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Development of shelf-stable brined vegetables by lactic acid fermentation

E. R. Suresh
Division of Post Harvest Technology
Indian Institute of Horticultural Research, Bangalore-560 089, India
E-mail: ers@iihr.ernet.in

ABSTRACT

Preservation of vegetables, viz., bitter gourd, carrot, capsicum, cucumber, French bean and gherkin by lactic acid fermentation was attempted. Properly prepared vegetables, packed in brine containing 2.5% equilibrated salt with additives, were allowed to undergo fermentation by their natural flora and this was compared with pure culture fermentation by *Lactobacillus plantarum*. Fermented vegetables had 0.5 to 1.31% lactic acid, with pH values ranging from 2.97 to 4.02, at the end of 4 weeks of fermentation at 20 ± 2°C. In general, fermentation by *L. plantarum* resulted in a slightly faster rate of acid production compared to that by natural flora. Mustard powder at 1% concentration was found to be useful as alternate preservative in vegetable fermentation. Fermented vegetables had acceptable quality in terms of colour, texture, flavour, taste and were microbiologically stable for six months of storage at room temperature (25 ± 8 °C).

Key words: Vegetables, lactic acid fermentation, natural flora, *Lactobacillus plantarum*

INTRODUCTION

Preservation of vegetables in brine of higher salt concentration as salt-stock is one of the traditional methods commercially practised in several countries (Costilow and Ubersax, 1982; Fleming, 1982; Daeschel and Fleming, 1984). However, fermentation of vegetables in low salt brine by natural flora or by pure cultures of lactic acid bacteria is known to produce stable products. This technique of low-salt fermentation is gaining greater importance in recent years as the finished product contains optimum level of salt and obviates cumbersome de-salting process. Several reports indicate possibility of preserving vegetables such as cucumber, cabbage, carrot, French bean, okra, turnip, etc. by fermentation (Pederson and Albury, 1969; Gordona *et al*, 1973; Fleming *et al*,1978; Kozup and Sistrunk, 1982; Fleming *et al*, 1983, Yamini, 1993). Reduction in pH due to lactic acid production and removal of sugars from vegetables during fermentation prevents growth of pathogenic microorganisms, thereby, providing prolonged shelf-life that extends over a year. The relative advantage of lower pH value (below 3.5) enables vegetables to be preserved as such by a simple heating process. Microbiological and physico-chemical changes occurring during fermentation are of great importance in terms of storage stability and quality of the fermented vegetable. It is well-established that raw vegetables improve in taste, aroma and flavor due to fermentation, resulting in a more palatable product (Fleming and Mc Feeters, 1981). Although fermented cabbage (Sauerkraut), cucumber (Pickle), carrot and mixed vegetable ‘Korean Kimchi’ are popular and commercially produced in several countries, these products are not common in India. A few attempts have been made to develop lactic fermented vegetables in this country (Anand and Das Lakshmi, 1971; Kohli and Kulkarni, 1973; Sethi and Anand, 1984; Ramdas and Kulkarni, 1987; Sethi, 1990; Suresh *et al*, 1996; Desai and Seth, 1997). These fermented vegetables have a good potential for use in pickling, salads and various culinary preparations, with or without addition of spices, condiments, etc. In traditional pickle products, spices are used as an essential ingredient and a few of these even exert inhibitory action against selected microorganisms (Pruthi, 1980). Mustard is a common ingredient in many indigenous, pickled products and its use in vegetable fermentation is suggested (Sethi and Anand, 1984). The present investigation deals with lactic fermentation of some vegetables by natural flora *L. plantarum* and use of mustard as an alternate preservative for vegetable fermentation.

MATERIAL AND METHODS

Microbial culture: A standard culture of *Lactobacillus plantarum* obtained from the Microbiology Department of Michigan State University, East Lansing, U.S.A., and maintained in our laboratory was used in this study. A loopful of culture grown in De Man Rogosa Sharpe (MRS) agar medium was transferred to MRS broth (Himedia, Mumbai) containing 2% sodium chloride and incubated at 30°C. At 48h of growth, cells were harvested by centrifugation at 10,000 rpm for 15 min, washed and the pellet was suspended in normal saline. Concentration of the cells in suspension was adjusted to 10^6 / ml and used as inoculum at 2% (v/w) for vegetable fermentation.

