ORIGINAL ARTICLE



# Effect of sucrose on the generation of free amino acids and biogenic amines in Chinese traditional dry-cured fish during processing and storage

Jinjie Zhang • Zhenfeng Liu • Yaqin Hu • Zhongxiang Fang • Jianchu Chen • Dan Wu • Xingqian Ye

Revised: 23 June 2010 / Accepted: 29 September 2010 / Published online: 11 November 2010  $\odot$  Association of Food Scientists & Technologists (India) 2010

**Abstract** The Chinese traditional dry-cured grass carp fish (Layú) was processed with (A) and without (B) sucrose. Higher levels of free amino acids (FAA) and biogenic amines were detected in the final products when compared to the fresh fish. In the presence of sucrose, Layú A had higher total free amino acids (39.9 g/kg DW) but lower total biogenic amines (112.5 mg/kg DW) than those in Layú B (35.4 g/kg DW and 143.7 mg/kg DW, respectively) after ageing. After 60 days, the products storaged at 4 °C had lower amount biogenic amines (Layú A: 112.2~579.5; Layú B: 144~593.8 mg/kg DW) than those at 20 °C (Layú A: 112.2~974.8; Layú B: 144~773.4 mg/kg DW), indicating that low temperature storage was a safer procedure.

Keywords Layú  $\cdot$  Dry-cured  $\cdot$  Free amino acids  $\cdot$  Biogenic amines  $\cdot$  Sucrose

## Introduction

Layú is the traditional name for the dry-cured grass carp (*Ctenopharyngodon idellus*) produced in China. It is popular not only for its special chewiness and flavor but also longer shelf life (Tan et al. 2006). In terms of nutrition,

Z. Fang School of Land, Crop and Food Sciences, The University of Queensland, Brisbane, Qld 4072, Australia cured fish is a rich source of high quality proteins as well as some vitamins and minerals (Liu et al. 2010).

Fish proteins have an excellent essential amino acid composition, which is widely recommended for a well balanced and healthy diet. The total amino acid composition of fish does not vary greatly in the raw state, however, the free amino acid composition does change considerably after processing and storage. When fermented fish age, the proteins undergo degradation and large peptides are produced initially. These large peptides are further degraded into smaller peptides and free amino acids. Finally, the free amino acids can be converted into even smaller compounds, such as ammonia,  $\alpha$ -ketoacids, methylketones and amines (Lopetcharat et al. 2001).

The biogenic amines are basic nitrogenous compounds usually formed by decarboxylation of their precursor amino acids from the action of amino acid decarboxylase enzymes (Halász et al. 1994; Kumudavally et al. 2005). In fish products, the formation of biogenic amines is closely related to the activity of the microorganisms present in the fish. In the case of cured products, large amount of specific biogenic amines can be produced as a consequence of poor quality raw materials, microbial contamination and adverse conditions during processing and storage. High temperatures, high pH values and low salt concentrations can favor the accumulation of free amino acids, but can also stimulate the formation of biogenic amines. However, during the dry-aging stage, the factors that affect enzymatic decarboxylation may be more important than the availability amount of their precursor amino acids in forming of biogenic amines (Xu et al. 2010).

The formation of biogenic amines in foods not only plays a significant role in producing off flavors, but is also important from a health and safety point of view.

J. Zhang · Z. Liu · Y. Hu · Z. Fang · J. Chen · D. Wu · X. Ye (⊠) Department of Food Science and Nutrition, School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310029, Zhejiang Province, China e-mail: psu@zju.edu.cn

Consuming foods with excessive levels of biogenic amines can affect blood pressure and cause migraines, gastric and intestinal problems and allergic responses (Lehane and Olley 2000; Stratton et al. 1991; Taylor 1985). These substances are especially dangerous for people being treated with inhibitors for the monoaminooxidase enzyme (Stratton et al. 1991). The European Union has set the maximum average value of histamine in fish and canned fish at 100 mg/kg; whereas in aged products like anchovies, the average histamine content must be lower than 200 mg/kg (EEC 1991). It is therefore very important to monitor the levels of biogenic amines in foodstuffs for health and safety reasons.

