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Enhancement of Shelf-stable Brined Vegetables using *Lactobacillus helveticus* as a Food Additive

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ABSTRACT

It was tried to preserve vegetables carrot, capsicum, cucumber, and cabbage by fermenting lactic acid. When vegetables were prepared correctly and put in a brine containing 2.5% equilibrated salt with additions, their natural flora fermented the vegetables. This fermentation process was compared to pure culture fermentation using *Lactobacillus helveticus*. After four weeks of fermentation at 20 ± 2o C, the pH of the fermented vegetables ranged from 2.97 to 4.02, with 0.5 to 1.31% lactic acid. When *Lactobacillus helveticus* fermented, acid production was often produced at a somewhat faster pace than when wild flora was used. In vegetable fermentation, mustard powder at 1% concentration was proven to be an effective substitute preservative. Fruits and vegetables' shelf life is extended by lactic acid fermentation, which also improves their nutritional content, flavors, and toxicity levels. Fermented vegetables had acceptable quality in terms of color, texture, flavor, and taste and were microbiologically stable for six months of storage at room temperature (25 ± 8 o C).

KEYWORDS

• Vegetables • Food additive • Normal flora • *Lactobacillus helveticus*

INTRODUCTION

One of the conventional techniques that are still used commercially in many nations is the preservation of vegetables in a brine with a higher salt content as salt-stock[1]. One straightforward and practical method of biotechnology for preserving and/or improving the safety, nutritional value, sensory appeal, and shelf life of fruits and vegetables is lactic acid fermentation[2]. Nonetheless, it is

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known that stable products can be produced when vegetables are fermented in low-salinity brine using either pure lactic acid bacteria cultures or native flora. Low-salt fermentation is becoming more and more popular since the final product has the ideal amount of salt and eliminates the need for laborious de-salting procedures. Numerous findings suggest that fermentation may be used to preserve crops like turnips, cucumbers, cabbage, carrots, French beans, okra, and more.[3-5] In addition to the antibacterial activity that the lactic acid fermentation of fruits and vegetables imparts due to the formation of numerous antimicrobial chemicals, lactic acid fermentation has been linked to medicinal values.[6,7] While pickled cucumbers (pickles), fermented cabbage (Sauerkraut), carrots, and mixed vegetables (Korean Kimchi) are widely consumed and manufactured internationally, they are uncommon in India. In this nation, there have been a few attempts to create lactic fermented vegetables.[8-12] Lactic acid bacteria produce a variety of metabolites with antimicrobial action during growth and fermentation, they are crucial to the biopreservation process. These metabolites include hydrogen peroxide, lactic acid, acetic acid, and low molecular weight substances like diacetyl, fatty acids, reuter in, and reutericyclin, as well as antifungal compounds like phenyl lactate, propionate, and hydroxyphenyl lactate, and bacteriocins[13]. Fermentation, the oldest technology in comparison to refrigeration, can extend the shelf life of perishable goods. One of the earliest methods of treating food to increase its shelf life is fermentation, which was crucial prior to refrigeration. For many years, lactic acid fermentation of cabbage to make sauerkraut has been extensively researched[14,15]. The present investigation deals with lactic fermentation of some vegetables by natural flora *lactobacillus helveticus* and use of mustard as an alternate food additives for vegetable fermentation.

MATERIAL AND METHOD

Microbial culture: Whey-derived *lactobacillus helveticus* culture. After being developed in De Man Rogosa Sharpe (MRS) agar medium, a loopful of the culture was moved to MRS broth (Himedia, Mumbai), which contained 2% sodium chloride, and it was incubated at 35°C. Cells were extracted after 48 hours of development by centrifuging them for 15 minutes at 10,000 rpm, washing them, and then suspending the pellet in regular saline. For vegetable fermentation, the concentration of the suspended cells was adjusted to 10^6/ml and utilized as an inoculum at 2% (v/w).

Preparation of vegetables: Freshly picked vegetables were gathered from the neighborhood market and the Institute's experimental fields at Noble University in Junagadh, Gujarat. They received a thorough cleaning under running water and a few pre-treatments. The carrot skin was scraped off and then chopped into 3-4 cm long, tiny pieces. When it comes to French beans, the pods were split into three or four parts and both ends were clipped. Similar to this, the cucurbit in the gherkin was divided into two longitudinal slices after the ends were taken off. After removing the stem and seeds, the capsicum was sliced into 1-2 cm-wide pieces. Cucumber and bitter gourd trimmings were cut into small, 2-3 cm pieces by cutting them lengthwise and removing the seed and placenta. All of these veggies were prepared in duplicate lots of 1 kilogram each for *lactobacillus helveticus* fermentation. The vegetables were blanched for 5 to 8 minutes at 80 °C and quickly cooled in water. Research on natural flora fermentation was conducted using unbranched lots.

