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Effect of Sodium Benzoate on the Fermentative Activity of *Lactobacillus plantarum* in Fermented Prawn Pickle

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Experiments were conducted to investigate the effect of sodium benzoate (0.1%) on the fermentative activity of *Lactobacillus plantarum* in fermented prawn pickle and in liquid medium. *L. plantarum* survived and grew in heavily spiced, salted Indian style prawn pickle. Both lactic acid bacterial growth and acid production were reduced significantly by sodium benzoate in prawn pickle. In liquid medium, sodium benzoate showed an inhibitory action on the growth of lactic acid bacteria, acid production and pH reduction. Addition of (0.1%) sodium benzoate in liquid medium increased the generation time of *L. plantarum*.

Preservation of food products in safe and wholesome condition by fermentation with lactic acid bacteria (LAB) depends on its rapid growth and subsequent acid production. (Raccach, 1986). In order to promote safe fermentation, it is important to ensure proper growth conditions as well as an utilisable substrate for acid production by LAB (Nes & Skjelkvale, 1982). Additives commonly added to fermented products may stimulate or inhibit the growth and acid production by LAB. The stimulatory or inhibitory effect of spices (Kissinger & Zaika, 1978; Zaika & Kissinger, 1979, 1981; Nes & Skjelkvale, 1982) and salt (Etchells, *et al.*, 1975; Arroyo *et al.*, 1978; Bartholomew & Blumer, 1980) on LAB have been well documented.

Pickling, using organic acids, is an easy and safe means for preservation of fish and shellfish. Since sodium benzoate is commonly used as a preservative agent in fermented and pickled fishery products it is of interest to know if it affects the starter culture used in the product. The present work deals with the effect of sodium benzoate on the fermentative activity of lactic starter culture in liquid medium and in fermented prawn pickle.

Material and Methods

Lactic acid bacterium, *Lactobacillus plantarum* supplied by the Department of Dairy Bacteriology, University of Agricultural Sciences, Bangalore was selected for the preparation of fermented prawn pickle, based on the initial screening with respect to growth response and acid production, in broth containing sodium chloride (0 to 15%). *L. plantarum* 89 supplied by National Dairy Research Institute, Karnal was used for the study in liquid medium.

MRS broth (Sharpe & Fryer, 1965) was used for studying the effect of sodium benzoate on the acid production and growth of *L. plantarum* 89. Initial pH of the medium was adjusted to 6.50.

The lactic culture, *L. plantarum* maintained in yeast glucose chalk milk, without litmus (Harrigan & McCance, 1976) was subcultured repeatedly, at least 3 times, in MRS broth before preparation of inoculum. Cultures, 20-24 h old, were centrifuged at 6,000 x g for 10 min and the supernatent discarded. The pellet was washed twice in sterile physiological saline (0.85% NaCl) and the cells were finally resuspended in physiological saline at $\geq 10^{10}$ cells/ml concentration

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and stored at 4°C till use.

Frozen, peeled and deveined prawns, *Parapenaeopsis stylifera*, was procured from Karnataka Fisheries Development Corporation, Mangalore. The thawed prawn was blanched in 5% sodium chloride containing 0.02% citric acid for 10 min at 100°C. After blanching, the liquid portion was decanted and used as blanch liquor. The prawn pickle recipe was as follows: Blanched prawn 50% (w/w), table salt 8%, chilli powder 5%, garlic 4.5%, mustard 0.5%, ginger 1.5%, pepper powder 0.65%, turmeric powder 0.2%, sucrose 4%, refined groundnut oil 12.5% (v/w) and blanch liquor 13.15%. Sodium benzoate where required was added at 0.1%. The *L. plantarum* suspension was inoculated to the pickle to attain a level of $\geq 1 \times 10^7$ cells/g. In the control sample with benzoate no LAB were added. The prawn pickle was then packed in glass bottles of 250 g capacity, covered with aluminium caps, sealed and kept at room temperature (30± 1 °C) for fermentation. Care was taken to cover the surface of the pickle with a layer of oil to avoid any contact with air. Samples for titratable acidity, pH and LAB counts were taken at 7 day intervals for 5 weeks.

LAB counts were made by conventional pour plate technique using MRS Agar (Sharpe & Fryer, 1965) and plates were incubated for 48 h at 30± 1°C.

For titratable acidity, prawn pickle (10.0 g) was blended well in a mortar with 30 ml of distilled water, made upto 100 ml and filtered. Aliquots (10.0 ml) were titrated against 0.1 N NaOH using phenolpthalein as indicator and expressed as percent lactic acid (AOAC, 1975). Prawn pickle (10.0 g) was blended thoroughly with 50 ml of distilled water in a mortar and the pH was recorded using a combined electrode pH meter (Horiba, Japan).

MRS broth with and without 0.1% sodium benzoate was prepared in flasks and sterilized at 121°C for 20 min. All flasks were inoculated with *L. plantarum* 89 at $\approx 10^7$ cells/ml medium. Flasks were incubated without agitation at 30 ± 1°C for 60 h. Samples for pH and titratable acidity were drawn at 6 h intervals. LAB count was estimated at every 12 h interval on MRS agar. Number of generations and generation times were calculated as described by Raccach & Baker (1978).