The traditional processing ingredients for Lavú are coarse salt and spices (such as illicium verum, wild pepper and ginger etc.), without sucrose. Recently, some factories are adding sucrose to improve the appearance and the quality of the final products. Studies on the contents of free amino acids and biogenic amines in sea-fish and the factors influencing the formation of biogenic amines are abundant in the literatures (Rabie et al. 2009; Mah et al. 2009; Mah and Hwang 2009a, b). However, to the best of our knowledge, no such information is available for the drycured freshwater fish products made from grass carp. The objectives of this study were to determine the contents of free amino acids and biogenic amines in Chinese traditional dry-cured fish during processing and storage, and to elucidate the effects of sucrose added. The results obtained in this study would contribute to the quality improvement of Chinese traditional dry-cured fish, Layú.

# Materials and methods

Sample preparation Fresh grass carp (*Ctenopharyngodon idellus*) was purchased from a local fish market (Hangzhou, Zhejiang province, China) and transported to the laboratory in ice within 30 min. Each fish (mean weight  $3\pm0.5$  kg) was deheaded, gutted, and deboned immediately for curing.

Cured fish were processed according to the traditional processing standard of Zhejiang Province. In Layú A, 20 kg of raw fish were salted with 2 kg NaCl, 80 g illicium verum, 80 g wild pepper, 200 g fresh ginger, and 400 g sucrose, forming piles alternating between fish and salt with spices. The temperature of the salting room was 2-5 °C and the relative humidity 80–90%. After curing for 15 days, the fish were taken out, brushed, hung on shelves and transferred to a room at 10 °C and relative humidity of 75%. There, the cured fishes undergo aging for 15 days. Layú B was treated similar to that of Layú A, except that no sucrose was added.

After 15 days of curing and another 15 days of aging as described above, the Layú became mature and can harvest.

Both of the mature Layú A and Layú B were divided into two groups for further storage under two different temperatures of 4 °C and 20 °C.

Two samples were taken during the curing and aging period, one sample right after curing for 15 days, the other after aging for 15 day. Three samples were taken during the storage, one at 20 days, the other at 40 and the third one at 60 days. All the samples were minced in a high-capacity mincer (TQ-5, Henglian Food Machinery Co., Ltd, China). Fresh fish was minced to obtain control sample. Minced samples obtained were stored at -80 °C for experimental analysis.

*Determination of dry weight (DW)* Dry weight (DW) was determined by taking 5 g samples, and dried in a constant temperature oven at 100 °C until constant weight was obtained, according to the Chinese National Standard (GB/T 9695. 15-2008).

*Free amino acid composition* Amino acid composition was analyzed using the Waters Associates AccQ·Tag method (Liu et al. 1995). This technique consists of three steps (1) extraction of free amino acids using Riat's method (Riat and Sadana 2009) (2) pre-column derivatization of samples with AccQ·fluor reagent and (3) analysis by (reverse phase) HPLC. The chromatographic separation was performed using Waters Allicance 2695 with heater, Waters 2475 Multi fluorescence detector (Ex: 250, Em: 395 nm) and Nova-PakTM C18 column ( $3.9 \times 150$  mm, particle size 4 µm). The mobile phase consisted of two solvents: (A) AccQ·Tag and (B) Acetonitrile in water. Amino acid standard (Sigma chemicals) was used. Identification of the amino acids in the sample was carried out by comparing their retention times with the standards.

*Biogenic amine analysis* The extraction of the biogenic amines was based on the method described by Vasundhara et al. (1998). Finely ground fish sample (5.0 g) was homogenized with 20 ml of 5% TCA solution for 2 min. The supernatant was collected by centrifugation  $(10,000 \times g, 4 \text{ °C}, 10 \text{ min})$  and the residue was homogenized again with another 20 ml of 5% TCA solution. Both supernatants were combined and filtered through Whatman No. 4 filter paper. The filtrate was made up to 50 ml with 5% TCA solution and stored at -4 °C for HPLC analysis. Using a Waters 2695 series liquid chromatograph (Milford, MA) equipped with a quaternary pump, on-line degasser, autosampler, automatic injector, column heater, and fluorescence detector (Model 2475) connected on-line.

