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Change in Microflora of Sauerkraut During Fermentation and Storage

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Abstract: Sauerkraut is defined as the clean sound product of characteristic flavour, obtained by full fermentation of properly prepared and shredded cabbage in the presence of not less than 2 to 3% salt, finally containing not less than 1 and 1.5% acid expressed as lactic acid. In the present study, change in bacterial and fungal flora of sauerkraut during fermentation and storage was observed. In the present study, The pH of the sauerkraut brine ranged between 3.0 ad 4.0 and showed decreasing trend from the day of preparation upto 120th day of storage. The total acidity expressed as percent lactic acid of sauerkraut ranged between 0.045 and 1.70 and showed an increasing trend from day of preparation upto 120th day of storage. The naturally occurring microbial (lactic acid bacteria) load was found to vary between 1.40 x10^{3}and 3.00x10^{7} CFU/ml. *Lactobacillus brevis, Lb. plantarum, Lb. fermentum* and *Leuconostoc mesenteroides* (lactic acid bacteria), *Bacillus* sp., *Staphylococcus aureus* (as aerobic bacteria), *Aspergillus luchuensis, A. niger, Scopulariopsis* sp. (molds) and *Saccharomyces* sp. (yeast) were identified. No regular trend was observed during the course of study. No fungal colony was formed upto 28th day of fermentation of sauerkraut. Fungal growth was observed superficially on sauerkraut after 60th day of storage and completely spoiled the sauerkraut on the 90th day, this may be due to aerial contamination.

Key words: Cabbage · Lactic acid bacteria · Microbial load · Naturally occurring · Sauerkraut

INTRODUCTION

Sauerkraut is an acidic cabbage which results from natural fermentation by bacteria indigenous to cabbage in the presence of 2 to 3% salt. The addition of salt restricts the activities of Gram negative bacteria, while the growth of lactic acid bacteria is favoured. The dominant lactic acid bacteria involved in sauerkraut production are *Leuconostoc mesenteroides, Leuconostoc fallax* and *Lactobacillus plantarum.* The activity of *Leuconostoc* cease when acid content increases to 0.7 to 1%. The final total acidity is generally 1.6 to 1.8%, with lactic acid at 1.0 to 1.3% and pH in the range of 3.1 to 3.7. The final stages of kraut production are affected by *Lb. plantarum* and *Lb. brevis. Pediococcus cerevisiae* and *Enterococcus faecalis* may also contribute to product development. Lactic acid bacteria are useful in producing fermented foods such as yoghurt, pickles and are also used as probiotics. The sole ingredient in sauerkraut, the cabbage, is rich in vitamins (C and K), boost the immune system and has other beneficial effects [1, 2].

Microbial spoilage of sauerkraut is generally categorized into soft kraut, slimy kraut, rotted kraut and pink kraut. Soft kraut results when bacteria that normally do not initiate growth until the late stages of kraut production actually grow earlier. Slimy kraut is caused by the rapid growth of *Lb. cucumeris* and *Lb. plantarum,* especially at elevated temperatures. Rotted kraut may be caused by bacteria, molds and/or yeast, whereas pink kraut is caused by the surface growth of *Torula glutinis.* Due to the high acidity, finished kraut is generally spoiled by molds growing on the surface [3]. Sauerkraut is not known as a food among the masses of India. Although sauerkraut is manufactured occasionally in small quantity

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by a few canners in India, they have conducted no research [4]. Literature search revealed that this kind of study has not been carried out in India. In lieu of the above justification, the objective of the present paper was to study the change in microflora of sauerkraut during fermentation and storage.

