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Changes in biogenic amines in fermented silver carp sausages inoculated with mixed starter cultures

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Abstract

The effects of three group mixed starter cultures (group one: *Lactobacillus plantarum*-15, *Staphylococcus xylosus*-12 and *Pediococcus pentosaceus*-ATCC33316, group two: *L. plantarum*-15, *S. xylosus*-12 and *Lactobacillus casei* subsp. *casei*-1.001, and group three: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316) and a batch without starter as control, on biogenic amine formation during the manufacture of silver carp sausage were investigated. Determination of seven different biogenic amines was carried out by reverse-phase high performance liquid chromatography (RP-HPLC) with fluorometric detection. Quality parameters of silver carp sausage, namely pH, water activity, microbial counts, α-amino nitrogen and total volatile base nitrogen, were determined. In addition to these, amino acid contents were analyzed to note changes of amino acids in silver carp sausages. Mixed starter cultures decreased the pH quickly, inhibited the growth of contaminant microorganisms present in the raw materials, and suppressed the accumulation of histamine, cadaverine, putrescine, tryptamine and tyramine. In order to prevent biogenic amine formation, mixed starter cultures with negative-decarboxylate activity should be inoculated into the manufactured food products.

Keywords: Biogenic amines; Mixed starter cultures; Lactic acid bacteria; *Staphylococcus xylosus*; Silver carp sausage

1. Introduction

In recent years, more concerns about food safety, together with the consumer's demand for safe and healthier products, have promoted studies of compounds with harmful effects on human health. Thus, the formation of biogenic amines in fermented foods has received considerable interest. Biogenic amines are organic molecules, of low molecular weight, which occur in a wide variety of foods, such as fish and fish products, meat, dairy products, wine and other fermented foods. They are formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Maijala, Nurmi, & Fischer, 1995). Biogenic amines have an important metabolic role in living cells; some of them are essential for growth and some of them are involved in nervous system functions (Santos, 1996). However, when high amounts of biogenic amines are consumed or normal pathways of amine catabolism are inhibited, various physiological effects, which are hypotension (in the case of histamine, putrescine, cadaverine) or hypertension (in the case of tyramine), and nausea, headache, rash, dizziness, cardiac palpitation and even intracerebral hemorrhage and death in very severe cases may occur (Rawles, Flick, & Martin, 1996). Biogenic amines are considered precursors of carcinogenic amines such as *N*-nitrosamines (Scanlan, 1983), and they are also indicators of food quality (Mietz & Karmas, 1978). Therefore, in the United States, the Food and Drug Administration (FDA) has ruled that histamine must be addressed in HACCP programmes for scombroid or scombroid-like fish and has set the maximum action level of 50 ppm (FDA, 1996). In Germany, maximum permissible limits of biogenic amines for fish and fish products is limited to 200 mg/kg of the food whereas it is only 100 mg/kg

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in Canada, Finland and Switzerland (Lange & Wittmann, 2002).

Fish fermentation is an old preservation technique in which both fresh water and marine fish are used. This preservation technique improves the sensory and hygienic qualities of end-product. According to Yin, Pan, and Jiang (2002), using starter cultures to ferment minced mackerel could suppress the growth of spoilage bacteria and pathogens, and substantially inhibit the development of volatile basic nitrogen. Rapid decline of pH not only gives the products unique lactic acid flavour, but also increases firmness, texture and palatability due to the acid denaturation of muscle protein (Mendonca, Molins, Kraft, & Walker, 1989). However, biogenic amines could be formed in the fermented food products since the fermentation process provides both microorganisms and free amino acids, together with environmental factors favouring bacterial growth and positive decarboxylase activity (Halasz, Barath, Simon-Sarkadi, & Holzapfel, 1994). Many authors have reported high biogenic amines in fermented fish products such as fish sauce and fish paste (Fardiaz & Markakis, 1979; Yongsawatdigul, Choi, & Udomporn, 2004).

In order to avoid formation of hazardous levels of biogenic amines during fermentation of sausages, raw materials must have low microbial counts, as one of the critical factors affecting amine formation in sausage (Pons-Sänche-Cascado, Vidal-Carou, Marineä-Font, & Veciana-Nogueäs, 2005; Veciana-Nogue's, Marine'-Font, & Vidal-Carou, 1997). Another important factor, suggested for preventing amine accumulation, is control of amine-positive bacteria by addition of amine-negative starter cultures. Hernadez-Jover, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, and Vidal-Carou (1997) reported reductions of tyramine and cadaverine during ripening of sausages with mixed starter consisting of *micrococcus* plus *Lactobacillus plantarum* and, likewise, Maijala et al. (1995) found significant decreases in tyramine, cadaverine and histamine levels during ripening of sausages with an amine-negative mixed starter culture. There are many published reports of the changes that occur in some physicochemical and microbiological parameters during the fermentation of fish sausages (Aryanta, Fleet, & Buckle, 1991; Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, 2004; Yin & Jiang, 2001). In contrast, few experiments have been conducted on biogenic amine levels during fermentation of fish sausages.

