

Preparation of Spiced Sauerkraut by Using Lactic Acid Bacteria and By Natural Fermentation

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Abstract: Fresh cabbage was procured from the market and sliced into thin shreds. The shredded cabbage was fermented by four treatments, i.e., (a) Sodium chloride (2.25%), (b) Sodium chloride (2.25%) + *Lactobacillus plantarum*, (c) Sodium chloride (2.25%) + mustard powder (1%) + spices (0.5%) (d) Sodium chloride (2.25%) + mustard powder (1%) + spices (0.5%) + *Lactobacillus plantarum*. During fermentation samples of brine were drawn at different time intervals for biochemical and microbiological analysis. In all the four treatments, total sugar and reducing sugar in the brine increased slowly to 3% and 2%, respectively upto 8th day and then decreased. Maximum total acidity (1.98-2.22%) was observed on 15th day which remained constant up to 90 days. The pH of the shredded cabbage was 6.9 and decreased to around 4 after 15 days and then remained constant. Around 60% of vitamin C was preserved upto 90 days. From cultural, morphological, physiological and molecular analysis, it was observed that *Bacillus*, *Pseudomonas* and *Micrococcus* were dominant at start of fermentation, but as the fermentation proceeded number of these microorganisms decreased and *Leuconostoc* and *Lactobacillus* became dominant. Coliforms were absent in all four treatments. Overall acceptability was higher in the Sauerkraut prepared by addition of spices whereas inoculation of *Lactobacillus plantarum* made no significant difference.

Keywords: Sauerkraut, Fermentation, Lactic acid bacteria, Cabbage, Vitamin C, *Lactobacillus plantarum*

1. Introduction

Fermentation is one of the oldest methods of food preparation and preservation. The fermented foods form an important component of diet in most of the parts of the world due to their high nutritive value and organoleptic characteristics. Fermentation of vegetables improves their digestibility, increase nutritive value; extend seasonal availability, overall acceptability in addition to prolonging their shelf life. A large number of vegetable fermentations have been studied extensively to determine the microbiological and biochemical changes occurring during fermentation[1]. These studies include fermentation of cabbage, cucumber, carrot, cauliflower and several other vegetable blends. Most of these fermentations are carried out by lactic acid bacteria (LAB) like *Leuconostocmesentroides*, *Lactobacillus plantarum* and *Lactobacillus brevis*[2]. Cabbage (*Brassica oleracea* var. Capitata) is grown in the tropical regions of world and is good source of vitamin A, B and C, minerals and carotene [3]. In many parts of the world, shredded cabbage is subjected to bacterial fermentation controlled with salt resulting in the production of acid cabbage also known as Sauerkraut [4]. During the fermentation, acid is produced that acts as preservative in addition to development of desired flavor[1]. Sauerkraut is very popular in America and European countries. It is often eaten as an adjuvant with other foods to make them more appetizing, digestive and enhance the flavor of other food. It has been reported that theisothiocyanates produced during Sauerkraut fermentation prevent the growth of cancer cells[5].

However in India it is not very popular so some innovation is needed to improve its taste that suits the Indian consumer. In India, cabbage is grown and available in most of the parts of the country. However, the availability of cabbage is for a short period and due to its perishable nature a large part of total production is destroyed due to lack of processing. Cabbage is used as either raw salad or cooked vegetable. During cooking, vitamin C which is an important component of cabbage is destroyed if not processed properly[6]. So through fermentation vitamin C and other nutrients can be preserved and availability of cabbage can be increased throughout the year. However, the natural fermentation of cabbage, depending on the natural microflora is a time consuming process and may result in spoilage, if desired lactic acid bacteria are not present in sufficient numbers. The development of specific starter cultures with desirable bacteria is of prime importance to achieve consistent quality of the Sauerkraut. So the present work was designed to prepare the Sauerkraut by natural fermentation and by inoculation of pure culture of *Lactobacillus plantarum* with and without addition of spices. The addition of spices was planned to popularize the Sauerkraut among Indian consumers.

2. Materials

2.1 Cabbage

Fresh white cabbage (*Brassica oleracea* var. capitata) was procured from the local market and washed thoroughly before further processing.

2.2 Chemicals and Microbiological Media Components

All chemicals and media components were of standard AR or GR grade and were manufactured by Glaxo Laboratories (India) Limited, Mumbai, E. Merck Limited, Worli, Mumbai and Hi-Media Laboratories, Pvt. Ltd., Mumbai.

