# NUTRITIONAL AND SENSORY EVALUATION OF SAUERKRAUT PREPARED BY MIXED FERMENTATION OF LACTIC ACID BACTERIA

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**Abstract:**
Fermentation is one of the most important methods of food preservation. Sauerkraut the fermented food traditionally used as a low cost ready to eat food originated approximately 2,000 years ago in China, where it is known as suan cai, with a literal translation of "sour vegetable". Now fermented cabbage also known as sauerkraut are consider as probiotic food that are full of probiotic lactic acid bacteria (LAB) and rich in Vitamins (especially A, B, C, some extent of vit-K) and minerals which ultimately boost our immune system and increase the digestibility.

Fresh white cabbages are collected from local market and head are selected after removing undesirable part and wash five time with water. The heads are chopped into 1-2mm in size and finally washed with hot water. The slat and lactic acid bacteria ate mixed aseptically and load in a food grade polythene vat for fermentation. In our study we ferment the white cabbage by different lactic acid bacteria solely and by combination (Lactobacillus plantarum and Lactobacillus brevis) in presence of different salt concentration (2.0%, 2.5% and 3.0%). During and after fermentation for 30 days at $25^0$C temperature we analyze change in pH level (by using pH meter), change in lactic acid (by measuring titrable acidity using 0.1N NaOH and phenolphthalein indicator) and Ascorbic acid content (by AOAC method using 2,6-dichlorophenolindophenol) as well as change in microbial load (LAB) (by growing in selective MRS media). Finally, we perform sensory evaluation by using ten-point Hedonic scale.

In our study we found that the pH level of fermented cabbage changes up to 4.2 for single and up to 3.0 for mixed fermentation and also found ascorbic acid content remain good level in mixed fermentation compare to others. The lactic acid bacteria also highest in mixed fermentation which may be due to synergistic effect. Finally, we proved that for sauerkraut production at $25^0$C is ideal at 2.5% salt concentration using mix fermentation by lactic acid bacteria.

**Index Terms**: Lactic acid bacteria, Mixed fermentation, Hedonic scale, Ascorbic acid

## I. INTRODUCTION

The food fermentation is one of the oldest technologies of world. In ancient it was traditionally used for homebased product development. Now fermented food become an important component of human diet and used in most part of the world due to its high nutritive value and organoleptic characteristics. The fermentation increases digestibility, nutrient content, vitamins, acceptability and self-life of vegetable; subsequently decrease any anti-nutrient and agro-pesticide (Pederson C.S.,1969; Bell V,2017).

India is the second highest producer of vegetable cabbage (Brassica oleracea var. Capitata). It is used either raw salad or cooked vegetable is good source of minerals, carotene and different vitamins viz. A, B, C some extent of vit-K (De and Rahman, 2014; Panday et al., 2006; Jahangir et al. 2009). In India about 30-40% of harvested cabbages are lost due to lack of proper preservation and other means. One way to minimize is to ferment the vegetable to produce such a product that increases the self-life (Caplice, E., 1999).

Most of the vegetable fermented using lactic acid bacteria (LAB) belong to both homo-fermentative and hetero-fermentative (Di Cagno, 2013 and Stamer J.R. et al., 1971). Many countries produce acidic cabbage by lactic acid bacterial fermentation with added salt concentration, traditionally known as Sauerkraut. It is highly popular in USA and European countries (Clarke T.C., 2015).

The most critical parameter for sauerkraut preparation is the salt concentration which determines the organoleptic characteristics of final product and producer organism which control the nutrition status of fermented cabbage (Holzapfed et al., 2003). Form literature it was shown that sauerkraut can be prepared with salt concentration varies from 0.6 to 3.0% sodium chloride. The increase salt concentrations are promoting the growth of some lactic acid bacteria but inhibitory for pathogenic bacteria (Viander et al., 2003).

So present research was designed to prepare sauerkraut by mixed fermentation of lactic acid bacteria with added different salt concentration to maintaining organoleptic characteristics and vitamin content.

