

## Research Article

### Biogenic Amines Formation and its Relation to Microbiological in air-Packaged Channel Catfish (*Clarias gariepinus*) Fillets stored at -0.7°C and 4°C

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**Abstract:** The biogenic amines formation in catfish (*Clarias gariepinus*) fillets with Air-Package (AP) kept for 15 days at -0.7°C and 10 days at 4°C were investigated using seven biogenic amines, Total Viable Counts (TVC) and psychrophilic Counts (PC). All amines, except spermidine and spermine in the fillets increased with time during storage at both temperatures. The change of putrescine, cadaverine, histamine and tyramine showed the best correspondence with microbial states of the samples suggesting the microbial pathways. Low storage temperature (-0.7°C) had a dominant effect, resulting in lower biogenic amine content. The observed shelf-life of catfish fillet were 7 days for 4°C sample and 10 days for -0.7°C sample. Hence, storage at -0.7°C can effectively extend the shelf life 3 days.

**Keywords:** Biogenic amines, channel catfish, microbiology, quality change

## INTRODUCTION

Biogenic Amines (BAs), a special category of basic nitrogenous compounds with low molecular weights, are mainly formed through the microbial decarboxylation of specific free amino acids in aquatic products (Hu *et al.*, 2012). Low levels of BAs in aquatic products do not present a serious risk to human health because the amine oxidases in human intestine can rapidly detoxify the amines. However, ingestion of large amounts may result in health problems (Mohamed *et al.*, 2010).

Fish are a rich source of high-quality protein, essential vitamins and healthful polyunsaturated fatty acids. But the high content of proteins represents a risk in the decomposition processes and yields peptides and amino acids, which are susceptible to further decay, resulting in biogenic amines.

The amount and type of biogenic amines formed is therefore influenced by the food composition, microbial flora and by several parameters, which promote bacterial growth during storage-such as temperature and packaging (Halasz *et al.*, 1994).

Channel catfish (*Clarias gariepinus*) consumption has grown rapidly due to its availability, good quality and healthy attributes (Ambardekar and Reigh, 2007). In contrast to the number of studies of changes in

biogenic amine contents in sea fish (Veciana-Nogues *et al.*, 1997; Poli *et al.*, 2001; Bakar *et al.*, 2010), there is little information on channel catfish. The objective of the present work was to determine how the temperature of storage can affect biogenic amine formation in channel catfish (*Clarias gariepinus*) fillets.

## MATERIALS AND METHODS

**Reagents:** Standard amines, containing tryptamine (TRY), 2-Phenylethylamine (PHE), Putrescine (PUT), Cadaverine (CAD), Histamine (HIS), Spermidine (SPD), Spermine (SPM) and Tyramine (TYR) and were obtained from Sigma (St. Louis, MO, USA). All chemicals used were of analytical grade.

**Sample preparation:** A total number of 20 live catfish (*Clarias gariepinus*) (average weight and length: 1250±25 g and 30±2 cm) were purchased from a local aquaculture farm at Hongqi (Tianjin, China). They were packed in insulated boxes and transferred to the laboratory within 15 min, then immediately processed in a walk-in cold room. Fish were dizzied in ice water (5-7°C for 10 min), deheaded, deskinning and eviscerated. Wash with sterile iced water, drained and cut the fillet into 4×3×1 cm<sup>3</sup> in size. Finally fillets were packaged by Air-Packaging (AP), two aliquots of

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fillets were stored separately in  $-0.7^{\circ}\text{C}$  and  $4^{\circ}\text{C}$  refrigerator (Department of Food Science, Tianjin Agricultural University, Tianjin, China) till the day they were considered spoiled judged by the presence of offensive odor. The fish quality during storage was evaluated at day 0, 1, 4, 7, 10 for  $4^{\circ}\text{C}$  samples and at day 0, 1, 4, 7, 10 and 15 for  $-0.7^{\circ}\text{C}$  samples.

**Microbiological analysis:** 10 g minced sample were homogenised with 90 mL sterile 0.85% saline to obtain 1:10 sample suspension. From this mixture, Series of decimal dilutions were made and spread onto Plate count agar (Aobox, 00-275). For Total Viable Count (TVC), plates were incubated at  $30^{\circ}\text{C}$  for 24-48 h; for psychrophilic Count (PC), plates were at  $7^{\circ}\text{C}$  for 10 days (Ariyapitipun *et al.*, 1999).