Preparation of vegetables: Freshly harvested vegetables were collected from the local market and from experimental fields of the Institute at Hessaraghatta, Bangalore. They were thoroughly washed under running tap water and subjected to specific pre-treatments. Carrot skin was removed by scraping and cut into small, longitudinal pieces of 2-3 cm. In the case of French bean, both the ends of the pod were trimmed and the bean pod cut into 3 to 4 pieces. Similarly, in gherkin, ends were trimmed off and the cucurbit was cut into two longitudinal slices. The stem and seeds of capsicum were removed and the vegetable cut into slices 1 cm wide. Trimmed bitter gourd and cucumber were sliced longitudinally, their seed and placenta portions removed and made into small pieces of 1-2 cm. Duplicate lots (1 kg. each) of all these prepared vegetables meant for *L. plantarum* fermentation were blanched at 80 °C for 5 min and rapidly cooled in water. Unblanched lots were used in studies on fermentation by natural flora.

Brining and fermentation: Prepared vegetables were packed in brine containing 2.5% each of sodium chloride, calcium chloride, acidifying agent acetic acid, texture improver and preservative, Potassium sorbate, with a pack-out ratio of 1:1. pH of the brine was adjusted to 4.5 with sodium hydroxide to maintain buffering capacity wherever needed. Fermentation was carried out by natural flora or by inoculation with *L. plantarum* for 4 weeks at 20 ± 2 °C. In experiments with mustard, vegetables viz., carrot, cucumber and French bean were fermented in brine by *L. plantarum* as described above in the presence of 1% mustard powder and were compared with treatments of 0.1% potassium sorbate, a common preservative used in vegetable fermentation.

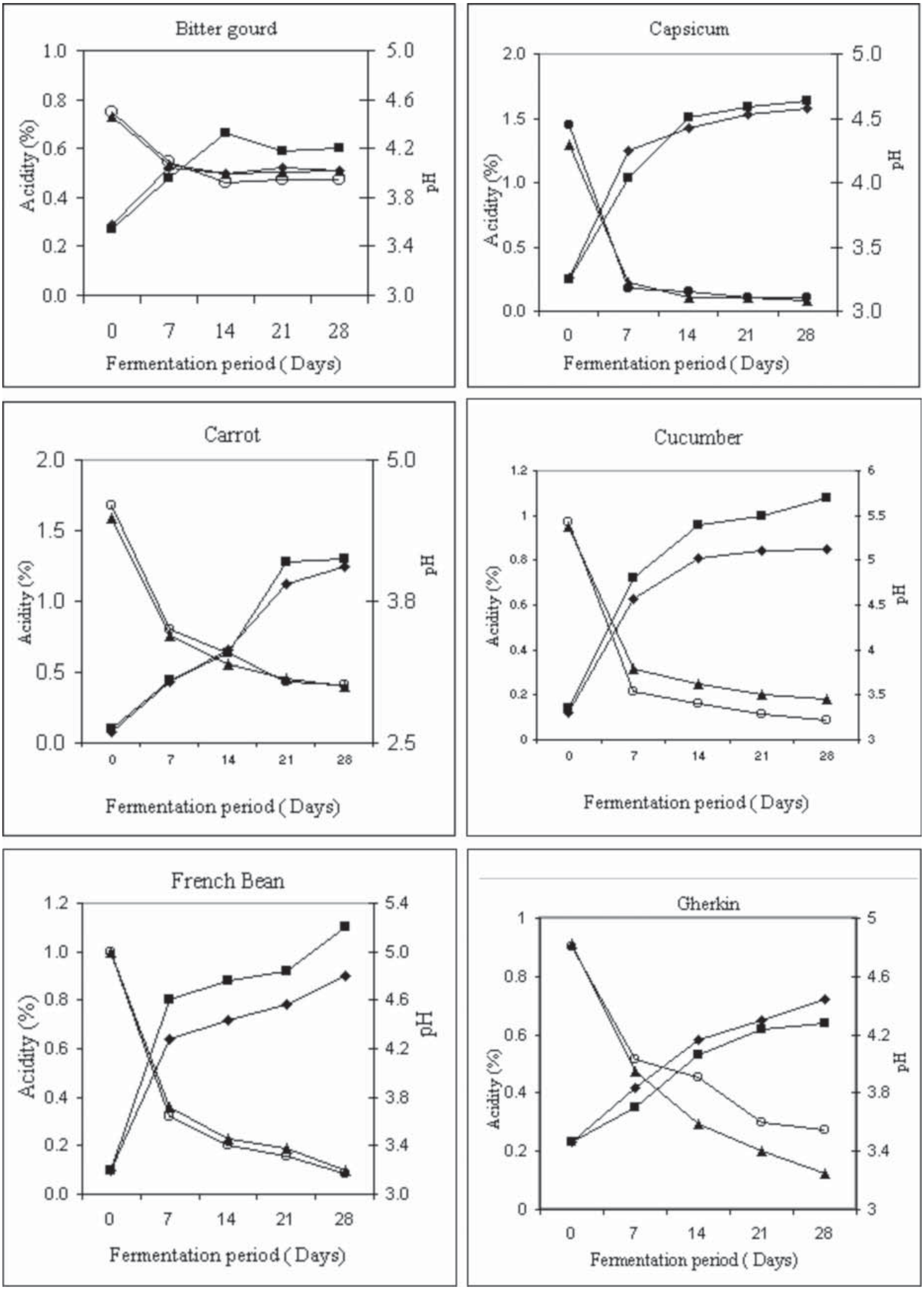
Preservation: At the end of 4 weeks, fermented vegetables were rinsed in water, re-packed in hot-filtered mother brine and stored at room temperature (25 ± 8 °C).

