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Novel starter cultures to inhibit biogenic amines accumulation during fish sauce fermentation

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ABSTRACT

Bacteria with amine oxidase activity have become a particular interest to reduce biogenic amines concentration in food products such as meat and fish sausages. However, little information is available regarding the application of these bacteria in fish sauce. Hence, our study was aimed to investigate the effect of such starter cultures in reducing biogenic amines accumulation during fish sauce fermentation. *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05 isolated from fish sauce which possess amine oxidase activity were used as starter cultures in this study. Fermentation was held for 120 days at 35 °C. The pH value increased in all samples, while salt concentration remained constant throughout fermentation. Aerobic bacteria count was significantly lower ($p<0.05$) in the control than in inoculated samples as a result of starter cultures addition. However, it decreased during fermentation due to the growth inhibition by high salt concentration. Proteolytic bacterial count decreased during fermentation with no significant difference ($p>0.05$) among samples. These bacteria hydrolyzed protein in anchovy to produce free amino acid precursors for amines formation by decarboxylase bacteria. The presence of biogenic amines producing bacteria in this study was considered to be indigenous from raw material or contamination during fermentation, since our cultures were negative histamine producers. Amino acid histidine, arginine, lysine and tyrosine concentration decreased at different rates during fermentation as they were converted into their respective amines. In general, biogenic amines concentration namely histamine, putrescine, cadaverine and tyramine increased throughout fermentation. However, their concentrations were markedly higher ($p<0.05$) in the control (without starter cultures) as compared to the samples treated with starter cultures. Histamine concentration was reduced by 27.7% and 15.4% by *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05, respectively. Both cultures could also reduce other amines during fermentation. After 120 days of fermentation, the overall biogenic amines concentration was 15.9% and 12.5% less in samples inoculated with *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05, respectively, as compared to control samples. These findings emphasized that application of starter cultures with amines oxidase activity in fish sauce fermentation was found to be effective in reducing biogenic amines accumulation.

1. Introduction

Biogenic amines are basic nitrogenous compounds produced mainly by bacterial decarboxylation activity toward amino acids in foods (Brink et al., 1990; Halasz et al., 1994). Biogenic amines can cause adverse health effect to consumers when ingested at considerable amounts or when the natural mechanisms for their catabolism are inhibited or genetically deficient in the human body. Histamine poisoning is often manifested by a wide variety of symptoms such as nausea, vomiting, diarrhea, abdominal cramp, rash, localized inflammation, headache, palpitation, and severe respiratory distress. Consumption of food with unusual tyramine levels can cause migraine and when it reacts with monoamine oxidase inhibitor (MAOI) drugs may lead to hypertensive crisis (Shalaby, 1996). Apart from being slightly toxic, putrescine and cadaverine also inhibit histamine metabolizing enzymes and therefore enhance histamine poisoning (Halasz et al., 1994; Shalaby, 1996). Moreover, putrescine, cadaverine, spermine and spermidine can react with nitrite in foods to form carcinogenic nitrosamine (Shalaby, 1996).

Fish sauce is a popular fermented fish product and commonly used as a condiment in Southeast Asian countries and now gaining acceptance worldwide. It is considered as an important dietary source of protein with 20 g/L nitrogen of which 80% is in the form of amino acids. Fish sauce is prepared by fermenting fish with salt (ratio 2–6:1) at ambient temperature for 4–12 months depending on the producing countries (Lopetcharat et al., 2001). Typically, no starter culture was applied in fish sauce fermentation, since it merely relies on indigenous bacteria from raw materials. At present, application of starter cultures

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with high proteinase activity was common practice to accelerate fermentation time (Fu et al., 2008; Yongsawatdigul et al., 2007). The end of fermentation was determined by color, aroma, flavor and clarity which are typical qualities of fish sauce (Wongkhalaung, 2004). Fish sauce was reported to contain considerable amounts of biogenic amines, although it also contains many nutritious compounds. Biogenic amine concentration in fish sauce was predominated by histamine, putrescine, cadaverine and tyramine. The highest ever reported histamine, putrescine, cadaverine and tyramine concentrations in fish sauce were 1220, 1257, 1429 and 1178 ppm, respectively (Zaman et al., 2009). Tryptamine and phenylethylamine are occasionally present at a low level, while spermine, spermidine and agmatine are trace amines in fish sauce.

Biogenic amines can be degraded through oxidative deamination catalyzed by amines oxidase with the production of aldehyde, ammonia and hydrogen peroxide (Ishizuka et al., 1993; Murooka et al., 1979; Yamashita et al., 1993). Monoamine and diamine oxidases had been described from some genus of the family *Enterobacteriaceae* namely *Klebsiella*, *Enterobacter*, *Eschericia*, *Salmonella*, *Serratia* and *Proteus* (Murooka et al., 1979; Yamashita et al., 1993). Histamine oxidase was found in *Staphylococcus xylosus* (Martuscelli et al., 2000), *Staphylococcus carnosus*, *Bacillus amyloliquefaciens* (Zaman et al., 2010), *Brevibacterium linens* (Leuschner et al., 1998), and *Lactobacillus sakei* (Dapkevicius et al., 2000). Other amines oxidase such as tyramine oxidase was found in *Micrococcus varians* (Leuschner and Hammes, 1998) and *Staphylococcus xylosus* (Martuscelli et al., 2000), putrescine oxidase in *Micrococcus rubens* (Ishizuka et al., 1993), as well as phenylethylamine oxidase in *Eschericia coli* (Parrot et al., 1987). In addition to amine oxidase, some strain of *Pseudomonas aeruginosa*, *Pseudomonas putida* and the methylotroph *Paracoccus versutus* utilize their amine dehydrogenase to oxidize amines (Hacisalihoglu et al., 1997). The potential role of microorganisms with amine oxidase activity had become a particular interest in the last few years to prevent or reduce biogenic amine accumulation in food products, especially fermented foods. Mah and Hwang (2009) investigated the effect of *Staphylococcus xylosus* No.0538 to inhibit biogenic amine formation in a salted and fermented anchovy. They observed that *Staphylococcus xylosus* No.0538 could reduce biogenic amines production by 16.0% as compared to a control. A huge reduction of tyramine during ripening of fermented sausages was achieved when *Micrococcus varians* LTH 1540 was applied as starter culture (Leuschner and Hammes, 1998). Inoculation of *Lactobacillus plantarum* in sauerkraut effectively suppressed the production of tyramine, putrescine and cadaverine (Kalac et al., 2000). Meanwhile, only little information is available on the effect of starter culture on biogenic amine reduction in fish sauce. Therefore, the objective of this study was to investigate the effectiveness of starter culture in inhibiting biogenic amine accumulation during fish sauce fermentation. In addition, the change of chemical and microbial properties of fish sauce during fermentation was investigated.