**Biogenic Amines (BAs) analysis:** Tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine were determined as

their dansyl derivatives, following the high-performance liquid chromatography. Sample preparation and extraction were done according to the method of Hu *et al.* (2012). The quantification of BAs was carried out using an Agilent 1200 HPLC unit (Agilent technologies Inc. Santa Clara, CA) consisting of a quaternary pump and a Diode Array Detector (DAD). BAs were separated using an Agilent Zorbax Extend-C18 column (4.6 mmI.D. $\times$ 150 mm) and were eluted with a binary solution consisting of ultrapure water (solvent A) and acetonitrile (solvent B) according to the optimised gradient elution program described as following: 0 min, 65% B; 5 min, 70% B; 20 min, 100% B; 24 min, 100% B; 25 min, 65% B; 30 min, 65% B. BAs were identified on the basis of retention time compared with standard solutions. The flow rate was 1.0 mL/min and the column temperature was set as  $30^{\circ}\text{C}$ . A sample volume of 20  $\mu\text{L}$  was injected and the detecting wavelength was 254 nm.

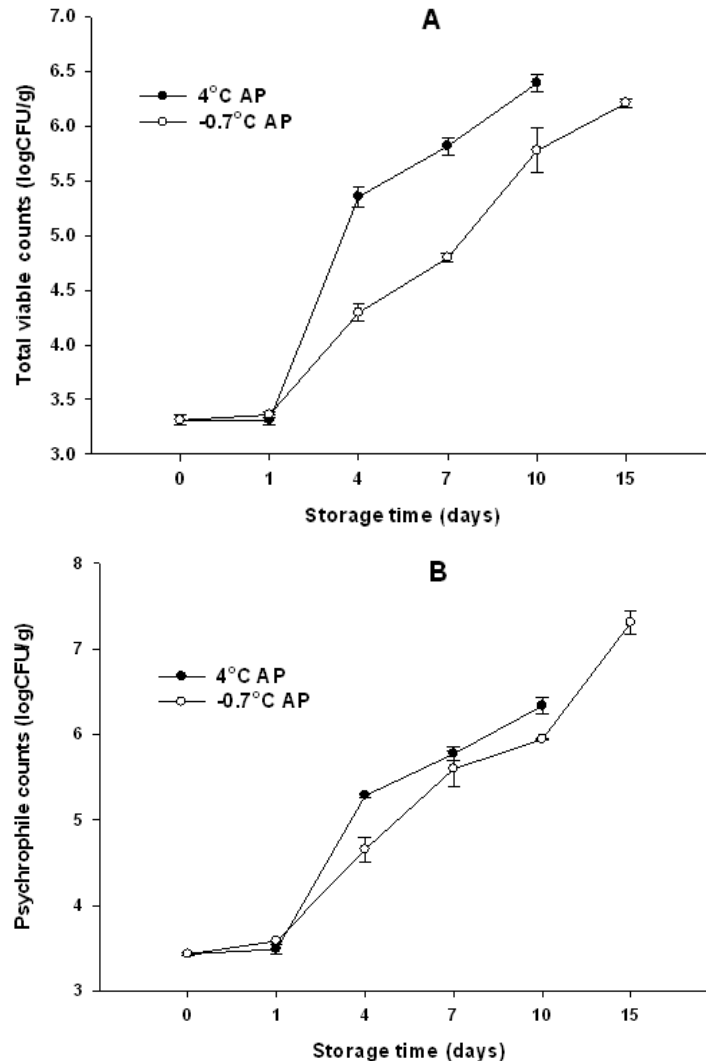


Fig. 1: Changes in total viable counts; (A): psychrophilic counts; (B): of catfish fillet, for stored at  $4^{\circ}\text{C}$  or  $-0.7^{\circ}\text{C}$ , with Air-Packaged (AP). The results are the mean of three replications and the bars indicate the standard deviation

**Quality index and biogenic amines index:** The quality index and the biogenic amine index were calculated according to the procedures described by Mietz and Karmas (1977) and Veciana-Nogues *et al.* (1997) respectively. The formulae used were as follows:

- Quality Index (QI) = 
$$\frac{(\text{histamine} + \text{putrescine} + \text{cadaverine})}{(1 + \text{spermidine} + \text{spermine})}$$
- Biogenic Amine Index (BAI) = (histamine + putrescine + tyramine)

**Statistical analysis:** Data from the different chemical measurements were subjected to one-way analysis of variance; comparison of means was performed using a Least-Squares Difference (LSD) method (Statsoft, 1994). The SPSS software (SPSS Inc., Chicago, IL) was also used to explore the statistical significance of the differences between batches, this including multivariate contrasts and multiple comparisons by the Scheffe' and Tukey tests. A confidence interval at the 95% level ( $p < 0.05$ ) was considered in all cases.