The derivatisation reagent was prepared by transferring 100 mg *o*-phthalaldehyde (OPA), 1 ml acetonitrile and 130  $\mu$ l 2-mercaptoethanol to a 10-ml volumetric flask, and then diluting with 0.4 M borate buffer (pH 10.2) to 10 ml.

The resulting solution was mixed well, stored at 4 °C, and used within 24 h. Pre-column derivatisation with OPA was performed automatically.

A reverse-phase Hypersil ODS C<sub>18</sub> ( $125 \times 4.60$  mm, particle size 5 µm) column was used for separation. The column temperature and flow rate were set at 40 °C and 1.0 ml/min, respectively. The mobile phase consisted of solvent A (pH 7.2), 7.35 mM sodium acetate solution: triethylamine:tetrahydrofuran (500:0.12:2.5  $\nu/\nu$ ), and solvent B (pH 7.2), 7.35 mM sodium acetate solution: methanol:acetonitrile (1:2:2  $\nu/\nu$ ). Fluorescence was monitored at an emission wavelength of 450 nm using an excitation wavelength of 340 nm.

All samples and standards repeated three times. Repeatability tests were performed by injecting a standard and sample consecutively six times a day. Reproducibility tests were also carried out by injecting the standard and the sample twice a day for 3 days under the same experimental conditions. Significant differences (p < 0.05) were not found between the results obtained in these tests.

*Statistical analysis* Data were analyzed by Duncan's multiple range tests using statistical package statistica V 5.5 software. A significant level was defined as a probability of 0.05 or less. Determinations were carried out in triplicate.

#### **Results and discussion**

*Changes in free amino acids content during salting and aging* Table 1 showed the contents of the amino acids during processing of dry-cured Layú made with (A) and without (B) sucrose. The fresh fish contained 12 free amino acids (FAA) with a total concentration of 2.7 g/kg DW and 6 essential amino acids with a concentration of 1.3 g/kg DW (Table 1).

After salting, Layú B contained higher content of FAA than Lavú A. In Lavú B, 15 free amino acids were detected while only 14 were detected in Lavú A. For Lavú B, the total contents of FAA and EAA increased 3.8-fold and 3.3fold respectively, while for Layú A, only 2.7-fold and 2.4fold increased, respectively (Table 1). Furthermore, more FAA (17 types) were detected in the aged samples for both batches, and Layú A contained higher FAA than that of Lavú B. After aging, compared with fresh fish, the total content of FAA and EAA in Lavú A increased 15-fold and 14-fold, respectively, and only a 13.2-fold and 12.5-fold increase for Lavú B. A possible explain of these phenomena is that salting was carried out at a low temperature room (2-5 °C), the sucrose added to Lavú A increase the osmotic pressure and inhibited the activity of bacteria and consequently decreased the content of bacterial proteolytic enzymes (Bover-Cid et al. 2001). On the other hand, the