Brining and fermentation: The prepared veggies were packed in a brine that had a packout ratio of 1:1 and contained 2.5% of calcium chloride, sodium chloride, acetic acid, texture enhancer, and preservative. Sodium hydroxide was used to bring the brine's pH down to 4.5 in order to preserve its buffering ability. For four weeks at 20 ± 2°C, fermentation was accomplished either by inoculating with *lactobacillus helveticus* or by using the indigenous flora. Carrot, cucumber, and French bean were the vegetables utilized in mustard tests. *Lactobacillus helveticus* fermented the vegetables in brine according to the previously described method, with 1% mustard powder present. Treatments with 0.1% potassium sorbate, a typical preservative used in vegetable fermentation, were compared.

Preservation: The fermented vegetables were kept at room temperature (25 ± 8°C) after being rinsed in water and repacked in hot-filtered mother brine after a 4-week period.

Analysis: During a month of fermentation, weekly measurements of the pH and acidity of the brine were made using established techniques[16]. Visual observations were made regarding color retention, texture softening, microbiological proliferation, and softening. Following CRD, the acquired data were subjected to ANOVA, and the means were evaluated at the 1 and 5% levels of significance.

RESULTS AND DISCUSSION

Figure 1 shows the accumulation of lactic acid and pH variations that occur as different vegetables ferment due to the action of both *lactobacillus helveticus* and natural flora. All vegetables (except from carrot and gherkin) generally displayed a somewhat higher lactic acid generation as a result of *lactobacillus helveticus* fermentation when compared to wild flora. Compared to other vegetables, fermented carrot and French bean had low pH levels of 3.0 to 3.1 and strong acidity, defined as more than 1.3%. The bitter gourd has the lowest acidity (Table 1). The type of vegetable utilized, the fermentation process, and the interaction between the fermentation method and the vegetable all contributed to the large difference in acidity. A notable fluctuation in the pH level was also noted. The employed fermentation technique did not, however, have a substantial impact. These differences can be linked to the particular vegetable that was fermented. However, the product was sufficiently shelf-stable due to the acidity and pH values reached at the end of the fermentation period. Traditional vegetable fermentation techniques primarily rely on the activities of yeast and lactic acid bacteria, which are part of the natural flora. In this work, *lactobacillus helveticus* and natural flora were employed to ferment blanched and unblanched vegetables, respectively. The process of blanching removes yeast and the naturally occurring homo- and hetero-lactics found in vegetables, promoting the growth of the inoculation culture, specifically *lactobacillus helveticus*. The brine's salt content prevents the growth of infections and other harmful microorganisms. Pure culture fermentations are often chosen over natural fermentations because they are known to produce products of higher and more consistent quality[17]. The results of this investigation showed that vegetables fermented with *lactobacillus helveticus* had a distinct flavor and a lovely scent. However, the product of satisfactory grade was obtained from both natural and *lactobacillus helveticus* infected fermentation. If the sugars in the vegetable are not fully utilized, yeast might cause secondary fermentation in the product. Therefore, it is crucial to create an ideal environment surrounding the vegetable so that during fermentation, the desired microflora can multiply and take center stage. It has previously been documented that brine acidity can be neutralized in cucumbers by adding sodium hydroxide or acetate (to ensure full fermentation)[18].