10.0 ml of filtered (0.45 µ pore size membrane, Millipore) liquid medium was diluted to 100 ml with distilled water and titrated against 0.1 N NaOH (AOAC, 1975). Results were represented as percent lactic acid. The pH of the medium was recorded using a combined electrode pH mater.

Results and Discussion

Titratable acidity, pH and LAB count in pickle samples are shown in Table 1. In the presence of 0.1% sodium benzoate, the titratable acidity increased to 0.27 from

Table 1. *Effect of sodium benzoate on Lactobacillus plantarum in prawn pickle*

Storage days	pH			Titratable acidity as percent latic acid			LogLAB count/g	
	without benzoate	with benzoate	control	without benzoate	with benzoate	control	without benzoate	with benzoate
0	7.05	6.95	6.95	0.13	0.13	0.09	7.27	7.15
7	6.77	6.81	ND	0.21	0.18	0.12	7.57	7.36
14	6.54	6.68	ND	0.24	0.21	ND	7.65	7.53
21	6.30	6.36	6.80	0.28	0.25	0.18	7.87	7.47
28	6.24	6.31	6.80	0.31	0.27	0.20	7.75	7.41
35	5.97	6.15	6.76	0.31	0.27	0.20	7.68	7.37

ND = Not done

Table 2. *Effect of sodium benzoate on Lactobacillus plantarum in liquid medium*

Time, h	Titratable acidity as percent lactic acid		pH	
	without benzoate	with benzoate	without benzoate	with benzoate
0	0.200	0.200	6.50	6.50
6	0.502	0.476	5.81	6.02
12	1.404	1.204	4.87	5.40
18	1.755	1.354	3.99	4.47
24	2.106	1.705	3.85	4.05
30	2.307	2.006	3.77	3.94
36	2.407	2.006	3.75	3.88
42	2.307	2.106	3.75	3.81
48	2.307	2.307	3.72	3.74
60	2.407	2.407	3.68	3.68

0.13% compared to 0.31% in the prawn pickle without benzoate. The pH decreased from 7.05 to 5.97 in the untreated sample and from 6.95 to 6.15 in the treated sample. The LAB count, though inoculated at high level ($\approx 10^7$ cells/g), did not show much increase during storage. When compared to the untreated sample, a significant reduction in LAB count ($t = 5.19$; $p < 0.01$) was observed in the treated sample; this corresponded with a significant inhibition of acid production ($t = 4.71$; $p < 0.01$) by *L. plantarum.*

Though the LAB survived and grew, the reduction in pH and increase in percent lactic acid over a period of 35 days was rather slow, presumbaly due to the slow growth of LAB in the heavily salted and spiced pickle samples. From the commercial and safety points of view, it is highly desirable to reduce the period of fermentation and also the pH considerably. However, proteinaceous foods with high buffering capacities will require a much greater growth of LAB and acid production to effect a rapid fall in pH value compared to vegetables, which have a low buffering capacity and require relatively little growth and acid production for preservation (Owens & Mendoza, 1985). The slow pH reduction in this study was, more or less, in agreement with Bartholomew & Blumer (1980), who observed a pH reduction of 0.5 units over a period of 30 days from an initial value of 5.31 in country style ham when inoculated with *L. plantarum.*

Several reports are available on the enhancement of acid production by LAB in fermented meat products and in liquid medium in the presence of spices (Kissinger & Zaika, 1978, Zaika & Kissinger, 1979, 1984; Nes & Skjelkvale, 1982). Zaika & Kissinger (1981) were of the opinion that LAB may be inhibited by spices added to foods at high concentration and may develop resistant strains. However, the use of high amount of spices for Indian pickles is inevitable as compared to western style pickles because of the consumer preferences.

One of the objectives of this study was to investigate the effect of benzoate on the fermentative activity of *L. plantarum* since the inhibitory effect of salt and spices are well documented by several authors. When the starter culture, *L. plantarum* 89 was incubated in a medium containing 0.1% sodium benzoate, it was observed that the

Table 3. *Effect of sodium benzoate on the growth of Lactobacillus plantarum 89 in liquid medium*

Time, h	Without benzoate			With benzoate		
	Log LAB count/ml	No.of generations	Generation time, min	LogLAB count/ml	No.of Generations	Generation time, min
0	7.140	-	-	6.912	-	-
12	9.057	6.33	144	7.908	3.29	219
24	9.681	8.39	172	8.959	6.79	213
36	9.756	8.63	250	9.560	8.74	247
48	Cell death began				Cell death began	

LAB showed a longer lag phase and decrease in acid production and pH reduction initially (Table 2). The generation time was found to be increased to 219 min from 114 min between 0 and 12 h in the presence of sodium benzoate (Table 3). However, the generation time became almost equal at 36 h. The observations reveal that sodium benzoate does have an inhibitory effect on LAB initially upto 24 h. Shenderyuk & Bykowski (1990) were of the opinion that in marinades the sodium and potassium salts of benzoic acid did not affect LAB when used as a preservative. Benzoate has been found to influence microbial enzymes controlling oxidative phosphorylation and intervenes at various points in the TCA cycle (Bosund, 1962). The longer generation time and the reduction in acid production in this study may be attributed to the inhibitory action of benzoate on the microbial enzymes controlling the metabolic pathways.

The results indicate that *Lactobacillus plantarum* may be inhibited by sodium benzoate (0.1%), a preservative commonly added in fermented fishery products.

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