MATERIALS AND METHODS

Preparation of Sauerkraut: For the preparation of sauerkraut, the cabbages (Band Gobi, *Brassica oleracea* var. *capitata*) were obtained from the local market of Kurukshetra. The spotted and defective cabbage heads were trimmed off and the cabbages were shredded with a sterile knife. The shredded cabbages were weighed one kg in three parts followed by the addition of 2.5% NaCl (non-iodized). The shredded cabbage (1kg) and salt (25g) were placed in alternating layers in wide mouthed jars. A heavy weight board was placed over the mixture put in the jars and was pressed gently to squeeze out the juice (brine). The jars were covered with sterile lids and incubated at 21 to 24°C for 28 days for the fermentation of the substrate [5, 6].

Determination of pH: The pH of the brine from sauerkraut at different stages of fermentation was determined by pH paper strips. pH meter could not be used for measuring the pH because amount of brine produced was not enough for sampling [5, 6].

Determination of Total Acidity: Total acidity, expressed as percent (%) lactic acid, was determined following the method given by Cappuccino and Sherman [5]. At each sampling time, ten ml undiluted brine was added to an Erlenmeyer's flask, followed by addition of 10 ml of distilled water. The contents were boiled for 1 minute to drive off the dissolved carbon dioxide. Five drops of phenolphthalein (1%) was added to the cooling contents. The titration with 0.1 N NaOH was carried out until a light pink colour persisted. The percent lactic acid was calculated by using the formula as given below:

$$\% \text{ Lactic acid} = \frac{\text{Vol. of alkali used x Normality of alkali x 9}}{\text{Vol. of sample taken (i.e. 10 ml)}}$$

Isolation of Microorganisms by Serial Dilution Agar Plate Technique: Serial dilution-agar plate technique was used for the quantitative qualitative determination of naturally occurring and aerobic bacteria and fungi (molds and yeasts) as described in the manual [3] from the sauerkraut at different stages of fermentation. Three media such as Rogosa agar, Plate count agar (PCA) and Malt extract agar (MEA) were used. In serial dilution agar plate technique, 1g mango slices and (1 ml brine samples) were suspended and agitated in 9ml water blank (to make the total volume to 10ml) to form a microbial suspension. Serial dilutions of 10^{-2}, 10^{-3} and 10^{-4} were made by pipetting 1 ml into 9 ml water blanks. To each of these inoculated plates, 15ml of sterile and cooled molten (45°C to 50°C) media (Rogosa agar for naturally occurring bacteria (lactic acid bacteria), PCA for aerobic bacteria and MEA, supplemented with streptopenicillin for fungi) were poured and incubated at 37°C for 24 hrs for bacteria and 25°C for 3 to 7 days for fungi, in an inverted position (in triplicate for each dilution/medium). The plates were observed for the appearance of colonies and number of colonies produced on each plate of different dilutions were recorded and characteristics of colonies were observed. Number of colonies per gram or ml (CFU/ml) was calculated by multiplying plate count with the dilution factor as given below:

$$\text{CFUs/ ml} = \frac{\text{Number of colonies (mean) x Dilution factor}^{1}}{\text{Volume plated (0.1ml)}}$$

[1]Dilution Factor: Reciprocal of the dilution (eg. $10^{-3} = 10^{3}$)

Purification and Identification of Microflora: Bacteria were purified by streak plate method by transferring on Rogosa agar and PCA and incubating at 37°C for 24 hrs. The pure colonies were streaked on Rogosa agar and PCA slants and incubated at 37°C for 24 hrs and slants maintained at 4°C in a refrigerator. Yeasts were also purified by streak plate method on MEA and incubated at 25°C for 5 days and transferred to MEA slants and incubated at 25°C for 5 days and slants maintained in refrigerator at 4°C. Molds were purified by needle inoculation and disc transfer methods on MEA plates and incubated at 25°C for 5 days and transferred to MEA slants and incubated at 25°C for 5 days and slants maintained in refrigerator at 4°C.

Colonial, morphological, staining and biochemical characteristics were recorded for all the bacterial isolates purified from sauerkraut. Bacteria were identified following the dichotomous keys of [4, 7,8]. Fungal colonies were grown on PDA, CDA and MEA media at 25°C for 7 days

and following characteristics: colony characteristics (i. e. colour, exudates produced, growth of the colony), sporulating structures (conidial head, types of conidiogenous cells, arrangement of conidia, sporangial head, types of spores, pycnidia, accervuli, sporodochia, ascocarps etc.) were recorded and identified by following various manuals and monographs [9-13].