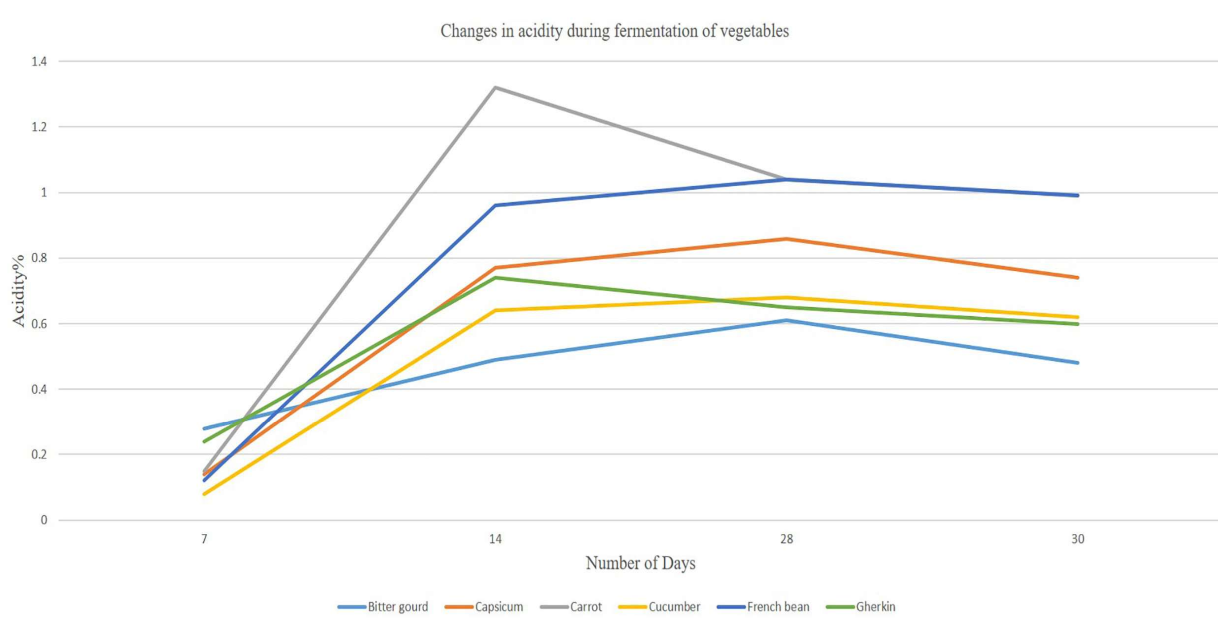

Figure 1: Changes in acidity during fermentation of vegetables

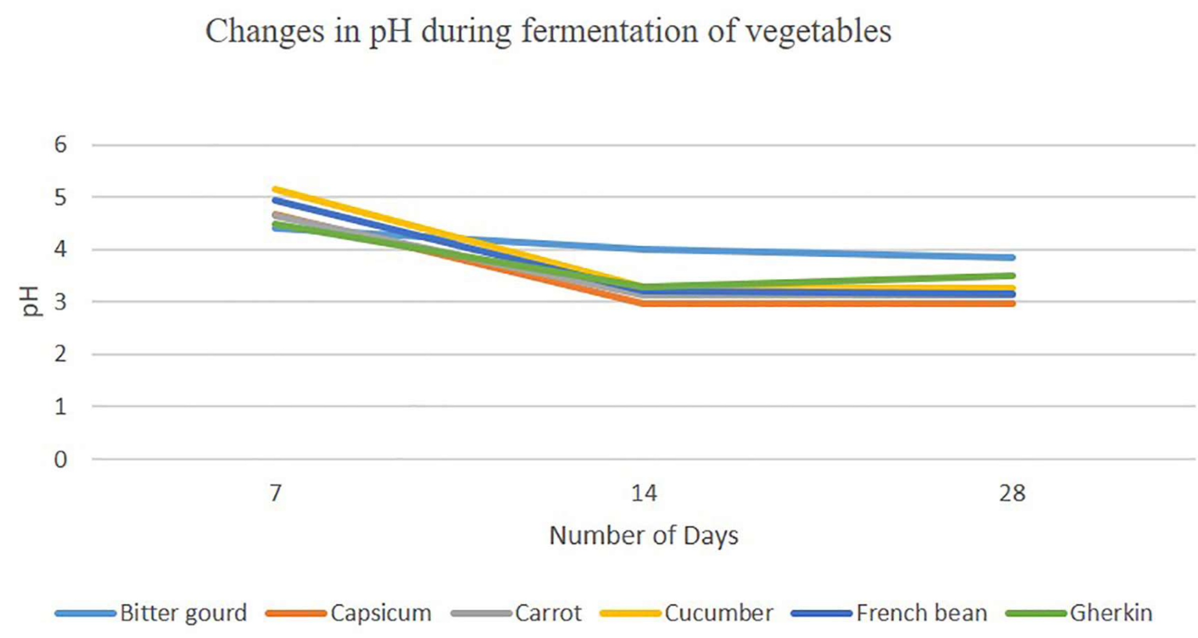

Figure 2: Changes in pH during fermentation of vegetables

Table 1: Variations in pH and brine acidity during vegetable lactic fermentation

Sampling period	Bitter gourd	Capsicum	Carrot	Cucumber	French bean	Gherkin	Mean
			Acidity (% Lactic acid)				
Initial	0.28	0.14	0.15	0.08	0.12	0.24	0.17
Natural (4 weeks)	0.49	0.77	1.32	0.64	0.96	0.74	0.82
L. helveticus (4 weeks)	0.61	0.86	1.04	0.68	1.04	0.65	0.82
Mean	0.46	0.59	0.84	0.47	0.71	0.55	
CD (P=0.05) Method 0.01 S.Em ± 0.00							
CD (P=0.01) vegetable 0.04 S.Em ± 0.01							
CD (P=0.01) interaction 0.06 S.Em ± 0.01							
			pH				
Initial	4.4	4.69	4.66	5.15	4.95	4.5	4.47
Natural (4 weeks)	4.00	2.96	3.14	3.28	3.20	3.29	3.31
L. helveticus (4 weeks)	3.84	2.96	3.13	3.27	3.17	3.51	3.32
Mean	4.2	3.83	3.9	4.22	3.89	4.01	
CD (P=0.05) Method NS S.Em ± 0.01							
CD (P=0.01) vegetable 0.05 S.Em ± 0.01							
CD (P=0.01) interaction 0.06 S.Em ± 0.01							

NS=Not Significance

Table 2: Impact of mustard or potassium sorbate on variations in the acidity and pH of vegetables fermented by ***Lactobacillus helviticus***

Vegetable	Fermentation period in days	0.1% mustard power		0.1% potassium sorbate	
		Acidity (%)	pH	Acidity (%)	pH
Carrot	0	0.12±0.01	4.50±0.01	0.20±0.01	4.55±0.01
	7	0.91±0.01	3.33±0.01	0.89±0.01	3.35±0.01
	14	0.98±0.01	3.23±0.01	1.18±0.01	3.26±0.01
	21	1.17±0.01	3.20±0.01	1.24±0.01	3.23±0.01
	28	1.02±0.01	3.10±0.01	1.27±0.01	3.00±0.01
Cucumber	0	0.13±0.01	4.80±0.01	0.10±0.01	4.92±0.01
	7	0.73±0.01	3.29±0.01	0.69±0.01	3.44±0.01
	14	0.84±0.01	3.25±0.01	0.83±0.01	3.24±0.01
	21	0.89±0.01	3.20±0.01	0.90±0.01	3.20±0.01
	28	0.80±0.01	3.23±0.01	0.91±0.01	3.12±0.01
French bean	0	0.13±0.01	4.75±0.01	0.14±0.01	4.83±0.01
	7	0.72±0.01	3.43±0.01	0.91±0.01	3.61±0.01
	14	0.83±0.01	3.41±0.01	1.11±0.01	3.30±0.01
	21	0.89±0.01	3.39±0.01	1.14±0.01	3.09±0.01
	28	0.93±0.01	3.32±0.01	1.11±0.01	3.18±0.01

SD value±

Stabilization of the products was also achieved in this investigation by maintaining the buffering capacity (by adding alkali) and adjusting the pH of the brine to 4.5. There have been previous reports on the effectiveness of calcium chloride/calcium acetate for maintaining firmness in fermented vegetables[19-21]. Based on this knowledge, calcium chloride was added to the brine