2.3 Spices

Garam masala prepared by MDH, New Delhi and common salt prepared by Tata were procured from market.

2.4 Bacterial Strain

The strain of *Lactobacillus plantarum* 201 LRR used as starter culture was procured from Culture Collection Centre, NDRI, Karnal.

3. Methods

3.1 Preparation of Cabbage for Fermentation

Cabbage was washed with water and damaged upper green leaves were removed. The cabbage heads were trimmed and sliced into 1-2mm thick shreds. The shredded cabbage were mixed with 2.25% (w/w) food grade salt (NaCl) and kept in four different 1L sterilized glass bottles in triplicate (Fig.1). The treatments given to shredded cabbage are shown in Table 1.

Table 1: Sauerkraut prepared by different treatments

Treatment no I: Cabbage + Sodium chloride (2.25%)
Treatment no II: Cabbage + Sodium chloride (2.25%) + <i>Lactobacillus plantarum</i> 201 LRR @ 10^5 - 10^6 cells/g of cabbage.
Treatment no III: Cabbage + Sodium chloride (2.25 %) + Spices @ 0.5% + Mustard powder @ 1%
Treatment no IV: Cabbage + Sodium chloride (2.25 %) + <i>Lactobacillus plantarum</i> 201 LRR @ 10^5 - 10^6 cells/g of cabbage.+ Spices @ 0.5% + Mustard powder @ 1%

3.2 Preparation of Bacterial Inocula for Controlled Fermentation

Lactobacillus plantarum strain 201 LRR was subcultured twice at 30°C in MRS broth. The culture was grown in MRS broth for 48 h before inoculation, the culture was centrifuged at 10,000 g and the pellet obtained was washed twice with sterilized normal, saline (0.85% w/v NaCl). Finally the pellet was resuspended in 200 ml sterile fresh normal saline for use or inoculum.

3.3 Fermentation Process

The fermentation was carried out in the month of December-January, when the room temperature was around 20°C for 15 days till the pH reached around 4 and maximum total acidity was achieved. When total acidity became constant, then bottles were kept in refrigerator for storage (6 to 8 °C).

3.4 Enumeration of Microorganisms

Samples of brine were withdrawn in triplicate from each bottle using one ml sterile glass pipettes. Samples were diluted by serial dilution technique and 0.1 ml aliquot of appropriate dilution was spread on two types of media in triplicate. The plates containing MRS medium were incubated at 37°C while the plates containing nutrient agar medium were incubated at 30°C in a incubator for 48 hr or till the appearance of visible colonies.

3.5 Chemical Analysis of Brine

Five gram of sample was taken and crushed in pestle and mortar, 10 ml of water was added to it. The crushed product was filtered through Whatman filter no. 20. The filtrate was then taken for analysis of total sugar, reducing sugar, total acids, volatile acids and vitamin C content. The total sugar was estimated colorimetrically by phenol sulphuric acid method as described by Dubois et al.[7]. The reducing sugar was estimated by DNSA method[8]. The pH was determined using a pH meter (PHS-25, Shanghai Precision Scientific Instruments Company, China).

3.6 Total Acids

The total acids were determined by titrating 5.0 ml of sample with a 0.1 N NaOH to pH 8.3 as examined by using pH meter. Total acid as percent of lactic acid were calculated using the formula:

$$1 \text{ ml of } 0.1 \text{ N NaOH} = 9 \text{ mg of Lactic acid}$$

3.7 Volatile Acids

To 5 ml sample in a round bottom flask, 20.0 ml of distilled water was added. The diluted samples were distilled and 10 ml distillate was collected. The distillate thus collected was titrated against standard 0.02 N NaOH solution using phenolphthalein as indicator. The volatile acid as percent acetic acid were calculated using the formula[9]:

$$1 \text{ ml of } 0.02 \text{ NaOH} = 7 \text{ mg of Acetic acid}$$

3.8 Ascorbic Acid / Vitamin C

One g of sample was macerated in pestle mortar with 5 ml of 3 percent metaphosphoric acid. It was filtered through Whatman filter paper 20 and volume was made to 10 ml with 3 percent metaphosphoric acid. Five ml of aliquot was titrated against 2,6-dichlorophenol-indophenol dye till light pink colour appeared [10].

