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Technical Study on Direct Vat of Fermentative Strains to Produce Cured Fish

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Abstract. Fermentative strains were chosen from the predominant strains of cured fish and then were inoculated. The optimal technological parameters for cured fish with fermentative strains were studied by the single factor test and the orthogonal test. *Pediococcus pentosaceus* and *Staphylococcus xylosus* were chosen as the fermentative strains, the total inoculum amount were 10^6CFU/g, inoculation ratio of *Pediococcus pentosaceus* and *Staphylococcus xylosus* was 1:1 and curing temperature was 10℃. Products with close texture, bright color, low salinity and rich unique cured fish flavor were obtained under these conditions, and the qualities were superior to non-vaccinated cured fish.

Key words: Direct vat; fermentative strains; cured fish

1. Introduction

Cured fish is warmly welcomed by consumers for its unique flavor.Traditional cured fish is mainly produced under high salinity and low temperature conditions, its flavor mainly depend on the microorganism infected in the curing process, its curing cycle is long and it's vulnerable to seasons, products under these conditions are usually unstable and with high salinity and low safety [1]-[3]. Lactic acid bacteria and Staphylococcus have been reported to be the predominant strains in the traditional cured fish [4], they can promote products to produce fragrancy and retain color [5]. Lactic acid bacteria can use carbohydrate to produce lactic acid, which can lower the pH of the products rapidly and inhibit the growth of harmful microorganism [6]. Staphylococcus has the activities of catalase and nitrate reductase, which can accelerate the speed of retaining colour and retain the particular flavor of cured products [7]. *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactobacillus casei*, et al are the predominant Lactic acid bacteria and *Staphylococcus xylosus*, *Staphylococcus carnosus*, et al are the predominant staphylococcus in the traditional cured fish. *Lactobacillus plantarum*, *Lactobacillus casei* and *Staphylococcus xylosus* were inoculated to silver carp surimi to suppress the increase of TVB-N, the growth of spoilage microorganisms and decrease the pH of the products by Hu [8]. Funatsu- Y inoculated lactic acid bacteria to fish sauce to remove the fishy odor [9]. In this study, in order to explore the optimal technological parameters for cured fish, fermentative strains were chosen from the predominant strains of traditional cured fish and then were inoculated, aiming at providing theoretical basises for the improving of traditional cured fish.

2. Materials and Methods

2.1. Materials

Lactobacillus plantarum(*Lp*), *Pediococcus pentosaceus*(*Pp*), *Lactobacillus casei*(*Lc*), *Staphylococcus xylosus*(*Sx*), *Staphylococcus carnosus*(*Sc*), MRS liquid broth, nutrient broth, MSA liquid broth were purchased from China General Microbiological Culture Collection Center. Grass carp and salt were purchased from the market. Methyl red, Methylene blue,Phenolphthalein were used as indicators. All the other reagents were of analytical grade.

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2.2. Instruments

A SW-CJ-2FD Double-sided Clean Bench(HengDa Purification Equipment Corporation, Suzhou) was used for sterile operation. A portable high-pressure steam sterilizer(SanShen Medical Devices Corporation, shanghai) was used for sterization. A UV-2600 UV-visible spectrophotometer(UNICO Instrument Corporation, Shanghai) was used for the measurement of nitrite and nitrate. A DHP-9082 electric incubator(YiHeng Technology Corporation, Shanghai) was used for the reculturing of strains.

2.3. Selection of fermentative strains

According to the references, fermentative strains should have salt tolerance, antibiotic activities, reductivities of hydrogen peroxide and nitrate [10]. In this study, salt tolerance, antibiotic activities, reductivities of hydrogen peroxide and nitrate of Lp, Pp, Lc, Sx, Sc were considered and compared to choose the more suitable fermentative strains for cured fish. Experiments of salt tolerance, antibiotic activities, reductivities of hydrogen peroxide and nitrate were performed according to the reference [11]-[14].

2.4. Preparation of suspension culture

Lp, Pp, Lc were recultured in MRS liquid broth according to the reference [15], Sx, Sc was respectively recultured in nutrient broth and MSA liquid broth according to the reference [16].

2.5. Preparation of Cured fish

Fresh grass carp was firstly purified, washed, cut into pieces about 10g and then was drained, after that, 5% salt was added and mixed well based on the weight of the fish meat, afterwards fermentative strains were inoculated and prepared fish was fermented under constant temperature for 4 days [17].

2.6. Testing of quality indicators

Acid value, Peroxide value were tested according to《Method for analysis of hygienic standard of edible vegetable oils》in GB/T5009.37-2003. TVB-N content was tested according to《Method for analysis of hygienic standard of meat and meat products》in GB/T5009.44-2003. Nitrite content was tested using hydrochloride naphthodiamide method in GB/T5009.33-2003. Nitrate content was tested using ultraviolet spectrophotometry method [18].