FAA	Fresh fish	After salting		After aging		
		Layú A	Layú B	Layú A	Layú B	
Aspartic acid	0.10±0.03 <sup>e</sup>	$0.43 \pm 0.01^{\circ}$	$0.67{\pm}0.04^{d}$	$2.5 {\pm} 0.06^{a}$	$2.3 {\pm} 0.07^{b}$	
Serine	$0.04{\pm}0.01^{e}$	$0.34{\pm}0.08^d$	$0.90 {\pm} 0.03^{\circ}$	$2.3 {\pm} 0.10^{a}$	$2.1 \pm 0.11^{b}$	
Glutamic acid	$0.12 {\pm} 0.02^{d}$	$0.18 {\pm} 0.03^{cd}$	$0.27{\pm}0.06^{\circ}$	$2.0 {\pm} 0.10^{\rm a}$	$1.6 {\pm} 0.08^{b}$	
Proline	nd	nd	nd	$1.1 \pm 0.11^{a}$	$0.68 {\pm} 0.15^{b}$	
Glycine	$0.51 {\pm} 0.09^d$	$1.01 \pm 0.13^{\circ}$	$1.2 \pm 0.17^{c}$	$4.5{\pm}0.10^{a}$	$4.1 \pm 0.27^{b}$	
Alanine	$0.58{\pm}0.06^d$	$1.15 \pm 0.06^{\circ}$	$1.4 {\pm} 0.13^{\circ}$	$3.8 {\pm} 0.11^{a}$	$3.2{\pm}0.25^{b}$	
Cysteine	nd	$0.04{\pm}0.01^{\circ}$	$0.12{\pm}0.04^{c}$	$1.4{\pm}0.09^{\mathrm{a}}$	$1.2 \pm 0.11^{b}$	
Tyrosine	nd	$0.22{\pm}0.05^{\mathrm{a}}$	$0.22{\pm}0.05^{a}$	$0.14{\pm}0.02^{b}$	$0.20{\pm}0.03^{b}$	
Histidine	$0.04{\pm}0.01^d$	$0.97{\pm}0.05^{\circ}$	$1.2 {\pm} 0.13^{b}$	$3.9{\pm}0.09^{a}$	$3.8{\pm}0.05^{a}$	
Arginine	nd	nd	nd	$0.70 {\pm} 0.04^{\mathrm{a}}$	$0.61 {\pm} 0.10^{a}$	
Essential amino ac	cids					
Threonine	$0.19{\pm}0.02^{d}$	$0.95 {\pm} 0.07^{\rm c}$	$1.3 {\pm} 0.05^{b}$	$3.4{\pm}0.12^{a}$	$3.3 \pm 0.13^{a}$	
Valine	$0.30{\pm}0.03^{e}$	$1.1 \pm 0.01^{\circ}$	$1.6 {\pm} 0.20^{d}$	$4.2{\pm}0.09^{a}$	$3.2{\pm}0.16^{b}$	
Methionine	$0.26{\pm}0.05^{b}$	$0.30{\pm}0.03^{b}$	$0.41 {\pm} 0.09^{b}$	$1.2{\pm}0.04^{\rm a}$	$1.2{\pm}0.16^{a}$	
Isoleucine	$0.05 {\pm} 0.01^{d}$	$0.19 {\pm} 0.02^{\circ}$	$0.22 {\pm} 0.04^{\circ}$	$2.2{\pm}0.08^{a}$	$2.0 {\pm} 0.10^{b}$	
Leucine	$0.37{\pm}0.05^{b}$	$0.31 {\pm} 0.06^{b}$	$0.57 {\pm} 0.07^{b}$	$3.0{\pm}0.32^{\mathrm{a}}$	$2.7{\pm}0.08^{a}$	
Phenylalanine	nd	nd	$0.02{\pm}0.01^{b}$	$1.3 \pm 0.11^{a}$	$1.3 \pm 0.06^{a}$	
Lysine	$0.11 {\pm} 0.01^{a}$	$0.13 {\pm} 0.02^{a}$	$0.18{\pm}0.01^{a}$	$2.2 \pm 0.11^{b}$	$2.1 \pm 0.11^{b}$	
$\sum$ FAA	2.7±0.76 <sup>e</sup>	$7.3 \pm 0.11^{\circ}$	$10.1 {\pm} 0.58^d$	$39.9 {\pm} 1.02^{a}$	$35.4 {\pm} 0.36^{b}$	
$\sum_{i=1}^{n}$ EAA	$1.3\!\pm\!0.08^e$	$3.0{\pm}0.24^{\circ}$	$4.2{\pm}0.18^d$	$17.6 {\pm} 0.72^{a}$	$15.9{\pm}0.36^{b}$	

 Table 1
 Main free amino acid

 contents (g/kg DW) during
 processing of Layú made with

 (A) and without (B) sucrose
 Sucrose

Means followed by different superscripts in a raw are significantly  $(P \le 0.05)$  (n=3)

FAA free amino acids, EAA essential amino acids, nd not detected

cured fish changed to a higher temperature room (about 10 °C) undergo ageing, an increase in the content of FAA during the aging period could be attributed to that sucrose served as a carbon source and promoted bacterial growth. The endogenous proteases would be another possible factor that increase and promote proteolysis which may result in a rapid increase in the content of FAA.