## II. MATERIAL AND METHODS Preparation of sauerkraut

For preparation of sauerkraut fresh white cabbage (Brassica olearaceae var. capitata) were collected from local market of Kolkata, West Bengal. It was washed thoroughly and removes undesirable upper part before further processing. The cabbage head was trimmed and shredded into 1-2mm with sterile knife and finally washed with hot water. The washed shred cabbages take for 1 kg and mixed with appropriate amount of salt and lactic acid bacteria ($10^2$-$10^3$ cell/gm); Lactobacillus plantarum and Lactobacillus brevis singly or combined (Harris LJ, 1992). The treatments are given in table 1. All treatments are performed in triplicate manner at $25^0$C for up to 30 days (Gardner NJ, 2001).

Table 1: Treatment method form sauerkraut preparation

| |
|---|
| **Treatment 1:** Cabbage + Salt (2.5%) |
| **Treatment 2:** Cabbage + Salt (2%) + Lactobacillus plantarum ($10^2$-$10^3$ cell/gm) |
| **Treatment 3:** Cabbage + Salt (2.5%) + Lactobacillus plantarum ($10^2$-$10^3$ cell/gm) |
| **Treatment 4:** Cabbage + Salt (3%) + Lactobacillus plantarum ($10^2$-$10^3$ cell/gm) |
| **Treatment 5:** Cabbage + Salt (2%) + Lactobacillus brevis ($10^2$-$10^3$ cell/gm) |
| **Treatment 6:** Cabbage + Salt (2.5%) + Lactobacillus brevis ($10^2$-$10^3$ cell/gm) |
| **Treatment 7:** Cabbage + Salt (3%) + Lactobacillus brevis ($10^2$-$10^3$ cell/gm) |
| **Treatment 8:** Cabbage + Salt (2%) + Lactobacillus plantarum ($10^2$ cell/gm) + Lactobacillus brevis ($10^2$ cell/gm) |
| **Treatment 9:** Cabbage + Salt (2.5%) + Lactobacillus plantarum ($10^2$ cell/gm) + Lactobacillus brevis ($10^2$ cell/gm) |
| **Treatment 10:** Cabbage + Salt (3%) + Lactobacillus plantarum ($10^2$ cell/gm) + Lactobacillus brevis ($10^2$ cell/gm) |

### Determination physiochemical properties

The different physiochemical properties like change in pH, lactic acid content, vitamin C content of sauerkraut are determined during fermentation as well as after fermentation. 5 gm of fermented sample was taken and crushed in presence of 10 ml phosphate buffer saline (PBS). The crushed product was filtered through Whatman filter paper 20. The filtrate was used for analysis the above parameters.

The change in pH during fermentation was determine using pH meter (PHS-25, Shanghai Precision Scientific Instruments Company, China). The pH meter was calibrated at pH 7 and pH 5 using citrate and phosphate buffer (Ranganna, 2000).

The titrable acidity (TA) of fermented sample was determined by titration method taking 5 ml of filtrate using 0.1N NaOH; phenolphthalein indicator and expressed as lactic acid content according to the method described by Yang et al. (2019). The TA as percent of lactic acid content were determine using the formula:

1 ml of 0.1 N NaOH = 9 mg of Lactic acid

The ascorbic acid content of sauerkraut was determined by the dye-titration method used was essentially that of the AOAC procedure (AOAC, 1990). In this procedure 1gm of fermented sample was crushed in motor pastel with 5ml of 3% metaphosphoric acid. The filtrate was collect using Whatman filter paper 20 and made volume 10 ml with 3% metaphosphoric acid. 5ml of metaphosphoric acid extract are taken and pH adjusted to about 1.2. The reduction capacity of extract was measure by titrating with 2, 6-dichlorophenol-indophenol (DCIP) using indophenol as indicator. In this procedure the ascorbic acid in extract was oxidized to DHAA and indophenol reduce to colorless compound. The end of the titration was detected by appearance of light pink color of the solution. The vitamin C content was measured using formula:
Ascorbic acid (mg/100g) = Titer value × Dye factor × Volume made up to/ Volume of filtrate taken × Weight or Volume of sample taken × 100, Whereas Dye factor = 0.5/Titer value

**Change in microbial load (LAB)**

To isolate LAB 1gram of different fermented sample are crushed in 10 ml phosphate buffer saline (PBS) and filtrates was collected. It was diluted serially using phosphate buffer saline (PBS). 0.1ml of each diluted solution was spreaded onto de Man, Rogosa and Sharpe (MRS) agar (Difco, USA) (De Man, 1960). The plates were incubated in anaerobic chest with AnaeroPack (AnaeroPack – Anaero, Mitsubishi Gas Chemical America) at 37°C for 48 h.