2.7. Sensory evaluation

Sensory evaluation team was constituted by teachers and students coming from food college. Cured fish were assessed according to color, salinity, umami, fishy odor and hardness, sensory evaluation criterias were shown in Table 1.

Table 1: Criteria for sensory evaluation

indicators	0—5 scores	6—10 scores	11—15 scores	16—20 scores
color	dark color	very light color	light color	bright color
salinity	very salty or no salinity	higher or lower salinity	slightly higher or lower salinity	appropriate salinity
umami	no umami	subtle umami	slightly less umami	rich umami
fishy odor	obvious fishy odor and off- flavor	obvious fishy odor	subtle fishy odor	no fishy odor
hardness	loosened into small pieces	loose	slightly loose	firm texture

2.8. Experiment design

Qualities of cured fish mainly depended on the total inoculum amount, inoculation ratio and fermentation temperature[17] when fermentative strains were applied to cured fish. The optimal technological parameters for cured fish were optimized using single factor test and orthogonal test. The levels of orthogonal factors were shown in Table 2.

Table 2: Factors and levels of orthogonal test

levels	factors		
	A inoculum amount /(CFU/g)	B inoculation ratio/（Pp：Sx）	C fermentation temperature /℃
1	10^5	1:1	5
2	10^6	1:2	10
3	10^7	2:1	15

3. Results and Analysis

3.1. Traits of fermentative strains

Results of salt tolerance, antibiotic activities, reductivities of hydrogen peroxide and nitrate of Lp, Pp, Lc, Sx, Sc were shown in Table 3.

Table 3: Traits of fermentative stains

	salt tolerance/(10%NaCl)	antibiotic activities	reductivities of hydrogen peroxide	reductivities of nitrate
Lp	+	+	_	_
Pp	+	++	_	_
Lc	+	+	_	_
Sx	++	+	+	+
Sc	+	+	+	+

Note: "+" indicates that the tested result was positive, and "-" indicates that the tested result was negative.

Seen from the Table 3, Lp, Pp, Lc, Sx can tolerate 10% NaCl, but the salt tolerance of Sx was stronger than that of Lp, Pp, Lc and Sc. The antibiotic activities of Pp was stronger when compared with with Lp, Lc, Sx and Sc. The reductivities of hydrogen peroxide and nitrate of Sx and Sc were positive while Lp, Pp and Lc were negative. Therefore, to compensate for the deficiencies of single fermentative strain, Pp and Sx were chosen as the fermentative strains.

3.2. Effect of total inoculum amount

Acid value, peroxide value, TVB-N content, nitrite content and nitrate content of products were detected after 4 days fermentation under the following conditions: total inoculum amount was respectively 10^5CFU/g, 10^6CFU/g, 10^7CFU/g, inoculation ratio of Pp and Sx was 1:1, fermentation temperature was 10℃. The results were shown in Table 4.

Table 4: Effect of total inoculum amount on the qualities of cured fish

total inoculum amount /(CFU/g)	acid value /(mgKOH/g)	peroxide value /(g/kg)	TVB-N content /(mg/100g)	nitrite content /(mg/kg)	nitrate content /(mg/g)
10^5	0.117	0.141	13.32	1.56	0.173
10^6	0.108	0.130	12.34	1.48	0.167
10^7	0.103	0.124	12.02	1.44	0.163
0	0.140	0.189	16.24	1.64	0.184

With the increase of total inoculum amount, acid value, peroxide value, TVB-N, nitrite content and nitrate content decreased gradually with a obvious trend, and the values detected in the inoculation groups were obviously lower than that in the non-inoculation group. When total inoculum amount was 10^5 CFU/g, the values were significantly higher than that whose inoculum amount were 10^6 CFU/g and 10^7 CFU/g, but there were little value differences when the inoculum amount were 10^6 CFU/ g and 10^7 CFU/g.

3.3. Effect of Inoculation Ratio

Acid value, peroxide value, TVB-N content, nitrite content and nitrate content of products were detected after 4 days fermentation under the following conditions: the total inoculum amount was 10^6CFU/g, inoculation ratio of Pp and Sx was respectively 1:1, 1:2, 2:1, fermentation temperature was 10℃.The results were shown in Table 5.