Effect of Storage on the Microbial Load of Sauerkraut: Sauerkraut was prepared as per the method described above. The jars were allowed to ferment at room temperature 21 to 24°C and screened for microbial load (CFUs/ml) on the day of preparation, 1st and 3rd day and were observed for microflora after 7th day of storage and repeated ever 7 day until 28 days and after 60th, 90th and 120th day of storage by using serial dilution agar plate technique [5, 6].

RESULTS AND DISCUSION

In the present investigation, the pH of sauerkraut brine ranged between 3.0 and 4.0. No change in pH of 4.0 was observed in the present study during the first two days of sauerkraut fermentation and declined till the 28th and remained constant (3.0) on the 60th day onwards.

The total acidity expressed as percent lactic acid of sauerkraut ranged between 0.045 and 1.70 and showed an increasing trend from day of preparation upto 120th day of storage. Our results are in agreement with Jones [14] who also reported the same initial percent lactic acid to be 0.045 and which increased upto 1.6 after 35 days of fermentation of sauerkraut. Our results also substantiate with Jay *et al.* [15] who suggested that the final acidity of sauerkraut approximately lies between 1.6 and 1.8% and pH in the range of 3.1 and 3.7. The floral succession is governed mainly by the pH of the growth medium. According to Das *et al.* [16] who suggested that low moisture and reduced pH are the two measure factors contributing to shelf stability of the pickle.

In the present study, the naturally occurring microbial (lactic acid bacteria) load was found to vary between 1.40 x10^3 and 3.00x10^7 CFU/ml. The lactic acid bacterial counts increased from the time of preparation till the 7th day of fermentation and started declining after 7th day of fermentation till the 120th day of storage. Our results are in accordance with Doyle *et al.* [4] who reported that the increase lactic acid bacterial counts are selectively favoured by sauerkraut fermentation by the complete lack of oxygen, lowered pH and elevated salt content.

In the present study, lactic acid bacterial counts declined at the later stage of fermentation which is in accordance with Doyle *et al.* [17] who reported that lactic acid bacteria in later stage of fermentation decreased because rapid increase in total acidity and drop in the pH value of brine. *Lactobacillus brevis*, *Lb. plantarum, Lb. Fermentum* and *Leuconostoc mesenteroides* (as lactic acid bacteria), were identified during the present study. According to Aneja *et al.* [2] and Doyle *et al.* [4], the activity of the some lactic acid bacteria usually cease when acid content increases to 0.7-1.0% (as lactic acid). Species of *Lactobacillus* (e.g., *L. brevis* and *L. plantarum*) and *Pediococcus pentosaceous* then proliferate with the production of additional lactic acid. The initial population and growth rate of microorganisms as well as salt and acid tolerance are important factors that influence the sequential development of various lactic acid bacteria in most vegetable fermentations. Leroi and Pidoux [18] reported that the presence of the lactic acid bacteria count in the olive and sauerkraut brine is important not only to assure continuous acidification of the medium, inhibiting the Gram-negative bacteria and some heterofermentative lactic acid bacteria, but also to inhibit the fermentative metabolism of yeasts that produce bloaters in olives and sauerkraut. According to Balatsouras [19], the control of pH and salt concentration in brine, mostly practiced in the olive industry, does not reduce the incidence of this spoilage in fruits and it has an influence on the fermentation process. Hence, the high acidification to pH of 3.5 and the addition of high amount of salt (10-12%) in brine would have an inhibitory effect on the lactic acid bacterial flora, mainly involved in the fermentation process.