**Sensory analysis**

The sauerkraut was evaluated for aroma, flavor, and after-taste using a flavor profile by a panel of ten judges using Hedonic scale. Various parameters were given score varying from 1 to 9. The judges were trained to evaluate for commercial sauerkraut and experimental sauerkraut. Panelists used a scale of 0 (not present) to 9 (strong) to notes their evaluations (Johanningsmeieret al. 2007 and Wu, C.2014).

## III. RESULT AND DISCUSSION

In this study the fermentation of cabbages was carried out using lactic acid bacteria Lactobacillus brevis and Lactobacillus plantarum individually and by co-culture with different salt concentration (2, 2.5 and 3%) for 0 to 30 days at $25^0$C and analysis different biochemical parameters was carried out in 5days interval after starting the fermentation process.

In this study we get the pH decrease gradually with time interval but after 25 days of fermentation no remarkable acid further produces so it was as equivalent as 30 days of fermentation. At the end of fermentation (30 days) pH change was highest in treatment-9 (Cabbage + Salt (2.5%) + Lactobacillus plantarum + Lactobacillus brevis) which more maximum acid and decrease the pH from 6.0 to 3.0 and treatment-1 (Cabbage + Salt (2.5%) produce lowest amount acid (pH change 6.0 to 5.6) whereas for single culture fermentation treatment-6 (Cabbage + Salt (2.5%) + Lactobacillus brevis) produce most acid (pH change 6.0 to 4.2) (Fig. 1).