Analysis: Changes in brine acidity and pH were measured using standard methods at weekly intervals during one month of fermentation (Ranganna, 1986). Visual observations on colour, retention of texture, softening and microbial growth were recorded. Data obtained were subjected to ANOVA following CRD, and the means were compared at 1 and 5% levels of significance.

RESULTS AND DISCUSSION

The build-up of lactic acid and changes in pH during progression of fermentation in various vegetables by natural flora and by *L. plantarum* are illustrated in Fig. 1. In general, all vegetables (except carrot and gherkin) showed a slightly increased production of lactic acid due to fermentation by *L. plantarum,* as compared to that by natural flora. Fermented carrot and French bean had high acidity, i.e., above 1.3%, and low pH of 3.0 to 3.1 compared to that in other vegetables. Lowest acidity was recorded in bitter gourd (Table 1). There was significant variation in acidity due to fermentation methods used the type of vegetable as well as interaction between fermentation method and the vegetable. Significant variation in pH value was also observed. However, the effect of fermentation method used was not significant. These variations can be attributed to the specific vegetable used, in fermentation. However, acidity and pH levels attained at the end of fermentation period was sufficient to make the product shelf-stable. Traditional methods of vegetable fermentation depend mainly on activity of the natural flora viz., lactic acid bacteria and yeast. In this study, unblanched and blanched vegetables were used for fermentation by natural flora and *L. plantarum,* respectively. Blanching treatment eliminates yeast and homo- and hetero-lactics naturally present in vegetables, and encourages growth of the inoculated culture i.e., *L. plantarum* only. Salt used in the brine inhibits growth of pathogens and other destructive micro-organisms. Pure culture fermentations are known to result in better and uniform quality of the product and are generally preferred over natural fermentation (Fleming *et al*, 1978, Daeschel and Fleming, 1984). The present study revealed that vegetables fermented with *L. plantarum* had special flavour and a pleasant aroma. However, both natural as well as *L. plantarum* inoculated fermentation resulted in product of acceptable quality.

Incomplete utilization of sugars in the vegetable makes the product susceptible to secondary fermentation by yeast. Hence, it is essential to set up suitable environment



Natural fermentation: Acidity; pH. *L. plantarum: Acidity; pH.*

Fig 1. Changes in acidity and pH during fermentation of vegetables

Table 1. Changes in brine acidity and pH during lactic fermentation of vegetables

Sampling period	Bitter gourd	Capsicum	Carrot	Cucumber	French bean	Gherkin	Mean
				Acidity (% lactic acid)			
Initial	0.29	0.13	0.14	0.07	0.13	0.23	0.17
Natural (4 weeks)	0.50	0.75	1.31	0.63	0.97	0.73	0.82
L. plantarum(4 weeks)	0.60	0.87	1.02	0.67	1.03	0.64	0.80
Mean	0.55	0.81	1.17	0.65	1.00	0.69	
CD (*P=0.05*)		Method (m)		0.01	S.Em ±	0.00	
CD (*P=0.01*)		Vegetable (v)		0.03	S.Em ±	0.01	
CD (*P=0.01*)		Interaction (m x v)		0.04	S.Em ±	0.01	
				pH			
Initial	4.5	4.71	4.68	5.17	4.97	4.8	4.81
Natural(4 weeks)	4.02	2.98	3.16	3.31	3.22	3.32	3.33
L. plantarum (4 weeks)	3.88	2.97	3.11	3.23	3.21	3.50	3.32
Mean	3.95	2.97	3.13	3.27	3.22	3.41	
CD (*P=0.05*)		Method (m)		NS	S.Em ±	0.01	
CD (*P=0.01*)		Vegetable (v)		0.05	S.Em ±	0.01	
CD (*P=0.01*)		Interaction (m xv)		0.07	S.Em ±	0.02	

