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Biogenic Amines and Endotoxin Content Associated with the Bacterial Numbers of Ground Meat Sold in Bangkok and Nearby Areas

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ABSTRACT

Ninety samples of ground meat; beef, pork and chicken were collected from fresh market and supermarket in Bangkok and nearby areas. Comparing the sources where the samples were collected, microbial loads appeared to be higher in samples from fresh market than supermarket.

Changes of biogenic amines concentrations, putrescine and cadaverine were studied in parallel with the development of the microbial population during the storage of ground pork at 0 and 8°C. Changes in sensory scores were also recorded. Pseudomonads and H_2S producing bacteria were found to be the dominant microorganisms. Significant changes in putrescine and cadaverine concentration did not occur until the total viable count and pseudomonads exceeded $\log_{10}$ 6 to $\log_{10}$ 7 CFU/g. Cadaverine concentration increased more rapidly than that of putrescine.

Endotoxins were determined by the Limulus amoebocyte lysate test (LAL). LAL titres increased from 10 to 10^5 during storage of ground pork and correlated with TVC, pseudomonads and Enterobacteriaceae counts were at both 0 and 8°C.

Key words : putrescine, cadaverine, endotoxin, ground meat

INTRODUCTION

The acceptability of fresh meat for consumption is usually assessed organoleptically and/or by the measurement of bacterial numbers. Time-consuming microbiological analyses may be replaced by analyses of chemical changes associated with microbial growth on meat. Quantifying chemical changes could provide information about the degree of spoilage. Microbial spoilage in meat leads to the formation of enzymes known as decarboxylases which, under appropriate conditions, can convert amino acids to their corresponding amines. Among chemical indicators, biogenic amines, particularly putrescine and cadaverine, have been proposed for determining meat quality. These compounds are found in very low levels in fresh meat, and their formation is associated with bacterial spoilage (Dainty, 1996).

Putrescine is the decarboxylation product of the amino acid lysine, and cadaverine arises from the decarboxylation of ornithine. Several studies have shown that pseudomonads are responsible for the decarboxylation of these two amino acid (Okozumi *et al*., 1990; Edwards *et al*., 1983).

The Limulus amoebocyte lysate (LAL) test is well established as a sensitive and rapid test for bacterial endotoxins (Jay *et al*., 1979). There are

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close relationship between endotoxin content and microbial quality (Jay, 1981; Sullivan *et al*., 1983). Most results concerning the relationship of bacterial number, endotoxin content and biogenic amines have been reported for fish species (Dalgaard, 1995; Koutsoumanis *et al*., 1999) while, no data are available on changes of biogenic amines during spoilage of ground pork. In addition, few studies have been made on the relation between bacterial counts and biogenic amine production. The objectives of the study therefore, were to investigate changes in biogenic amines during storage of ground pork and to compare these changes with bacterial counts and endotoxin production.

MATERIALS AND METHODS

Meat samples

All ground meat (90 samples) were obtained commercially from fresh market and supermarket in Bangkok and nearby areas. It was consisted of ground beef (n = 30), ground pork (n = 30) and ground chicken (n = 30). The samples were transported to the laboratory in ice box and the experiments were performed immediately.

Microbiological analysis

1. Total Viable Count was determined according to AOAC (1990) section 966.23 (C).

2. Enterobacteriaceae were determined according to ICMSF (1978) by using Violet Red Bile Glucose Agar (VRBGA; Merck, Darmstadt), incubated at 30°C for 24h. The large colonies with purple halos were counted.

3. Lactic acid bacteria were determined by using MRS Agar (Merck, Darmstadt), incubated at 30°C for 3 days (De Man *et al*., 1960).

4. Pseudomonads were determined by using Pseudomonas Selective Agar Base (Merck, Darmstadt) (ICMSF, 1978).

5. H_2S producing bacteria were determined by using Iron Agar (Oxoid, Basingstoke), incubated at 30°C. Black colonies formed by the production of H_2S were enumerated after 48 h (ICMSF , 1978).

Analysis of biogenic amines by HPLC

Five grams of pork were homogenized with 10 ml 6% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged (12,000 rpm, 20 min, 4°C) and filtered through Whatman No. 2 filter paper. The filtrate was placed in a volumetric flask and enough 6% TCA was added to make 20 ml. Each extract (2 ml) was derived with benzoyl chloride according to the modified method of Yen and Hsieh (1991).