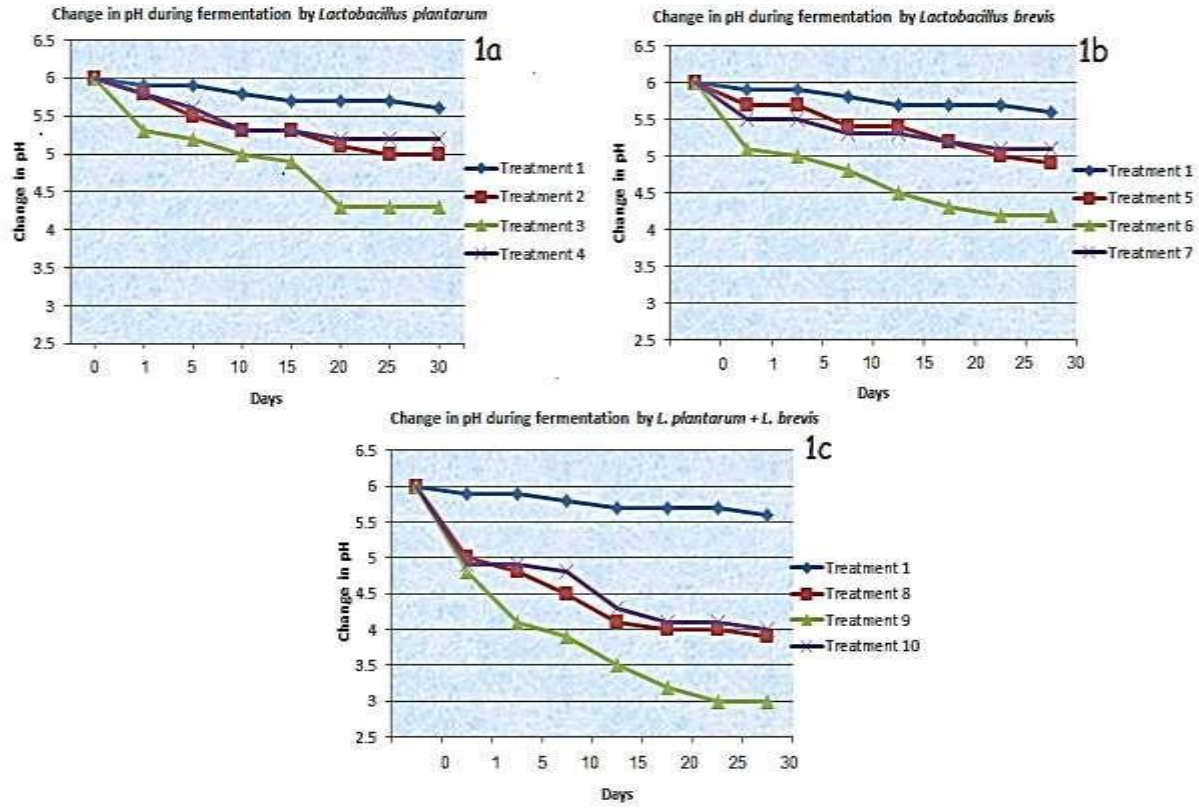


**Fig. 1: Change in pH of the fermented broth during fermentation; 1a, by *L. plantarum*; 1b by *L. brevis* and 1c, by co-culture**.

Cabbage is the one of the most important sources of ascorbic acid (vitamin C) that needs to be preserved. The initial vitamin C content of shredded cabbage was 38 mg/100mg of cabbage and which is decrease depending fermentation procedure and paraments. In our study we show that the ascorbic acid content maintaining optimum level (30.0mg/100mg) form double fermentation method (treatment-9) whereas without added culture (treatment-1) fermentation vitamin-C content decrees to 9.0mg/100mg. For single culture we found the treatment-3 (Cabbage + Salt (2.5%) + *Lactobacillus plantarum*) maintain the vitamin-C content highest (24mg/100mg) (Fig. 2).

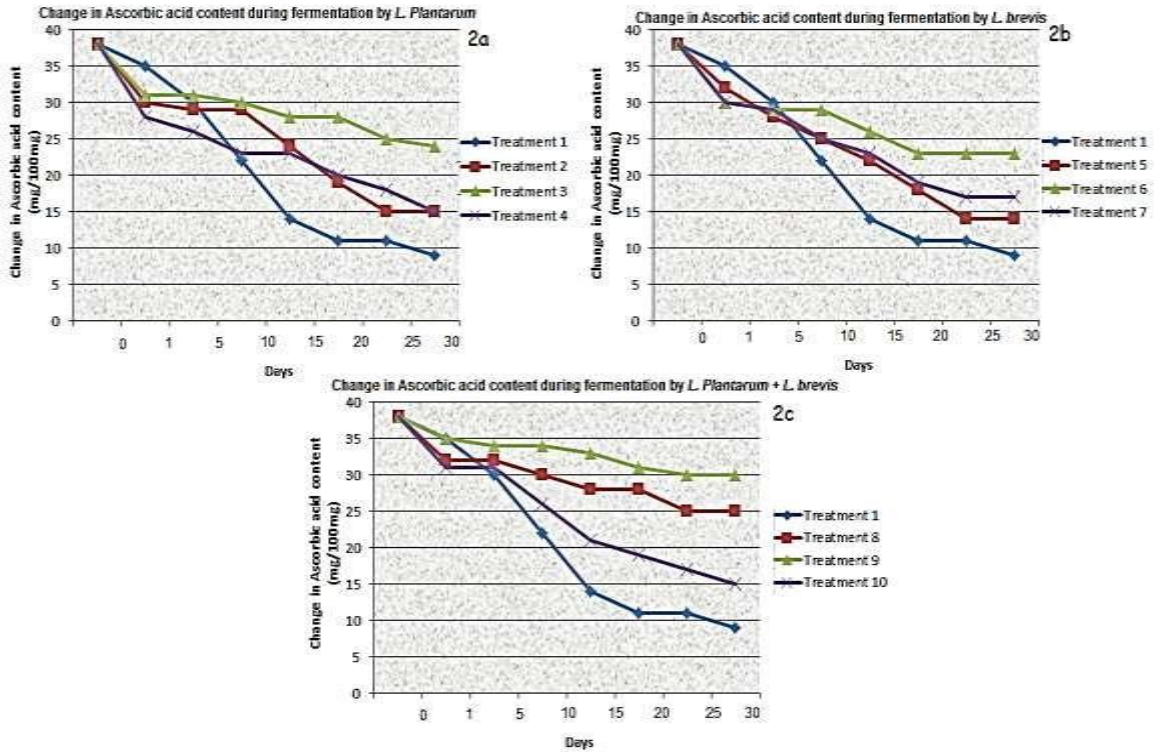


**Fig. 2: Change in Ascorbic acid content during fermentation; 2a, by *L. plantarum*; 2b by *L. brevis* and 2c, by co-culture**.