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made upto}}{\text{Volume of filtrate taken} \times \text{Weight or Volume of sample taken} \times 100}, \text{ Whereas Dye factor} = \frac{0.5}{\text{Titre value}}$$

3.9 Biochemical Tests

Bacterial isolates were characterized by methods described in Manual of Microbiological Methods (Conn and Pelczar, 1957), Microbiological Laboratory Manual (Cappuccin and Sherman, 1983) and Microbiological methods (Collins and Lyne, 1976). The biochemical tests used were Indole production, Methyl red reaction, Voges-proskauer test, Catalase test, Gelatin liquefaction, Nitrate reduction, Citrate utilization, Sugar fermentation (acid and gas production), oxidase test, Esculin hydrolysis, Growth on nutrient agar with 7.5% NaCl.

3.10 Molecular Identification

The DNA was extracted from selected isolates by using ZR Fungal/Bacterial DNA kit (Zymo Research, California, USA). The extracted DNA was used as the template for PCR amplification of the 16S rRNA gene using universal 16S rRNA gene primers pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGCA-3') to obtain a product of approximately 1500 bp [11]. The PCR program used was as follows: initial incubation at 94°C for 5 min followed by 35 cycles (94°C for 50s, 55°C for 1 min and 72°C for 90s) and final extension at 72°C for 10 min using thermal cycler (BioRad). The 16S rRNA gene was sequenced by Xcelris Labs (Ahmedabad, India) using Sanger's di-deoxy nucleotide sequencing method. A similarity search for the sequence was carried out using the BLAST program of the National Centre of Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). The phylogenetic tree was drawn with Mega 5.0 [22]. In order to statistically evaluate the confidence of branching, bootstrapping was carried out with data resampled 1,000 times.

3.11 Determination of coliforms

To ensure the safety of the final product coliform bacteria were determined by MPN. The selective media used was MacConkey medium that contain bile salt inhibitory for the growth of non-intestinal lactose fermenting bacteria. Statistical method was employed to estimate most probable number of coliforms.

3.12 Enumeration of coliform Bacteria

Samples of brine were withdrawn in triplicate from each bottle using 1 ml sterile glass pipettes. Coliforms were determined by the most probable number (MPN) method after inoculating the MacConkey's broth tube (using 10, 1 and 0.1 ml of brine as inoculum in triplicate). These samples were further examined to confirm the test by serial dilution technique and 0.1 ml aliquot of appropriate dilution was spread on EMB media in triplicates. The plates were incubated at 37 °C in a desiccator for 48 hr.

3.13 Sensory Evaluation

The final product Sauerkraut was evaluated by a panel of ten judges using Hedonic scale. Various parameters were given score varying from 1 to 9. The acceptability of

product was determined by one factorial statistical analysis.

3.14 Nucleotide Sequence Accession Numbers

The sequences generated in this study were deposited in NCBI GenBank. The 16S rRNA gene sequences retrieved from the cultures isolated from Sauerkraut were assigned accession numbers KP862662, KP862661, KP862660, KP872765 and KP862657.

4. Results

4.1 Fermentation of Sauerkraut

The fermentation was carried out for 15 days till the maximum acidity was achieved. During the course of fermentation it was found that brine was released from the shredded cabbage after addition of Sodium chloride and other ingredients. In general, the cabbage was observed to be fresh without any change in natural colour except that of spices and remained crispy during fermentation and storage till 90 days (Fig.1).



Figure 1: Sauerkraut prepared by four different treatments

4.2 Biochemical changes during Sauerkraut fermentation

During the fermentation and storage, the brine released was analyzed for total sugar, reducing sugar, total acidity, volatile acidity, pH and ascorbic acid.

4.3 Total Sugar

The soluble sugar was released slowly into the brine solution and increased from 1.8% to 3.8% after 6 days of fermentation. The amount of total sugar decreased to 1.1% after 15 days of fermentation and then remained constant in the Sauerkraut prepared by addition of Sodium chloride only (Fig. 2). Almost a similar type of pattern was followed in other treatments where spices and/ or *L. plantarum* were added (Fig.2). Sugar content was found to be higher in the treatments where *Lactobacillus plantarum* was inoculated for e.g total sugar was 4.2 and 4.1 percent in treatment no. II and IV after 6 days of fermentation (Fig. 2) in comparison to 3.8 and 3.6 in treatment number I

and II. The final sugar content after 90 days of fermentation and storage was found to be in the range of 0.8 to 0.95 percent in different treatments.

