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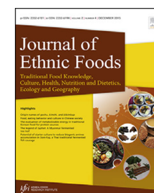

Original article

Potential of starter culture to reduce biogenic amines accumulation in *som-fug*, a Thai traditional fermented fish sausage

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ABSTRACT

This study assessed the effects of starter cultures (*Lactobacillus sakei* KM5474 and *Lactobacillus plantarum* KM1450) on the accumulation of biogenic amines (BAs) in Thai traditional fermented fish sausage. BAs formation (cadaverine, putrescine, histamine, tryptamine, phenylethylamine, and tyramine) was significantly higher in *som-fug* fermentation without starter culture. Accumulation of these BAs in *som-fug* could reduce significantly by the incubation with of *L. sakei* KM5474 and *L. plantarum* KM1450. The influence of the availability of free amino acids (FAAs) on BA formation during the fermentation of *som-fug* with and without starter culture was also investigated. The significant differences in the amount of some FAAs among batches would be partially attributed to the distinct proteolytic and BAs accumulation of specific microbiota of each batch. In general, amounts of FAA were related with their corresponding BAs. In batch control of the availability of practically all FAA precursors, BA formation could be observed. The formation of some BAs (histamine, putrescine, cadaverine, and phenylethylamine) occurring in batch control was related to the amounts of FAA precursors (histidine, arginine, lysine, and phenylalanine). Starter culture *L. plantarum* KM1450 was more efficient in reducing BA accumulation in *som-fug* than that of starter culture *L. sakei* KM5474, while mixed starter cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 showed the highest effectiveness in reducing BA accumulation during *som-fug* fermentation compared with the sample sausage without starter culture or with monostarter culture.

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1. Introduction

Biogenic amines (BAs) are nonvolatile low-molecular weight nitrogenous organic bases, derived through the decarboxylation of corresponding amino acids, in which they are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines. High concentrations have been found in fermented foods as a result of a contaminating microflora with amino acid decarboxylase activity [1]. However, BAs can also trigger human health problems leading to palpitations, hypertension, vomiting, headaches, and flushing, if food containing high concentrations are ingested. In fermented foods, certain microorganisms are able to convert available amino acid precursors into BAs via decarboxylase or deiminase activities during or following fermentation processes. For this reason, amino acid catabolism can affect both the quality and safety of fermented foods [2]. The quantity and kind of BAs formed depends on the nature of food and particularly on the kind of microorganisms present. Enterobacteriaceae are particularly active in the production of BAs [3]. These microorganisms may form part of the food-associated population or contamination before, during, or after processing of the food product. Therefore, microorganisms naturally present in raw materials introduced throughout the processing can critically influence BA production during the manufacture of fermented products [4].

Som-fug is a Thai traditional fermented food, composed of minced fish meat, cooked rice, minced garlic, and sodium chloride. The ingredients are mixed thoroughly and tightly packed in banana leaves or plastic bags and let to ferment for 2–5 days at ambient temperature (25–30°C) [5]. *Som-fug* can be consumed as uncooked *som-fug* or cooked *som-fug* in the form of fried *som-fug* either as a main dish or as a snack with vegetables. Uncooked *som-fug* contains live microflorae some of which are probiotics including *Lactobacillus* and *Bifidobacterium*. These microorganisms offer some form of health benefits to the host such as regulating proper

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intestinal function and digestion and offering significant protection against certain pathogenic bacterial infection [6,7]. Consuming uncooked *som-fug* with active live cultures confer a health benefit on the host. *Som-fug* contains high nutritional values with an excellent source of protein. Freshwater fish are generally used rather than sea fish [5,8]. The species of fishes are included rohu (*Labeo rohita*), spotted featherback (*Notopterus chitala*), smallscale mud carp (*Cirrhinus microlepis*), grey featherback (*Notopterus notopterus*), and giant snake-head fish (*Ophicephalus micropeltes*) [8,9].

During meat fermentation, microbial growth, acidification, and proteolysis provide favorable conditions for BA production. BAs can reach concentrations higher than 1,000 mg/kg [10], which constitutes a health hazard. There is limited research on the toxicity of BAs and most focus on histamine. Moreover, it is notable that intolerance levels depend on the characteristics of the individual. It is assumed that the intake of foods with histamine concentrations higher than 400 mg/kg is hazardous to health [11]. It was demonstrated that 75 mg of oral histamine provoke symptoms in 50% of healthy individuals [12], and approximately 1,000 mg of histamine intake is associated with severe intoxications [13]. Tyramine concentrations higher than 125 mg/kg have effects in healthy individuals, and an intake of 6 mg/kg is potentially toxic to patients treated with monoamine oxidase inhibitors [14]. High concentrations of tyramine in the brain have been associated with depression, schizophrenia, Parkinson's disease, and Reye's syndrome [15]. Secondary amines including putrescine and cadaverine can react with nitrite to form carcinogenic nitrosamines [16]. There is increasing evidence that putrescine could have a role in promoting the malignant transformation of cells. Dietary putrescine increased the malignancy grade of adenomas in a murine model [17]. High levels of putrescine have also been detected in gastric carcinomas caused by *Helicobacter pylori* and the putrescine levels are maintained though the microbial infection is eliminated [18]. It is known that putrescine and cadaverine enhance histamine toxicity [19]. Elevated levels (50–100 mg/L) of cadaverine and putrescine also exert a considerable impact on the organoleptic properties. BA production is a characteristic of certain microorganisms such as Micrococcaceae, Enterobacteriacece, Enterococci, and *Pseudomonas* species by microbial substrate-specific enzyme decarboxylation of amino acids [20]. Therefore, BAs are also related to food spoilage due to decarboxylase activity of spoiling microorganism during fermentation [21]. Maijala and Eerola [22] concluded that contaminant microbial spoilage plays an important role in tyramine and histamine formation during the fermentation of sausages. Putrescine is the major diamine produced by *Pseudomonas*, whereas cadaverine is produced by Enterobacteriaceae [23].

BAs can be degraded through oxidative deamination catalyzed by amines oxidase (AO) with the production of aldehyde, ammonia, and hydrogen peroxide. Monoamine oxidases and diamine oxidases have been described from some genus of the family Enterobacteriaceae [24]. The potential role of microorganisms with AO activity has become a particular interest to prevent or reduce BA accumulation in fermented foods. Mah and Hwang [25] investigated the effect of *Staphylococcus xylosus* to inhibit BAs formation in a salted and fermented anchovy. Reduction of tyramine during fermentation of fermented sausages was achieved when *Micrococcus varians* was applied as a starter culture [26]. Inoculation of *Lactobacillus plantarum* in sauerkraut effectively suppressed the production of tyramine, putrescine, and cadaverine [27].

The sequential action (in the presence of an electron acceptor, such as O_2) of an AO and aldehyde dehydrogenase leads to the production of an acid and ammonia, which can be used to support microbial growth. Diamine oxidases can oxidase several BAs, such as putrescine and histamine. Leuschner and Hammes [26] tested *in vitro* the potential amine degradation by strains belonging to the genera *Lactobacillus*, *Pediococcus*, *Micrococcus*, as well as *S. carnosus* and *Brevibacterium linens*. They found that this enzymatic activity can be present at very different quantitative levels. Tyramine oxidase activity was strictly dependent on pH (with an optimum at 7.0), temperature, and NaCl, as well as glucose and hydralazine concentration. Moreover, this enzyme was characterized by a higher potential activity under aerobic conditions [28]. The AO responsible for this degradation has its optimum temperature at 37°C and retains about 50% of its maximum activity at 20°C [29]. Many *S. xylosus* strains isolated from artisanal fermented sausages in southern Italy showed the ability to degrade BA *in vitro* [30]. Among the strains tested, *S. xylosus* S81 completely oxidized histamine, but it degraded, under the adopted conditions, a part of tyramine as well. Reduction of histamine in dry sausages has been observed in the presence of AO-positive staphylococcal starter cultures [26]. In addition, important reduction of the concentration of tyramine and putrescine in the presence of AO positive *S. xylosus* starter cultures have been observed by Gardini et al [31]. So, the presence and relative activity of AO should be considered as an important characteristic in the selection of starter cultures used in the production of fermented foods.

