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Studies on the Optimization of Salt and Lactic Starter Inoculum Levels for the Development of Fermented Shrimp Pickle

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Abstract

A study was conducted to develop a new fermented shrimp pickle using lactic starter cultures, viz., *Lactobacillus plantarum* and *L. acidophilus*, and to optimize the salt and inoculum levels. Salt concentration at 8% and 10% and inoculum at $\geq 10^4$ and $\geq 10^7$ cells·g^{-1} of pickle were tried. In the salted spicy pickle, the lactic acid bacteria (LAB) acquired resistance and grew after initial inhibition. The generation time and number of generations of LAB were affected in pickles with higher salt and lower inoculum levels. Salt at lower level (8%) and LAB inoculum at higher level ($\geq 10^7$ cells·g^{-1}) resulted in a better product in terms of fermentative and sensory character-istics. *L. plantarum* was more resistant than *L. acidophilus* and showed better fermentative activity.

Introduction

Preservation of food can be done by physical, chemical and biological means. One of the earliest applications of biological means was the use of acid-producing bacteria in preparing food products with the addition of salt and sugar to enhance flavor and retard spoilage. The suitability and consistency of these products primarily depend upon the controlled conversion of sugar to lactic acid by lactic acid bacteria (LAB). The natural fermentation caused by LAB or

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by inoculating a portion of recently fermented products into the fresh batch is commonly practiced even today.

The use of LAB has been reported in the preservation of fish products such as fermented crucian carp (Matsushita 1937), "burong-dalag" (fermented mudfish) (Orillo and Pederson 1968), marinades (Blood 1975), fermented rice-shrimp mixture (Arroyo et al. 1978) and cooked rice-fish mince (Mendoza and Owens 1986). Reports on the enzymically hydrolyzed and bacterially fermented fish products (Owens and Mendoza 1985) and LAB fermented fish silages (Rao and Gildberg 1982) have been reviewed. Studies on the application of LAB cultures for the fermentation of shrimp/fish pickles have been limited. The objective of the present study was to develop fermented shrimp pickle using lactic starter cultures and to optimize the salt and inoculum levels in the preparation of pickle.

Materials and Methods

Fresh shrimp, *Parapenaeopsis stylifera*, was procured from Mangalore (India) fish landing center and brought to the laboratory in iced condition. In the laboratory the shrimps were washed, peeled, deveined and blanched in 5.0% sodium chloride (NaCl) and 0.02% citric acid solution at 100°C for 10 min. Lactic acid bacteria, *Lactobacillus plantarum* and *L. acidophilus* 1899, supplied by the Department of Dairy Bacteriology of the University of Agricultural Sciences, Mangalore (India) were used. Sterile 250-g glass bottles were used to pack the shrimp pickle.

Lactic acid bacterial cell suspensions were prepared as described by Abraham et al. (1990). The LAB cells were suspended in sterile physiological saline (0.85% NaCl) at $\geq 10^{10}$ cells·ml^{-1} concentration and kept at 4°C until used.

Preliminary studies with blanched shrimp and the two lactic starter cultures *L. plantarum* and *L. acidophilus* 1899, indicated that the rate of pH reduction increased with the reduction in salt levels from 15% to 5%. Salt at 12% and 15% levels was found to be too high for the growth of lactic cultures. Although 5% salt gave a higher acid production, it was not preferred, since it is too low a concentration for pickling and at the same time favors the growth of spoilage and food poisoning organisms. Keeping this in view, 8% and 10% salt levels were selected, since it is the maximum tolerable salt concentration for organisms like *Clostridium botulinum* and

Vibrio parahaemolyticus. However, *Staphylococcus aureus* can tolerate up to 18% salt (Owens and Mendoza 1985). Addition of carbohydrate is necessary to promote satisfactory fermentation since shrimp flesh is low in free carbohydrate. The concentration of sucrose in this experiment was kept at a 4% level to avoid unsuitable acidification due to lack of carbohydrate. A desirable level of pH reduction using 4% (w/w) sugar has been reported in krill sausage maturation (Christians 1980) and in minced fish-salt-glucose systems (Adams et al. 1987).

Shrimp pickles with 8% and 10% salt were prepared separately according to the method developed by Chandrasekhar (1979) with slight modifications. Commercial sugar (sucrose) at 4% (w/w) was added to the pickle as a fermentable carbohydrate source for LAB. The prepared pickles were allowed to cool to room temperature and LAB cell suspensions were inoculated into the pickles placed in sterile glass bottles. The cell suspensions were inoculated into the pickles in such a way as to obtain a LAB population of $\geq 10^4$ and $\geq 10^7$ cells·g^{-1} of pickle. After inoculation, the pickles in the bottles were mixed well and the bottles sealed with aluminium caps. The pickles were stored at 30°C for fermentation. No lactic acid bacteria were added in control samples.

LAB were enumerated on MRS agar plates by the standard pour plate technique (Sharpe and Fryer 1965).