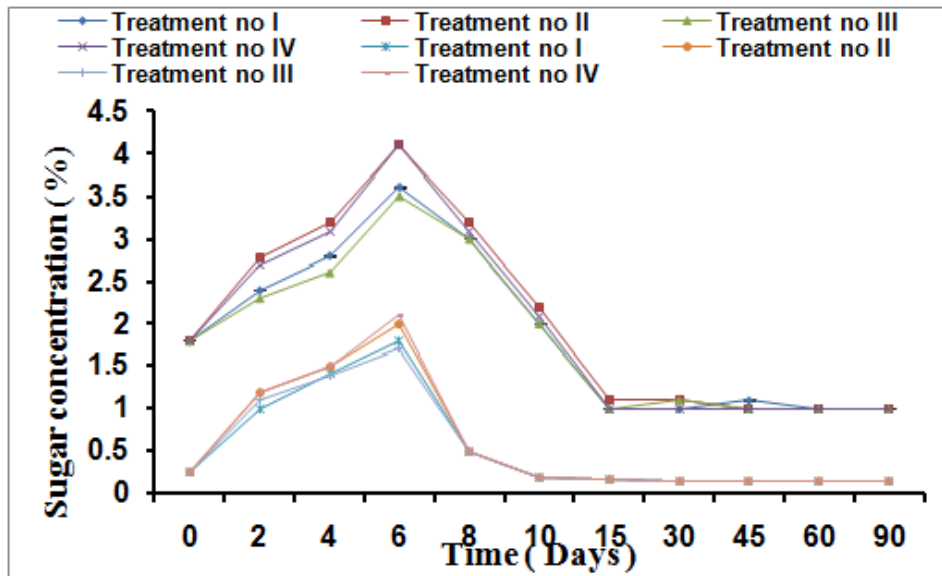


Figure 2: Changes in total sugar and reducing sugar in all four treatments during Sauerkraut fermentation

4.4 Reducing Sugar

The reducing sugar of Sauerkraut prepared by different treatments was determined at different intervals of time. At different interval after the start of fermentation and during storage, in all the four treatments reducing sugar increased from 0.3 percent to in a range of 1.9-2.1 percent after 6 days of start of fermentation (Fig. 1). The reducing sugar decreased thereafter and was found to be in the range of 0.18 to 0.23 percent after 90 days of storage. No difference in decrease in pattern in reducing sugar was found in different treatments (Fig. 2).

4.5 Total Acidity

The amount of total acids present in brine drawn during Sauerkraut fermentation and storage was analyzed. In the Sauerkraut prepared by addition of Sodium chloride only, the initial total acid was found to be 0.45 % that increased to 2% on 15th day and 2.18% after 90 days of fermentation and storage (Fig. 3). The increase in acids was faster when the fermentation was carried out by the inoculation of *Lactobacillus plantarum*.g total acids were 1% after 2 days of start of fermentation when *Lactobacillus plantarum* was inoculated, whereas, it was 0.6% in the Sauerkraut prepared without inoculation of *L. plantarum* (Fig.3). However, after 15 days of fermentation there was not much difference in total acidity.

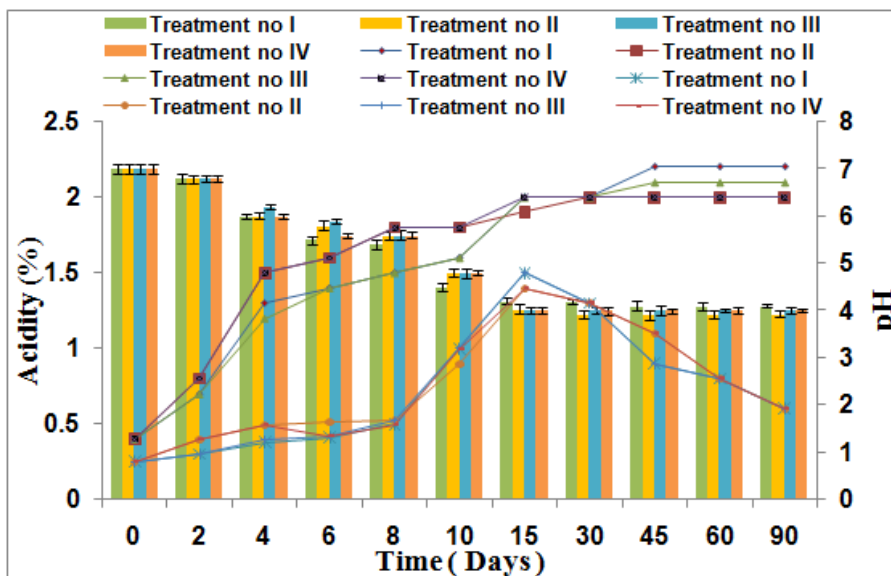


Figure 3: Changes in total acidity, volatile acidity and pH in all four treatments during Sauerkraut fermentation

