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Effect of autochthonous starter cultures on microbiological and physico-chemical characteristics of Suan yu, a traditional Chinese low salt fermented fish

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ABSTRACT

In this study, three groups of mixed starter cultures (S1: *Lactobacillus plantarum* 120, *Staphylococcus xylosus* 135 and *Saccharomyces cerevisiae* 31; S2: *L. plantarum* 145, *S. xylosus* 135 and *S. cerevisiae* 22; S3: *Pediococcus pentosaceus* 220; *S. xylosus* 135 and *S. cerevisiae* 22), isolated from Suan yu, were inoculated to produce the traditional fermented fish. After 42 days fermentation at 24 °C, Suan yu inoculated with different mixed starter cultures underwent rapid growth of lactic acid bacteria (LAB), declined pH, suppressed increase of thiobarbituric acid (TBARS) and total volatile base nitrogen (TVB-N) as well as growth of spoilage bacteria and pathogens. Besides, Suan yu had higher contents of non-protein nitrogen (NPN) and total free amino acids (FAA) compared to the control ($P < 0.05$). The muscle proteins were severely hydrolyzed during fermentation (SDS-PAGE). Moreover, the sensory evaluation indicates the fermented fish was more widely accepted than the control. The results suggest that the inoculation with S1, S2 and S3 reduced the lag time that fermentation began and improved the quality of Suan yu.

1. Introduction

Suan yu, which refers to a traditional fermented fish pieces or a whole fish, is widely consumed and accepted as a snack due to its characteristic flavor. Such fermented fish products retain almost all the special nutrition of fish and are free of fishy odor and taste. They are commonly manufactured by spontaneous fermentation that is a small-scale traditional technique without adding starter cultures.

The spontaneous fermentation of meat is characterized by the participation of lactic acid bacteria (LAB), Gram-positive, catalase-positive cocci, yeasts and molds (Buckenhüskes, 1993), among which LAB mainly contribute to acidification that ensures the safety of products by reducing pH through the fermentation of sugars (Lücke, 2000). Meanwhile, staphylococci and yeast dominate the development of aroma, flavor and color of fermented products (Cocconcelli & Fontana, 2010). Selecting autochthonous microflora as a starter formulation can effectively preserve the typical characteristics of these products. It has been previously reported that some food products with autochthonous starters were more competitive than those with other sourced starters in the formation of similar products (Gelman, Drabkin, & Glatman, 2000). Therefore, adding the starter cultures isolated from local products has been recommended as a common strategy in manufacturing several fermented fish (Andrighetto, Zampese, & Lombardi, 2001; Holzapfel, 2002). In our previous work (unpublished), six autochthonous microflora (*Lactobacillus plantarum 120*, *L. plantarum 145*, *Pediococcus pentosaceus 220*, *Staphylococcus xylosus 135*, *Saccharomyces cerevisiae 31 and S. cerevisiae 22*) were isolated from the traditional Suan yu, which were the most appropriate strains owing to their technological and antimicrobial characteristics. The phylogenetic analysis of LAB based on partial 16S rRNA gene sequences shows that isolates were closely related to *L. plantarum* and *P. pentosaceus*, respectively. Similarly, *Staphylococcuses* were identified as *S. xylosus*. While for yeast, the isolates based on the D1/D2 domain sequences of 26S rRNA were identified as *S. cerevisiae*.

Complex biochemical and physical reactions take place during fish fermentation, which significantly change the initial features. A rapid decline of pH not only produces a unique lactic acid flavor, but also increases the texture firmness and mouth-feel due to the acid denaturation of muscle proteins (Palumbo et al., 1993). Yin, Pan, and Jiang (2002a, 2002b) reported that adding 5 LAB strains into fermented mackerel mince could inhibit the accumulation of TVBN, suppress the growth of main microflora, and improve consumer acceptability. Appling LAB and *S. xylosus-12* in fermented silver carp

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sausages could also rapidly decrease pH, suppress the increase of TBARS, TVB-N and trimethylamine (TMA), and whiten the product compared with those of the control (without any starters) (Hu, Xia, & Ge, 2007, 2008).

Thereby motivated, this study aims to evaluate the effects of differently combined *L. plantarum 120*, *L. plantarum 145*, *P. pentosaceus 220*, *S. xylosus 135*, *S. cerevisiae 22* and *S. cerevisiae 31* isolated from Suan yu on its microbiological and physico-chemical characteristics.

2. Materials and methods

2.1. Starter cultures

The strains of *L. plantarum 120*, *L. plantarum 145*, *P. pentosaceus 220*, *S. xylosus 135*, *S. cerevisiae 22* and *S. cerevisiae 31*, which are the most appropriate strains for starter cultures owing to their technological and antimicrobial characteristics, were previously isolated from Suan yu. Their biochemical characteristics are shown in Table 1. LAB strain, *S. xylosus 135* as well as *S. cerevisiae 22* and *S. cerevisiae 31* were separately subcultured twice according to the methods of Hu et al. (2007, 2008). All the cell pellets were harvested by a high speed refrigerated centrifuge (Sigma Laborzentrifugen, Model 4K15, Osterode, Germany) at 10,000 *g* for 15 min at 4 °C. Subsequently, they were washed by saline water (0.9% NaCl) and then were resuspended therein. Finally, the cell concentrations were adjusted to 7–9 log cfu g^{-1}.