The lactic acid is the product of anaerobic fermentation of sugar by lactic acid bacteria. This acid provides sourness and also are inhibitory for other pathogenic bacteria. The lactic acid content also acts as preservatives and prevent the spoilage of cabbage after fermentation. It also effects the test of the sauerkraut. In this experiment maximum lactic acid is produce during treatment-9 for co-culture fermentation and for single culture fermentation treatment-6. The salt concentration for maximum lactic acid production was 2.5%, lowering or increasing of which decrease lactic acid production in both case of treatments (Fig. 3).

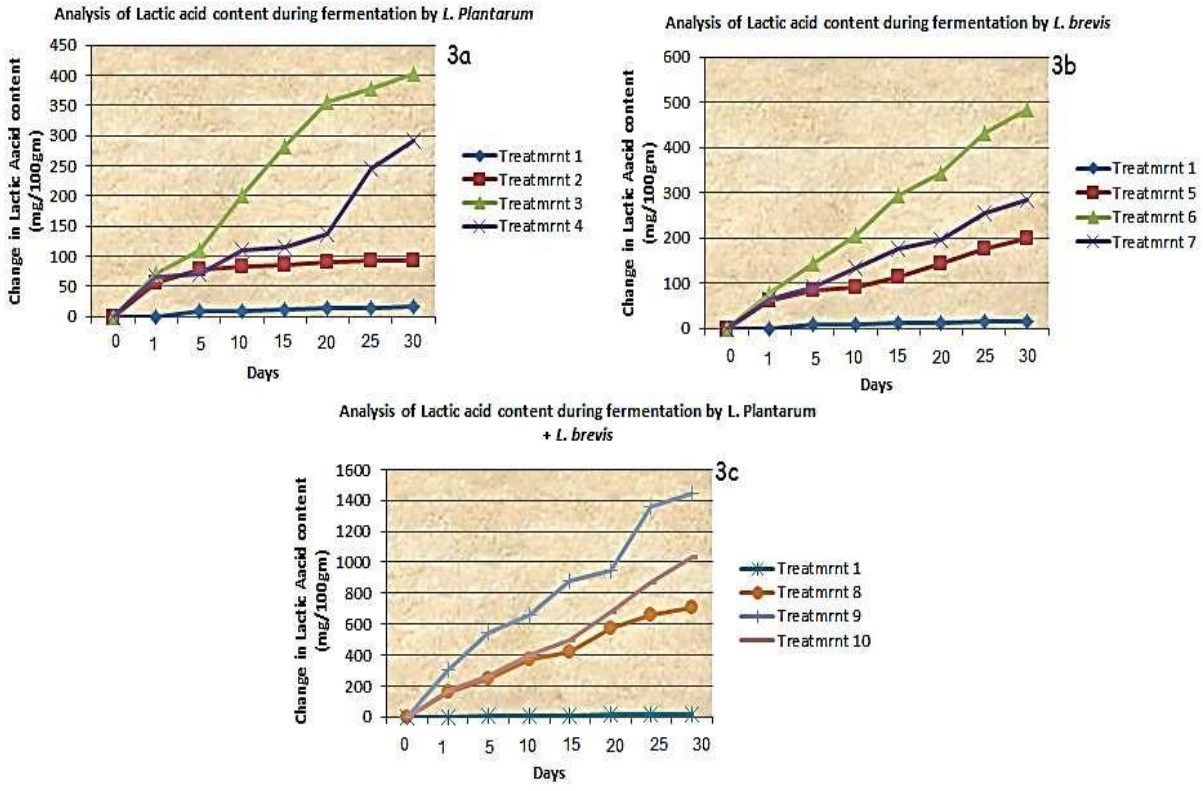


**Fig. 3: Change in lactic acid content during fermentation; 3a, by *L. plantarum*; 3b by *L. brevis* and 3c, by co-culture.**