The main aim of the present study was to determine the effects of the three group mixed starter cultures (group one: *L. plantarum*-15, *Staphylococcus xylosus*-12 and *Pediococcus pentosaceus*-ATCC33316, group two: *L. plantarum*-15, *S. xylosus*-12 and *Lactobacillus casei* subsp. *casei*-1.001, and group three: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316) on biogenic amine formation in fermented silver carp sausage. Quality parameters of fermented silver carp, namely pH, water activity, microbial counts, α-amino nitrogen and total volatile base nitrogen (TVB-N), were also determined. In addition to this, amino acid content was analyzed, which might provide further information on the formation of the biogenic amines.

2. Materials and methods

2.1. Preparation of starter culture

Lactobacillus casei subsp. *casei*-1.001 and *P. pentosaceus*-ATCC 33316 were purchased from China General Microbiological Culture Collection Center, Beijing; *L. plantaum*-15, and *S. xylosus*-12 were taken from the technology centre of the Shuanghui group, Luohe, Henan, China as a starter culture. The lactic acid bacteria (LAB) were subcultured twice in deMan Rogosa Sharpe (MRS) broth at 30 °C for 2 days. Finally, cell pellets were harvested by a high speed refrigerated centrifuge (Model HSC-20RA, China) at 10,000*g* for 15 min at 4 °C and washed with 20 mM phosphate buffer, pH 7.0, and then the cell pellets were resuspended in the same buffer. Similarly, *S. xylosus*-12 was subcultured twice in Nutrient Broth at 30 °C for 3 days and cells were harvested by the same centrifuge at 2000*g* for 15 min at 4 °C, washed with saline water (0.9% NaCl), then resuspended in the same saline solution. Finally, the number of bacterial cells in each suspension was adjusted to reach the range of 7–8 log CFU/ml of saline solution by using a spectrophotometer (Model WFZ-UV-2100, UNICOTM) (Fadda, Vignolo, Holgado, & Oliver, 1998).

2.2. Silver carp sausage preparation

Silver carp was thawed in running tap water and then gutted, eviscerated and deboned, after which the scale, pin bones, debris and connective tissues were removed. The processed samples were then mixed with 1 volume of sterile water, 3% NaCl and 3% glucose. The cell suspension of the mixed starter cultures (10 ml/kg) was added to the meat mixture and well mixed using a sterile glass rod. Four separated batches of fermented sausage were prepared with different mixed starter cultures, namely: batch S-PXP (*L. plantarum*-15, *S. xylosus*-12, and *P. pentosaceus*-ATCC 33316 [1:1:1]), batch S-PXC (*L. plantarum*-15, *S. xylosus*-12 and *L. casei* subsp. *casei*-1.001 [1:1:1]), batch S-XCP (*S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316 [1:1:1]) and a batch without any starter (S-NS) as control. Each of the sausage batters was stuffed into collagen casings (ø 38 mm) and incubated at 30 °C, RH 85%, and taken out at every 12 h for analysis. Samples were thoroughly cut up and ground in a meat grinder (Osterizer, USA) until a homogeneous sample was obtained.

2.3. Microbiological analyses

Silver carp sausage sample (25 g) was aseptically transferred to a sterile plastic bag and stomached for 1 min in a stomacher, with 225 ml sterile peptone water.

Appropriate decimal dilutions of the samples were prepared using the same diluents and 0.1 ml of each dilution was plated in triplicate on different growth media. MRS agar incubated at 30 °C for 1–2 days for LAB count, Mannitol Salt Agar (MSA), incubated at 30 °C for 2–3 days for *staphylococci/micrococci* count, Violet red bile glucose agar (VRBG), incubated at 37 °C for 24 h for *enterobacteriaceae* count, and *pseudomonas–aeromonas*-selective (GSP) agarbase, incubated at 26 °C for 72 h for *pseudomonas* count, were used. Results were expressed as colony forming units per gramme (CFU/g).