## RESULTS AND DISCUSSION

**Total Viable Count (TVC) and Psychrophilic Counts (PC):** Figure 1 shows the Total Viable Counts (TVC, A) and Psychrophilic Counts (PC, B) in AP catfish fillets stored at 4°C and -0.7°C. Higher counts were observed at 4°C as compared to the counts at -0.7°C. At day 0, the initial aerobic colony counts were 3.31 log CFU/g. At day 10 TVC were to 6.39 and 5.78 log CFU/g for samples stored at 4°C and -0.7°C, respectively. If 6 log CFU/g is taken as the TVC limit of acceptability, therefore, the shelf-life of catfish was approximately 7 and 10 days at 4°C and -0.7°C, respectively. There were significant differences ( $p < 0.05$ ) in TVC of fillets stored at -0.7°C and at 4°C after storage of day 4. This indicates storage at -0.7°C was better in inhibiting microbial reproduction than storage at 4°C. El Marrakchi *et al.* (1990) also reported that the total viable counts in sardines stored at ambient temperature exceeded the acceptable limit faster as compared to those kept on ice.

Figure 1 also shows the changes of PC in catfish fillets during storage at -0.7°C and 4°C. The PC grew faster in catfish fillets stored at 4°C, as compared to at -0.7°C. As a result, the microbial growth and reproduction was inhibited at low temperature (-0.7°C) in this study.

**Comparative Biogenic Amines (BAs) development:** The concentrations of seven biogenic amines (putrescine, cadaverine, tyramine, phenylethylamine, spermidine, spermine and histamine) present in the muscle of catfish stored at -0.7°C for 15 days and 4°C for 10 days are given in Table 1 and tryptamine haven't be detected in all samples.

For 4°C storage, four amines were detected on the day 1 and seven amines were detected on the day 10 of storage. A significant increase in histamine, putrescine, cadaverine and tyramine were observed throughout the storage period ( $p < 0.05$ ) (Table 1). At the day 10, these amines concentrations reached maximum levels of 36.4 mg/kg (putrescine), 13.1 mg/kg (cadaverine), 6.3 mg/kg (histamine) and 6.2 mg/kg (tyramine) in descending order and became the dominant amines. Valle *et al.* (1996) found that when herring (*Clupea harengus*) was inedible, putrescine and cadaverine contents of the herring stored at 0°C were at 10.1 and 23 mg/kg, respectively. They also reported that plaice (*Pleuronectes platessa*) and whiting (*Merlangius merlangus*) contained 15.7 and 58 mg/kg putrescine and 9.1 and 9.2 mg/kg cadaverine, respectively.

Although spermidine, spermine and 2-phenylethylamine contents of catfish showed fluctuations, they increased to 3.9, 4.9 and 5.1 mg/kg, respectively, at the end of the storage. These findings are generally in agreement with the amine values reported by Ritchie and Mackie (1979) for mackerel (*Scomber scombrus*).

For -0.7°C storage, a similar change were observed and putrescine, tyramine histamine and cadaverine became the dominant amines after storage of day 7 and increased to maximum levels of 38.9 mg/kg (putrescine), 21.0 mg/kg (tyramine), 16.9 mg/kg (histamine) and 12.7 mg/kg (cadaverine) on the day 15, respectively. On the whole, samples stored at -0.7°C had lower concentrations of the biogenic amines as compared to those stored at 4°C. Higher storage temperature seemed to produce a higher rate of formation for these amines. Middlebrooks *et al.* (1988) also reported that the levels of histamine, cadaverine and putrescine and the time and temperature of decomposition in Spanish mackerel (*Scomberomorus maculatus*) showed a strong correlation ( $p < 0.05$ ).