In the present study, *Bacillus* sp. and *S. aureus* (as aerobic bacteria) were identified. The aerobic bacterial count was 1500 CFU/ml on the 0 day that increased on the 1st and 3rd day and decreased from 7 thday of fermentation till the 120th day of storage. Doyle *et al.* [4] and Jay *et al.* [15] reported that when cabbage is tightly packed for fermentation to begin, the number of strictly aerobic bacteria decreases immediately owing to the lack of oxygen and overgrown by the facultatively anaerobic lactic acid bacteria.

Table 1: Types of microorganisms and their viable counts (CFU/ml) in sauerkraut observed by serial dilution agar plate technique

Lactic acid bacterial load CFU/ml		Aerobic bacteria CFU/ml		Fungi CFU/ml	
Leuconostoc mesenteroides	8.00 X 10^5	*Bacillus* sp.	1.80X10^3	*A. luchuensis*	3.00 X 10^2
Lactobacillus brevis	2.00 X 10^5	*S. aureus*	1.50X10^3	*A. niger*	2.00 X 10^2
Lactobacillus plantarum	1.30 X 10^5			*Scopulariopsis* sp.	2.00 X 10^2
Lb. fermentum	1.30 X 10^5			*Saccharomyces* sp.	1.00 X 10^2

Table 2: Physicochemical (pH and percent lactic acid and microbial load (CFU/ml) analysis of sauerkraut during fermentation and storage

	Sauerkraut					
Fermentation/ Storage time (in days)	pH	%Lactic Acid	Lactic acid bacteria (CFU/ml)	Aerobic bacteria (CFU/ml)	Fungi (CFU/ml)	Microorganisms
0	4.0	0.045	1.40X10^3	1.50X10^3	7.00x10^2	*Lactobacillus*
1	4.0	0.10	8.00X10^5	1.80X10^3	-	*brevis,*
3	3.8	0.19	1.00X10^6	3.00X10^3	-	*Lb. plantarum*
7	3.6	0.50	3.00X10^7	1.50X10^3	-	*Lb. fermentum*
14	3.4	1.40	2.00X10^7	1.25X10^3		*Leuconostoc*
21	3.1	1.60	1.30X10^6	1.00X10^2	-	*mesenteroides*
28	3.1	1.60	1.00X10^6	1.80X10^1	-	*Bacillus* sp.,
60	3.0	1.67	1.00X10^5	1.50X10^1	4.50x10^{5*}	*S. aureus,*
90	3.0	1.69	3.00X10^3	1.40X10^1	6.00x10^{6*}	*A. luchuensis,*
120	3.0	1.70	2.00X10^2	1.00X10^1	8.50x10^{7*}	*A. niger,* *Scopulariopsis* sp., and *Saccharomyces* sp.

- No growth; *On the surface of spoiled sauerkraut

No fungal colony was formed upto 28th day of fermentation of sauerkraut. Fungal growth (*A. luchuensis, A. niger, Scopulariopsis* sp. (molds) and *Saccharomyces* sp. (yeast) was observed superficially on sauerkraut after 60th day of storage and completely spoiled the sauerkraut on the 90th day (Table 1,2). Frazier and Westhoff [20] have given an explanation that owing to the high acidity, finished kraut may be spoiled by molds growing on the surface. Sauerkraut is especially subjected to spoilage at its surface, where it is exposed to air. The surface film yeasts and molds destroy the acidity, permitting other microorganisms to grow and causing softening, darkening and bad flavours. According to Asehraou *et al.* [21], bloater spoilage is the main defect attacking the olives and sauerkraut during fermentation and storage. The control of brine salt concentration and pH, practices widely used in the Moroccan table olive industry, are not sufficient to avoid or reduce the incidence of this defect. The control of other factors, such as the use of starters and antimicrobial compounds as well as the control of temperature, is necessary. Excessive mold growth origin a moldy taste in olives and sauerkraut and can cause spoilage by consuming the acids produced during fermentation.

From the present study, it may be suggested that sauerkraut is good for consumption up to 28 days of fermentation and dangerous for the health after microbial contamination and spoilage.

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