Lysine, tyrosine and histidine are the main precursors of biogenic amines (Bauza et al. 1995). All the three amino acids showed a gradual increasing trend during salting and aging (Table 1). After aging, the content of lysine, tyrosine and histidine were 2.2, 0.14 and 3.9 g/kg DW respectively in Layú A, and 2.1, 0.20 and 3.8 g/kg DW respectively in Layú B. There was no significant difference of the content of lysine, tyrosine and histidine between Layú A and Layú B except that the contents of all these three amino acids showed an increasing trend throughout storage.

Changes in total free amino acids content during storage The shelf life of dry-aged Layú is about 2 months according to our pre-test. Figure 1a shows that the total FAA of Layú A and B increased gradually during storage at 4 °C and 20 °C, which is similar to the Egyptian salted-fermented fish (Feseekh) (Rabie et al. 2009). During the first 40 days, Layú A stored at 20 °C had a higher total FAA than that stored at 4 °C, but after 60 days the results



Fig. 1 Changes in the content of total free amino acids (a) and biogenic amines (b) of Layú made with (Layú A) and without (Layú B) sucrose at different storage temperatures. (n=3)

were reversed and the samples stored at 4 °C were higher. In contrast, Layú B stored at 20 °C had a higher total FAA than that stored at 4 °C during the whole 60 days of storage.

FAA is a major contributor to the flavor of fish products (Hashimoto 1965). Figure 1a showed that, both in low (4 °C) and high (20 °C) the temperature was, the total content of FAA in Layú A was higher than that of Layú B during the first 40 days of storage, which suggests that the addition of sucrose during the processing of Layú can improve its flavor.

Changes in biogenic amines during salting and aging As shown in Table 2, the fresh grass carp contained low levels of the six investigated biogenic amines. Among them, putrescine had the highest content (3.0 mg/kg DW), followed by tyramine (2.6 mg/kg DW), spermine (2.0 mg/kg DW), spermidine (1.4 mg/kg DW), and cadaverine (1.1 mg/kg DW), but no histamine was detected. The results were similar to those of Liu et al. (2010), except that small amount of histamine (0.28 mg/kg) but no cadaverine was detected in their samples. The slight differences may be due to the different fish culture locations of the grass carp (Zhejiang Province vs Hunan Province). Both the salted and dry-aged fish, all the six biogenic amines understudy were detected. The main biogenic amines were putrescine, cadaverine, histamine and tyramine. The content of each biogenic amine increased gradually during the salting and aging periods (Table 2).

After salting, it was found that the biogenic amines content in Layú A was 8.5 mg/kg DW lower than that in Layú B (Table 2). The difference was even lower (31.2 mg/kg DW) after aging. The results indicated that the addition of sucrose could inhibit the production of biogenic amines during the processing of Layú, which was consistant with the findings from Sara in fermented sausage (Bover-Cid et al. 2001).

*Changes in biogenic amines during storage* Figure 2 shows the contents of biogenic amines both in Layú A and B during 4 °C and 20 °C storage. In the content of putrescine, cadaverine and histamine increased gradually, while the levels of spermine, spermidine and tyramine did not change significantly under the storage temperatures in current study.

After 60 days of storage, cadaverine was found to be the main biogenic amine, representing about 54.3% and 45.1% of total biogenic amine content in Layú A at 4 °C and 20 °C respectively and about 55.1% and 48.6% respectively in Layú B. Cadaverine originated from the decarboxylation of lysine and was associated with the decarboxylase enzymes metabolized by *Enterobacteriaceae* microbe (Halász et al. 1994).