Histamine has been known as the contributory toxin of fish poisoning (Önal, 2007). In this study, the level of histamine didn't exceeded the 50 mg/kg legal limit set by the US Food and Drug Administration (FDA). The content of histamine in fillets of channel catfish was significantly lower than marine fish species such as herring (Özogul *et al.*, 2002) or sardines (Özogul and Özogul, 2006) associated with histamine poisoning. Other freshwater fish species such as oilfish, Nile perch, pangasius catfish and African catfish available in polish market did not show high levels of histamine (Polak-Juszczak, 2007) that might suggest that freshwater fish species are less susceptible for the growth of the histamine level.

Contents of cadaverine, putrescine, histamine and tryptamine, seem to be good quality markers, they were in the best correlation with storage time in both storage temperature ( $r = 0.766 \sim 0.933$ ,  $p < 0.01$ ) (Table 2) within all experiment.

Table 1: Concentration of biogenic amines in samples of catfish fillets, for storage at 4°C or -0.7°C, with Air-Packaged (AP). The results are the mean of three replications± the standard deviation

|           |    | PUT                   | PHE                   | CAD                   | HIS                   | TYR                   | SPD                   | SPM                    | TBA                    |
|-----------|----|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|
| 4°C-AP    | 0  | 3.1±0.1 <sup>C</sup>  | 1.0±0.04 <sup>B</sup> | ND <sup>C</sup>       | ND <sup>C</sup>       | ND <sup>B</sup>       | 4.58±1.4 <sup>A</sup> | 4.1±0.3 <sup>B</sup>   | 12.6±1.8 <sup>C</sup>  |
|           | 1  | 4.4±0.9 <sup>C</sup>  | 7.3±0.8 <sup>B</sup>  | 0.1±0.1 <sup>C</sup>  | ND <sup>C</sup>       | ND <sup>B</sup>       | 7.1±0.7 <sup>A</sup>  | 4.5±2.0 <sup>B</sup>   | 23.4±1.2 <sup>BC</sup> |
|           | 4  | 9.0±0.5 <sup>B</sup>  | 5.1±0.1 <sup>B</sup>  | 1.1±0.1 <sup>C</sup>  | ND <sup>C</sup>       | 4.5±0.2 <sup>A</sup>  | 3.2±3.0 <sup>A</sup>  | 20.6±20.5 <sup>B</sup> | 30.0±3.3 <sup>B</sup>  |
|           | 7  | 3.6±0.4 <sup>C</sup>  | 6.3±0.3 <sup>B</sup>  | 3.8±0.7 <sup>B</sup>  | 3.5±0.2 <sup>B</sup>  | 5.0±1.7 <sup>A</sup>  | 1.6±1.1 <sup>A</sup>  | 6.2±0 <sup>B</sup>     | 30.0±4.1 <sup>B</sup>  |
|           | 10 | 36.4±4.8 <sup>A</sup> | 5.1±0.3 <sup>A</sup>  | 13.1±0.1 <sup>A</sup> | 6.3±0.1 <sup>A</sup>  | 6.2±0.4 <sup>A</sup>  | 3.9±1.5 <sup>A</sup>  | 4.9±0.5 <sup>A</sup>   | 119.3±3.7 <sup>A</sup> |
| -0.7°C-AP | 0  | 3.1±0.1 <sup>C</sup>  | 1.0±0.04 <sup>C</sup> | ND <sup>C</sup>       | ND <sup>D</sup>       | ND <sup>D</sup>       | 4.5±1.4 <sup>AB</sup> | 4.1±0.3 <sup>B</sup>   | 12.6±1.8 <sup>D</sup>  |
|           | 1  | 3.4±0.4 <sup>C</sup>  | 5.0±0.1 <sup>B</sup>  | ND <sup>C</sup>       | ND <sup>D</sup>       | ND <sup>D</sup>       | 6.0±2.7 <sup>AB</sup> | 4.1±0.2 <sup>B</sup>   | 18.5±2.1 <sup>CD</sup> |
|           | 4  | 3.6±0.5 <sup>C</sup>  | 4.5±0.7 <sup>B</sup>  | 0.3±0.1 <sup>C</sup>  | ND <sup>D</sup>       | 3.9±0.2 <sup>CD</sup> | 0.37±2.9 <sup>B</sup> | 6.5±0.4 <sup>A</sup>   | 19.2±2.9 <sup>CD</sup> |
|           | 7  | 4.2±1.1 <sup>C</sup>  | 4.6±0.04 <sup>B</sup> | 2.8±0.1 <sup>B</sup>  | 1.4±0.4 <sup>C</sup>  | 6.7±3.4 <sup>C</sup>  | ND <sup>B</sup>       | 6.4±0.2 <sup>A</sup>   | 26.1±2.8 <sup>C</sup>  |
|           | 10 | 9.5±0.4 <sup>B</sup>  | 5.0±0.2 <sup>B</sup>  | 3.9±0.7 <sup>B</sup>  | 3.3±0.1 <sup>B</sup>  | 13.4±0.2 <sup>B</sup> | ND <sup>AB</sup>      | 6.7±0.4 <sup>A</sup>   | 64.6±2.9 <sup>B</sup>  |
|           | 15 | 38.9±2.2 <sup>A</sup> | 13.6±0.9 <sup>A</sup> | 12.7±0.4 <sup>A</sup> | 16.9±0.3 <sup>A</sup> | 21.0±0.4 <sup>A</sup> | 9.8±0.2 <sup>A</sup>  | 7.6±0.4 <sup>A</sup>   | 149.8±4.4 <sup>A</sup> |

