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Effect of fermentation temperature on the microbial and physicochemical properties of silver carp sausages inoculated with *Pediococcus pentosaceus*

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ABSTRACT

The effect of fermentation with *Pediococcus pentosaceus* at different temperatures ranging from 15 to 37 °C on the quality characteristics of silver carp sausages was investigated. Higher temperature stimulated the rapid growth of lactic acid bacteria, resulting in a rapid decline in pH, and consequently suppressed the growth of *Pseudomonas*, *Micrococcaceae* and *Enterobacteriaceae*. However, increasing fermentation temperature gave a progressive increase in total volatile basic nitrogen and biogenic amines in fermented silver carp sausages. Histamine was the main biogenic amine, exceeding 100 mg/kg after 48 h of fermentation at temperatures above 30 °C. Higher content of non-protein nitrogen and α-amino nitrogen correlated with the electrophoretic studies, which showed that proteolysis of high molecular weight myofibrillar and sarcoplasmic proteins was more prominent at higher fermentation temperatures. Products fermented at 23–30 °C showed greatest consumer preference and most favourable textural properties.

1. Introduction

In China, freshwater fishes account for about 45% of total aquacultural production, reaching 18 million tons in 2004. Silver carp (*Hypophthalmichthys molitrix*) is one of the main freshwater fish species, due to its fast growth rate, easy cultivation, high feed efficiency ratio as well as high nutritional value (Luo, Shen, Pan, & Bu, 2008). However, freshwater fish including silver carp often have strong earthy/musty taste and odour, due to significant concentrations of geosmin or 2-methyl-isobomeol (MIB) in fish flesh (Howgate, 2004). Furthermore, silver carp contains many intramuscular small bones. Therefore, the consumption of silver carp has been limited and the price of the fish is low.

Lactic acid fermentation is an important method of preserving perishable fish and marine products in developing countries (Adams, Cooke, & Twiddy, 1987). Lactic acid bacteria (LAB) could cause rapid acidification of the raw material, through the production of organic acids, mainly lactic acid and acetic acid, and also produce a variety of antimicrobial substances, which can consequently prevent the growth of most hazardous food microorganisms (Hu, Xia, & Ge, 2008).

Fermented minced fish, which is an excellent source of protein, is widely consumed throughout Southeast Asia. It is typically composed of freshwater fish species, salt (2–7%), a carbohydrate source and spices (Paludan-Müller, Huss, & Gram, 1999). Nevertheless, most fish fermentation is still conducted as spontaneous processes at household or small-scale levels in developing countries. Initiation of a spontaneous fermentation process takes a relatively long time, and the quality of the products varies considerably, limiting their acceptability and commercial importance (Twiddy, Cross, & Cooke, 1987).

The use of starter cultures in food fermentation has been studied widely and introduced into commercial practice to increase processing rates and product consistency in recent years. Using starter cultures to develop novel fish products, which would be free of fishy odour and taste, is attracting increasing interest (Hu et al., 2008). A minced fish-salt-glucose system with *Lactobacillus plantarum* and *Pediococcus pentosaceus* was investigated for its ability to promote rapid fermentation at 30 °C (Twiddy et al., 1987). *P. pentosaceus*, *L. plantarum* and *Leuconostoc mesenteroides* were inoculated into the minced frozen fillet of yellowfin tuna and fermented at 8 °C to develop novel fish food products with desired meat properties (Glatman, Drabkin, & Gelman, 2000). *L. plantarum*, *Pediococcus acidilactici*, and *P. pentosaceus* BT520 have been used for the production of Som-fug, a Thai fermented fish at 30 °C (Riebroy, Benjakula, & Visessanguan, 2008). Yin, Pan, and Jiang (2002) used several LAB, including *L. plantarum* CCRC10069, *Lactococcus lactis* subsp. *lactis* CCRC 12315 and *Lactobacillus helveticus* CCRC 14092, as starter cultures to ferment mackerel mince at 37 °C, which could substantially inhibit the development of volatile basic nitrogen, suppress the growth of spoilage microflora and improve the organoleptic qualities and digestibility of the product. Mixed starter cultures were

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also reported to substantially suppress the accumulation of biogenic amines, and improve the flavour and nutritional value of fish muscle at 30 °C (Hu, Xia, & Liu, 2007b; Hu et al., 2008). A fermented cold-smoked fish with lactic acid bacteria was prepared at 20–22 °C (Petäjä, Eerola, & Petäjä, 2000).

Although these widely varying temperatures are used on different types and batches of fermented fish, there are only a few publications concerning the effect of fermentation temperature on the characteristics of fermented fish products (Adams et al., 1987). Temperature is one of the key environmental parameters that affect the microbial growth and the rate of fermentation. Better understanding of the temperature effects on the fermentation process will facilitate improvement of product quality and safety.

The objectives of this study were to develop a value-added product, a sausage using silver carp meat, and to investigate the effect of temperature on the characteristics of the sausages inoculated with *P. pentosaceus*, in terms of microbial and physicochemical changes during fermentation.

2. Materials and methods

2.1. Preparation of starter culture

P. pentosaceus was obtained from the Technology Center of the Shuanghui Group (Luohe, Henan, China). *P. pentosaceus* was subcultured twice in de Man Rogosa Sharpe (MRS) broth at 30 °C for 24 h. Cells were harvested by centrifugation at 10,000*g* for 15 min at 4 °C, and washed twice with saline water (0.85% NaCl); then the cell pellets were resuspended in the same saline water. After adjusting the level of cells to 7–8 log CFU/ml by using a UV-2100 spectrophotometer (Unico, Shanghai, China), the resulting cell suspension was stored at 4 °C for the inoculation of fish mince on the same day.