BAs in fermented foods are a potential risk for consumers [32]. BA formation has been found in fermented sausages [33]. BAs, especially histamine, putrescine, tyramine, and cadaverine, have been suggested as indicators of spoilage of some foods, such as fresh fish, meat, and vegetables by contaminating microbial spoilage [34]. *Som-fug* is a popular domestic fermented food in Thailand. Because of increasing demands in the markets *som-fug* has been continuously produced in higher amounts.

In addition, since *som-fug* production is important to determine the quality and safety of *som-fug* for consumption. Depending on the initial number of contamination, the occurrence of microbial spoilage and pathogens such as *Salmonella* species, *Staphylococcus aureus,* and *Listeria monocytogenes* was found especially in *som-fug* with a pH higher than 4.6. Owing to inconsistency of product quality and ambiguous product safety, improved processes of *som-fug* fermentation have been developed. Starter cultures could be applied to improve and stabilize, as well as to protect certain pathogens and reduce BA content of the fermented product.

There is limited information on BA formation in *som-fug* and only some information is obtainable on the effect of starter cultures on BA reduction in *som-fug*. Therefore, the objectives of this study were to investigate BA formation and the reduction of BA contents in *som-fug* fermenting by starter cultures by comparing the effectiveness of AO-positive lactic acid bacteria (LAB) as a monostarter culture and mixed starter cultures in reducing BA accumulation during *som-fug* fermentation. In addition, the changes of chemical and microbial properties of *som-fug* during fermentation were investigated.

2. Materials and methods

2.1. Selection of AO-exhibiting strains of starter culture

Overnight-tested cultures were harvested, washed with 0.05M phosphate buffer (pH 7), and the cell pellet resuspended in 0.05M phosphate buffer supplemented with tyramine, histamine, tryptamine, phenylethylamine, putrescine, and cadaverine. The cell concentration was adjusted to 10^7 CFU/mL. The cell suspensions (20 mL) were incubated in a 100 mL flask and shaken at 200 rpm at 30°C for 24 hours. Samples were taken and added to an equal amount of 1M HCl. The mixture was boiled for 10 minutes and centrifuged at 9,000*g*. The supernatant was frozen at −15°C until high-performance liquid chromatography (HPLC) analysis.

2.2. Som-fug preparation

Minced fresh fish (smallscale mud carp or *Cirrhinus microlepis*) 1 kg (58.8%), garlic 100 g (5.9%), cooked rice 500 g (29.4%), and sodium chloride 100 g (5.9%) were mixed thoroughly, packed into a plastic casing, and sealed before fermentation. Four separated batches of fermented sausage were prepared without a starter culture (naturally fermented), with *Lactobacillus sakei* KM5474, with *L. plantarum* KM1450, and with mixed starter cultures (*L. plantarum* KM1450 and *L. sakei* KM5474) of approximately 10^7 cell/g. All treatment batches were incubated at 30°C for 0 hours, 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours for analyses.

2.3. Preparation of starter culture

The *som-fug* starter, *L. plantarum* KM1450 and the tyraminogenic *L. sakei* KM5474 were obtained from the Department of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Thailand. Each starter was grown separately in de Man, Rogosa, and Sharpe broth at 30°C for 24 hours. Cells of *L. plantarum* KM1450 and *L. sakei* KM5474 were collected by centrifugation at 12,000 rpm at 4°C for 10 minutes. The bacterial cells were harvested and washed three times with peptone solution (0.1% w/v) and adjusted to cell concentrations of 10^7 CFU/mL and 10^9 CFU/mL using the same diluent, respectively. *Som-fug* samples were prepared in four separate batches according to the bacterial strains used: (1) *som-fug* naturally fermented without starter (as control); (2) with inoculation of 10^7 CFU/g of *L. plantarum* KM1450; (3) with inoculation of 107 CFU/g of *L. sakei* KM5474; and (4) with inoculation of 10^7 CFU/g of *L. plantarum* KM1450 and 10^7 CFU/g *L. sakei* KM5474. Each batch preparation was stuffed into casings and incubated at 30°C.

2.4. Microbiological analysis

Using aseptic techniques, 20 g of each sample (three replicates) was homogenized in 180 mL of sterile peptone saline (sterile 0.1% peptone saline). Following mixing at 250 rpm for 10 minutes inside a stomacher, the suspension was serially diluted (1:10) in peptone saline in triplicate. Total aerobic bacteria were determined by plating sample on plate count agar (Merck, Darmstadt, Germany) at 30°C for 24–48 hours. LAB enumeration was determined with inoculation of diluted suspensions in de Man, Rogosa, and Sharpe agar [35] and incubated anaerobically at 37°C for 48 hours. Bacteria numbers were expressed as logarithms of colony forming units per gram (log CFU/g). Means and standard deviations were calculated.

2.5. Determination of trichloroacetic acid soluble peptides

Trichloroacetic acid (TCA) soluble peptide of the fermented sausages was measured with a method described previously [36]. A sample (3 g) was homogenized with 27 mL of 5% TCA (w/v). The homogenate was kept in ice for 1 hour and centrifuged at 12,000*g* for 5 minutes. The soluble oligopeptide content in the supernatant was determined according to by the method of Lowry et al [37]. Results were expressed as μmol/g (dry matter).

2.6. Extraction of amino acids and BAs

Ten milliliters of 10% (w/v) TCA were added to 3-g samples, and homogenization of the mixture was effected via shaking for 1 hour. The extract was then filtered through Whatman No. 1 filter paper. To remove any fat, the samples were kept at −20°C for 1 day, and then centrifuged at 7,000*g* for 15 minutes. The supernatants were collected and filtered through a 0.25-μm membrane filter.

2.7. Determination of BAs

Amines were determined using the HPLC method described by Hernández-Jover et al [38]. The method is based on the formation of ion pairs between amines extracted with 0.6M perchloric acid from 5–10 g of sample, and octanesulfonic acid present in the mobile phase. Separation was performed using a reversed phase column, and then postcolumn derivatization with *o*-phthalaldehyde is followed by spectrofluorimetric detection. The method allows one to quantify, by an external standard procedure, 6 BAs, i.e., tyramine, histamine, tryptamine, phenylethylamine, putrescine, and cadaverine. Samples for BA determination were stored at −15°C until required.

2.8. Determination of free amino acids

Free amino acids (FAAs) in samples were determined using HPLC according to the method proposed by Rozan et al [39]. A 20-μL aliquot of amino acid standard and digested sauce samples were transferred into vials and dried under vacuum. Then 20 μL of drying reagent containing methanol, water, and triethylamine (ratio 2:2:1 v/v) was added. Then 20 μL of derivatizing reagent containing methanol, triethylamine, water, and phenylisothiocyanate (ratio 7:1:1:1 v/v) was added. The derivatized samples were then dissolved in 100 mL of buffer A that was used as a mobile phase for HPLC. A Purospher STAR RP-18e, 5-μm column was used with buffer A (0.1M ammonium acetate, pH 6.5) and buffer B (0.1M ammonium acetate containing acetonitrile and methanol, 44:46:10 v/v, pH 6.5) as a mobile phase set for linier gradient at the flow rate of 1 mL/min. The injected sample volume was 20 μL and monitored at 254 nm of wavelength.

2.9. Statistical analysis

All data from the analyses were calculated to obtain their mean values and standard deviation of three determinations. All statistical analyses were done using one-way analysis of variance. Tukey's multiple range tests was employed to make confidence intervals for the differences between means at $p < 0.05$.

