and 10 µl of each of dansylated sample extracts. The plate was developed in chloroform/ benzene/ triethyl amine (6: 4: 1, v/v/v) for 15cm. The plate was taken out of the jar and allowed to dry. Then it was developed in the same direction in benzene/acetone/ triethyl amine (10: 2: 1, v/v/v) for 15cm. The plate was allowed to dry at room temperature until the excess of the solvent disappeared before interpretation.

5- Interpretation of the chromatogram

The chromatogram after the second development was examined under long wave (360 nm) of ultraviolet light to establish weather or not the dansyl amines of interest are present in the sample.

6- Quantification of dansyl amines

The developed TLC plates was placed under a chromatogram scanner and the absorbance value for each separated spot is recorded at wave length of 254nm.

7- Calculation

The concentration of each amine in the sample is made using the following formula:

µ gm/ gm (mg / kgm) = S Y V/ X W

S: amine standard equal to unknown (µl).

Y: concentration of amine standard (µ gm/ ml).

V: final dilution of sample extract (ml).

X: sample extract given a spot intensity equal to S (µl).

W: weight of the sample represented by the final extract (in grams).

Table (1) Concentration of biogenic amines(mg/100g) in minced meat mixed with

spices and estimated at room temperature (32ºC) without storage (0 day).

Biogenic amine Minced meat	No.	Cadaverine				Histamine				Putrescine			
		Min.	Max.	**Mean**	S.E.	Min.	Max.	Mean	S.E.	Min	Max	Mean	S.E
0.0%*	10	3.2	15.6	9.4	0.34	2.0	20.2	11.1	0.34	13.1	29.3	21.2	0.47
0.1%	10	3.0	15.4	9.2	0.37	5.8	13.8	9.8	0.35	11.7	30.1	20.9	0.59
0.3%	10	2.6	16.4	9.1	0.42	6.3	13.1	9.7	0.29	9.3	32.3	20.8	0.72
0.5%	10	2.9	15.1	9.0	0.38	3.4	14.9	9.4	0.52	8.4	32.2	20.3	0.78

No.: Number of samples **Min: Minimum concentration of biogenic amines**
*** : Concentration of spices** **Max: Maximum concentration of biogenic amines**
S.E.: Standard error

Table (2) Concentration of biogenic amines(mg/100g) in minced meat mixed with spices and stored at 4ºC for 1 day.

Biogenic amine Minced meat	No.	Cadaverine				Histamine				Putrescine			
		Min.	Max.	**Mean**	S.E.	Min.	Max.	Mean	S.E.	Min	Max	Mean	S.E
0.0%*	10	6.2	13.4	9.8	0.43	2.3	20.1	11.2	0.82	9.6	34.6	22.1	0.68
0.1%	10	5.3	12.1	8.7	0.62	5.7	13.5	9.6	0.57	7.3	34.1	20.7	0.71
0.3%	10	3.9	12.5	8.3	0.81	2.3	16.1	9.2	0.71	10.4	29.2	19.8	0.52
0.5%	10	4.1	12.1	8.1	0.76	3.9	12.3	8.1	0.63	5.3	33.1	19.2	0.83

No.: Number of samples **Min: Minimum concentration of biogenic amines**
***: Concentration of spices** **Max: Maximum concentration of biogenic amines**
S.E.: Standard error

Table (3) Concentration of biogenic amines(mg/100g) in minced meat mixed with spices and stored at -5ºC for 2 weeks.

Biogenic amine Minced meat	No.	Cadaverine				Histamine				Putrescine			
		Min.	Max.	**Mean**	S.E.	Min.	Max.	Mean	S.E.	Min	Max	Mean	S.E
0.0%*	10	3.2	14.2	8.7	0.91	7.3	12.4	9.7	0.43	9.8	32.4	20.1	0.48
0.1%	10	5.1	10.7	7.9	0.53	5.9	10.7	8.3	0.47	7.6	29.6	18.6	0.57
0.3%	10	2.9	10.1	6.5	0.73	3.7	10.5	7.1	0.71	8.2	22.4	15.8	0.73
0.5%	10	0.0	11.6	5.8	1.08	1.3	11.1	6.2	0.93	5.8	18.8	12.3	0.62

No.: Number of samples **Min: Minimum concentration of biogenic amines**
***: Concentration of spices** **Max: Maximum concentration of biogenic amines**
S.E.: Standard error

Table (4) Frequency of biogenic amine contents in the examined samples that higher than the regulatory limit (10mg / 100g) obtained by FDA(2001).

Biog.amine	**No**	Cadaverine			Histamine			Putrescine		
Minced Meat		Room temp. No(%)	4°ºC No(%)	-5ºC No(%)	Room temp. No (%)	4°ºC No(%)	-5ºC No(%)	Room temp. No(%)	4°ºC No(%)	-5ºC No(%)
0.0%*	10	6(60%)	5(50%)	3(30%)	8(80%)	7(70%)	5(50%)	10(100 %)	9(90%)	8(80%)
0.1%	10	4(40%)	5(50%)	2(20%)	7(70%)	6(60%)	3(30%)	10(100 %)	8(80%)	6(60%)
0.3%	10	3(30%)	4(40%)	1(10%)	5(50%)	6(60%)	1(10%)	9(90%)	8(80%)	4(40%)
0.5%	10	3(30%)	3(30%)	0(00%)	4(40%)	4(40%)	1(10%)	7(70%)	6(60%)	3(30%)

No.: Number of samples
*** : Concentration of spices**

Table (5) Incidence of biogenic amine contents in the examined samples that higher than permissible limit (20mg / 100g) obtained by EOS (1996).

Biog.amine	**No**	Cadaverine			Histamine			Putrescine		
Minced Meat		Room temp. No(%)	4°ºC No(%)	-5ºC No(%)	Room temp. No (%)	4°ºC No(%)	-5ºC No(%)	Room temp. No(%)	4°ºC No(%)	-5ºC No(%)
0.0%*	10	0(0%)	0(0%)	0(0%)	1(10%)	2(20%)	0(0%)	6(60%)	7(70%)	4(40%)
0.1%	10	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	5(50%)	4(40%)	3(30%)
0.3%	10	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	6(60%)	4(40%)	2(20%)
0.5%	10	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	4(40%)	3(30%)	0(0%)

No.: Number of samples *** : Concentration of spices**

Conclusion

High levels of biogenic amines in foods are of public health significance because of their potential toxic effects. Toxic levels of biogenic amines cause reddening of the skin, stomach trouble and migraine. Histamine and other food amines as; putrescine and cadaverine have vaso- active properties and, in some cases, they can reach concentrations in foods, which are dangerous for the most sensitive consumers (Rice et al., 1976 and Maijala et al., 1993).

Moreover the quality of the raw material in minced meat appears to be one of the main factors affecting biogenic amine formation whose levels increase in conjunction with microbial spoilage under favorable conditions (Edwards and Sandine, 1981 and Marino et al. 2000).

It is concluded that the high biogenic amine levels found in the tested samples indicate poor handling and/or processing of these products. Therefore, education on hygienic handling and manufacturing of raw materials and proper process control is recommended for local health authorities and producers. Moreover, the addition of natural spices (as; cumin, coriander, black pepper and red pepper) during processing of meat products has an inhibitory effect on the biogenic amine formation and improve the keeping quality of these products