To prepare standard amine solutions, dissolved putrescine –2HCl (182.9 mg) and cadaverine –2HCl (171.4 mg) in 10 ml deionized water . The final concentration of each amine (free base) was 10 mg/ml solution.

The presence of amines was determined using a Jasco (Japan) Liquid Chromatograph consisting of a Model PU-980 Intelligent pump, a Model LG-980-02 ternary gradient unit, and a MD-910 multiwavelength detector. A LiChrospher 100 RP-18 reverse – phase column (5 mm, 125× 3 mm interior diameter E. Merck, Germany) was used for separation.

The gradient elution program was at 1.1 ml/min, starting with a 70:30 (v/v) water – acetonitrile mixture for 4 min. The program proceeded linearly to 50:50 (v/v) water – acetonitrile with the same flow rate over 2 min. This composition and flow rate were maintained for 3 min, then changed to 70:30 (v/v) water – acetonitrile at 1.1 ml/min for 2 min. The whole spectra (190-800 nm) of the chromatograms were analyzed. The solvents were HPLC grade. To identify peaks, solutions of reference substances were analyzed using the same program, and their retention times and spectra were compared. The precision of the results was always better than ± 5%.

Sensory evaluation

A scoring scale with three categories was used : Class 1 high – quality without any off – odors

or off – flavors; Class 2, to slight off – odors or – flavors but acceptable quality (initial decomposition stage); Class 3, to unacceptable quality (advanced decomposition stage).

Determination of endotoxin

Endotoxin was determined by using of E-toxate® (Limulus Amoebocyte Lysate) (Sigma Chemical Company, St. Louis, MO, U.S.A) and used according to the directions of the manufacturer. The sensitivity of the endotoxin was 0.05 endotoxin units (EU) per ml. *Escherichia coli* O55 : B5 (lipopolysaccharide) were used as endotoxin standard. A 1.0 g sample was homogenized and 9.0 ml of distill water were added. Tenfold dilutions (0.1 ml) of the prepared homogenate were incubated with LAL (0.1 ml) at 37°C for 60 min. Endotoxin level (nanograms of endotoxin per gram of sample) was calculated by multiplying the reciprocal of the highest sample dilution that resulted in formation of a solid gel by the determined sensitivity of LAL. In this study, LAL sensitivity was consistently 0.05 ng of *E. coli* endotoxin per ml.

RESULTS AND DISCUSSION

There were no obvious differences between ground beef, pork and chicken in the initial loads of TVC and enterobacteriaceae except when the results were compared between supermarket and fresh market. Fresh market samples tended to have microbial loads more than supermarket which might be due to the difference in sanitation as well as the storage temperatures as shown in Table 1.

The changes in the microbial flora of ground pork during storage under aerobic conditions at 0 and 8°C are shown in Table 2 and 3 respectively. Total viable counts reached approximately 9.0 $\log_{10}$ cycles (CFU/g) by the end of the storage periods, regardless the temperature. Pseudomonads were the dominant population of ground pork stored at all temperatures, followed by H_2S producing bacteria. These findings agreed with those of Drosinos and Nychas (1998), who studied fish and reported that the microbial population of fish stored aerobically consisting almost exclusively of pseudomponads and H_2S producing bacteria. The counts of the H_2S producing bacteria were always lower than those of pseudomonads at the end of the storage period. Enterobacteriaceae and lactic acid bacteria were also members of the microbial population, but these groups remained at low numbers (< $\log_{10}$ 5 and < $\log_{10}$ 6 CFU/g) during storage of ground pork at 0 and 8°C respectively.

Table 1 Comparison of Total viable count[a] and Enterobacteriaceae[b] isolated from ground meat between supermarket and fresh market.