3. Results and discussion

3.1. Som-fug preparation

Som-fug was obtained from fermented mixture of minced fresh fish (smallscale mud carp), minced garlic, cooked rice, and sodium chloride in plastic casing as shown in Fig. 1A. Raw *som-fug* after fermentation and fried *som-fug* are shown in Figs. 1B and 1C, respectively.

3.2. AO exhibiting strains of starter cultures

Study of AO-exhibiting strains of LAB for use as starter cultures is shown in Table 1. AO-exhibiting strains of LAB were investigated by determination of BAs in buffer system by HPLC with *o*-phthalaldehyde derivatization. Among the seven LAB strains tested, five lactobacilli (in particular, *L. curvatus*) were amine producers and *L. plantarum* KM5474 and *L. sakei* KM1450, were nonamine-forming strains with exhibiting AO activity. Therefore, in this study, these two strains were used as starter cultures of *som-fug* fermentation to investigate the ability of AO activity to degrade amines *in vivo* during sausage fermentation.

Fig 1. Pictures of *som-fug*. (A) Fermented in closed transparent plastic bag. (B) Raw *som-fug*. (C) Fried *som-fug*.

Table 1
Strains exhibiting the potential to degrade biogenic amines in a buffer system within 24 hours at 30°C.

Strain	Percent degradation (%)					
	Trym	Phem	Put	Cad	Him	Tym
Lactobacillus curvatus KM2633	0	0	0	0	0	0
Lactobacillus farciminis KM7642	0	0	0	0	0	4.5
Lactobacillus plantarum KM5474	2.1	3.5	11.9	4.7	6.9	17.2
Lactobacillus kandleri KM2416	0	0	0	2.7	0	0
Leuconostoc mali KM2725	0	0	0	0	0	0
Lactobacillus sakei KM1450	0	1.6	9.3	6.9	2.7	8.4
Lactobacillus reuteri KM8124	0	0	3.6	0	0	0

Cad, cadaverine; Him, histamine; Phem, phenylethylamine; Put, putrescine; Trym, tryptamine; Tym, tyramine.

3.3. Microbiological analysis

The differences between *som-fug* in counts of LAB during fermentation are shown in Fig. 2. LAB in *som-fug* increased until 120 hours of fermentation. Counts of LAB in *som-fug* with *L. sakei* KM5474, *L. plantarum* KM1450, and mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 at 120 hours of fermentation at 30°C (8.1 log CFU/g, 8.7 log CFU/g, and 9.6 log CFU/g, respectively) were higher ($p < 0.05$) than in *som-fug* control (7.3 log CFU/g), while the highest LAB observed at 72 hours of fermentation were 8.4 log CFU/g, 9.0 log CFU/g, 9.8 log CFU/g, and 7.5 log CFU/g for *som-fug* with *L. sakei* KM5474, *L. plantarum* KM1450, and mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 and control, respectively. LAB counts in *som-fug* increased steadily during fermentation. In the present study, total LAB counts in *som-fug* with starters on 120 hours of fermentation were higher ($p < 0.05$) in comparison with the *som-fug* control. An increase of LAB in *som-fug* with starters and control consecutive increase until 120 hours of fermentation was significant. The differences between *som-fug* in counts of total plate count (TPC) during fermentation are shown in Fig. 3. TPC in *som-fug* increased until 120 hours of fermentation. TPC in *som-fug* with *L. sakei* KM5474, *L. plantarum* KM1450, and mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 120 hours of fermentation at 30°C (8.74 log CFU/g, 9.42 log CFU/g, and 9.8 log CFU/g, respectively) were higher ($p < 0.05$) than in *som-fug* control (7.8 log CFU/g), while the highest TPC observed at 72 hours of fermentation were 9.12 log CFU/g, 9.65 log CFU/g, 10.1 log CFU/g, and 8.6 log CFU/g for *som-fug* with *L. sakei* KM5474, *L. plantarum* KM1450, and mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 and control, respectively. TPC counts in *som-fug* increased steadily during fermentation. In the present study, TPC in *som-fug* with starters on 120 hours of fermentation were higher ($p < 0.05$) in comparison with the *som-fug* control. An increase of TPC in *som-fug* with starters and control consecutively increased until 120 hours of fermentation was significant.

3.4. TCA-soluble peptides analysis

Fig. 4 shows that TCA-soluble peptide content of *som-fug* samples, the initial content was 15.4 µmol/g dry matter. It then gradually increased throughout the fermentation process. The TCA-soluble peptide content of *som-fug* control and *som-fug* with *L. sakei* KM5474, *L. plantarum* KM1450, and mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 reached 82.4µmol/g, 63.5µmol/g, 55.3µmol/g, and 49.7 µmol/g dry matter, respectively, at the end of fermentation (hour 120). However, there was not significant difference ($p < 0.05$) between the TCA-soluble peptide content of samples inoculated with starter culture after 24 hours of fermentation. The results was shown that *som-fug* control

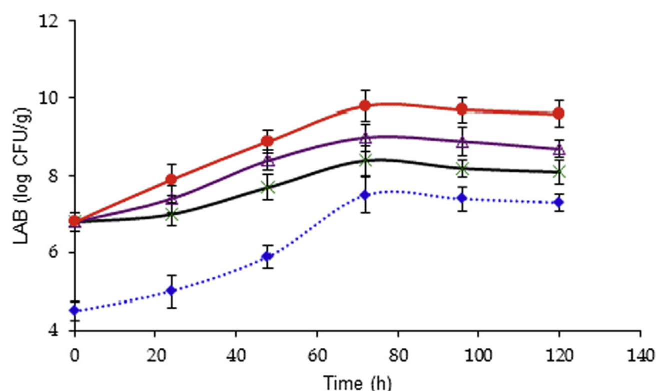

Fig. 2. Total count of lactic acid bacteria during fermentation of *som-fug* control (◆) and *som-fug* with *Lactobacillus sakei* KM5474 (×), *Lactobacillus plantarum* KM1450 (Δ), and mixed cultures of *L. plantarum* KM1450 and *L. sakei* KM5474 (●). LAB, lactic acid bacteria.

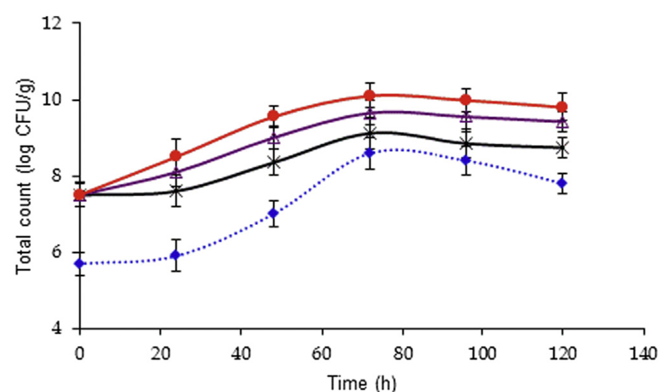

Fig. 3. Total plate count during fermentation of *som-fug* control (◆) and *som-fug* with *Lactobacillus sakei* KM5474 (×), *Lactobacillus plantarum* KM1450 (Δ), and mixed cultures of *L. plantarum* KM1450 and *L. sakei* KM5474 (●).