2.4. Determination of pH, α-amino nitrogen, and total volatile basic nitrogen and free amino acids analysis

pH measurement was carried out according to the procedure of Wang (2000). Ten grams of sample were homogenized with 90 ml of deionized water (pH ≈ 7) and the pH was measured with a digital pH meter (Mettler Toledo 320-s). The α-amino nitrogen (AAN) analysis was done by titration with formaldehyde (AOAC, 2002). TVB-N was determined by the Conway micro-diffusion technique (Cobb & Thompson, 1973).

Free amino acids (FAAs) were determined by using a reversed phase high-performance liquid chromatograph (RP-HPLC) (Agilent 1100 Series, USA), equipped with a UV detector. Detection wavelength was 338 nm and the column used was Hypersil ODS C_{18} (4 × 125) mm; the column temperature was 40 °C and flow rate of the analysis 1.0 ml/min. Extract of the muscle was prepared according to the method of Aristoy and Toldra (1991). First, the amino acids extraction (1:10) was done by using 0.1 N hydrochloric acid and deproteinizing was by using 5% (w/v) sulfosalicylic acid (SSA). Then precolumn amino acid derivatization was done by using OPA (orthophthalaldehyde), following the method of Antoine et al. (2001). The concentrations of the different amino acids were calculated from the standard curves of the pure amino acids prepared and derivatized simultaneously with the samples and run under identical conditions. The concentration of amino acids was expressed as mg/100 g sausage.

2.5. Determination of biogenic amines

Biogenic amines in silver carp sausages were extracted according to the procedure of Koutsoumanis, Lampropoulou, and Nychas (1999) with a slight modification; 2 g of sample from finely ground meat were mixed with 10 ml of 5% (w/v) TCA solution in mortar and extracted for about 2 min, then filtered through two folds of filter paper. After that, 1 ml of filtrate was placed in a centrifuge vial, then centrifuged at 10,000 rpm for 10 min. Finally, 0.5 ml supernatant fluid was transferred into a glass vial for HPLC (Agilent 1100 Series, USA) analysis.

The HPLC column was Hypersil ODS C_{18} (4 × 125 mm) and the column temperature was 40 °C and flow rate 1.0 ml/min. Pre-column amine derivatization with OPA (orthophthalaldehyde) was done according to the method of Krishnamurti, Heindze, and Galzy (1984). The amines peaks were detected by using a fluorescence detector at 340 nm wavelength and emission wavelength was 450 nm.

Standard amines, namely, tyramine, putramine, cadaverine and tryptamine were purchased from Sigma (USA) and histamine, spermidine, and spermine from Fluka chemical (Switzerland).

2.6. Statistical analyses

Duncan's multiple range test was employed to determine the significance of difference within treatments for each analysis; three replicates were performed and the mean values were calculated. Values were considered significantly different when $p < 0.05$. Data were analyzed for degree of variation and significance of difference using analysis of variance (ANOVA) (Ramsey & Schafer, 1997). All statistical analyses were performed using the SPSS statistic programme (Version 10.01 for windows, SPSS, 1999).

3. Results and discussion

3.1. Microbial flora

The changes in the microbial flora of silver carp sausages during fermentation are shown in Fig. 1.

Initial LAB counts in the batches were significantly higher than in the control samples ($p < 0.05$), due to the inoculation of starter strains (Fig. 1a). During 36 h of fermentation, LAB numbers reached levels up to 9.5 log CFU/g of sausage in all batches inoculated with starters, which indicated that silver carp sausage is a suitable for the growth of LAB. Likewise, the initial counts for *micrococcaceae* among samples ranged from 4 to 7 log CFU/g depending upon the starter addition. The counts of *micrococcaceae* were significantly increased in the batches inoculated with mixed starters to a level of 7–8 log CFU/g at 12 h; thereafter the counts were gradually decreased in all batches except the control (Fig. 1b). The higher acidity, as well as the anaerobic conditions in the silver carp sausage inoculated mixed cultures, might have such inhibitory action against *micrococcaceae* (Hugas & Monfort, 1997).