Type of samples	Supermarket (n = 15)		Fresh market (n = 15)	
	Total viable count (Mean)	Enterobacteriaceae (Mean)	Total viable count (Mean)	Enterobacteriaceae (Mean)
ground beef (n = 30)	3.2 ± 0.1	2.1 ± 0.2	5.0 ± 0.5	4.6 ± 0.3
ground pork (n = 30)	3.0 ± 0.3	2.7 ± 0.3	5.9 ± 0.3	4.8 ± 0.2
ground chicken (n = 30)	3.4 ± 0.1	2.3 ± 0.1	5.3 ± 0.5	4.0 ± 0.5

[a] $\log_{10}$ CFU/g
[b] $\log_{10}$ CFU/g

Table 2 Changes in microbial flora[a], concentration of biogenic amine[b] during the storage of ground pork at 0°C.

	Storage time (day)						
	0	2	4	6	8	10	12
Total viable counts	3.6 ± 0.2	5.0 ± 0.5	5.6 ± 0.5	7.3 ± 0.5	8.0 ± 0.5	8.5 ± 0.7	9.1 ± 0.6
Pseudomonads	3.2 ± 0.3	4.3 ± 0.3	5.0 ± 0.3	6.2 ± 0.5	7.3 ± 0.7	8.0 ± 0.5	8.9 ± 0.6
H_2S-producing bacteria	2.8 ± 0.5	3.4 ± 0.3	3.9 ± 0.2	4.5 ± 0.7	5.7 ± 0.5	6.3 ± 0.7	7.0 ± 0.3
Enterobacteriaceae	2.0 ± 0.0	2.1 ± 0.2	2.5 ± 0.2	2.5 ± 0.6	3.7 ± 0.8	4.0 ± 0.3	4.8 ± 0.5
Lactic acid bacteria	1.2 ± 0.3	1.5 ± 0.0	1.4 ± 0.3	1.7 ± 0.5	2.0 ± 0.7	2.4 ± 0.5	3.5 ± 0.7
Biogenic amines							
Putrescine	0	0	0	0.15 ± 0.1	0.36 ± 0.3	0.72 ± 0.3	1.40 ± 0.2
Cadaverine	0	0	0	0.28 ± 0.0	0.41 ± 0.1	0.84 ± 0.2	1.38 ± 0.5
Sensory rating	1	1	1	2	2	3	3

[a] $\log_{10}$ CFU/g
[b] mg/100g
sensory rating 1 = without any off-odors
2 = slight off-odors
3 = unacceptable quality

Table 3 Changes in microbial flora[a], concentration of biogenic amine[b] during the storage of ground pork at 8°C.

	Storage time (day)				
	0	2	4	6	8
Total viable counts	3.6 ± 0.2	6.5 ± 0.3	8.2 ± 0.2	8.9 ± 0.3	9.6 ± 0.5
Pseudomonads	3.2 ± 0.3	6.0 ± 0.5	7.0 ± 0.5	7.7 ± 0.1	8.3 ± 0.2
H_2S-producing bacteria	2.8 ± 0.5	5.1 ± 0.5	7.0 ± 0.5	7.1 ± 0.1	7.5 ± 0.2
Enterobacteriaceae	2.0 ± 0.0	3.9 ± 0.6	5.3 ± 0.3	5.4 ± 0.5	5.5 ± 0.3
Lactic acid bacteria	1.2 ± 0.3	2.7 ± 0.3	4.1 ± 0.7	4.3 ± 0.3	4.6 ± 0.2
Biogenic amines					
Putrescine	0	0.16 ± 0.2	0.62 ± 0.1	1.13 ± 0.4	1.32 ± 0.2
Cadaverine	0	0.19 ± 0.1	0.87 ± 0.1	1.48 ± 0.2	1.61 ± 0.1
Sensory rating	1	2	3	3	3

[a] $\log_{10}$ CFU/g
[b] mg/100g
sensory rating 1 = without any off-odors
2 = slight off-odors
3 = unacceptable quality

In samples stored at 0°C, putrescine and cadaverine were not detected before 6 days, which sensory evaluation defined as the begining of the initial decomposition stage (Table 2). In particular, these diamines were produced only when pseudomonads reached approximately $\log_{10}6$ CFU/g. Ground pork stored at 8°C could obtain putrescine and cadaverine within 2 days, with the initial decomposition stage observable, while numbers of pseudomonads reached around $\log_{10}6$ CFU/g (Table 3). It could be noted that as decomposition progressed, putrescine and cadaverine increased. The cadaverine concentration increased more rapidly than that of putrescine. Okozumi et al., (1990) showed that pseudomonads were dominant and putrescine and cadaverine were at high levels in the bacterial flora of spoiled horse mackerel stored at 5°C. Suzuki *et al.* (1988) also confirmed the production of putrescine by pseudomonads.