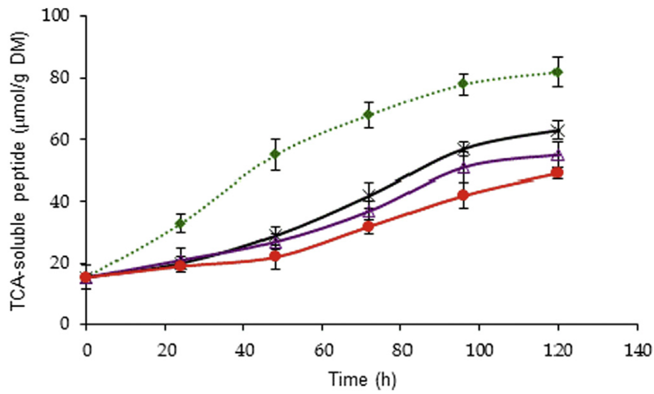

Fig. 4. Total plate count-soluble peptide content during fermentation of *som-fug* control (◆) and *som-fug* with *Lactobacillus sakei* KM5474 (×), *Lactobacillus plantarum* KM1450 (Δ), and mixed cultures of *L. plantarum* KM1450 and *L. sakei* KM5474 (●). DM, dry matter; TCA, total plate count.

fermented contained TCA-soluble peptide content higher than the other *som-fug* samples after fermentation for 120 hours.

Proteolysis leads to an increasing number of soluble peptides in all *som-fug* samples during fermentation and leads to an increase of amino acid content. Some of these FAAs can be subjected to decarboxylation reactions, which are catalyzed by specific bacterial decarboxylases and result in the formation of BAs. Staphylococci are strongly related to proteolytic processes during meat fermentation, although LAB have also shown to have proteolytic or aminopeptidase activity on meat proteins [40]. However, *som-fug* with starter cultures liberated soluble peptides at a lower rate than that of the control. A decrease in soluble peptides of *som-fug* with starter cultures (Fig. 4) showed lower proteolysis from the growth of spoilage microorganisms. These results might be due to the presence of a wide range of proteolytic enzyme systems in microbial spoilage than starter cultures. In *som-fug* with starter cultures, lactic acid produced by LAB starters increased the firm texture of fermented meat and inactivated the growth of spoilage microorganisms resulting in a decrease in proteolysis rate and also resulting in the reduction of BA formation. BAs have been found in several cheeses including Swiss, Emmental, Cheddar, Gouda, Blue, Ras, processed cheese, and Hungarian hard cheese [41,42]. Kebary et al [43] reported a positive correlation between an increase in total FAAs with the total BAs in cheese. This indicated that lower proteolysis caused lower BAs formation. Steidlova and Kalac [44] found high levels of BAs in silages prepared from forages with high content of proteins and high levels of BAs in maize silages. BAs are also present in all natural products in which protein degradation has occurred. Thus, treatments with high proportion of soluble peptides (highly degradable protein) were observed to be also high in BAs. These results corresponded to this study that was also found positive correlation between total FAAs and total BAs that came from lower proteolysis causing lower FAAs and BAs formation. Besides the importance of presence of FAAs, this kind of bacteria is crucial for the formation of BAs in *som-fug*. The presence and accumulation of BAs depends on many factors such as presence of specific bacteria and enzymes, availability of substrate (FAAs), presence of suitable cofactor, existence of a proper environment in fermented food (higher pH, higher moisture, higher temperature, and lower salt content), fermentation, and storage period [45].

The contents of putrescine, tryptamine, cadaverine, tyramine, phenylethylamine, and histamine in *som-fug* during fermentation are shown in Fig. 5. Highest contents of histamine and putrescine were BAs found in *som-fug* control. Lowest BA contents were found in *som-fug* with mixed starter culture. It was reported that histamine, putrescine, and tyramine were indicated as spoilage in meat [46]. It might be that proteolysis provides the nutrients for growth of spoilage microorganisms during fermentation. Decreasing proteolysis of *som-fug* with starter cultures (Fig. 4) involved in decreasing of BA contents.

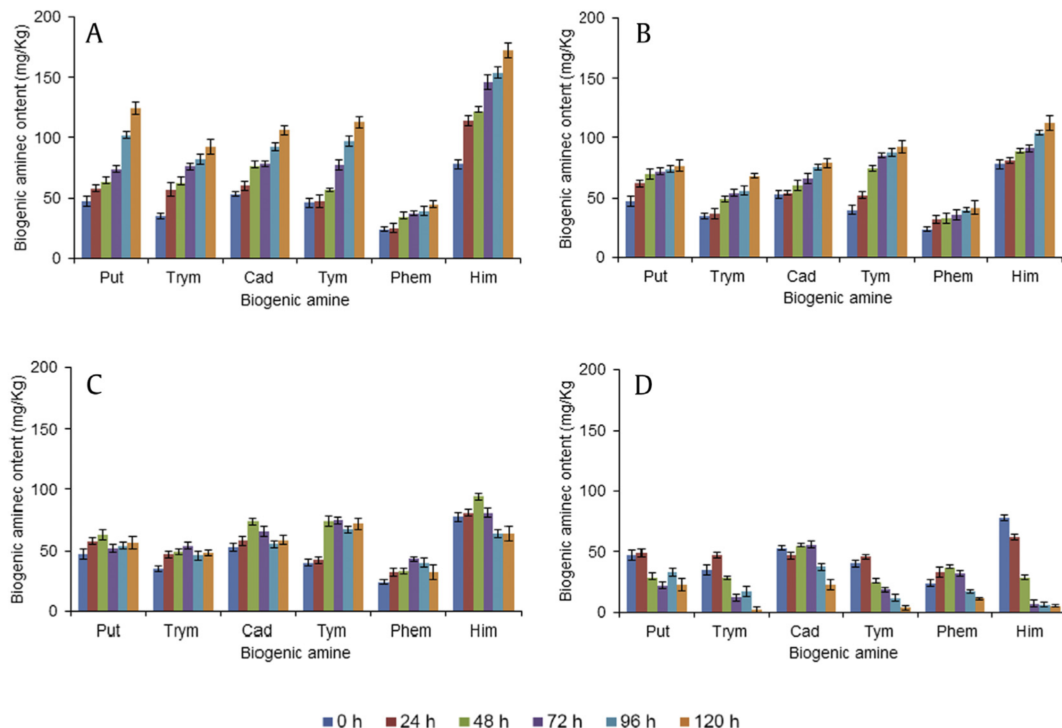

Fig. 5. Biogenic amines accumulation. (A) *Som-fug* control. (B) *Som-fug* with *Lactobacillussakei* KM5474. (C) *Lactobacillus plantarum* KM1450. (D) Mixed cultures of *L. plantarum* KM1450 and *L. sakei* KM5474 during fermentation 0–120 hours. Cad, cadaverine; Him, histamine; Phem, phenylethylamine; Put, putrescine; Trym, tryptamine; Tym, tyramine.

3.5. BA contents of som-fug

Occurrence of toxic compounds such as BAs is favored a high concentration of substrates (i.e., FAAs) together with environmental and technological factors (e.g., NaCl content, chemicophysical variables, hygienic procedure adopted during production) promoting microbial growth and the decarboxylase activity of microorganisms [1]. In this study, a high correlation among total BA and total FAA content was observed. Temperature markedly influences the formation of BAs, and at 30°C decarboxylases might be still active [47]. During fermentation, the higher temperature exceeds 14–15°C the higher decarboxylase activities might release BAs from FAAs. In this respect, processing procedures for *som-fug* based on low salt addition and high fermentation temperatures (over 20°C), may favor proteolytic and decarboxylase activities. The high values of histamine, putrescine, and tyramine detected in some *som-fug*, may be ascribed to inadequate microflora and LAB producing BA reduction occurring in *som-fug* control (Fig. 5).

The toxicological level of BAs depends on the individual characteristics and the presence of other amines [48]. Toxic doses of tyramine in foods were reported in the range 100–800 mg/kg, but average amounts of tyramine detected in analyzed samples (Fig. 5) were below this range, even though in few samples the 100 mg/kg value was exceeded. Putrescine has been regarded as not toxic by itself, but as a potentiator for the toxic effect of tyramine and histamine if present. However, it was probable to demonstrate a significant relationship between the concentration of a specific FAA and its corresponding BA in meat products [49]. Fig. 5 shows the BA content of *som-fug* evidence of the effect of starters on the decrease of the BA occurrence in *som-fug* after fermentation. Histamine was always below the minimum detectable, in spite of the abundance of their precursors (histidine) released during the process.