An increasing of cadaverine during the whole 60 days' storage were also detected (Fig. 2). The increase of

Table 2 Biogenic amines           content (mg/kg DW) during	Biogenic amine	Fresh fish	After salted		After ripened	
processing of Layú made with sucrose (A) and without			Layú A	Layú B	Layú A	Layú B
sucrose (B)	Putrescine	3.0±0.11 <sup>c</sup>	9.5±0.18 <sup>c</sup>	9.7±0.16 <sup>c</sup>	$16.4 {\pm} 0.71^{b}$	22.1±0.95 <sup>a</sup>
	Cadaverine	$1.1{\pm}0.05^d$	$28.2{\pm}0.96^{b}$	$32.0{\pm}1.28^{b}$	$55.7 {\pm} 1.5^{b}$	$77.1 \pm 1.9^{a}$
	Spermidine	$1.4{\pm}0.46^{d}$	$1.6 {\pm} 0.06^{\circ}$	$1.7{\pm}0.07^{b}$	$2.1{\pm}0.17^{a}$	$2.1 {\pm} 0.08^{a}$
	Spermine	$2.0{\pm}0.31^{b}$	$2.2{\pm}0.11^{a}$	$2.3{\pm}0.07^{a}$	$1.8 {\pm} 0.18^{\circ}$	$1.5 \pm 0.09^{\circ}$
Means followed by different	Histamine	nd	$3.2{\pm}0.17^{d}$	$5.3 \pm 0.29^{\circ}$	$21.4 \pm 1.4^{b}$	$25.6 {\pm} 0.67^{a}$
superscripts in a raw are significantly ( $P \le 0.05$ ) ( $n=3$ ) <i>nd</i> not detected	Tyramine	$2.6{\pm}0.78^{\circ}$	$13.2 {\pm} 0.49^{b}$	$15.4{\pm}0.69^{a}$	$15.2{\pm}0.77^{a}$	$15.3 {\pm} 0.68^{a}$
	Total	$10.1 \pm 1.7^{d}$	$50.9 {\pm} 0.10^{\circ}$	$59.4 \pm 1.44^{c}$	$112.5 \pm 0.25^{b}$	$143.7 \pm 0.37^{a}$

cadaverine was found to be 5.6-fold (56.2–315.9 mg/kg DW) and 7.8-fold (56.2–435.4 mg/kg DW) in Layú A stored at 4 °C and 20 °C, respectively. However, the increase of cadaverine in Layú B was only 4.2-fold (77.2–326.1 mg/kg DW) and 4.9-fold (77.2–376.5 mg/kg DW) at 4 °C and 20 °C, respectively. The final content of cadaverine in Layú A and B was much lower than Rabie et al. (2009) reported in Egyptian salted-fermented fish (Feseekh), which was 997 mg/kg DW after 60 days' storage. The content were also lower than the cadaverine levels (1,083–1,205 mg/kg DW) reported by Mah et al. (2002), in the Korean salted and fermented fish products stored for 20 days. These variations may be attributed to the fish species, process method and storage conditions.



Fig. 2 Changes in the content of biogenic amine of Layú made with (Layú A) and without (Layú B) sucrose at different storage temperatures. (n=3)

Putrescine was another dominant biogenic amine produced in Layú during storage (Fig. 2). The putrescine generated in Layú A stored at 4 °C and 20 °C were increased from an initial level of 16.2 mg/kg DW to 107.2 mg/kg DW and 172.2 mg/kg DW, respectively. For Layú B, the same trend was found where the initial level of 22.2 mg/kg DW increased to 67.2 mg/kg DW (4 °C) and 149.3 mg/kg DW (20 °C). Vallé et al. (1996) found that when fresh fish became inedible, the putrescine content of herring stored at 0 °C was 110 mg/kg. Krizek et al. (2002) suggested that putrescine values should be lower than 10 mg/kg for good quality, values between 10 and 20 mg/kg for acceptable quality and values over 20 mg/kg for poor quality, based on the sensory scores of carp meat.