4.6 Volatile Acids

Volatile acids measured as per cent of acetic acid, increased from 0.2% to 1.02- 1.50% in 15 days. The increase in volatile acids was found to be more in presence of *L. plantarum* in treatment no. II and IV (Fig.3). Sauerkraut prepared by addition of salt only, the volatile acids was found to be 1.25% after 15 days of fermentation whereas volatile acids increased to 1.5% in Sauerkraut prepared by inoculation of *Lactobacillus plantarum*. The concentration of volatile acids was found to be in the range of 1.0 percent to 1.32 percent in other treatments (Fig. 3). The volatile acids decreased during storage and the final content ranged between 0.65 to 0.78 percent in all four treatments (Fig. 3).

4.7 Effect of Fermentation on pH

The pH of the Sauerkraut which was almost neutral (6.9) at the start of fermentation decreased slowly to approximately 4 after 15 days of fermentation and then it became constant in all four treatments (Fig. 3). The pH of Sauerkraut was found to be 4.1, 3.9, 4.0 and 3.8 in treatment no. I, II,III andIV respectively (Fig.3).

4.8 Effect of Fermentation on Vitamin C Content of Sauerkraut

Vitamin C is one of the most important nutrients of cabbage that needs to be preserved. The initial vitamin C content of shredded cabbage was 27.5 mg/100mg of cabbage. Vitamin C of cabbage decreased from 27.5mg/100g to 17.5mg/100g when cabbage was fermented and stored for 90 days by addition of Sodium chloride only (Fig.4). The decrease in vitamin C content was also observed in other three treatments (Fig.4). The decrease was found to be statistically significant. Although there was decrease in all the four treatments, the amount of vitamin C was found to be slightly higher in the Sauerkraut prepared by addition of spices and inoculation of *Lactobacillus plantarum*. The difference in vitamin C contents in treatment no. II, III and IV were statistically significant in comparison to Treatment no. I.

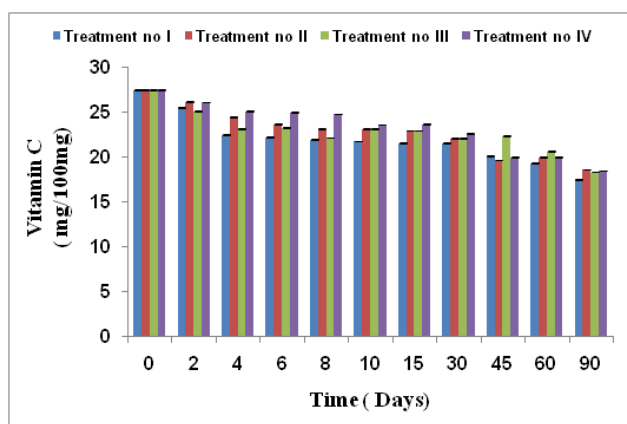


Figure 4: Effect of fermentation on Vitamin C content in different treatments

4.9 Enumeration of Microorganisms during Fermentation

The number of various microorganisms which were present at the start of the fermentation process or developed during fermentation were determined by spreading appropriate dilution of brine on two types of media namely de Man Rogosa Sharpe (MRS) and nutrient agar media. The number of colonies that appeared on two types of media after 48 hours of incubation were counted from a plate containing 100-300 colonies. The number of bacteria on nutrient agar media at the start of fermentation was found to be $(1.2-1.5) \times 10^4$ (Table not given) in the Sauerkraut prepared without inoculation of *L. Plantarum*. After that number of bacteria decreased initially to $(2.0-2.6) \times 10^3$ per ml on 6th day. Thereafter it increased to $(1.2-2.1) \times 10^6$ after 60 days and then remained constant upto 90 days. Whereas, in the Sauerkraut prepared by inoculation of *Lactobacillusplantarum*, number of bacteria at the start of fermentation was 1.2×10^5 which increased to 2.3×10^6 and then remained constant upto 90 days. The number of microorganisms on MRS media at the start of fermentation was found to be 2.0×10^4 in the Sauerkraut prepared without inoculation of *Lactobacillus plantarum* which increased exponentially to $(1.8-2.8) \times 10^7$ on 15th day and remained constant thereafter. In the Sauerkraut prepared by inoculation of *Lactobacillus plantarum*, number of bacteria at the start of fermentation was $(2.3-2.5) \times 10^6$ and then remained constant up to 90 days.