2. Materials and methods

2.1. Preparation of starter culture

Starter cultures used in this study were *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05, which were isolated from fish sauce of northeastern Malaysia. As reported in our previous study, both strains have the ability to degrade histamine, putrescine and cadaverine in a buffer system (Zaman et al., 2010). A loop from a slant tryptic soy agar culture of each culture was inoculated in 10 mL of tryptic soy broth and incubated at 37 °C for 24 h. Five milliliters of the culture was then transferred to 100 mL of tryptic soy broth and incubated at 37 °C for another 24 h. The culture was centrifuged at 10,000 *g* for 10 min at 4 °C and then washed with fish broth. Fish broth was prepared by homogenizing 1 part of anchovy with 9 part of distilled water, filtered, adjusted to pH 7.0 and then autoclaved at 121 °C for 15 min. After centrifugation, the cell pellet was resuspended in sterile fish broth, adjusted to approximately 1×10^7 cell/mL and used as starter culture in fish sauce fermentation.

2.2. Preparation of fish sauce fermentation

Anchovies were obtained from Tumpat, Kelantan, Malaysia, and transported in ice to the Centre of Excellence for Food Safety Research (CEFSR) at Faculty of Food Science and Technology, Universiti Putra Malaysia within 10 h. Upon arrival, samples were immediately kept for two days in a freezer (−20 °C) prior to fermentation. Solar salt used in the study was collected from the same place as the anchovies were obtained. Anchovies were thawed in room temperature for five hours. After thawing, anchovies were mixed thoroughly with 15% salt (w/w) and divided into three equal lots of 1500 g. Two lots were treated separately with 150 mL (10% of anchovy weight) of starter culture suspension. Another lot was treated with the same volume of sterile fish broth serving as control. Each lot was prepared in triplicate. The mixture of all treatments were transferred into glass jars and kept in an incubator (Memmert, Germany) at 35 °C for 120 days. Samples were drawn periodically for chemical and microbiological analysis.

2.3. Determination of pH value and salt concentration

The pH value of fish sauce was determined by direct measurement with electronic pH meter (Mettler Toledo 8603, Switzerland). The salt concentration of each sample was determined with a salt meter (Atago ES-421, Japan) after a tenfold dilution.

2.4. Microbiological analysis

Samples (25 mL) were aseptically transferred into a stomacher bag (Bagmixer 400, Model L, Interscience, France), with 225 mL of peptone (0.85% of sodium chloride added) and then homogenized for two minutes. Further decimal dilutions were made and then 100 μL of each dilution was spread onto agar plates. Aerobic plate count agar and skim milk agar, both supplemented with 3% of sodium chloride were used to determine total aerobic and proteolytic bacteria count, respectively. Biogenic amine producing bacteria were counted using differential media supplemented with amino acids as precursor of biogenic amines (Joosten and Northolt, 1989). The media contained of tryptone (0.5%), yeast extract (0.5%), sodium chloride (0.5%), glucose (0.1%), tween 80 (0.05%), $MgSO_4\cdot7H_20$ (0.02%), $CaCO_3$ (0.01%), $MnSO_4\cdot4H_2O$ (0.005%), $FeSO_4\cdot7H_2O$ (0.004%), bromocresol purple (0.006%), amino acid (2%) and agar (2%). Histidine, ornithine and lysine were used as precursor of histamine, putrescine and cadaverine, respectively. All plates were then incubated for 48 h at 37 °C. Bacterial colonies which developed on each agar were then enumerated and expressed as log colony forming unit (CFU)/mL. Only bacterial colonies with purple halo in the differential media were counted as biogenic amines producing bacteria.

2.5. Determination of amino acids

Amino acids in samples were determined using HPLC according to the method proposed by Rozan et al. (2000). Briefly, a 20 μL aliquot of amino acid standard and digested fish sauce samples were transferred into vials and dried under vacuum (37 °C, 20 mmHg) for 20 min. Then 20 μL of drying reagent containing methanol, water and triethylamine (ratio 2:2:1 v/v) was added. After mixing, the sample was dried under vacuum for 20 min. Then 20 μL of derivatizing reagent containing methanol, triethylamine, water and phenylisothiocyanate (PITC) (ratio 7:1:1:1 v/v) was added and incubated at room temperature for 20 min. Samples were then dried under vacuum for 20 min to remove the excess PITC. The derivatized samples were then dissolved