Enterobacteriaceae and *pseudomonas* counts of sausages, with or without starters, were between 2.1 and 2.5 log CFU/g at the beginning of fermentation, and no differences between the batches were observed. After 48 h of fermentation, the sausages inoculated with starter cultures significantly inhibited the growth of *enterobacteriaceae* and *pseudomonas* (Fig. 1c and d). This result was agreement with the result of Ayhan, Kolsarici, and Ozkan (1999) who reported that *Enterobacteriaceae* counts were significantly decreased in starter culture with added Turkish Soudjoucks (a fermented meat product) after fermentation. This result was also similar to that obtained by Yin et al. (2002) in a study on mackerel fermented with LAB. The inhibition of the growth of *pseudomonas* and

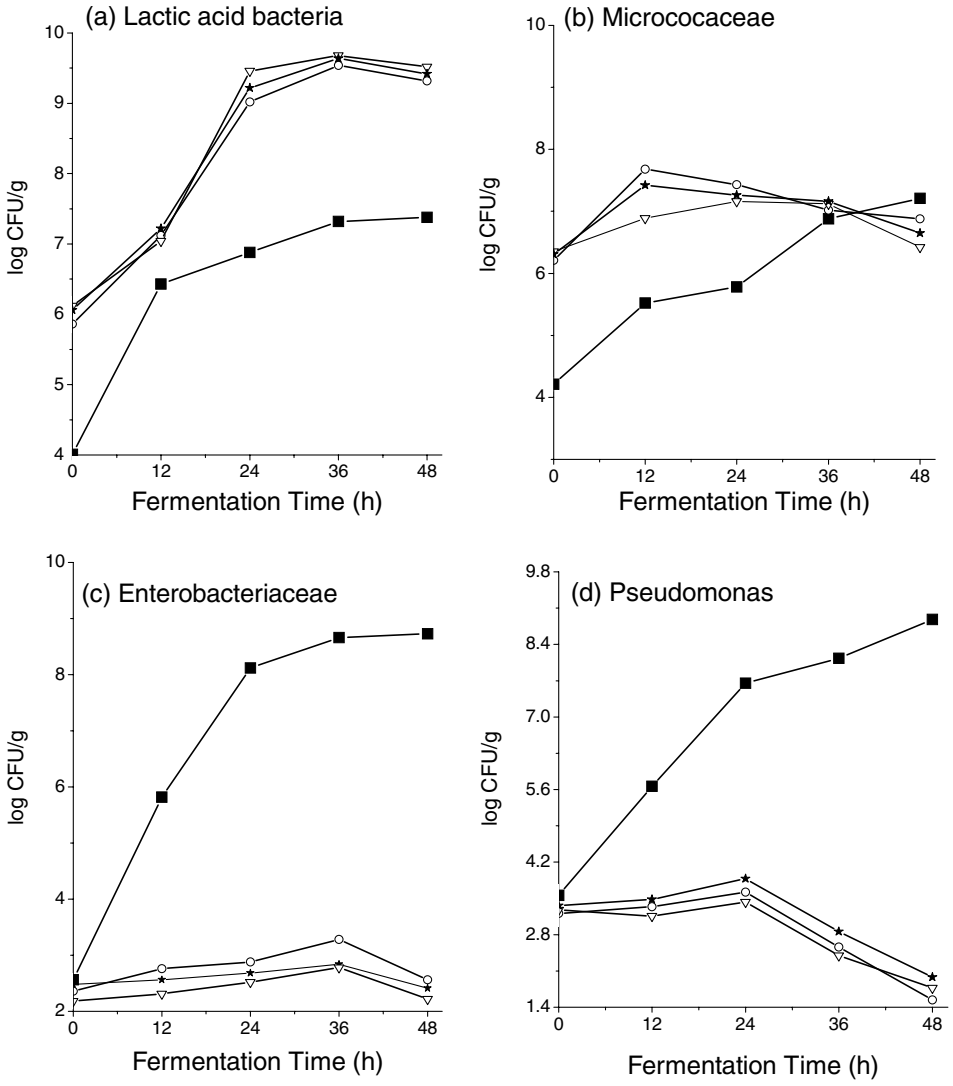

Fig. 1. Microbiological changes in Silver carp sausage during fermentation. (■) S-NS: No starter added; (∇) S-PXP: *L. plantarum*-15, *S. xylosus*-12 and *P. pentosaceus*-ATCC33316 mixed culture; (○) S-PXC: *L. plantarum*-15, *S. xylosus*-12 and *L. casei* subsp. *casei*-1.001 mixed culture; (★) S-XCP: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316 mixed culture.

enterobacteriaceae on silver carp sausages with mixed starters might be due to the actions of bacteriocins and the rapid drop of pH.

3.2. Biogenic amines

The silver carp sausages inoculated with different mixed starter cultures and the control were analyzed for biogenic amines by RP-HPLC methods. A typical HPLC chromatogram for biogenic amines in silver carp sausages inoculated with mixed S-PXC cultures is given in Fig. 2 and biogenic amine contents in silver carp sausage are shown in Table 1.