4.10 Morphological, Cultural and Biochemical characteristics

After counting, microorganisms were isolated from the plates on the basis of colony morphology and colour of colonies on both types of media. The morphological characteristics of five major types of colonies observed during different stage of fermentation were 1. Wrinkled, white and large (HAU-6) 2. Circular, white and small (HAU-4) 3. Reddish, Circular and small (HAU-3)4. Circular, raised, white and small (HAU-7) 5. Circular, raised white and large(HAU-6) (Table 2). The morphological, cultural and biochemical characteristics observed are described in Table no 2. From morphological, cultural and biochemical characteristics, it was concluded that different bacterial groups belonged to *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Leuconostoc* sp. and *Lactobacillus* sp. Table no 2.A partial 16S rRNA gene sequence of all bacterial isolates were submitted in Genebank. The phylogenetic analysis revealed that the closest phylogenetic neighbor of HAU-5, HAU-4, HAU-3, HAU-7 and HAU-6 were *Bacillus subtilis*, *Pseudomonas* sp., *Micrococcus flavus*, *Leuconostocmesenteroides* and *Lactobacillus plantarum*(Fig.5).

Table 2: Morphological, Cultural and Physiological characteristics of different bacteria isolated from Sauerkraut during fermentation and storage

Colony characteristics	Wrinkled, white and large (HAU-5)	Circular, white and small (HAU-4)	Reddish, Circular and small (HAU-3)	Circular, raised white and small (HAU-7)	Circular, raised white and large (HAU-6)
Cell morphology	Large rods	Small rods	Cocci	Coccobacilli	Large rods
Gram reaction	+	-	+	+	+
Spore formation	+	-	-	-	-
Growth in 5% salt concentration	-	-	+	+	-
Production of dextran in presence of 5% sucrose	-	-	-	+	-
Sugar fermentation					
Glucose	+	-	+	+	+
Fructose	+	-	-	+	+
Sucrose	-	-	+	+	+
Maltose	+	-	+	+	+
Lactose	-	-	-	+	+
Catalase test	+	+	=	-	-
Oxidase test	+	=	=	-	-
Indole production test	-	-	-	-	-
Methyl red	+	-	-	+	+
Vogesproskauer reaction	+	-	-	-	-
Gelatin hydrolysis	+	+	-	-	-
Esculin hydrolysis	+	+	-	+	+
Growth on Simmons Citrate agar	+	+	-	+	+
Nitrate reduction	+	+	-	-	+
	<i>Bacillus</i> species	<i>Pseudomonas</i> species	<i>Micrococcus</i> species	<i>Leuconostoc</i> species	<i>Lactobacillus</i> species