The concentration of tyramine was high in *som-fug* control while low concentration of their precursors (tyrosine) was released during the process. Moreover, tryptamine was absent in all the investigated samples. The sum of vasoactive BAs (VBAs) (tyramine, phenylethylamine, histamine, and tryptamine) lower than 200 mg/kg has been suggested by Eerola et al [49] as a quality index (VBA index) for fermented meat products. It is interesting to note that the computed VBA index of *som-fug* with mixed starter cultures with differently processed resulted appreciable samples at 120 hours of fermentation (21.78 ± 2.46 mg/kg). These results could be related to the specific characteristics of the product as well as to the process conditions adopted that could, in general, have limited the growth and activity of amino acid decarboxylase-positive microorganisms [50]. Histamine, putrescine, and tyramine were found in high amounts in *som-fug* control. However, the occurrence of BAs in *som-fug* control and after the fermentation could be due to the microflora and LAB-producing BAs that could have favored their formation during fermentation. During fermentation of *som-fug* control, histamine, putrescine, and tyramine show a marked increase with high amounts of their precursor, histidine, arginine and tyrosine, respectively, were detected. In fact, arginine may generate putrescine both via arginine deiminase pathway (ADI) leading to ornithine [51] and their subsequent decarboxylation to putrescine, and via arginine decarboxylation to agmatine followed by deamination to putrescine and removal of urea [52]. It seems reasonable to postulate that the large amounts of arginine could be the source of putrescine, which subsequently may be converted in spermine and spermidine by transamination reactions [53].

3.6. FAA contents of som-fug

FAAs were reported in Table 2 as net amounts (mmol/g dry matter) in order to investigate the differences in contents due to starters in *som-fug* during fermentation. FAAs were compared to evaluate if the extended fermentation times gave a similar increase in all of them or if different patterns were detectable. Most single FAAs increased during fermentation with particular reference to the lipophylic ones) a rise in lypophilic valine, phenylalanine, and tryptophan processed following a traditional prolonged way [54]. In the present study, *som-fug* showed a FAA pattern enriched with glutamic acid, alanine, arginine, cysteine, serine, threonine, and glycine, and most FAAs displayed a rise during the extended fermentation. Arginine found in the most fermented *som-fug* was increased, due to changes of its content by proteolysis and rise in arginine in fermented *som-fug* control was higher than fermented *som-fug* with starters. Arginine hydrolysis could be hydrolyzed via the ADI leading to ammonia and ornithine. It seemed reasonable to postulate that ADI pathway enzymes (arginine deiminase and ornithine transcarbamylase) could be still active during fermentation times. Arginine catabolism may be regarded as a source of putrescine both via ADI ornithine generation [51] and subsequent decarboxylation to putrescine, and via arginine decarboxylation to agmatine followed by deamidation to putrescine and removal of urea [52]. The presence in *som-fug* of environmental conditions suitable for decarboxylase activities together with large amounts of arginine may be consistent with the increase in putrescine.

The evolution during fermentation of the total FAA content, in both the *som-fug* control and after inoculation with either of the two *Lactobacillus* strains selected, is shown in Table 2, and encompassed 17 different amino acids. The control *som-fug* showed the highest concentration of total amino acids at a 17.9% level of significance. The contents of total amino acids in *som-fug* inoculated with *L. sakei* KM5474, *L. plantarum* KM1450, and mixed cultures of *L. plantarum* KM1450 and *L. sakei* KM5474 increased throughout time, but at lower rates than the control at 12.6%, 11.9%, and 7.3% level of significance, respectively.

The contents of FAAs and BAs in control and experimental *som-fug* increase significantly throughout fermentation. However, specific lactic acid strains of the *Lactobacillus* genus can effectively prevent BAs from building-up excessively, putrescine and histamine (for quantitative reasons, owing to its level). This may lead to a favorable contribution to public health, especially in regions where *som-fug* is frequently included in the diet. To have an overall evolution index of the proteases action in the *som-fug* during processing the TCA-soluble peptide was evaluated (Fig. 4) [55]. More intense proteolytic activity occurred in the *som-fug* control. The TCA-soluble peptide values of *som-fug* control are quite high compared to those generally observed in other *som-fug* with starters. This could be attributed to the microflora in *som-fug* control slightly higher proteolytic activity during the process, in comparison to *som-fug* applied with starters. Proteolysis contributes to texture by breakdown of the muscle structure [56].

The FAA content of *som-fug* during fermentation arginine, glutamic acid, and histidine were the FFAs most representative. Table 2 shows the effect of the starters treatment on the evolution of the FAA pattern of the *som-fug* investigated during the fermentation: a significant increase in the concentration of FAAs with respect to their initial occurred in *som-fug* control and *som-fug* with starters, resulting from the aminopeptidases activity of meat [53] as well as microbial proteases [57].

Moreover, *som-fug* control seems to affect the production of some amino acids (Table 2). After fermentation a marked increase of alanine was observed in *som-fug* control. It was noticed that *som-fug* with mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 starters contained FAAs content lower than that of *som-fug* with monoculture starter and *som-fug* with *L. plantarum* KM1450 was observed FAAs content lower than that of *L. sakei* KM5474.

Table 2
Amino acid content of *som-fug* without and with starter cultures at 0 hours and 120 hours of fermentation at 30°C.