During 48 h of fermentation at 30 °C, a sharp rise in biogenic amine production occurred in the control, except spermine and spermidine. Histamine was the main amine formed, followed by cadaverine, putrescine and tyramine, their concentrations increased from 38.2 to 843, 2.63 to 501, 14.7 to 318, 32.2 to 67.8 mg/kg, respectively. The results obtained from this study were in agreement with the results of other authors, who have reported that biogenic amines were highly accumulated in spontaneously fermented sausages (Durlu-Ozkaya, Ayhan, & Vural, 2001). The production of biogenic amines is a characteristic of several groups of microorganisms, such as *enterobacteriaceaea*, *pseudomonas*, *micrococcaceae* and LAB (Halasz et al., 1994). Many *enterobacteriaceae* and *pseudomonas* species possess histidine, lysine and ornithine decarboxylase activities, that can produce considerable levels of histamine, cadaverine, and putrescine, e.g. *E. cloacae*, *E. aerogenes*, *H. alvei* and *Klebsiella oxytoca* (Özogul & Özogul, 2005), as well as *Ps. Aeroginosa*, *Ps. Cepaciae* and *Ps. flourescens* (Jørgensen, Huss, & Dalgaard, 2000). Some *micrococcaceae* and LAB also have high amino acid decarboxylase activities and can produce putrescine, histamine and tyramine (Maijala & Eerola, 1993; Komprda, Neznalova, Standara, & Bover-Cid, 2001). Although these

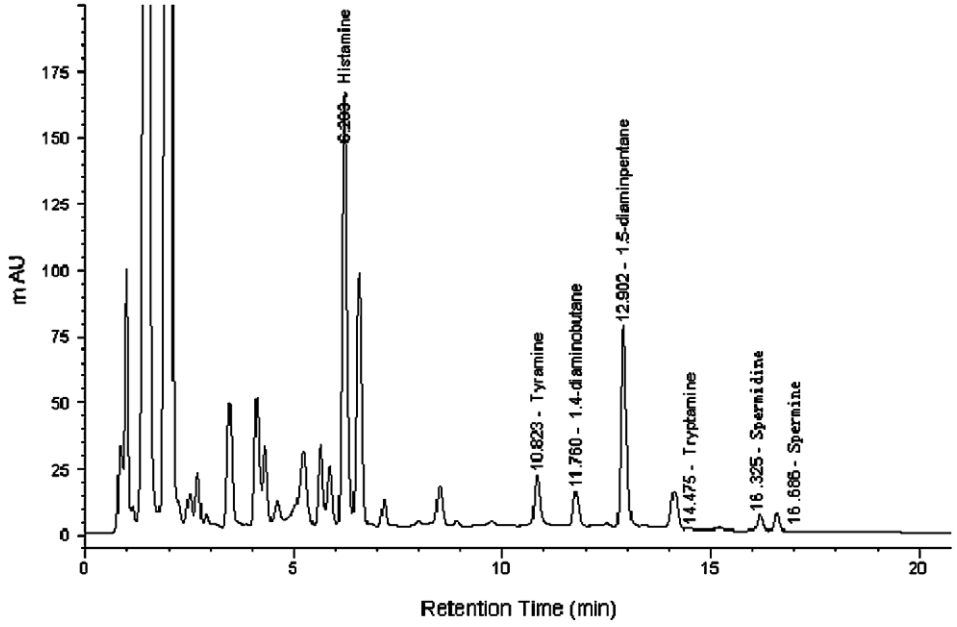

Fig. 2. A typical HPLC chromatogram of biogenic amines produced in silver carp sausage inoculated with mixed starter cultures (S-PXC).

Table 1
Changes in biogenic amines in silver carp sausages inoculated with/without mixed starter cultures

Time (h)	Starters[a]	Biogenic amines (mg/kg)						
		Histamine	Tyramine	Tryptamine	Putrescine	Cadaverine	Spermidine	Spermine
0	S-NS	38.22	32.18	1.48	14.67	2.63	1.22	1.83
	S-PXC	46.18	32.92	1.88	14.23	2.87	1.32	1.92
	S-PXP	45.24	30.84	1.87	12.13	2.45	1.50	2.16
	S-XCP	44.38	30.43	1.56	15.73	2.42	1.43	1.96
24	S-NS	461.3	52.48	12.20	137.25	216.47	1.28	2.08
	S-PXC	47.92	51.02	5.28	68.57	41.89	1.46	2.14
	S-PXP	51.43	62.48	5.56	67.17	50.69	1.54	2.60
	S-XCP	50.48	61.78	4.88	65.78	54.86	1.66	2.38
48	S-NS	842.55	144.03	28.45	318.16	501.12	1.60	2.02
	S-PXC	65.28	67.79	6.19	58.45	75.23	1.42	1.86
	S-PXP	50.48	61.78	4.88	75.78	54.86	1.53	1.94
	S-XCP	110.68	85.62	6.88	68.42	79.46	1.62	2.12