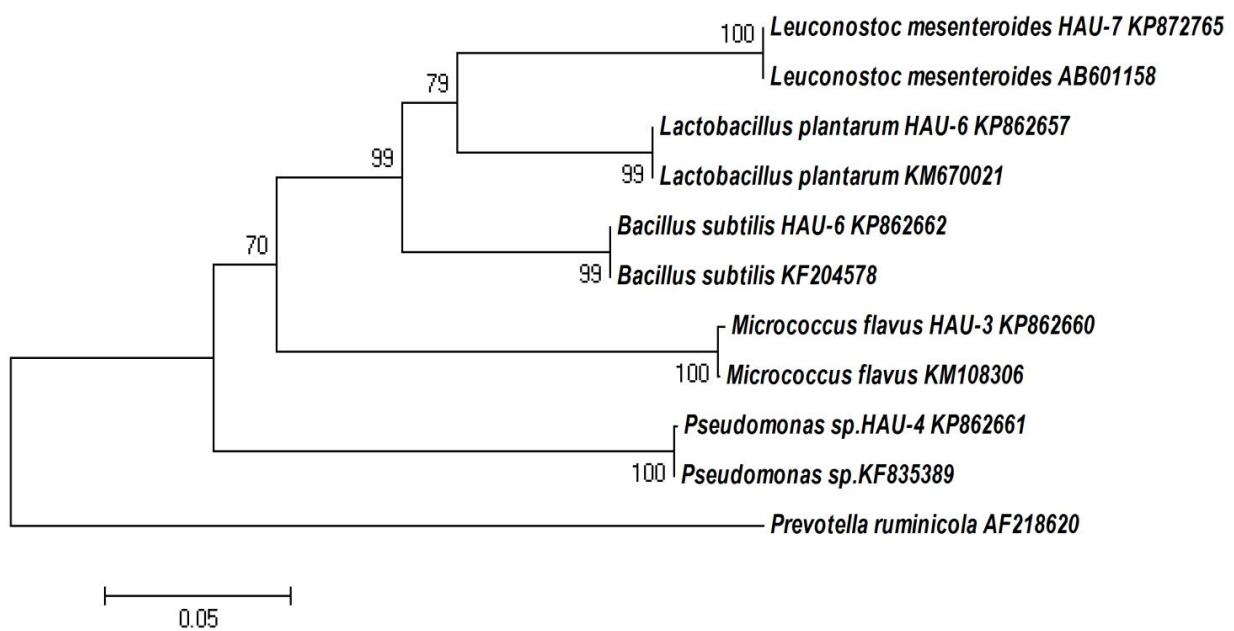


Figure 5: Phylogenetic tree of culturable bacteria based on the 16S rRNA gene sequence isolated during Sauerkraut fermentation

4.11 Determination of Coliforms

No acid and gas formation was observed in the MacConkey's broth tubes (using 10, 1 and 0.1 ml of brine as inoculum in triplicate), indicating the absence of coliforms. Similarly, the confirmatory test on EMB agar plate gave negative results. No colony having Green metallic sheen was observed on the EMB agar plates.

4.12 Sensory Evaluation

The sensory evaluation of the Sauerkraut prepared by different treatments was done by a panel of judges using 9-point Hedonic scale. The significant difference was observed in appearance and colour of the Sauerkraut prepared in presence and absence of spices and mustard powder. For taste, treatment number II and IV were awarded score 7.3 and 6.9, respectively, whereas treatment no I and IV were awarded 4.8 and 4.2. Treatment no III and IV were awarded 7.3 and 6.9 whereas, treatment no I and II were awarded 6.3 and 5.8 for sourness. For odour, treatment number III and IV were awarded 7.3 and 6.4, whereas, I and II were awarded 5.4 and 4.9. There was no significant difference in pungency was found among four treatments. Sauerkraut prepared without addition of spices and mustard powder were more crispy than other treatments and treatment number I and II were awarded 7.2 and 6.8, whereas, treatment number III and IV were awarded 4.0 and 4.1. The overall acceptability was higher in the Sauerkraut prepared by addition of spices and mustard powder and points awarded was 7.2 and 6.8, whereas, inoculation of *Lactobacillus plantarum* made no significant difference (Table no 3).

Table 3: Sensory evaluation of Sauerkraut using ten point Hedonic scale

Parameters	Treatment no I	Treatment no II	Treatment no III	Treatment no IV	C.D
Appearance	6.5	6.1	4.8	4.5	0.978
Colour	6.4	6.3	4.4	4.4	1.044
Taste	4.8	4.2	7.3	6.2	0.624
Sourness	6.3	5.8	7.0	6.6	0.646
Odour	5.4	4.9	7.3	6.4	0.793
Pungency	6.3	6.1	5.8	6.2	N.S
Crispiness	7.2	6.8	4.0	4.1	0.588
Overall acceptability	5.7	5.2	7.2	6.8	0.634

5. Discussion

The fermentation of vegetable is one of the important method of food preservation and retention of nutritional qualities of vegetables during offseason. Fermented cabbage is very popular in European countries. But, to make it more acceptable among Indians, spices and mustard powder were added to bring taste and flavor to the fermented product. The trend of biochemical changes in terms of total sugar, reducing sugar, total acids, volatile acids, pH and vitamin C contents were almost in