Table 5: Effect of inoculum ratio on the qualities of cured fish

inoculation ratio /(Pp：Sx)	acid value /(mgKOH/g)	peroxide value /(g/kg)	TVB-N content /(mg/100g)	nitrite content /(mg/kg)	nitrate content /(mg/g)
1:2	0.119	0.138	12.99	1.58	0.171
2:1	0.122	0.134	12.83	1.54	0.173
1:1	0.108	0.130	12.34	1.48	0.167

When the inoculation ratio of Pp and Sx was 1:1, acid value, peroxide value, TVB-N, nitrite content and nitrate content were lower than the groups whose inoculation ratio were 1:2, 2:1, but the values showed little difference between the groups whose inoculation ratio were 1:2 and 2:1, this may due to the inhibition of one strain to the other when the former strain took a bigger proportion than the later.Therefore, in order to improve the qualities of the products, inoculation ratio of Pp and Sx should be 1:1.

3.4. Effect of Curing Temperature

Acid value, peroxide value, TVB-N content, nitrite content and nitrate content of products were detected after 4 days fermentation under the following conditions: the total inoculum amount was 10^6CFU/g, inoculation ratio of Pp and Sx was 1:1, fermentation temperature was respectively 5℃, 10℃, 15℃. The results were shown in Table 6.

Table 6: Effect of curing temperature on the qualities of cured fish

fermentation temperature /℃	acid value /(mgKOH/g)	peroxide value /(g/kg)	TVB-N content /(mg/100g)	nitrite content /(mg/kg)	nitrate content /(mg/g)
5	0.092	0.113	11.21	1.16	0.153
10	0.108	0.130	12.34	1.48	0.167
15	0.128	0.172	14.24	1.81	0.196

The qualities of products were worse when the fermentation temperature was higher. Acid value, peroxide value, TVB-N content, nitrite content and nitrate content of products fermented under 15℃ were higher than those under 5℃, 10℃.The qualities of products can be ensured under low temperature, so 5℃, 10℃ can be chosen as fermentation temperature.When fermentation temperature was higher than the ambient temperature, more energy will be consumed and the cost of the production will increase, so 10℃ can be chosen as fermentation temperature.

3.5. Optimization of the optimal technological parameters

Table 7: Optimization results of technological parameters

tested number	A	B	C	sensory scores
1	1	1	1	75.8
2	1	2	2	78.3
3	1	3	3	69.8
4	2	1	2	83.3
5	2	2	3	76.9
6	2	3	1	80.3
7	3	1	3	71.9
8	3	2	1	75.5
9	3	3	2	74.2
K_1	223.9	231.0	231.6	
K_2	240.5	230.7	235.8	
K_3	221.6	224.3	218.6	
R	6.30	2.23	5.73	

The order arranged according to the importance of every factor to the sensory scores were A>C>B, that was to say, inoculum amount was of the most importance, followed by fermentation temperature, inoculation ratio was of the least importance.The best combination of process conditions were $A_2B_1C_2$, inoculum amount was 10^6 CFU/g, inoculation ratio of Pp and Sx was 1:1 and fermentation temperature was 10℃, products under these conditions got the highest sensory scores, with bright color, close texture, low salinity and rich unique cured fish flavor.

3.6. Quality Comparison

Prepared fish meat was divided into experimental group and control group averagely, Pp and Sx were inoculated to experimental group, the inoculum amount was 10^6 CFU/g, inoculation ratio was 1:1, fermentation temperature was 10℃, the control group was handled with the same method but without

inoculation. After 4 days fermentation, acid value, peroxide value, TVB-N content, nitrite content and nitrate content were detected and compared, the results were shown in Table 8.

Table 8: Comparison between inoculated group and non- inoculated group

	acid value /(mgKOH/g)	peroxide value /(g/kg)	TVB-N /(mg/100g)	nitrite content /(mg/kg)	nitrate content /(mg/g)
Experimental group	0.108	0.130	12.34	1.48	0.167
Control group	0.14	0.189	16.24	1.64	0.184
The amount of change(%)	—22.9	—31.2	—24.0	—9.76	—9.24

Acid value, peroxide value, TVB-N content, nitrite content and nitrate content detected in the experimental group were lower than those in the control group, the values reduced by 22.9%, 31.2%, 24.0%, 9.76%, 9.24% respectively when compared with the control group. The experimental group products had bright color, close texture and low salinity,while the control group products had dark color, loose texture and fishy odor.The qualities of inoculated products were superior to non-inoculated products.

4. Conclusion

Pp and Sx were chosen as the fermentative strains.The optimal levels of the parameters were optimized to be 10^6 CFU/g, inoculation ratio 1:1, fermentation temperature 10℃. After 4 days fermentation under these conditions, producteds with bright color, close texture, low salinity and rich flavor of cured fish were produced, acid value, peroxide value, TVB-N content, nitrite content and nitrate content were respectively 0.108mgKOH/g, 0.130g/kg, 12.34mg/100g, 1.48mg/kg, 0.167 mg/g, the qualities were superior to non-inoculated products.

5. Acknowledgment

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