[a] S-NS: No starter added; S-PXP: *L. plantarum*-15, *S. xylosus*-12 and *P. pentosaceus*-ATCC33316 mixed culture; S-PXC: *L. plantarum*-15, *S. xylosus*-12 and *L. casei* subsp. *casei*-1.001 mixed culture; S-XCP: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316 mixed culture.

microorganisms are usually present in low numbers in the raw materials, incorrect storage, as well as uncontrolled fermentation, can induce their proliferation, which can release their decarboxylases during the production of sausages. In this present study, the high contents of histamine, cadaverine, putrescine and tyramine in the control might be produced from the decarboxylation of endogenous decarboxylase enzymes naturally occurring in fish or high levels of *enterobacteriaceae*, *pseudomonas*, and wild LAB at the end of fermentation.

Biogenic amines (approximately at 1000 ppm in food) are supposed to elicit toxicity in humans (Nout, 1994). Histamine is the most toxic amine which is frequently associated with health problems after fish consumption, and its potential toxicity can be enhanced by other biogenic amines, such as the diamines putrescine and cadaverine, or monoamines, such as tyramine. After 48 h of fermentation, the silver carp sausage inoculated with different mixed starter cultures showed significantly decreased contents of histamine compared with the control. Histamine varied from 46.2 to 95.3 mg/kg for S-PXC, 45.2 to 50.5 mg/kg for S-PXP and 44.4 to 111 mg/kg for S-XCP. Ruiz-Capillas and Jimenez-Colmenero (2004) reported that histamine concentration varied from 0 to 200 mg/kg in dry-cured sausages. Fardiaz and Markakis (1979) reported that histamine was the major amine found, with maximal amounts of 64 mg/100 g in fermented fish paste. Nout (1994) pointed out that histamine contents should be in the range

50–100 mg/kg in sausages processed according to "Good Manufacturing Practice". In this study, histamine accumulation was significantly inhibited (90–95%) in starter-mediated sausages compared to the control without starters. Tyramine concentration increased gradually during the fermentation process, reaching levels in the range of 1.75–213 mg/kg at the end of fermentation. Starter cultures reduced the tyramine formation compared with the control without starters. The allowable level of tyramine in foods is 100–800 mg/kg whilst 1080 mg/kg is toxic (Shalaby, 1996). In this study, its concentrations were well below to this level. The concentrations of putrescine and cadaverine also increased during the fermentation of silver carp sausages inoculated with starters. However, compared to the control, the accumulations of putrescine and cadaverine were significantly inhibited in starter-mediated sausages.

Addition of amine-negative starter cultures has been suggested to prevent amine formation in dry sausages. Mixed starter cultures (*L. sakei*, *S. carnosus* and *S. xylosus*) greatly reduced (about 90%) the presence of putrescine, cadaverine and tyramine in Spanish sausages (Bover-Cid, Hugas, Izquierdo-Pulido, Vidal-Caro, & Vidal Carou, 2000). Maijala et al. (1995) found significant decreases in tyramine, cadaverine and histamine levels during ripening of sausages with an amine-negative mixed starter culture (*Staphylococci* plus *Lactobacilli*). Slight reductions of tyramine, cadaverine and putrescine were also observed in fermented sausages treated with *M. carnosus* plus *L. plantarum* and *M. carnosus* plus *P. pentosaceus* by Hernadez-Jover et al. (1997). In the present work, the combinations of amine-negative LAB plus *S. xylosus* resulted in drastic inhibitions of the accumulations of histamine, putrescine, cadaverine and tyramine compared to the control. The starter bacteria inoculated did not change amine-negative character during fish sausage fermentation. Moreover, starters were able to outgrow the usually amine-positive wild microbial flora and inhibit their growth, thus reducing the production of biogenic amines during fermentation.

The levels of spermine and spermidine varied slightly during the fermentation, and ranged between 1.68 and 2.12 mg/kg and 1.42 and 1.62 mg/kg, respectively. Small changes in polyamine contents are usually found during sausage fermentation, since these amines are naturally present in raw material and they are not formed by microbial decarboxylation of amino acids (Hernadez-Jover et al., 1997). Since polyamines can be consumed as a nitrogen source by the microorganism (Bard'ocz, 1995), a decrease in their levels might be found throughout sausage fermentation. In this study, a slight decrease was observed for spermine in all batch sausages.

