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Characterization of *Lactobacillus pentosus* as a starter culture for the fermentation of edible oyster mushrooms (*Pleurotus* spp.)

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ABSTRACT

In this study, *Lactobacillus pentosus* was used as a starter culture for the lactic acid fermentation of three kinds of oyster mushrooms (*Pleurotus cornucopiae*, *Pleurotus sajor-caju*, *Pleurotus ostreatus*). The population of LAB in the three kinds of mushroom products was above 7.5 log cfu/ml throughout the fermentation process. In addition, the LAB rapidly controlled the spoilage and pathogenic microorganisms, and Enterobacteriaceae were not detected in the three kinds of final fermented mushroom products. The total concentrations of organic acids of the three kinds of oyster mushroom products increased during fermentation, and the levels of lactic acid ranged from 3.72 mg/ml to 4.49 mg/ml on the 18th day. The final products had lower concentrations of nitrite than that required under the current regulations, plus acceptable sensory characteristics. Our results thus suggest that *L. pentosus* has the potential to be used as a starter culture in the production of fermented oyster mushrooms.

1. Introduction

Pleurotus spp., the oyster mushroom, is the third most cultivated edible mushroom in the world (Cardoso, Demenjour, & Paz, 2013). Oyster mushrooms have a high nutritional value and contain several bioactive compounds, including polysaccharides, dietary fiber, ergosterol, B vitamins and minerals (Kalač, 2009; Manzi, Aguzzi, & Pizzoferrato, 2001). In addition, oyster mushrooms require a shorter growth time when compared to other edible mushrooms, their fruiting bodies are highly resistant to diseases and pests, and they can be cultivated in a simple and cheap way (Bonatti, Karnopp, Soares, & Furlan, 2004). As a result, oyster mushrooms have become increasingly popular from a commercial point of view (Fernandes, Barros, Martins, Herbert, & Ferreira, 2015).

However, fresh mushrooms are highly perishable, mainly due to their high water content, high respiration rate and the presence of microflora (Burton & Noble, 1993; Villaescusa & Gil, 2003). Therefore, it is necessary to apply preservation technologies to extend the shelf life of these mushrooms. The preservation method used is mainly determined by the final use of the product and the estimated storage time (Liu et al., 2014). Canning and salting are two methods that are frequently used to preserve mushrooms for ready-to-eat foods. However, these methods can alter the physical and chemical properties of the mushrooms, resulting in color darkening, flavor loss, and a decrease in nutritional value (Bernaś & Jaworska, 2012; Jaworska, Bernaś, & Mickowska, 2011).

Because the organic acids produced by lactic acid bacteria (LAB) inhibit the growth of undesirable microorganisms, lactic acid fermentation is one of the oldest preservation methods for extending the shelf life of vegetables (Caplice & Fitzgerald, 1999). LAB are the main epiphytic bacteria responsible for the fermentation of vegetables, although natural fermentation typically results from the competitive activities of LAB together with yeasts and gram-negative bacteria (Maifreni, Marino, & Conte, 2004; Tassou, Panagou, & Katsaboxakis, 2002). When the natural LAB flora present is able to grow and dominate the rest of the microflora, a predictable fermentation can be sustained. In this case, for LAB consume the sugars of the vegetables and produce mainly lactic

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acid and, to a lesser extent, other acids, leading to a drop in pH (Panagou & Katsaboxakis, 2006). The developed acidity and drop in pH are the determining factors for the success of fermentation and safety of the final product since suppression of both spoilage and pathogenic microorganisms take place and decrease the concentration of nitrite (Tassou et al., 2002). Today, the use of autochthonous or pure starter cultures were the two main options to be pursued for controlling lactic acid fermentation in vegetables (Di Cagno et al., 2008; Johanningsmeier, McFeeters, Fleming, & Thompson, 2007).

Lactobacillus is widely used as a starter to manufacture a variety of fermented products such as cheese, meat, and vegetables (de Castro, Montaño, Sánchez, & Rejano, 1998; El-Ghaish et al., 2011; Gardner, Savard, Obermeier, Caldwell, & Champagne, 2001). However, little is known about the effects of *Lactobacillus* used as a starter to produce fermented edible oyster mushrooms. Thus, the aim of this study was to investigate the effects of *Lactobacillus pentosus* as a starter culture on the microbiological quality, organic acid content, nitrite depletion and sensory quality of three kinds of oyster mushrooms.

2. Material and methods

2.1. Raw materials

Fresh *Pleurotus cornucopiae*, *Pleurotus ostreatus* and *Pleurotus sajor-caju* mushrooms were collected from the Institute of Applied Mycology (Huazhong Agricultural University).