Amino acid	*L. sakei* + *L. plantarum*		*L. plantarum*		*L. sakei*		Control	
	0 h	120 h	0 h	120 h	0 h	120 h	0 h	120 h
Ala	3.32 ± 0.12^{Aa}	3.41 ± 0.15^{ABa}	3.44 ± 0.26^{Aa}	3.63 ± 0.12^{Ba}	3.37 ± 0.24^{Aa}	4.36 ± 0.14^{Ca}	3.59 ± 0.12^{Ba}	4.42 ± 0.28^{Ca}
Arg	16.26 ± 0.14^{Ah}	17.43 ± 0.21^{Bb}	16.43 ± 0.23^{Ab}	17.24 ± 0.37^{Bb}	16.21 ± 0.32^{Ab}	18.16 ± 0.43^{BCb}	16.32 ± 0.17^{Ab}	18.59 ± 0.36^{Cb}
Asp	0.94 ± 0.15^{Ac}	1.02 ± 0.08^{Ac}	1.18 ± 0.04^{Ac}	1.84 ± 0.07^{Bc}	1.07 ± 0.09^{Acj}	1.61 ± 0.28^{Bcf}	1.41 ± 0.05^{Cc}	1.84 ± 0.12^{Bci}
Cys	0.76 ± 0.09^{Ac}	1.21 ± 0.14^{Bcm}	0.87 ± 0.08^{Ad}	1.02 ± 0.14^{Bd}	1.26 ± 0.15^{Bc}	1.28 ± 0.22^{BCd}	1.57 ± 0.17^{Cch}	2.05 ± 0.26^{Dc}
Glu	4.82 ± 0.21^{Ad}	5.22 ± 0.12^{Bd}	4.52 ± 0.27^{Ae}	5.27 ± 0.23^{BCe}	5.04 ± 0.16^{Bd}	5.61 ± 0.26^{Ce}	4.73 ± 0.22^{Ad}	5.72 ± 0.24^{Bd}
Gly	6.39 ± 0.23^{Ae}	6.14 ± 0.16^{ABe}	5.91 ± 0.22^{BCf}	5.48 ± 0.26^{Ce}	6.17 ± 0.18^{Be}	$5.55 \pm 0.\ 21^{Ce}$	5.46 ± 0.15^{Ce}	5.16 ± 0.22^{Ce}
His	3.14 ± 0.16^{Af}	3.67 ± 0.17^{Bf}	3.72 ± 0.15^{Bg}	4.26 ± 0.09^{Cf}	3.23 ± 0.15^{Af}	4.37 ± 0.14^{Ca}	3.22 ± 0.08^{Af}	3.91 ± 0.15^{Bf}
Ile	0.82 ± 0.07^{ABc}	0.66 ± 0.08^{Ag}	0.95 ± 0.07^{Bd}	1.52 ± 0.04^{Cg}	1.24 ± 0.06^{Dc}	1.71 ± 0.06^{Ef}	1.43 ± 0.14^{Cc}	1.88 ± 0.14^{Eci}
Leu	2.32 ± 0.09^{Ag}	1.56 ± 0.14^{Bh}	2.45 ± 0.18^{Ah}	1.89 ± 0.06^{Cc}	2.75 ± 0.12^{Dg}	3.26 ± 0.12^{Eg}	2.16 ± 0.17^{Fg}	3.23 ± 0.08^{Eg}
Lys	3.25 ± 0.07^{Af}	3.43 ± 0.12^{ACf}	2.76 ± 0.14^{Bh}	3.72 ± 0.18^{Ch}	3.67 ± 0.12^{Ch}	4.29 ± 0.17^{Da}	3.26 ± 0.16^{Af}	4.46 ± 0.18^{Da}
Met	1.22 ± 0.04^{ADh}	1.14 ± 0.07^{Ac}	1.47 ± 0.12^{BDi}	0.76 ± 0.02^{Ci}	1.16 ± 0.08^{Ac}	1.33 ± 0.12^{Dci}	1.35 ± 0.08^{Dc}	1.45 ± 0.06^{Dh}
Phe	1.26 ± 0.08^{Ah}	1.76 ± 0.08^{Bb}	1.42 ± 0.09^{ADi}	1.98 ± 0.04^{Ec}	1.54 ± 0.06^{Di}	2.08 ± 0.14^{Eh}	1.72 ± 0.06^{Bh}	2.32 ± 0.12^{Ec}
Pro	2.14 ± 0.17^{Ag}	2.15 ± 0.04^{Ai}	2.31 ± 0.12^{Ah}	2.75 ± 0.08^{Bj}	2.52 ± 0.24^{Bg}	2.09 ± 0.15^{Ah}	$2.56 \pm 0.27^{Bg}i$	2.16 ± 0.14^{Ac}
Ser	0.67 ± 0.09^{Ac}	0.79 ± 0.06^{ABj}	0.82 ± 0.07^{ABd}	1.59 ± 0.14^{CEk}	0.91 ± 0.08^{Bj}	1.14 ± 0.08^{Di}	1.22 ± 0.16^{CDc}	1.68 ± 0.18^{Ei}
Thr	2.56 ± 0.16^{Ai}	2.72 ± 0.05^{Ak}	2.68 ± 0.12^{Ah}	2.03 ± 0.17^{Bc}	2.23 ± 0.14^{Bg}	3.16 ± 0.14^{Cgj}	2.51 ± 0.18^{Agi}	3.12 ± 0.12^{Ccg}
Tyr	2.32 ± 0.12^{Aj}	3.04 ± 0.02^{Bl}	2.61 ± 0.08^{Ch}	3.16 ± 0.11^{Bl}	2.28 ± 0.16^{Ag}	2.9 ± 0.16^{Bj}	2.67 ± 0.05^{Ci}	2.41 ± 0.16^{ACc}
Val	0.73 ± 0.05^{Ac}	1.42 ± 0.07^{Bm}	0.24 ± 0.03^{Ci}	1.04 ± 0.23^{Dd}	0.96 ± 0.04^{Dj}	1.06 ± 0.12^{Di}	0.34 ± 0.09^{Cj}	1.02 ± 0.08^{Dj}
Total	52.92 ± 0.12^{Ak}	56.77 ± 0.10^{Bn}	53.78 ± 0.13^{Cj}	60.17 ± 0.14^{Dm}	55.61 ± 0.14^{Ek}	63.90 ± 0.17^{Fk}	55.48 ± 0.14^{Ek}	65.42 ± 0.17^{Ak}

Mean values and standard deviations with different letters (a, b, c) in the same column indicate significant differences ($p < 0.05$) during fermentation, and different letters (A, B, C) in the same row indicate significant differences ($p < 0.05$) among *som-fug* formula.
Ala, alanine; Arg, arginine; Asp, aspartate; Cys, cysteine; Glu,. glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

A higher concentration of glutamic acid, alanine, histidine, phenylalanine, lysine, and threonine was detected, after fermentation, in *som-fug* processed. Arginine was the most abundant amino acid in all the final products, and its level was significantly lower in subjected with starters to *som-fug* than those in *som-fug* control.

The different profile of FAAs observed in *som-fug* control and *som-fug* with starters may be due to a different evolution of reactions and processes involving both production and consumption of amino acids that occur simultaneously during the various steps of the fermentation process and fermentation and whose combined effects could give rise to an increase or, on the contrary, to a decrease of their concentration. The aminopeptidase activity is considered the main process implied in the FFA release in meat. Moreover, FAA concentration could be decreased either by chemical and enzymatic reactions where they act as substrates leading to the formation of secondary products [54] and/or by microbial amino acid decarboxylase activity with consequent BA production [58].

In *som-fug* control, an effect due to higher concentration of decarboxylase than that of *som-fug* with starters, thus, their reaction with the FAAs causing an increase of their BA concentration in these samples. The fermented taste could be related to lysine and glutamic acid, while isoleucine and aspartic acid are implied in acid taste and unpleasant aroma [59]. In this study, the increase in concentration of lysine and glutamic acid was observed. The changes in the contents of FAAs observed in fermented sausages during fermentation are given in Table 2. The total FAAs contents of the *som-fug* control and *som-fug* with *L. sakei* KM5474 and *L. plantarum* KM1450 and mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 starter constituted 52.92 mmol/g, 53.78 mmol/g, 56.77 mmol/g, and55.48 mmol/g dry matter, respectively (before fermentation) on 0 hours. An increase in the contents of amino acids of *som-fug* with starters and *som-fug* control were observed 56.77 mmol/g, 60.17 mmol/g, 63.90 mmol/g, and 65.42 mmol/g dry matter during the fermentation at 120 hours, respectively. The data showed the highest total FAA concentration of 1,867.2 mmol/g was observed with *som-fug* control, whereas the lowest total FAAs concentration of 359.6 mmol/g was observed with *som-fug* with mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 starters.

The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and FAAs. This degradation can be produced by endogenous and microbial enzymes [60]. The increase in the total FAAs concentration was detected in all batches [60]. The main differences in the content of total FAAs among batches were detected during 120 h of fermentation. The amino acids in which differences, which were primarily responsible for the increase in total FAAs during fermentation, observed were arginine, glutamic acid, histidine, and phenylalanine, in *som-fug* with mixed cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 starters, while alanine and lysine were also observed increase in *som-fug* with *L. plantarum* KM1450 and threonine was another one was also observed increase in *som-fug* with *L. sakai* and control.

Verplaetse et al [61] reported an increase in the total FAA content during fermentation. The change occurred during the fermentation process indicating that the highest enzymatic activity took place during these stages. A major release of FAAs at the beginning of the process has been studied in coincidence with the fermentation stage. This increase has been attributed to the higher temperatures applied during fermentation compared to the low temperature. The most significant increases occurred in the content of arginine in the sample. The decrease in the content of amino acids may indicate their metabolism by bacteria [62].