The content of histamine also increased obviously during the storage period. In the first 40 days, the content of histamine increased from 21.2 mg/kg DW to 37.3 mg/kg DW (4 °C) and 117.2 mg/kg DW (20 °C) in Layú A, and from 25.7 mg/kg DW to 47.7 mg/kg DW (4 °C) and 97.9 mg/kg DW (20 °C) in Layú B (Fig. 2). When stored at 4 °C for 60 days, the content of Layú A and Layú B increased to 127.2 mg/kg DW and 174.5 mg/kg DW, respectively. However, when stored at 20 °C for 60 days, the content of Layú A and Layú B increased to 332.0 mg/kg DW and 214.1 mg/kg DW, respectively, which exceeded the maximum tolerance level (200 mg/kg DW) of the EEC (1991). Based on these results, Layú would be safe when stored at either 4 °C for 60 days or 20 °C for 40 days.

As illustrated in Fig. 2, the tyramine value in both Layús did not change significantly under the two different storage temperatures. Yen and Hsieh (1991) also reported low content and slight changes in tyramine in canned tuna. The values of tyramine in Layús were very low (from 15.1 to 21.6 mg/kg DW), compared to the possible toxic limits of 100–800 mg/kg (Ten Brink et al. 1990).

Spermine and spermidine were present in low amounts in Layús and did not change much during the storage period (Fig. 2). The spermine content varied from 1.6 to 8.4 mg/kg DW and spermidine content varied from 2.1 to 6.0 mg/kg DW. The contents of spermine and spermidine for Layú A and Layú B showed no significant differences. These results are similar to those in the Egyptian saltedfermented fish of Feseekh (Rabie et al. 2009).

In a word, the total content of biogenic amines (sum of the individuals) increased obviously in Layú A and Layú B during storage, especially at 20 °C (Fig. 1b). The total biogenic amines in Layú A stored at 20 °C for 60 days was 974.8 mg/kg DW, higher than that stored at 4 °C (579.5 mg/kg DW). Similarly, the total content of biogenic amine in Layú B at 20 °C was 773.4 mg/kg DW, higher than that stored at 4 °C (593.8 mg/kg DW). The results indicate that high temperatures were more suitable for the accumulation of biogenic amines during the storage of fish products, and sucrose addition (Layú A) in the processing can cause greater biogenic amines formation during the storage time although it improved the flavor.

# Conclusion

In the present study, we investigated the effect of adding sucrose on the generation of FAA and biogenic amines in Layú during the processing and storage. FAA and biogenic amine contents in Lavú increased gradually during processing and storage. Sucrose added during processing contributed to the increase of FAA but decrease of biogenic amines contents in aged Layú. Sucrose could also give rise to the accumulation of FAA during the storage period (60 days), especially at the pro-40 days. The storage temperature plays an important role on the biogenic amines accumulation in Lavú. The formation of biogenic amines was inhibited at a low storage temperature (4 °C) because of the sucrose, however, at a high storage temperature (20 °C), the sucrose showed a synergetic effect with the temperature on its accumulation, especially in the middle and latter storage period (20-60 days). That might be attributed to the effect of sucrose on the growth of microorganism. So it is recommended that Layú should be stored at low temperatures for its safety and long shelf-life.

Acknowledgments This work was supported by the National Scientific and Technological Supporting Program (2008BAD91B00), NSFC (30972282) and the National High Technology Research and Development Program ("863" Program) (2007AA091802), in China.