2.2. Preparation of starter cultures

The commercial starter culture used in this study was Vege-Start 60 (Chr. Hansen's Biosystems, Horsholm, Denmark), which consists of a pure freeze-dried starter culture of *L. pentosus* appropriate for vegetable fermentation. Vege-Start 60 was activated on MRS agar for 2 days at 37 °C. Approx 20 colonies of Vege-Start 60 were used to inoculate MRS broths and the cultures were then incubated for 24 h at 37 °C under anaerobic condition. After centrifugation using a Avanti® J-E refrigerated centrifuge (BECKMAN COULTER, USA) at 4500 g for 25 min, the microbial cells were harvested and washed twice in a sterile saline solution (0.9% NaCl) before mushroom inoculation.

2.3. Preparation of fermented mushrooms

Fresh mushrooms were first sorted, cleaned, and washed under cold running water. The mushrooms were then blanched in boiling water (96–98 °C) for 2 min. 500 g of the treated mushrooms were placed in 1000 ml glass jars to which 2% NaCl (w/w) and 3% sucrose were added with a solid–liquid ratio of 2:1 (w/v). The commercial starter cultures were added with an initial population of LAB approximately 7.00 log cfu/ml and mixtures were incubated at 20 °C for 18 days under anaerobic conditions.

2.4. Microbial counts

The numeration of viable LAB, yeasts and Enterobacteriaceae in pickles was determined by plate count method. Pickle juice (1 ml) sterilely taken from pickle jars was subjected to sequential decimal dilutions after which 0.1 ml of appropriate decimal dilutions were spread onto agar media. MRS for LAB was incubated at 37 °C for 48 h; potato dextrose agar (PDA) for yeasts was incubated at 25 °C for 48 h; Violet Red Bile Glucose agar (VRBGA, Oxoid England) for Enterobacteriaceae was incubated at 37 °C for 24 h (Li et al., 2015).

2.5. Organic acids content analysis and pH measurement

Organic acids of fermented mushrooms were analyzed using a modified method of Belguesmia et al. (2014). Two milliliters of fermented medium were diluted in 8 ml of phosphoric acid solution (pH = 2.65) and left for 25 min at 75 °C. After being centrifuged for 20 min at 10,000 g at 4 °C, the supernatant was filtered with a syringe filter (Waters, Milford, MA, USA) for HPLC analysis.

The organic acids were analyzed using a Waters e2695 HPLC system equipped with a photodiode array detector, and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck, Darmstadt, Germany). The mobile phase was a phosphoric solution (pH = 2.65), and the flow rate was 0.5 ml/min. All samples were detected at 214 nm.

At each sampling point, pickle juice (10 ml) was sterilely taken from pickle jars, and pH values were measured using a digital pH-meter (Mettler-Toledo Instruments Co., Shanghai, China).

2.6. Nitrite content analysis

The nitrite content of fermented mushrooms was determined by a modified method described by Zhang, Kong, and Xiong (2007). Samples (fermented mushrooms) were homogenized in distilled water using a Waring® disintegrator (Karl Kolb, Dreieich, Germany). The homogenate was then centrifuged at 5000 g for 10 min for collection of the supernatant. The nitrogenous compound in the supernatant was developed by reacting with 0.2% (w/v) sulfanilamide and 0.1% (w/v) N-1-naphtyethylene diamine dihydrochloride for 15 min. Next the absorbance (538 nm) of the solution was measured. The different concentrations of standard liquid of sodium nitrite (equivalent to 0, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10 μg of sodium nitrite) were used to obtain the calibration curve. Nitrite content was expressed as: $NaNO_2 = (C \times 2000)/(M \times V)$, where C = concentration of sodium nitrite (1 μg/ml) from the calibration curve that corresponded to the sample absorbance, M = sample weight (g), and V = volume (ml) of the extraction solution.

2.7. Color measurement

The color of fermented mushrooms was measured using an UltraScan® XE meter (HunterLab, USA). The color values were expressed as *L* values (lightness), *a* values (redness) and *b* values (yellowness) units.

2.8. Sensory evaluation

Sensory evaluations of fermented (for 18 days) mushrooms were done by 10 faculty and students from the College of Food Science and Technology, Huazhong Agricultural University. Samples were coded with random numbers and acceptability ratings for appearance, flavor, texture and overall on a 10-point hedonic scale (10 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely).

2.9. Statistical analysis

The data collected reflect the results of three repetitions. The results were expressed as mean values and standard deviation (SD). Analysis of variance was performed using a one-way analysis of variance (ANOVA), and differences between the means of samples were analyzed by Duncan's test at a significance level of 0.05.