Lactic acid bacteria are found throughout the nature and those found in milk and on fruit, vegetable can be used for fermentation. Most of the vegetable fermentation can be carried by indigenous bacteria but some time special culture was added to improve its flavor, aroma and digestibility and also act as probiotic microorganisms. The sauerkraut fermentation carried out by submerge technique under microaerophilic condition using sealed container. In the present study, the naturally occurring lactic acid bacteria (treatment1) found $3.9x10^5$ to $4.2x10^5$ cell/gm at the end of fermentation process (30 days). In case of added culture fermentation double starter (treatment 8-10) shows synergistic effect to each other and the count of MRS media shows LAB count in between $3.3x10^7$ and $4.5x10^7$ cell/gm whereas single starter fermentation (treatment 2-7) the LAB count varies $1.1x10^7$ and $3.5x10^7$ cell/gm. In this experiment, the highest bacteria count found in treatment-9 and for single culture fermentation with *Lactobacillus brevis* maintain highest count in MRS media. Above both treatments were carried out with 2.5% added salt concentration. In this study we represent that added salt concentration is vital force for fermentation of sauerkraut (Fig. 4).

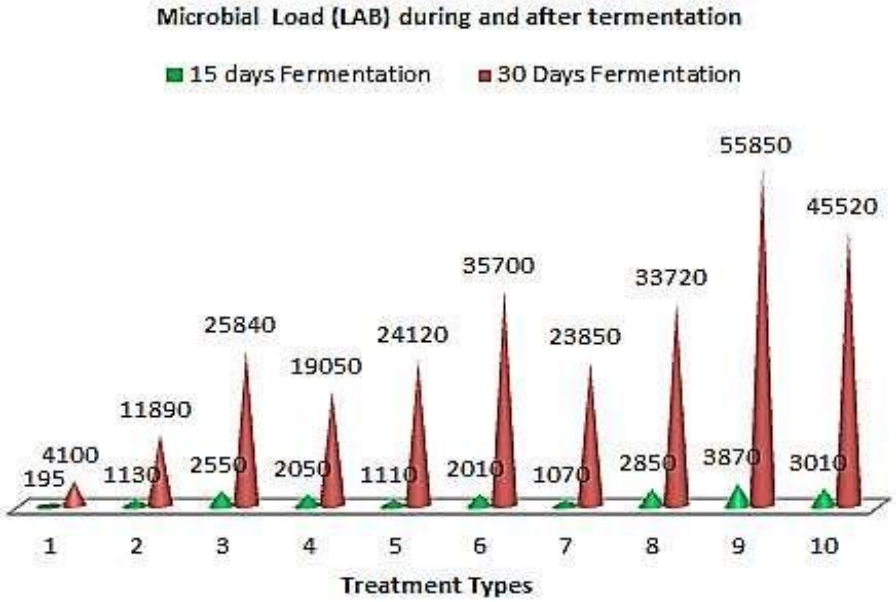


**Fig 4: Change in lactic acid bacterial count on MRS media during (15 days) and after fermentation (30days)**

The sensory parameters (color, texture, odor, overall acceptability etc.) of sauerkraut prepared by different treatments was evaluated by a panel of judge using 9-point Hedonic scale (Table 2). Significant difference was observed in sensory parameters after fermentation carried out for 30 days with different salt concentration and added starter culture. For appearance, treatment-9 and treatment-8 were sensory awarded record score 7.9 and 6.3 respectively whereas for single starter culture treatment- 3 and treatment-6 awarded 5.8 and 5.6 respectively, the control treatment-1 get lowest score 2.1. For color, the highest score for double culture were 7.3 (treatment-9) where for single culture it was 5.5 (treatmen-6) and for no added culture 4.4 (treatment-1). Treatment-9 and treatment-6 also get highest test sensory score 8.2 and 4.7 and sour sensory score 6.7 and 4.8 with respect to co-culture and single fermentation respectively. For odor, treatment-9 and treatment-7 were awarded 7.2 and 5.1, whereas pungency score highest for treatment-9 and treatment-6; 7.9 and 6.2 respectively. Crispiness is a good sensory parameter, treatment-9 awarded 6.9 sensory score whereas for single culture highest was 6.9 (treatment-6) and lowest was 2.1 with no added culture (treatment-1). The overall acceptability of fermented sauerkraut was highest for *Lactobacillus brevis* and *Lactobacillus plantarum* with 2.5% salt (treatment-9) and awarded score 7.3 out of 9-point Hedonic scale (Fig. 5).