The pH and titratable acidity of LAB-inoculated and control pickles were determined at 0, 15 and 30 days of fermentation. The pH of the shrimp pickle was measured using a combined electrode pH meter by blending a 10-g sample in a mortar with 50 ml distilled water. Titratable acidity was determined by the methods of AOAC (1975).

The following formulae were used to calculate generation time and number of generations:

Number of generations (M) = 3.3 $\log_{10}$ b/B; where b = final number of bacteria; B = initial number of bacteria.

Generation time (G) = T/M, where T = time intervals in days; M = number of generations.

Pickle samples were subjected to sensory evaluation a month after preparation. Appearance, color, taste, texture, odor and overall acceptability were evaluated and the average scores presented. The limit of acceptability was fixed at a minimum of 5.0 on a 9 point hedonic scale.

Results and Discussion

Effect of Inoculum Levels on the Fermentation of Shrimp Pickle

In the present study, LAB cell suspensions were inoculated into the pickles at two levels. The added quantity of lactic starters guaranteed LAB levels of $\geq 1.0 \times 10^4$ and $\geq 1.0 \times 10^7$ cells·g^{-1} pickle. The speed and efficiency of the lactic fermentation is generally monitored by the rate of pH decrease and the balance between LAB inoculum and competing microbes. Most of the studies showing successful inhibition of pathogens or spoilage organisms with at least 10^7 lactics/g (Raccach and Baker 1979; Raccach 1986). Table 1 shows that in the shrimp pickle with 8% salt and *L. plantarum*, the titratable acidity increased by 2.8 and 4.4 times at 10^4 and 10^7 levels resulting in the reduction of pH to 6.30 and 5.85, respectively, from neutral pH in 30 days. The LAB count was increased to log 5.236 and 8.716 from log 4.30 and 7.30, respectively. At the same time, in pickle with 10% salt, the pH remained around 6.50 throughout the incubation period. The increase in LAB count and the titratable acidity were negligible at both levels. In control samples, the pH was above 6.80 and the increase in titratable acidity was very negligible (0.09 to 0.12 per cent lactic acid) in 30 days. These findings are, by and large, in accordance with those of Bartholomew and Blumer (1980), who observed a pH reduction of 0.5 units in *L. plantarum* inoculated country style ham in 30 days.

Higher salt concentrations require a longer fermentation period because of the inability of LAB to convert much sugar to acid at high salt concentration (Arroyo et al. 1978; Adams et al. 1987). Rapid acid production early in the fermentation is desirable for controlling the growth of pathogens that may be present in the product. Increased acidity in the product would have the advantage of providing protection against *S. aureus* and other pathogenic bacteria (Zaika and Kissinger 1984).

More or less similar observations were made in shrimp pickle inoculated with *L. acidophilus* 1899 (Table 1). The reduction of pH and the increase in LAB count in *L. plantarum* inoculated pickle were higher than that of *L. acidophilus* inoculated pickle.

Table 1. Effect of inoculum levels on the rate of fermentation in shrimp pickle.

Salt concentration	Storage days	pH			Titratable acidity as % lactic acid			Log LAB count/g	
		Inoculum level/g			Inoculum levels/g			Inoculum levels/g	
		No LAB	10^4	10^7	No LAB	10^4	10^7	10^4	10^7
a. *L. plantarum*									
	0	6.95	7.00	6.90	0.09	0.09	0.09	4.301	7.303
8%	15	6.90	6.50	6.25	0.12	0.22	0.26	4.431	7.511
	30	6.80	6.30	5.85	0.12	0.25	0.40	5.236	8.716
	0	6.95	6.90	6.95	0.09	0.09	0.09	4.332	7.380
10%	15	ND	6.80	6.75	ND	0.13	0.14	ND	ND
	30	6.90	6.60	6.40	0.12	0.18	0.23	4.394	7.445
b. *L. acidophilus*									
	0	6.95	7.00	6.90	0.09	0.09	0.09	4.182	7.279
8%	15	6.90	6.60	6.30	0.12	0.18	0.25	4.301	7.518
	30	6.80	6.50	5.95	0.12	0.22	0.38	5.041	8.290
	0	6.95	6.95	6.90	0.09	0.09	0.09	4.130	7.236
10%	15	ND	6.80	6.70	ND	0.13	0.17	ND	ND
	30	6.90	6.60	6.45	0.12	0.18	0.22	4.182	7.303

ND = Not done

Generation Time and Number of Generations

The effects of salt and inoculum levels on the generation time and number of generations of LAB in shrimp pickle are presented in Tables 2 and 3. The generation time of LAB in shrimp pickles between zero and 15th day was longer at the 10^4 level than at 10^7 level. The growth rate was faster between 15 and 30 days of storage (Table 2). This might have been due to the initial inhibition caused by salt and spices on LAB and the adaptation of LAB in the later periods of fermentation. Investigations of Zaika and Kissinger (1981) indicated that *L. plantarum* and *P. cerevisiae* could be completely inhibited by appropriate concentrations of oregano. The microorganisms could adapt to the toxic effects after first being exposed to sublethal concentrations. Several reports suggest that LAB are relatively resistant to the toxic effects of spices (Kissinger and Zaika 1978; Zaika and Kissinger 1979; Zaika et al. 1983). In all cases, after a growth lag, acid production increased with the increase in spice concentration. Yet the mechanism by which the lactic cultures acquire resistance is not known.