Histamine and tyramine contents in *som-fug* samples varied from 5.02–172.27 mg/kg and 3.62–112.73 mg/kg, respectively. Histamine contents should be in the range of 50–100 mg/kg in sausage processed according to Good Manufacturing Practice [63]. Among all samples tested, histamine and tyramine levels were highest in *som-fug* control (172.27 mg/kg and 112.73 mg/kg, respectively). Şenöz et al [64] determined histamine content in Turkish style sausages of 6.72–362 mg/kg. The allowable maximum level of tyramine in foods is 100–800 mg/kg and tyramine with a level of 1080 mg/kg is toxic [32]. Tyramine is usually the major amine found in fermented sausages. Its production is mainly associated with tyrosine-decarboxylase activity of microbial spoilage [34].

In *som-fug* control, tryptamine was a minor BA. High content of BAs can be found in fermented products derived from raw materials with high protein content. The microbial spoilage often leads to high concentrations of BAs [1]. Also, the higher proteolysis in *som-*

fug control sample might favor the decarboxylase activity of microorganisms [65].

In contrast, the formation of some BA (tyramine) occurring in control batch, was related to the low contents of their FAA precursors (tyrosine). Despite the high formation of tyramine and depletion of tyrosine could have been a limiting factor for further additional increase. However, amount of tyrosine were enough for the strong formation of tyramine by decarboxylase positive in control batch. In this study, *L. sakei* KM5474 and *L. plantarum* KM1450 were decarboxylase-negative microorganisms and contained AO to degrade different types of BAs. Therefore, they could both in the presence of high FAAs not effect to the increase amount of BAs and in the same time BAs formation during fermentation could be degraded by their AO.

Mixed starter cultures have more benefits than monostarter cultures in different ways as shown in this study including increase growth rate. In mixed starter cultures, one microorganism may produce essential growth factors to another microorganism. Compounds made by mixed starter cultures might complement the others or exclude the contaminant microorganisms by producing certain inhibitors to eliminate undesirable microorganisms. Mixed starter cultures might produce enzymes to protect several toxic substances. In addition, mixed starter cultures have a variety of genetic bases to eliminate. Therefore, the prospects of mixed starter culture fermentations could be exploited rather than monostarter cultures.

4. Conclusion

In summary, the levels of BAs increased significantly in *som-fug* without starter during fermentation for 120 hours. Histamine, putrescine, and tyramine were BAs mostly found in *som-fug*. Monostarter culture *L. sakei* KM5474 could reduce BA accumulation in *som-fug*, while monostarter culture *L. plantarum* KM1450 had higher efficiency in reduction of BAs accumulation than that of *L. sakei* KM5474. In this study, it was found that mixed starter cultures of *L. sakei* KM5474 and *L. plantarum* KM1450 enhanced the highest effectiveness on reduce BAs accumulation during *som-fug* fermentation especially histamine. Therefore, the appropriate starter culture could reduce BAs accumulation during the process of *som-fug* fermentation.

Conflicts of interest

All authors have no conflicts of interest to declare.

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References

[1] Silla-Santos MH. Biogenic amines: their importance in food. Int J Food Microbiol 1996;29:213–21.
[2] Verges CMC, Zuniga M, Morel-Deville F, Perez-Martinez G, Zagorec M and Ehrlich SD. Relationships between arginine degradation, pH and survival in *Lactobacillus sake*. FEMS Microbiol Lett 1999;180:297–304.
[3] Beutling D. Biogenic amines in nutrition. Biogene Amine in der Ernaehrung. Berlin (Germany): Springer–Verlag; 1996. p. 59–67.
[4] Bover-Cid S, Izquierdo-Pulido M and Vidal-Carou MC. Influence of hygienic quality of raw materials on biogenic amine production during ripening and storage of dry fermented sausage. J Food Prot 2000;63:1544–50.
[5] Saisithi P, Wonkhalsung C, Boonyaratanakornkit M, Yongmanitchai P, Chimanage P and Maleehuan S. Improvement of Thai traditional fermented fish product: *som-fug*. Bangkok (Thailand): Institute of Food Research and Product Development, Kasetsart University; 1986.
[6] Vieira LQ, dos Santos LM, Neumann E, da Silva AP, Moura LN and Nicoli JR. Probiotics protect mice against experimental infections. J Clin Gastroenterol 2008;42:S168–9.
[7] Lin JS, Chiu YH, Lin NT, Chu CH, Huang KC, Liao KW and Peng KC. Different effects of probiotic species/strains on infections in preschool children: a double-blind, randomized, controlled study. Vaccine 2009;27:1073–9.
[8] Phitakpol B. Fish fermentation technology in Thailand. In: Lee CH and Steinkraus KH, editors. Fermentation technology. Tokyo (Japan): United Nation University Press; 1993. p. 155–66.
[9] Ostergaard A, Emberk PKB, Yamprayoon J, Wedell-Neergarrd W, Huss HH and Gram L. Fermentation and spoilage of som fak, a Thai low-salt fish product. Trop Sci 1998;38:105–12.
[10] Fernández M, Flórez AB, Linares DM, Mayo B and Alvarez MA. Early PCR detection of tyramine-producing bacteria during cheese production. J Dairy Res 2006;73:18–21.
[11] Taylor SL. Histamine food poisoning: toxicology and clinical aspects. CRC Crit Rev Food Sci Nutr 1986;17:91–128.
[12] Wöhrl S, Hemmer W, Focke M, Rappersberger K and Jarisch R. Histamine intolerance-like symptoms in healthy volunteers after oral provocation with liquid histamine. Allergy Asthma Proc 2004;25:305–11.
[13] Rauscher-Gabernig E, Grossgut R, Bauer F and Paulsen P. Assessment of alimentary histamine exposure of consumers in Austria and development of tolerable levels in typical foods. Food Control 2009;20:423–9.
[14] McCabe-Sellers BJ. Dietary tyramine and other pressor amines in MAOI regimens: a review. J Am Diet Assoc 1986;86:1059–64.
[15] Ladero V, Calles-Enriquez M, Fernández M and Alvarez MA. Toxicological effects of dietary biogenic amines. Curr Nutr Food Sci 2010;6:145–56.
[16] ten Brink B, Damink C, Joosten HMLJ and Huis in't Veld JHJ. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 1990;11: 73–84.
[17] Ignatenko NA, Besselsen DG, Roy UK, Stringer DE, Blohm-Mangone KA, Padilla-Torres JL, Guillen-R JM and Gerner EW. Dietary putrescine reduces the intestinal anticarcinogenic activity of sundilac in a murine model of familial adenomatosus poluposis. Nutr Cancer 2006;56:172–81.
[18] Shah P and Swiatlo E. A multifaceted role for polyamines in bacterial pathogens. Mol Microbiol 2008;68:4–16.
[19] Lehane L and Olley J. Histamine fish poisoning revisited. Int J Food Microbiol 2000;58:1–37.
[20] Halász A, Baráth Á, Simon-Sarkadi L and Holzapfel W. Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 1994;5: 42–50.
[21] Vidal-Carou MC, Izquierdo-Pulido M, Martín-Morro MC and Marine-Font A. Histamine and tyramine in meat products: relationship with meat spoilage. Food Chem 1990;37:239–49.
[22] Maijala R and Eerola S. Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. Meat Sci 1993;35:387–95.
[23] Jay JM. Microorganisms in foods. Modern food microbiology. New York (NY): Chapman & Hall; 1996. p. 69–191.
[24] Yamashita M, Sakaue M, Iwata M, Sugino H and Murooka Y. Purification and characterization of monoamine oxidase from *Klebsiella aerogenes*. J Ripen Bioeng 1993;76:289–95.
[25] Mah JH and Hwang HJ. Inhibition of biogenic amine formation in a salted and fermented anchovy by *Staphylococcus xylosus* as a protective culture. Food Control 2009;20:796–801.
[26] Leuschner RGK and Hammes WP. Tyramine degradation by micrococci during ripening of fermented sausages. Meat Sci 1998;49:289–96.
[27] Kalac P, Spicka J, Krizek M and Pelikanova T. The effect of lactic acid bacteria inoculants on biogenic amines formation in sauerkraut. Food Chem 2000;70: 355–9.
[28] Dapkevicius MLNE, Nout MJR, Rombouts FM, Houben JH and Wymenga W. Biogenic amine formation and degradation by potential fish silage starter microorganisms. Int J Food Microbiol 2000;57:107–14.
[29] Schomburg D and Stephan D. Enzyme Handbook. Berlin (Germany): Springer-Verlag; 1993.
[30] Martuscelli M, Crudele MA, Gardini F and Suzzi G. Biogenic amine formation and oxidation by *Staphylococcus xylosus* strains from artisanal fermented sausages. Lett Appl Microbiol 2000;31:228–32.
[31] Gardini F, Martuscelli M, Caruso MC, Galgano F, Crudele MA and Favati F. Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of *Enterococcus faecali*. Int J Food Microbiol 2001;64:105–17.
[32] Shalaby AR. Significance of biogenic amines to food safety and human health. Food Res Int 1996;29:675–90.
[33] Şenöz B, Isýklý N and Çoksöyler N. Biogenic amines in Turkish sausages (sucuk). J Food Sci 2000;65:764–7.
[34] Bover-Cid S, Miguélez-Arrizado MJ and Vidal-Carou C. Biogenic amine accumulation in ripened sausages affected by the addition of sodium sulphite. Meat Sci 2001;59:391–6.
[35] Strelec I, Kučko L, Roknić D, Mrša V and Ugarčić-Hardi Z. Spectrofluorimetric, spectrophotometric and chemometric analysis of wheat grains infested by *Sitophilus granarius*. J Stored Prod Res 2012;50:42–8.
[36] Greene DH and Babbitt JK. Control of muscle softening and protease–parasite interactions in arrowtooth flounder, *Atersthes stomias*. J Food Sci 1990;55:579–80.
[37] Lowry QH, Rosebrough NJ, Farr LA and Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:256–75.