agreement with several published literature [12-14]. Cabbage typically contains 4 to 5% sugar, consisting of about 2.5% glucose and 2% fructose[15]. During fermentation sugar present in cabbage diffuses in brine and concentration of sugar increases in the brine[16]. The same trend of sugar change was observed in present investigation. The initial total sugar was found to be low, but as the fermentation proceeded the soluble sugars of cabbage released in the brine and total sugar increased from 1.8% to 3.9-4% in all four treatments after 6 days of fermentation. As the total sugar degraded, the amount of reducing sugar increased from 0.3% to 2% after 6 days of fermentation. The reducing sugar was metabolized by the microorganisms to organic acids and thus both reducing sugar as well as total sugar decreased to 0.8% and 1.1%, respectively. During storage of sauerkraut in the refrigerator both types of sugar remained constant up to 90 days. This may be due to static nature of growth of microbes at low temperature. The increase in total acidity was accompanied by decrease in pH. The pattern of changes observed in terms of total acidity and pH are typically similar found in most of the vegetable fermentation. The total acidity attained during cauliflower fermentation is 0.39% with a pH of 4.1 [17]. Likewise in the fermentation of whole carrots an acidity of 1.1 to 1.3 % with a pH of 3.3 has been reported by Niketic-Aleksic et al [18]. The addition of spices and *L. plantarum* made no difference in the pattern of amount of total sugar and reducing sugar released and amount of total and volatile acids produced. The vitamin C, one of the important nutrient of cabbage is generally lost during cooking and storage[19]. However, vitamin C of vegetables can be preserved by microbiological fermentation[20]. In the present investigation, decrease in vitamin C content was significant but still 60% of the vitamin C was preserved during fermentation and storage of Sauerkraut. It was also observed that Vitamin C content was higher in Sauerkraut prepared by inoculation of *Lactobacillus plantarum*. This finding need further investigation that whether *Lactobacillus* has some role in preservation of vitamin C. It has been reported that carbon dioxide produced during fermentation makes the environment anaerobic and thus helps in stabilization of ascorbic acid and natural colour of the vegetables [21]. The morphological, cultural, physiological and molecular studies of bacteria isolated during fermentation of cabbage revealed that *Bacillus*, *Pseudomonas* and *Micrococcus* constituted the natural surface microflora of cabbage. It has been reported that prior to fermentation, fresh fruits and vegetables harbor a variety of microorganisms, including aerobic microorganisms responsible for spoilage like *Pseudomonas*, *Micrococcus*, *Bacillus*, *Erwinia* and *Enterobacter* as well as yeasts and molds[22]. As the fermentation proceeded the LAB became dominant in both natural and controlled fermentation. Brining vegetables for fermentation results in the production of organic acids by lactic acid bacteria (LAB) and variety of microbial compounds [23,24]. Because LAB are more resistant to acid than the spoilage microbiota, they dominate the brined vegetable fermentation. *Leuconostoc* sp, are important in the initiation of the fermentation of vegetables. *L. mesenteroides* grow faster than most other LAB over a range of temperature (5 to 35°C) and NaCl

brine concentrations (0 to 5%) [1]. *L.mesenteroides* carries out a heterolactic fermentation of vegetable sugars, typically fructose and glucose and produces carbon dioxide and acids (lactic and acetic). The production of acid quickly lowers the pH and inhibits the growth of undesirable microorganisms and activity of their enzymes. The carbon dioxide produced creates an anaerobic conditions making the environment unsuitable for growth of aerobic ones like *Bacillus*, *Pseudomonas* and *Micrococcus*. The high acidity produced by *L.mesenteroides* and other heterofermentative LAB inhibits the growth of these heterofermentative microbes in favour of more acid tolerant homofermentative LAB. The *Lactobacillus plantarum*, a homofermentative LAB finally dominated the fermentation. It has been reported that *L. plantarum* produces only lactic acid from the remaining fermentable sugars[25]. In agreement with present investigation, it has been reported that *L.plantarum* eventually outcompete the other LAB in most of the vegetable fermentations due to its high acid tolerance[1]. These microbial successions are well known and form the basis of fermentation and preservation of vegetables. Pederson and Albury[17] have reported that Sauerkraut fermentation is initiated by *Leuconostocmesentroides* followed by *Lactobacillus brevis*, *Pediococcus cerevisiae* and *L. plantarum*. Similarly, Stamer et al[26] reported that *Leuconostocmesentroides* depicted a shorter lag period and fast generation time when grown in cabbage juice as compared to any other microorganisms. Coliforms such as *Escherichia coli* which form the natural microflora of irrigation water are generally present on the surface of the most of the vegetables. Since the presence of coliforms was determined only after completion of fermentation, the coliforms were not present in the Sauerkraut and the Sauerkraut prepared by fermentation was safe for human consumption. Addition of spices increased the overall acceptability of the product. The Sauerkraut prepared by different treatments was crispy and possessed a pleasant aroma and flavor.

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