3.3. Water activity (A_w), pH, and proteolysis

Some authors have reported that the main reason for low levels of biogenic amines is the low pH during the manufacturing process (Maijala & Eerola, 1993). In contrast, other researchers have found that low pH accelerates biogenic amine production (Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001; Maijala et al., 1995). These authors have pointed out that bacteria increase the decarboxylase activity to defend themselves against acidic pH. However, in this work, no significant correlation was found between pH and level of the amines detected. The control, which had the highest pH values, showed higher amine contents than did the starter batch. During 48 h of fermentation, the pH of samples with mixed starter cultures decreased rapidly from around 6.49 to 4.2–4.4 (Fig. 3); low pH values substantially suppressed the activity of amine-positive microorganisms, particularly *Enterobacteriaceae*. This could explain why the lower pH did not result in a higher amine yield in the end-products. Similar results were also reported in studies of dry-fermented sausages by Bover-Cid, Izquierdo-Pulido, and Vidal-Caro (2005).

Initially, the A_w for sausages was in the range 0.92 to 0.93, which decreased significantly ($p < 0.05$) with the fermentation time to a final range 0.82–0.89 (Fig. 3). Mixed starter culture batches showed slightly higher A_w values than did the control, but no differences were observed within the batches and there was no significant correlation between A_w and formation of biogenic amines.

Total volatile basic nitrogen is the most useful index for spoilage in fresh and fermented fish products. The initial TVB-N values were in the range 1.11–1.14 mg/100 g, which significantly increased with fermentation time. After fermentation for 48 h, its concentration reached 56.7 mg/100 g in the control (Table 2). In contrast, slight increase in TVB-N value was observed in the silver carp sausages inoculated with mixed starter cultures. This result agreed with the result of Yin et al. (2002), who reported that the use of LAB in meat fermentation could subsequently inhibit the accumulation of TVB-N by producing lactic acid and

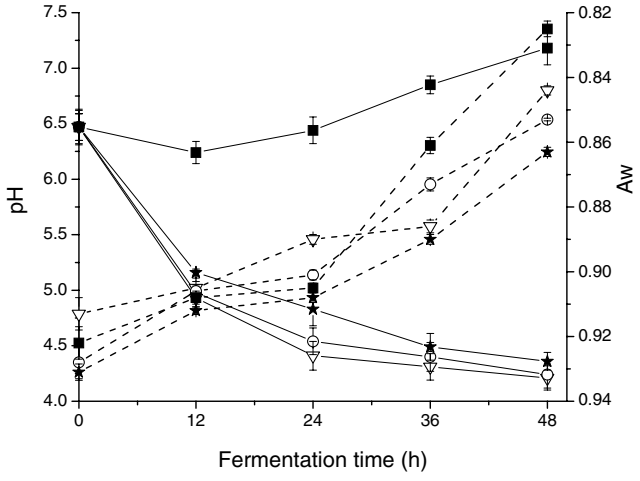

Fig. 3. Changes in pH and A_w during fermentation of silver carp sausages with/without mixed different starter cultures. (■) S-NS: No starter added; (▽) S-PXP: *L. plantarum*-15, *S. xylosus*-12 and *P. pentosaceus*-ATCC33316 mixed culture; (○) S-PXC: *L. plantarum*-15, *S. xylosus*-12 and *L. casei* subsp. *casei*-1.001 mixed culture; (★) S-XCP: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316 mixed culture.

Table 2
Changes in TVB-N, and AAN in silver carp sausages inoculated with/without starter cultures

Fermented time (h)	TVB-N (mg/100 g)				AAN			
	S-NS	S-PXC	S-PXP	S-XCP	S-NS	S-PXC	S-PXP	S-XCP
0	1.15^{aA}	1.11^{aA}	1.14^{aA}	1.12^{aA}	$0.02\%^{aA}$	$0.02\%^{aA}$	$0.02\%^{aA}$	$0.02\%^{aA}$
12	9.54^{bA}	1.24^{aB}	1.29^{aB}	1.29^{aB}	$0.04\%^{aA}$	$0.07\%^{bA}$	$0.08\%^{bA}$	$0.07\%^{bA}$
24	16.35^{cA}	1.89^{bB}	1.88^{bB}	1.93^{bB}	$0.08\%^{bA}$	$0.14\%^{cB}$	$0.16\%^{cB}$	$0.15\%^{cB}$
36	38.66^{dA}	3.64^{cB}	3.24^{cB}	3.75^{cB}	$0.10\%^{bA}$	$0.17\%^{cB}$	$0.19\%^{cB}$	$0.16\%^{cB}$
48	56.71^{eA}	5.01^{dB}	5.78^{dB}	5.92^{dB}	$0.21\%^{cA}$	$0.33\%^{dB}$	$0.38\%^{dB}$	$0.37\%^{dB}$

*S-NS: No starter added; S-PXP: *L. plantarum*-15, *S. xylosus*-12 and *P. pentosaceus*-ATCC33316 mixed culture; S-PXC: *L. plantarum*-15, *S. xylosus*-12 and *L. casei* subsp. *casei*-1.001 mixed culture; S-XCP: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316 mixed culture.