Furthermore, the spices used in this study were not sterilized and the LAB had to compete with the spoilage flora in neutral pH

Table 2. Effect of 8% salt on the growth rate of LAB in shrimp pickle.

Storage days between		*L. plantarum* inoculum level/g		*L. acidophilus* 1899 inoculum level/g	
		10^4	10^7	10^4	10^7
0 and 15	A	0.429	0.686	0.392	0.789
	B	34.960	21.860	38.260	19.010
15 and 30	A	2.657	3.976	2.442	2.548
	B	5.640	3.770	6.140	5.880
0 and 30	A	3.086	4.663	2.835	3.336
	B	9.720	6.430	10.580	8.990

A = Number of generations
B = Generation time in days

environment to effect its fermentative activity. When the concentration of salt increased from 8% to 10%, the growth of LAB was negligible. The generation time increased more than 15 times at all inoculum levels at 10% salt concentration that at 8% salt (Table 3). This confirms the inhibitory action of salt together with spices on the growth and acid production of LAB in shrimp pickle. Among the two starter cultures tried, *L. plantarum* was relatively more resistant than *L. acidophilus* 1899.

Table 3. Effect of 10% salt on the growth rate of LAB in shrimp pickle.

Storage days between 0 and 30	*L. plantarum* inoculum level/g		*L. acidophilus* 1899 inoculum level/g	
	10^4	10^7	10^4	10^7
Number of generations	0.2046	0.2475	0.1716	0.2211
Generation time in days	146.60	121.20	174.80	135.60

Sensory Evaluation of Shrimp Pickle

The sensory evaluation of shrimp pickle was conducted a month after preparation and the average panel scores are presented in Tables 4 and 5. The scores for overall acceptability and the individual scores for the five attributes were higher at 10^4 and 10^7 levels than the control. Between the two salt concentrations, LAB inoculum at $10^7 \cdot g^{-1}$ level had a slightly higher rating. The panelists also pointed out that the taste and flavor of fermented shrimp pickles were superior to those of traditionally prepared ones available in the market. Increasing the inoculum level of LAB to a higher level of $\geq 10^7$ was reported to have reduced the fermentation period and the growth of *S. aureus* in sausage (Raccach 1986). In this study, though the higher level of inoculum resulted in a better product, the expected acid production and the consequent pH reduction were not achieved. This may be because of the stronger inhibitory effect of salt and unsterile spices on LAB in the pickle and the inability of LAB to compete with the spoilage microorganisms.

Table 4. Average panel scores for shrimp pickle inoculated with *L. plantarum*.

Salt concentration	8% salt			10% salt		
Inoculum level/g	10^4	10^7	No LAB	10^4	10^7	No LAB
Attributes						
Appearance	8.4	8.0	6.6	8.0	8.2	6.8
Color	8.4	8.6	7.0	8.0	8.2	7.0
Taste	7.6	7.6	7.6	7.6	7.4	6.8
Texture	7.8	7.8	7.4	7.8	7.8	7.0
Odor	7.8	8.2	7.0	7.8	7.4	7.2
Overall acceptability	8.0	8.0	7.0	7.9	7.8	7.0

Table 5. Average panel scores for shrimp pickle inoculated with *L. acidophilus* 1899.

Salt concentration	8% salt			10% salt		
Inoculum level/g	10^4	10^7	No LAB	10^4	10^7	No LAB
Attributes						
Appearance	8.0	7.8	6.6	8.0	8.0	6.8
Color	8.0	8.0	7.0	7.8	7.8	7.0
Taste	7.6	7.8	7.6	7.6	7.4	6.8
Texture	7.4	7.6	7.4	7.6	7.6	7.0
Odor	7.6	8.0	7.0	7.8	7.6	7.2
Overall acceptability	7.6	7.8	7.0	7.6	7.6	7.0

Conclusion

The LAB survived and grew in the heavily salted and spicy shrimp pickle. Although the 8% salt and the inoculum at 10^7 level gave a better product, acid production and pH reduction were very slow. Since the safety of lactic fermented foods has been attributed to the production of acid and consequent pH reduction, a rapid reduction in pH is desirable. This could be achieved either by reducing the salt level or by fermenting the shrimps separately and then mixing the fermented shrimps with the spice mixture. Future studies on these aspects would provide more information on the development of fermented shrimp pickle and would assure safety of the product from undesirable bacteria, especially food poisoning species.

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