2.2. Suan yu preparation

Carps (*Cyprinus carpio* L.) were purchased from a local market (Wuxi, Jiangsu, China), gutted and eviscerated. The scales were removed. Then they were cut in 3–5 cm pieces and cleaned using tap water. The samples were prepared according to the traditional techniques by mixing the carps pieces (94% w/w) with the following ingredients: sucrose (2% w/w), salt (3% w/w), cinnamon (0.3% w/w), star anise (0.3% w/w), and wild pepper (0.3% w/w). They were cured at 3–4 °C for 1–2 days. Then they were dried for 6 h at 60 °C and 65% RH till constant weights (76% w/w). Thereafter, the above fish pieces (76% w/w) were mixed with ground roasted corn (24% w/w) for 15 min by hand. The resulting fish pieces and corn were then divided into 4 batches and prepared with different mixed starter cultures that suspended in saline water, including S1-LSS (L.P120, S135 and S31 [1:1:1]), S2-LSS (L.P145, S135 and S22 [1:1:1]), S3-PSS (P.P220, S135 and S31 [1:1:1]) and a batch without any starter (NS) as the control. Then the fish pieces, roasted corn and starter cultures were completely mixed in a ceramic container. Each starter culture (6–7 log cfu g^{-1}) was inoculated to the fish pieces. Then each fish batter was placed in small-sized jars with lids and then sealed tightly with water surrounded. These products were fermented at ambient temperature (24 °C) for 42 days. The samples were withdrawn and analyzed on days 0, 7, 14, 21, 28, 35 and 42. Prior to analyses, the bone and corn flour of Suan yu were removed with a sterilized knife. The samples were cut up and ground in a meat grinder (MX-T2G National, Tokyo, Japan) for 3 min and kept at 4 °C for further analysis.

2.3. Microbiological analysis

The samples (duplicates of 25 g) and 225 ml of normal saline (0.9% NaCl) were weighed aseptically into sterile plastic pouches and homogenized (Ultra Turrax homogenizer, IKA Labortechnik, Selangor, Malaysia) at 25 °C for 120 s. Serial 10-fold dilutions were prepared in sterile normal saline (0.9% NaCl), and 0.1 ml of each dilution was inoculated in appropriate growth media to estimate the microbial counts: LAB on MRS agar in anaerobic conditions at 30 °C for 2 days; *Staphylococcus* on Manitol Salt Agar (MSA) plates at 30 °C for 2–3 days; Enterobacteriaceae on Violet red bile glucose agar (VRBG) at 37 °C for 24 h; Yeasts on rose bengal chloramphenicol agar at 25 °C for 3–4 days. The results were expressed as colony-forming units per gram (log cfu g^{-1}).

Table 1
Biochemical characteristics of starter cultures isolated from "Suan yu" of China.

Characteristic	*L. plantarum* P120	*L. plantarum* P145	*P. pentosaceus* P220	*S. xylosus* S135	*S. cerevisiae* Y22	*S. cerevisiae* Y31
Gram	+	+	+	+	NT	NT
Catalase	−	−	−	+	NT	NT
Gas production	−	−	−	NT	+	+
Ammonia from arginine	−	−	−	−	NT	NT
Growth at 10 °C	+	+	+	+	+	+
Growth at 6% NaCl	+	+	+	NT	+	+
Urease	NT	NT	NT	NT	−	−
Ester-forming	NT	NT	NT	NT	+	+
Acidification activity[a]						
0 h	6.78 ± 0.23	6.78 ± 0.23	6.78 ± 0.23	NT	NT	NT
24 h	4.16 ± 0.12	4.28 ± 0.16	4.21 ± 0.14	NT	NT	NT
Proteolytic activity[b]						
Myofibrillar	−	−	−	12.4 ± 0.43	NT	NT
Sarcoplasmic	−	−	−	7.64 ± 0.21	NT	NT
Skim milk	NT	NT	NT	NT	+	+
Lipolytic activity	−	−	−	+	+	+
Antimicrobial activity[b]						
Escherichia coli	10.3 ± 0.41	10.1 ± 0.34	10.8 ± 0.43	−	NT	NT
Listeria monocytogenes	21.6 ± 0.77	18.3 ± 0.74	17.2 ± 0.61	−	NT	NT
Staphylococcus aureus	13.4 ± 0.54	12.7 ± 0.62	12.6 ± 0.62	−	NT	NT
Decarboxylase activity						
Lysine	−	−	−	−	NT	NT
Tyrosine	−	−	−	−	NT	NT
Ornithine	−	−	−	−	NT	NT
Histidine	−	−	−	−	NT	NT
Nitrate-reductase activity[b]	−	−	−	11.3 ± 0.33	NT	NT

Values are expressed as mean ± standard deviation ($n = 3$).
NT: not tested; +: positive; −: negative.
[a] Acidification activity was checked by measuring the pH values.
[b] The diameter of a clear zone surrounding the well (mm) was measured according to agar well diffusion assay.