2.2. Preparation of silver carp sausages

Frozen silver carp (2–3 kg/fish), purchased from a local market (Wuxi, Jiangsu, China), was thawed in running tap water and then beheaded, gutted, and scaled. Prepared fish was then manually filleted. The fillets were passed through a deboner (Model 694, Baader North America, New Bedford, MA) with a drum having 5 mm-diameter perforations, to remove many intramuscular small bones and to obtain the mince. The fish mince was then mixed uniformly with 2% NaCl, 1% glucose, 4% sucrose, 0.05% sodium ascorbate, and 0.5% seasoning mix. Finally, an appropriate amount of *P. pentosaceus* suspension was inoculated to a final level of 6–7 log CFU/g fish mince, and well mixed using a sterile glass rod. The resulting mince was then stuffed into collagen casings (38 mm diameter) and incubated at 15, 23, 30 and 37 °C, RH 90–93% for 2 days. Sausage samples were taken every 12 h for analysis.

2.3. Microbiological analysis

Ten grams of sausage sample were aseptically transferred into a sterile plastic bag and stomached for 2 min in a stomacher (Lab-Blender 400; Seward Medical, London, UK) with 90 ml sterile peptone saline diluent (containing 0.1% peptone and 0.85% NaCl). Appropriate decimal dilutions of the samples were prepared with the same diluent and 0.1 ml aliquots of appropriate dilution were spread on the different agar plates and incubated as follows: total aerobic bacteria on plate count agar (PCA, Oxoid, CM325), incubated at 37 °C for 48 h; lactic acid bacteria on de Man Rogosa Sharpe agar (MRS, Oxoid, CM0361), incubated anaerobically at 30 °C for 48 h; *Enterobacteriaceae* on violet red bile dextrose agar (VRBD, Oxoid, CM485) with double layer, incubated at 37 °C for 24 h; *Micrococcaceae* on mannitol salt agar (MSA, BD Difco), incubated at 30 °C for 48 h; *Pseudomonas* on *Pseudomonas aeromonas* selective agar base (GSP Agar, Merck, Nr 10230), incubated at 26 °C for 72 h. Bacteria counts were expressed as log CFU/g sample.

2.4. Determination of pH and water content

Each of five-gram sausage samples was homogenised (Ultra Turrax homogeniser, IKA Labortechnik, Selangor, Malaysia) with 45 ml of boiled distilled water at 11,000 rpm for 1 min, and the pH was measured (Mettler Toledo 320-s, Shanghai, China). Water content was determined by drying 3–5 g of sausage sample at 100–102 °C to a constant weight (AOAC, 1998).

2.5. Determination of non-protein nitrogen (NPN), α-amino nitrogen (AAN) and total volatile basic nitrogen (TVB-N)

NPN was extracted from 5–10 g of sausage sample with 5% (w/v) trichloroacetic acid (TCA) for Kjeldahl procedure. The α-amino nitrogen (AAN) was analysed according to the Sorensen method by titration with formaldehyde (AOAC, 1998). TVB-N content was determined by the Conway micro-diffusion technique (Cobb, Alaniz, & Thompson, 1973).

2.6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

Sarcoplasmic proteins and myofibrillar proteins were extracted according to the method of Joo, Kauffman, Kim, and Park (1999) with slight modification. Using a homogeniser (Ultra Turrax), 2 g of sausage sample were homogenised (12,000 rpm, 1 min) with 20 ml of chilled 0.025 M potassium phosphate buffer (pH 7.2) and centrifuged at 5000*g* for 20 min. The supernatant was used as sarcoplasmic proteins. The resulting precipitate was homogenised (Ultra Turrax homogeniser) with 20 ml of chilled 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2) at 12,000 rpm for 1 min. The supernatant was collected by centrifugation at 5000*g* for 20 min and used as myofibrillar proteins.

SDS–PAGE was carried out in a vertical gel electrophoresis unit (Mini-Protean-3 Cell, Bio-Rad, Richmond, CA) according to the method of Laemmli (1970), using 10% separating gel and 4% stacking gel. Aliquots of 10 μl were injected in each well including standard protein marker. Electrophoresis was done at 90–120 V. After electrophoresis was completed, gels were stained with 0.125% Coomassie Brilliant Blue R-250 in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

2.7. Determination of biogenic amines

Finely ground sausage sample (5 g) was transferred to 50-ml centrifuge tubes and homogenised (Ultra Turrax homogeniser) with 20 ml of 5% TCA solution for about 2 min. The supernatant was collected by centrifugation (10,000*g*, 4 °C, 10 min) and the residue was extracted again with an equal volume of TCA solution. Both supernatants were combined and filtered through a filter paper (Whatman No. 4). The filtrate was made up to 50 ml with 5% TCA solution and stored at 0–4 °C for high-performance liquid chromatography (Agilent 1100 Series; Agilent, Santa Clara, CA) analysis within a week.

The derivatisation reagent was prepared by transferring 100 mg *o*-phthalaldehyde (OPA), 1 ml acetonitrile and 130 μl 2-mercaptoethanol to a 10-ml volumetric flask, and then diluting with 0.4 M borate buffer (pH 10.2) to 10 ml. The resulting solution was mixed well, and stored refrigerated and used within 24 h. Pre-column derivatisation with OPA was performed automatically.

A reverse-phase Hypersil ODS C_{18} (125 × 4.60 mm, particle size 5 μm) column was used for separation. The column temperature