

# A COMPREHENSIVE STUDY OF DIFFERENT TRADITIONAL FERMENTED FOODS/BEVERAGES OF HIMACHAL PRADESH TO EVALUATE THEIR NUTRITION IMPACT ON HEALTH AND RICH BIODIVERSITY OF FERMENTING MICROORGANISMS

NIVEDITA SHARMA, SHWETA HANDA & ANUPAMA GUPTA

Microbiology Research Laboratory, Department of Basic Science, Dr Y S Parmar University of Horticulture and Forestry,  
Nauni, Solan, Himachal Pradesh, India

## ABSTRACT

Himachal Pradesh, a state with diverse culture and tradition is located between 30°22'40" north latitudes and 75°45'55" to 79°04'20" east longitude. The state revealed a wide range of uniqueness and variability in traditional fermented foods made of cereal, pulses and milk. Most of these items are prepared by boiling, smoking, roasting, frying and fermentation. These food items are explored for their biochemical analysis and microbial profile and main nutritional aspects. Biochemical analysis of these food items showed an acidic nature with pH in the range of 3.25-4.20. Total soluble solids were in the range of 8°B-16°B. Microbial examination of these food items, their morphological and biochemical identification and 16S rRNA of some of important microorganisms revealed the presence of *Lactobacillus*, *Bacillus* and yeast species predominantly.

**KEYWORDS:** Traditional Foods, Fermentation, Himachal Pradesh

## INTRODUCTION

Fermented foods make up an important contribution to human diets in Himachal Pradesh because fermentation is an expensive technology which preserves food, improves its nutritional value and enhances its sensory qualities. Traditional fermentation food products containing probiotics, such as seera, bhaturu, bari, jhol, chhang etc., are quite popular foods in Himachal Pradesh (Sharma and Singh, 2012). These foods are not only traditionally fermented but are also functional foods and thus experiencing a burst of popularity as health foods not only within the state but also in the other parts of country. A rich legacy of consuming varieties of fermented food in different districts of Himachal Pradesh since ages might be due to its geographical as well as climatic conditions. Being a hilly state and having a cold weather for most of the year, probably renders this pre-digested food easier for digestion besides providing high nutritional value to the hard working people of Himachal Pradesh. Within the state, one can find local variation due to the pattern of food production and altitudinal variation, e.g. in the barren region of Lahaul Spiti and Kinnaur, people consume products having coarse grains (buckwheat, millet, barley) as the main substrate, while in the lower areas of the state i.e., Mandi, Kangra, Hamirpur, Bilaspur, people prefer roties made mostly of wheat and rice or maize flour. These traditional/indigenous foods are prepared according to the traditional methods using simple equipment's and under natural conditions from the staple material and other ingredients (Savitri and Bhalla, 2007).

Fermentation is the oldest known form of food biotechnology, which has been practiced for thousands of years by the ancient man as the potent tool for imparting longevity to foods and beverages (Borgstrom, 1968). It is a process by which useful products are made by the application of microorganisms. The product may be one which is synthesized by the

organism(s), the organism themselves or a combination of two (Wood, 1997). It is well established that the process of fermentation enhances the nutritional quality of any product by enhancing the amount of vitamins and protein solubility and thus fermented food products act as nutraceutical agents to impart beneficial health effects. Knowledge of the microorganisms associated during natural fermentation would help to establish genetic resources of fermentation processes. So far, microbiota associated with natural fermentation has not been assessed and hence an attempt has been made in present study to assess the microbiota of the fermented food and its sources of traditional food items.

## **MATERIAL AND METHODS**

The map of Himachal Pradesh depicts two clear topographical areas – the central and outer area. The study was carried out on some of the traditional fermented food items of the states viz. Bilaspur, Mandi, Kullu, Hamirpur, Kangra, Chamba, Lahaul Spiti and Kinnaur of Himachal Pradesh. Special consideration was given to nature of food, usual composition/ingredients and method of cooking. These foods have been described below:

### **Preparation of Cereal Based Fermented Food**

A number of cereal based fermented food preparations have been developed and consumed by the natives of the selected area. Babroo and Bhaturu (Figures 1 & 2) are fermented products (roti) made up of wheat flour. Bhaturu was prepared by mixing wheat flour with water. Malera (traditional inoculum) was added to the mixture and kneaded to form constant dough. The dough was kept at room temperature for 10 h for fermentation. Then the fermented dough was baked to prepare bhaturu. Another cereal based preparation is Seera.

It is a starchy white dried solid prepared from wheat grains. The grains are soaked in water for 4-5 days so as to allow fermentation. The water is decanted off in between many times and replaced with fresh water. The fermented grains are ground and steeped so as to allow the starch grains to settle down. The surface water is discarded and the remains (starch grains) at the bottom are collected and sundried in the form of small balls. These balls are stored in air tight containers for use throughout the year. When required the dried balls are soaked in water with addition of an equal amount of sugar to make a slurry. The prepared slurry is then poured in hot ghee and is cooked by continuous stirring till it becomes brown in colour and leaves the sides/surface of skillet. Babroo/gulgule is also prepared by mixing wheat flour with water and sugar also. Malera (traditional inoculum) was added to the mixture and kneaded to form constant dough. Then the fermented dough was baked to prepare babroo/gulgule.

Jalebi, mouth tempting sweet is prepared by mixing wheat flour with water and malera is added to ferment this batter. It is prepared by deep-frying a fermented wheat flour batter in pretzel or circular shapes, which are then soaked in sugar syrup.

Samples of dough, seera and jalebi batter were taken and stored at 4°C for further analysis. For microbiological analysis, the sample was processed immediately.