<sup>a</sup>: Mean value (mg/kg); <sup>b</sup>: Means within the same column with different superscripts are significantly (p<0.05) different; <sup>c</sup>: ND-not determined; <sup>d</sup>: PHE phenylethylamine; PUT putrescine; CAD cadaverine; HIS histamine; TYM tyramine; SPD spermidine; SPM spermine

Table 2: Pearson's correlation coefficients between biogenic amine and storage time, total viable counts, psychrophilic counts

| Biogenic amine              | Storage time | Total viable counts | Psychrophilic counts |
|-----------------------------|--------------|---------------------|----------------------|
| 2-phenylethylamine          | 0.667*       | 0.591               | 0.595                |
| Putrescine                  | 0.766**      | 0.718*              | 0.724*               |
| Cadaverine                  | 0.867**      | 0.821**             | 0.832**              |
| Histamine                   | 0.866**      | 0.781**             | 0.790**              |
| Tyramine                    | 0.933**      | 0.857**             | 0.871**              |
| Spermidine                  | 0.064        | -0.054              | -0.041               |
| Spermine                    | 0.590        | 0.643*              | 0.638*               |
| Total Biogenic Amines (TBA) | 0.907**      | 0.822**             | 0.830**              |
| Quality Index (QI)          | 0.948**      | 0.881**             | 0.900**              |
| Biogenic Amine Index (BAI)  | 0.936**      | 0.859**             | 0.870**              |

<sup>a</sup>N = 30; <sup>b</sup>ns, not significant; <sup>c</sup>\*p<0.05, <sup>d</sup>\*\*p<0.01

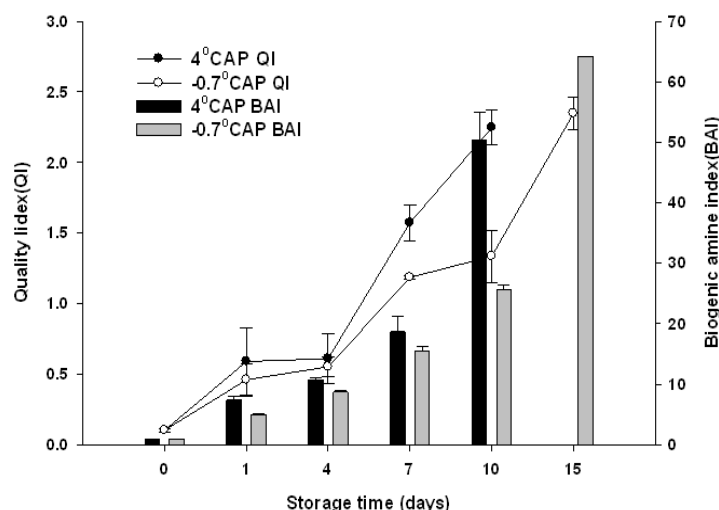


Fig. 2: Changes in Quality Index (QI) and Biogenic Amine Index (BAI) of catfish fillet, for stored at 4°C or -0.7°C, with Air-Packaged (AP). The results are the mean of three replications and the bars indicate the standard deviation

2-phenylethylamine was detected in all samples (Table 1) and fluctuated between 1.0~5.1 mg/kg during 0~10 days storage time and reached a maximum of 13.6 mg/kg (in -0.7°C samples) on the day 15. A positive correlation was shown between 2-phenylethylamine concentrations and storage time ( $r = 0.667$ ,  $p < 0.05$ ).