NS= Non Significant

Table 2. Effect of potassium sorbate/mustard on changes in pH and acidity of vegetables fermented by *L.plantarum*

Vegetable	Fermentation Period in days)	0.1% Potassium sorbate		0.1% Mustard powder	
		Acidity (%)	pH	Acidity (%)	pH
Carrot	0	0.19 ± 0.01	4.75 ± 0.03	0.13 ± 0.01	4.94 ± 0.04
	7	0.86 ± 0.01	3.42 ± 0.03	0.92 ± 0.02	3.35 ± 0.02
	14	1.12 ± 0.05	3.24 ± 0.01	0.97 ± 0.02	3.23 ± 0.02
	21	1.17 ± 0.05	3.21 ± 0.02	1.11 ± 0.03	3.22 ± 0.03
	28	1.20 ± 0.05	3.19 ± 0.01	1.04 ± 0.04	3.12 ± 0.02
Cucumber	0	0.09 ± 0.01	4.95 ± 0.02	0.15 ± 0.03	4.81 ± 0.01
	7	0.70 ± 0.02	3.45 ± 0.03	0.72 ± 0.02	3.31 ± 0.01
	14	0.85 ± 0.01	3.30 ± 0.01	0.81 ± 0.01	3.26 ± 0.02
	21	0.93 ± 0.02	3.22 ± 0.03	0.87 ± 0.01	3.22 ± 0.03
	28	0.94 ± 0.02	3.15 ± 0.01	0.82 ± 0.02	3.24 ± 0.02
French Bean	0	0.15 ± 0.02	4.86 ± 0.01	0.14 ± 0.01	4.76 ± 0.02
	7	0.92 ± 0.02	3.64 ± 0.02	0.73 ± 0.02	3.46 ± 0.29
	14	1.13 ± 0.02	3.34 ± 0.01	0.81 ± 0.01	3.44 ± 0.01
	21	1.18 ± 0.05	3.11 ± 0.02	0.87 ± 0.01	3.41 ± 0.01
	28	1.13 ± 0.05	3.20 ± 0.01	0.91 ± 0.03	3.35 ± 0.03

SD value ±

around the vegetable to allow desirable microflora to proliferate and predominate in the course of fermentation. Neutralization of brine acidity by adding sodium acetate or sodium hydroxide (for assuring complete fermentation) in cucumbers was reported earlier (Etchells *et al*, 1973; Fleming *et al*, 1978). In this study too, adjustment of brine pH to 4.5 and maintenance of buffering capacity (by adding alkali) resulted in stability of the products. Usefulness of calcium chloride/calcium acetate for retention of firmness in fermented vegetable has been reported earlier (Fleming *et al*, 1978; Thompson *et al*, 1979; Buescher *et al*. 1979, Buescher and Burgin, 1988). Based on this information, calcium chloride as an additive was incorporated into the brine solution, and this helped retain firm texture in fermented products during storage.

Microbial stability of fermented and stored vegetables was ascertained by absence of gas formation or visual microbial growth, supplemented with no changes in acidity and pH at the end of 6 months of storage. Hence, this study indicated the possibility of preserving seasonal vegetables by fermentation, due to which these can be made available throughout the year. Traditional pickled products of individual or mixed vegetables are popular in different parts of our country. Use of raw vegetables, as such, in pickles results in loss of texture and colour during storage.

Hence, it is suggested that these fermented vegetables can be a good replacement for raw vegetables in pickle production.

In pure culture fermentation of vegetables, use of the preservative potassium sorbate is essential to ensure growth of the introduced culture by suppressing competing microbial groups naturally present in vegetables. Since mustard is commonly used in traditional pickles, its use as a substitute for potassium sorbate in fermentation of some vegetables, viz., carrot, cucumber and French bean was tested. It is evident from Table 2 that there is only a marginal difference in acidity and pH of the vegetables during fermentation done in the presence of potassium sorbate or mustard. No difference in the quality of these fermented vegetables was noticed, except for a faint flavour wherever mustard was used. Mustard powder is reported to stimulate acid production during fermentation by lactic acid bacteria (Zaika and Kissinger, 1979). No such clear-cut observations were recorded in the present study. Sethi and Anand (1984) recommended the use of mustard in cauliflower fermentation.

Based on results of this study, it is concluded that microbiologically stable, fermented vegetables of acceptable quality can be derived either by natural flora or *L. plantarum* fermentation. Mustard can replace potassium sorbate as a selective preservative in vegetable fermentation stimulating the growth of Lactic Acid Bacteria.

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