## References

Bauza T, Blaise A, Daumas F, Cabanis JC (1995) Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection. J Chromatogr A 707:373–379

- Bover-Cid S, Izquierdo-Pulido M, Carmen Vidal-Carou M (2001) Changes in biogenic amine and polyamine contents in slightly fermented sausages manufactured with and without sugar. Meat Sci 57:215–221
- EEC (1991) Council directive 91/493/EEC, of 22nd July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Official Journal of European Communities (NrL268), pp 15–32
- Halász A, Baráth A, Simon-Sarkadi L, Holzapfel W (1994) Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5:42–49
- Hashimoto Y (1965) Taste-producing substances in marine products. In: Kreuzer R (ed) The technology of fish utilization: contributions from research. Fishing News (Books) Ltd, London, pp 57– 61
- Krizek M, Pavlicek T, Vacha F (2002) Formation of selected biogenic amines in carp meat. J Sci Food Agric 82:1088–1093
- Kumudavally KV, Padmini S, Radhakrishna K, Bawa AS (2005) Effect of surface treatment of fresh mutton with spices on lipolytic bacteria, Eenterobacteriaceae and their degradation products during storage. J Food Sci Technol 42:249–253
- Lehane L, Olley J (2000) Histamine fish poisoning revisited. Int J Syst Microbiol 58:1–37
- Liu HJ, Chang BY, Yan HW, Yu FH, Liu XX (1995) Determination of amino acids in food and feed by derivatization with 6aminoquionolyl-N-hydroxysuccinimidyl carbamate and reversedphase liquid chromatographic separation. J AOAC Int 78:736– 744
- Liu ZY, Li ZH, Zhong PP, Zhang P, Zeng MQ, Zhu CF (2010) Improvement of the quality and abatement of the biogenic amines of grass carp muscles by fermentation using mixed cultures. J Sci Food Agric 90:586–592
- Lopetcharat K, Choi YJ, Park JW, Daeschel MA (2001) Fish sauce products and manufacturing: a review. Food Rev Int 17:65– 68
- Mah JH, Hwang HJ (2009a) Effects of food additives on biogenic amine formation in Myeolchi-jeot, a salted and fermented anchovy (*Engraulis japonicus*). Food Chem 114:168–173
- Mah JH, Hwang HJ (2009b) Inhibition of biogenic amine formation in a salted and fermented anchovy by *Staphylococcus xylosus* as a protective culture. Food Control 20:796–801
- Mah JH, Han HK, Oh YJ, Kim MG, Hwang HJ (2002) Biogenic amines in Jeotkals, Korean salted and fermented fish products. Food Chem 79:239–243
- Mah JH, Kim YJ, Hwang HJ (2009) Inhibitory effects of garlic and other spices on biogenic amine production in Myeolchi-jeot, Korean salted and fermented anchovy product. Food Control 20:449–454
- Rabie M, Simon-Sarkadi L, Siliha H, El-Seedy S, El Badawy AA (2009) Changes in free amino acids and biogenic amines of Egyptian salted-dermented fish (Feseekh) during ripening and storage. Food Chem 115:635–638
- Riat P, Sadana B (2009) Effect of fermentation on amino acid composition of cereal and pulse based foods. J Food Sci Technol 46:247–250
- Stratton JE, Hutkins RW, Taylor SL (1991) Biogenic amines in cheese and other fermented foods: a review. J Food Prot 54:460–470
- Tan RC, Zhao SM, Xiong SB (2006) Effect of content of major compounds on texture quality of cured fish. Mod Food Sci Technol 22:14–16
- Taylor SL (1985) Histamine poisoning associated with fish, cheese, and other foods. World Health Organization. WPH/FOS 85: 1–47
- Ten Brink B, Damink C, Joosten HMLJ, Huis in't Veld JHJ (1990) Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 11:73–84

- Vallé M, Malle P, Bouquelet S (1996) Liquid chromatographic determination of fish decomposition indexes from analyses of plaice, whiting and herring. J AOAC Int 79:1134–1140
- Vasundhara TS, Kumudavally KV, Jayathilakan K, Jeyashakila R (1998) HPLC analysis of the biogenic amines in some processed foods of Indian origin. J Food Sci Technol 35:551–556
- Xu Y, Xia W, Yang F, Kim JM, Nie X (2010) Effect of fermentation temperature on the microbial and physicochemical properties of silver carp sausages inoculated with *Pediococcus pentosaceus*. Food Chem 118:512–518
- Yen GW, Hsieh CL (1991) Simultaneous analysis of biogenic amines in canned fish. J Food Sci 56:158–160