solution as an addition, which helped the fermented goods maintain their firm texture throughout storage. After fermenting and storing vegetables for six months, the microbiological stability of the vegetables was determined by looking for visible microbial growth or gas generation, as well as by observing no changes in pH or acidity. So, this study suggested that seasonal vegetables could be preserved through fermentation and so made available all year round. There are regions of our nation where traditional pickled products made of single or mixed vegetables are highly favored. When raw veggies are used in pickles, they lose their color and texture over time. Therefore, it is proposed that these fermented veggies may serve as a suitable substitute for fresh vegetables for making pickles. The preservative potassium sorbate is necessary for pure culture fermentation of vegetables because it inhibits competing microbial groups that are naturally present in the produce. This ensures that the introduced culture grows. Given that mustard is frequently used in traditional pickles, it was investigated whether it may be used in place of potassium sorbate during the fermentation of certain vegetables, including cucumber, carrot, and French beans. Table 2 clearly shows that the vegetables' acidity and pH very little vary when fermentation is carried out in the presence of mustard or potassium sorbate. Apart from a slight flavor whenever mustard was employed, there was no discernible difference in the quality of these fermented veggies. It has been observed that lactic acid bacteria will produce more acid when fermenting mustard powder[22]. In the current investigation, no such definitive observations were made. Mustard is suggested for use in the fermentation of cauliflower by[23].

CONCLUSION

To sum up, the addition of *Lactobacillus helveticus* to food additives has the potential to greatly enhance the flavor and nutritional value of functional foods. Nevertheless, more investigation is required to completely comprehend its modes of action, maximize its use in food products, and guarantee its stability and safety throughout processing and storage. With continued research and development, *L. helveticus* might play a bigger role in the food industry's endeavors to create enticing and nutrient-dense goods for consumers.

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