Microbial numbers and endotoxin content of ground pork held at 0 and 8°C over a 10 day period are shown in Table 4 and 5. The TVC for both 0 and 8°C showed similar increases in bacterial numbers from days 0 to 10. The endotoxin level increased parallel to the TVC from days 0 to 10. The same results could be obtained from pseudomonads numbers and Enterobacteriaceae. The LAL titre increased from 10 to 10^4 and 10 to 10^5 in ground pork held at 0°C and 8°C respectively. Sensory evaluation also showed that LAL titre 10^3

Table 4 Microbial numbers and endotoxin content of ground pork held at 0°C.

Days held	Total viable count ($\log_{10}$ CFU/g)	Pseudomonads ($\log_{10}$ CFU/g)	Enterobacteriaceae ($\log_{10}$ CFU/g)	LAL titre	Endotoxin[a] (ng/g)	Sensory rating
0	3.5 ± 0.1	3.4 ± 0.1	2.2 ± 0.1	10	0.5	1
3	5.0 ± 0.3	4.7 ± 0.2	2.5 ± 0.2	10^2	5	1
5	5.7 ± 0.2	5.2 ± 0.2	2.3 ± 0.1	10^2	5	1
7	7.1 ± 0.5	6.8 ± 0.3	3.4 ± 0.3	10^3	50	2
10	8.3 ± 0.5	8.0 ± 0.5	4.1 ± 0.3	10^4	500	3

[a] sensitivity of endotoxin was 0.05 ng
sensory rating 1 = without any off-odors
2 = slight off-odors
3 = unacceptable quality

Table 5 Microbial numbers and endotoxin content of ground pork held at 8°C.

Days held	Total viable count ($\log_{10}$ CFU/g)	Pseudomonads ($\log_{10}$ CFU/g)	Enterobacteriaceae ($\log_{10}$ CFU/g)	LAL titre	Endotoxin[a] (ng/g)	Sensory rating
0	3.7 ± 0.1	3.3 ± 0.1	3.0 ± 0.2	10	0.5	1
3	7.0 ± 0.3	6.1 ± 0.1	4.2 ± 0.5	10^3	50	2
5	8.1 ± 0.3	7.2 ± 0.3	5.2 ± 0.3	10^4	500	3
7	8.5 ± 0.5	8.0 ± 0.5	5.8 ± 0.5	10^5	5,000	3
10	9.8 ± 0.8	8.7 ± 0.5	5.9 ± 0.6	10^5	5,000	3

[a] sensitivity of endotoxin was 0.05 ng
sensory rating 1 = without any off-odors
2 = slight off-odors
3 = unacceptable quality

was the begining of the initial decomposition stage. It appeared from the above data that good quality, stored ground pork might be expected to produce LAL titres below 10^3.

This confirms the role of TVC, pseudomonads and Enterobacteriaceae in the response of LAL as fresh pork spoiled since it is well established that the refrigerator spoilage of fresh meats such as ground pork caused by these organisms, especially when packed in a manner that allows gas exchange. Sullivan *et al*., (1983) have demonstrated that endotoxin level can be used to predict TVC level and therefore freshness of lean fish.

Overall, fresh ground pork that had low endotoxin levels had low TVC, pseudomonads and Enterobacteriaceae counts. Because LAL produces results in only 1 h, the finding of low levels of endotoxin would suggest a product of excellent microbial quality.

CONCLUSION

Putrescine and cadaverine detected in this study, could be used as freshness indicators of ground pork. They were detected before initial decomposition, and increased markedly during storage at all temperatures. Endotoxin content could also be used as freshness indicator of ground pork and result could be obtained within 1 h. Correlation of TVC, pseudomonads and Enterobacteriaceae counts with putrescine, cadaverine and endotoxin content were noted. It indicated that the determination of putrescine, cadaverine and endotoxin content could be used to assess freshness in ground pork.

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