| Table 2: Sensory evaluation (Using ten-point Hedonic scale) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | Appearance | Color | Taste | Sourness | Odor | Pungency | Crispiness | Overall acceptability |
| **1.** | 2.1 | 4.4 | 2.0 | 2.1 | 4.3 | 6.3 | 2.1 | 3.3 |
| **2.** | 4.5 | 4.5 | 3.1 | 3.3 | 4.6 | 5.8 | 4.3 | 4.3 |
| **3.** | 5.8 | 4.9 | 3.3 | 4.2 | 5.2 | 5.9 | 4.8 | 4.8 |
| **4.** | 4.3 | 4.2 | 4.2 | 3.5 | 4.9 | 6.1 | 4.1 | 4.5 |
| **5.** | 4.8 | 4.5 | 3.9 | 3.9 | 5.1 | 4.5 | 5.1 | 4.5 |
| **6.** | 5.6 | 5.5 | 4.7 | 4.8 | 5.9 | 6.2 | 5.9 | 5.5 |
| **7.** | 5.1 | 4.3 | 4.5 | 4.1 | 5.1 | 6.1 | 4.9 | 4.8 |
| **8.** | 6.3 | 6.4 | 6.8 | 6.2 | 6.3 | 7.3 | 6.8 | 6.5 |
| **9.** | 7.9 | 7.3 | 8.2 | 6.7 | 7.2 | 7.9 | 6.9 | 7.4 |
| **10.** | 6.2 | 6.1 | 7.2 | 6.3 | 5.8 | 7.1 | 6.1 | 6.4 |

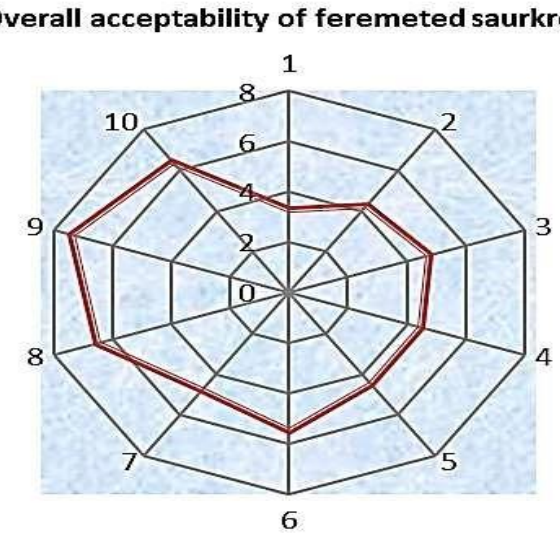


**Fig. 5: Radar diagram representation of overall acceptability of fermented product based on 9-point Hedonic scale.**

## IV. CONCLUSION

From the present experiment, it may be suggested that fermentation of sauerkraut should be done for 30 days before consumption to get optimum nutritional benefit and sensory properties. We found addition of optimum salt is one of the critical points for sauerkraut production because amount of salt affects sensory properties and microbial load as well as nutritional status of fermented product. The salt inhibits growth of different pathogen and spoilage microorganism in fermented food (Pederson al.,1975) and favoring growth of lactic acid bacteria (Mary,1994). In most literature, salt concentration 2.0-2.5% was shown ideal for fermentation and which coincide with our finding (Chauhan et al., 2008; Srivastava, 1998 and Holzapfel et al., 2003).

In present investigation it was found, mixed culture fermentation with 2.5% added salt concentration for 30 days produce good quality sour cabbage (sauerkraut) with high nutritional value (vitamin-C content), good sensory parameters and high content of probiotic microorganisms (lactic acid bacteria).

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