[38] Hernandez-Jover T, Izquierdo-Pulido M, Veciana-Nogues MT, Marine-Font A and Vidal-Carou MC. Biogenic amine sources in cooked cured shoulder pork. J Agric Food Chem 1996;45:2089–102.
[39] Rozan P, Kuo YH and Lambein F. Free amino acids present in commercially available seedlings sold for human consumption: a potential hazard for consumers. J Agric Food Chem 2000;48:716–23.
[40] Freiding S, Gutsche KA, Ehrmann MA and Vogel RF. Genetic screening of *Lactobacillus sakei* and *Lactobacillus curvatus* strains for their peptidolytic system and amino acid metabolism, and comparison of their volatilomes in a model system. Syst Appl Microbiol 2011;34:311–20.
[41] Abd Alla EAM, El-Shafei L, Ibrahim GA and Sharaf OM. Changes in microflora and biogenic amines of some market processed cheese during storage. Egyptian J Dairy Sci 1996;24:217–26.
[42] El-Sonbaty AH, Badawi RM and Kebary KMK. Nutritional quality of Egyptian processed cheese. Egyptian J Dairy Sci 1998;26:139–50.
[43] Kebary KMK, El-Sonbaty AH and Badawi RM. Effects of heating milk and accelerating ripening of low fat Ras cheese on biogenic amines and free amino acids development. Food Chem 1999;64:67–75.
[44] Steidlova S and Kala P. Levels of biogenic amines in maize silages. Anim Feed Sci Technol 2002;102:197–205.
[45] Petridis KD and Steinhart H. Biogenic amines in hard cheese production: 1. Factors influencing the biogenic amine content of the finished product using Emmental cheese as an example. Deut Lebensm-Rundsch 1996;92:114–20.
[46] Koutsoumanis K, Lampropoulou K and Nychas GE. Biogenic amines and sensory changes associated with the microbial flora of Mediterranean gilt-head sea bream (*Sparus aurata*) stored aerobically at 0, 8, and 15 °C. J Food Prot 1999;62:398–402.
[47] Bover-Cid S, Hugas M, Izquierdo-Pulido M and Vidal-Carou MC. Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. Int J Food Microbiol 2001;66:185–9.
[48] ten Brink B, Damink C, Joosten HMLJ and Huis in't Veld JHJ. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 1990;11:73–84.
[49] Eerola S, Roig-Saguè AX and Hirvi TK. Biogenic amines in Finnish dry sausages. J Food Saf 1998;18:127–38.
[50] Suzzi G and Gardini F. Biogenic amines in dry fermented sausages: a review. Int J Food Microbiol 2003;88:41–54.
[51] Montel MC and Champomier MC. Arginine catabolism in *Lactobacillus sake* from meat. Appl Environ Microbiol 1987;53:2683–5.
[52] Moreno-Arribas MV, Polo MC, Jorganes F and Munoz R. Screening of biogenic amine production by acid lactic bacteria isolated from grape must and wine. Int J Food Microbiol 2003;84:117–23.
[53] Lehninger AL, Nelson DL and Cox MM. Principi di biochimica. Bologna (Italy): Zanichelli; 1999.
[54] Ruiz J, Garcia C, Diaz MC, Cava R, Tejeda FJ and Ventanas J. Dry cured Iberian ham non-volatile components as affected by the length of the curing process. Food Res Int 1999;32:643–51.
[55] Toldrá F. Dry-cured ham. In: Hui YH, Culbertson JD, Duncan S, Guerrero-Legarreta I, Li-Chan ECY, Ma CY, Manley CH, McMeekin TA, Nip WK, Nollet LML, Rahman MS, Toldrá F and Xiong YL, editors. Handbook of food science, technology and engineering. Boca Raton (FL): Marcel-Dekker Inc./CRC Press; 2005. p. 1.
[56] Monin G, Marinova P, Talmant A, Martin JF, Cornet M and Lanore D. Chemical and structural changes in dry-cured hams (Bayonne hams) during processing and effects of the deharing technique. Meat Sci 1997;47:29–47.
[57] Durà MA, Flores M and Tolrà F. Effect of *Debaryomyces* spp. On the proteolysis of dry-fermented sausages. Meat Sci 2004;68:319–28.
[58] Virgili R, Saccani G, Gabba L, Tanzi E and Soresi Bordini C. Changes of free amino acids and biogenic amines during estende ageing of Italian dry-cured ham. LWT-Food Sci Technol 2007;40:871–8.
[59] Flores M, Spanier AM and Toldrá F. Flavor analysis of dry-cured ham. In: Shahidi F, editor. Flavor of meat product and seafood. London (UK): Blackie; 1998. p. 320–41.
[60] Hughes MC, Kerry JP, Arendt EK, Kenneally PM, McSweeney PLH and O'Neill EE. Characterization of proteolysis during the ripening of semidry fermented sausages. Meat Sci 2002;62:205–16.
[61] Verplaetse A, De Bosschere M and Demeyer D. Proteolysis during dry sausage ripening. Proceedings of the 35th international congress on Meat Science and Technology. Copenhagen (Denmark). 1989.
[62] Sekikawa M, Kawamura T, Fujii H, Shimada K, Fukushima M and Mikami M. Effect of arginine on growth of lactic acid bacteria for fermented sausage under high concentration of salt. Hokkaido Anim Sci Agric Soc 2003;45:17–23.
[63] Nout MJR. Fermented foods and food safety. Food Res Int 1994;27:291–4.
[64] Şenöz B, Isýklý N and Çoksöyler N. Biogenic amines in Turkish sausages (sucuk). J Sci Food 2000;65:764–7.
[65] Kebary KMK, El-Sonbaty AH and Badawi RM. Effects of heating milk and accelerating ripening of low fat Ras cheese on biogenic amines and free amino acids development. Food Chem 1999;64:67–75.