Spermine and spermidine are usually the major amines present in fresh muscle at concentration of less than 10 mg/kg flesh, but depending on the fish species, the free amino acids present in the tissue and the conditions of exposure to spoilage bacteria (Yassoralipour *et al.*, 2012; Hong *et al.*, 2013). In this

study, Spermidine concentrations in the samples for both temperatures were lower than spermine and the concentrations of both amines fluctuated during storage. This could be due to the fact that no significant differences in spermidine or spermine were found depending on the storage time ( $r = 0.064$ ,  $r = 0.590$ , respectively).

The changes in QI and BAI are shown in Fig. 2. During days 0-4, the QI of -0.7°C samples was not significantly different to that of 4°C samples ( $p > 0.05$ ). With increasing storage time, the QI of all samples increased. At day 7, the QI of 4°C samples greatly

increased to 1.62, indicating large amounts biogenic amine formation such as putrescine, histamine and cadaverine while the QI of -0.7°C samples increased slightly to 1.28 ( $p < 0.05$ ). Change of BAI were similar to the change of QI in catfish fillet stored at -0.7°C and 4°C during storage time. Therefore, storage at -0.7°C managed to extend the shelf-life (approximately 3 days) of catfish fillet by inhibiting biogenic amine formation compared to 4°C

**Relation of biogenic amines formation to microbial changes:** As mentioned previously, biogenic amines are usually formed through bacterial enzyme activity, while the types and levels of biogenic amines will depend on the specific microbial flora and count (Halasz *et al.*, 1994; Veciana-Nogues *et al.*, 1997). Biogenic amine production, with the exception of spermine and spermidine, was evident when bacterial load (total viable count) approached 7 log cfu/g as also reported by Dainty (1996).

Variance analysis was carried out and the results show that putrescine, cadaverine, histamine and tyramine has significant and positive influence to TVC and PC, with correlation coefficient ( $r$ ) ranged between 0.718 and 0.857 (Table 2). The correlations suggested that these amines were mainly generated by the activities of spoilage microorganisms.

Spermidine and spermine occur naturally in food and Spermidine formation is not related to bacterial spoilage in this study ( $r = -0.041 \sim -0.054$ ,  $p > 0.05$ ), however, significant correlation was shown between spermine and time ( $r = 0.638 \sim 0.643$ ,  $p < 0.05$ ).

Microbial growth and reproduction lead to a increase in the biogenic amine. The correlations between bacteria and TBA were found positive (for TVC,  $r = 0.741$ ,  $p < 0.001$ ; for PC,  $r = 0.774$ ,  $p < 0.001$ ), because BAs are formed via the microbial-mediated decarboxylation pathways. As a result, microbial quantity of samples was in accordance with TBA.

Variance analysis showed that TVC and PC have significant linear correlation with QI and BAI ( $p < 0.01$ ). Correlation coefficient that found between QI and TVC, PC was 0.881, 0.900 ( $p < 0.01$ ) and between BAI and TVC, PC was 0.859, 0.870 ( $p < 0.01$ ), respectively, suggesting QI and BAI was mainly determined by the metabolic product from microbial bacteria and could be used to determine the degree of spoilage of catfish. These values were high compared to the values reported for tuna (*Thunnus thynnus*) ( $r = 0.34$ ,  $r = 0.63$ ) by Mietz and Karmas (1977) and by Veciana-Nogues *et al.* (1997), respectively.

## CONCLUSION

The biogenic amines analyses revealed that the storage of catfish fillets at -0.7°C allowed a better maintenance of quality with an extended shelf-life up to 10 days than 4°C. Accordingly, it significantly lowered total viable counts and psychrophilic counts and

lowered increases in biogenic amines contents, these positive results obtained clearly confirmed that storage of catfish fillets with air-packaged at -0.7°C could inhibit biogenic amine formation and extend the shelf life 3 days.

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