[a] Means with different lower case letter (a–d) with in the same column stand for significant difference ($p < 0.05$).

[b] Means with different upper case letter (A, B) with in the same row stand for significant difference ($p < 0.05$).

bacteriocins, which could neutralize the volatile basic nitrogen and hence lower its value in products. Likewise, AAN contents showed a gradual increase over the processing. The starter-mediated batches showed higher ANN values than did the control, likely as a result of proteolytic activity of the starter cultures, but no differences were observed within the batches and there was no significant correlation between ANN and formation of biogenic amines.

Significant increases in total FAA and FAAs were observed in sausages among the control and samples inoculated with starters, comparing with that before fermentation (Table 3). The sweetening amino acids, such as glycine, alanine, leucine, valine, serine, threonine, methionine and tryptophan, and strong-tasting amino acids, such as arginine, increased several-fold, compared with those before fermentation (Table 3). However, there was no significant correlation between FAAs and formation of biogenic amines. This result was in agreement with those of Hortos and García-Regueiro (1991) who described the values of biogenic amines and no significant correlation with amino acid contents, and also in agreement with Bover-Cid, Izquierdo-Pulido, and Vidal-Carou (1999) who reported no direct correlation between proteolytic activity of *S. xylosus* (used as a starter culture) and biogenic amines production. The release of free amino acids as amine precursors occurred after the early amine production. High temperature and low pH can accelerate amino acid accumulation and stimulate amine formation but, during fermentation processes, the factors affecting the activity of the decarboxylating enzyme could be more important than the precursor availability (Edwards & Sandine, 1981; Joosten, 1988).

Table 3
Changes in free amino acids (FAAs) in silver carp sausages inoculated with/without starter cultures

Free amino acids (mg/100 g)	BF	S-NS	S-PXC	S-PXP	S-XCP
Asp	0.9	12.1	9.1	24.1	16.6
Glu	3.3	78.8	112.3	123.6	96.6
Ser	2.3	6.8	62.8	71.2	48.8
His	212.0	353.2	334.2	430.1	350.2
Gly	3.0	46.6	435.3	222.4	285.8
Thr	2.7	20.1	22.9	60.2	73.5
Ala	8.9	32.2	108.3	112.6	122.6
Arg	1.9	21.4	125.5	36.8	46.8
Tyr	0.4	44.8	14.6	20.1	18.9
Cys	1.2	21.1	53.5	17.85	32.6
Val	5.3	18.8	32.2	49.6	31.8
Met	2.3	25.2	78.8	63.2	58.1
Phe	2.2	66.4	53.5	71.2	62.3
Ile	2.2	62.2	17.1	62.2	73.2
Leu	3.3	28.6	30.3	56.3	41.8
Lys	8.6	12.6	38.9	31.6	22.3
Pro	1.9	36.1	50.6	35.4	42.6
Tyr	3.6	110.4	90.6	131.1	112.8
Total	266	997.4	1660.5	1619.55	1537.3

BF: Before fermentation; S-NS: No starter added; S-PXP: *L. plantarum*-15, *S. xylosus*-12 and *P. pentosaceus*-ATCC33316 mixed culture; S-PXC: *L. plantarum*-15, *S. xylosus*-12 and *L. casei* subsp. *casei*-1.001 mixed culture; S-XCP: *S. xylosus*-12, *L. casei* subsp. *casei*-1.001 and *P. pentosaceus*-ATCC33316 mixed culture.

4. Conclusion

This study determined the effect of the mixed starter cultures on biogenic amine formation in fermented silver carp sausage. Mixed starter cultures decrease pH quickly, inhibit the growth of contaminant microorganisms present in the raw materials, and suppress the accumulations of histamine, cadaverine, putrescine, tryptamine and tyramine. To avoid the formation of high concentrations of biogenic amines in silver carp sausage, it is advisable to inoculate mixed starter cultures with negative-decarboxylate activity and use to top-quality raw meat materials for the manufactured food products.

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