### **Preparation of Pulse Based Fermented Food**

For preparation of Mash bari, overnight soaked mash (*Phaseolus radiata*) is grounded to soft dough and batter is whipped till fluffy or left to ferment for 1-2 days. Small round balls of the batter are deposited on cotton/polythene sheets and are sundried. In Dangan bari, tender stems of *Colocasia esculanta* smudged with fermented mash batter are allowed to dry in hanging position. Dried stems are being cut into small pieces and stored in air tight containers/bags. Sepu bari preparation is more laborious and is being adjudged one of the most delicious cuisines of Himachal Pradesh. Fermented

mash batter is rolled in fresh leaves of *Butia* sp. and is being cooked in boiling water for 15 to 20 min. Steamed cooked rolls are cut into cubes of about 5×5" cm and deep fried for further use.

To assess various attributes of these food items, samples of fermented soft dough and batter were taken randomly from different places and stored at 4°C for further analysis. For microbiological analysis, the sample was processed immediately.

### **Preparation of Buttermilk Based Fermented Food**

Very popular by product of milk is chaa/lassi (buttermilk). It is a liquid leftover after extracting butter from churned yogurt. This is used for preparing delectable cuisine named kadi. It is prepared by simmering a mixture of butter milk and besan/gram flour in spluttered onion and spices. While, Jhol/Khoru is made without adding onion and gram flour in buttermilk simply by warming it up on gentle heat and adding some spices in it.

Chaa/lassi (buttermilk) was taken from different places and stored at 4°C for further analysis.

### **Preparation of Cereal Based Fermented Beverage**

Rice or wheat grains are washed and soaked in water (1:1) for 8-10 h. Then these soaked grains are cooked for 1-2 h in an open vessel. This preparation involves solid-state fermentation of cooked rice or wheat grains with phab (the traditional inoculum) for 4-5 days. After 4-5 days, it is filtered and filtrate is called chhang/lugri.

Phab (traditional inoculum) and undistilled chhang (100 ml) were taken and stored at 4°C for further analysis. For microbiological analysis, the sample was processed immediately.

### **Biochemical Analysis**

Food samples were analysed for various biochemical parameters viz., moisture content by oven drying method, acidity by using pH meter (Cyberscan 510), total soluble solids (TSS) (Rangana, 1997). Total proteins were estimated by Lowry method (1951), total carbohydrates by phenol-sulphuric acid method (Dubois et al., 1956) and reducing sugars by DNSA method (Miller, 1959). Results were expressed in mg/ml of the sample.

### **Microbial Profile of Fermented Food Items**

One gram of each sample was plated on nutrient agar. Czapek Malt agar, de Man, Rogosa, Sharpe (MRS) agar and Czapek Dox agar plates were used for isolation of bacteria, lactic acid bacteria, yeasts and fungi, respectively. 10 gm of samples was blended in 90 ml of autoclaved distilled water in a lab-blander for 10 min. Dilution series were prepared in sterile distilled water blanks of 9 ml each and 1 ml of appropriate diluted suspension was spreaded over pre-poured plates of respected media. The plates were then incubated at 37°C for 48 h to check the growth of microorganisms. The number of colonies of bacteria, yeasts and moulds that appeared on the plates were counted and expressed as CFU g<sup>-1</sup> of the sample. The colonies of bacteria exhibiting different morphology were selected and purified by culturing them on their respective media. The purified isolates were identified on the basis of morphology i.e., type, shape, colony, gram reaction (Gram, 1884) and biochemical characteristics viz. carbohydrate utilization, citrate utilisation, casein hydrolysis, catalase test, H<sub>2</sub>S production and MRVP test (Aneja, 2003).

### **Molecular Identification**

Some of the isolates were identified at genomic level by using 16S rRNA gene technique and sequence so obtained was analysed using BLASTN tool ([www.ncbi.nlm.nih.gov:80/BLAST/](http://www.ncbi.nlm.nih.gov:80/BLAST/))

### Antimicrobial Activity and Bacteriocin Production

Since most of the fermenting microorganisms have probiotic attributes and they are capable of secreting special antimicrobial compounds viz. bacteriocin so suppress growth of harmful microflora. Antimicrobial activity and bacteriocin production by LAB/*Bacillus* sp. was checked by bit/disc method (Barefoot and Klanhammer, 1983) and well diffusion method (Kimura et al., 1998) against pathogenic microorganisms. Bacteriocin production is measured in terms of inhibition zone size. The test pathogens selected for bacteriocin screening were *Listeria monocytogenes* MTCC 839, *Leuconostoc mesenteroides* MTCC 107, *Enterococcus faecalis* MTCC 2729, *Lactobacillus plantarum*, *Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus*.

### RESULTS AND DISCUSSIONS

The state of Himachal Pradesh has a vast basket of traditional foods of its own. People of the state have been following these methods of preparation from their ancestors and these dishes have continued like a tradition in maximum parts of Himachal. Some of the traditional foods still form a staple diet while rest are prepared during special occasions. Most of the foods identified in the study were prepared by boiling, roasting, smoking, frying and fermentation. The food patterns of the state are highly influenced by the availability of raw materials as well as geographical and climatic conditions. The traditional fermented items prepared by the local people are generally associated either with festivals or with the seasons.

A number of selected fermented food items of Himachal Pradesh were identified and the traditional methods of their production, nature of food, ingredients used, method of preparation and area wise distribution had been documented in the Table 1.

The nutritive value of selected food items per 100 gm of material used in various traditional recipes has been discussed (Table 2). All the fermented food items were found to be acidic in nature. pH of the food items was evaluated and found to be in between 3.0 to 4.5. The acidic nature of these products is probably due to the production of organic acids during fermentation by acid producing microorganisms, as the fermentation is carried out under unhygienic and uncontrolled dominance of these genera in other cereal based beverages has also been discussed earlier (Bassapa, 2002; Tamang et al., 1988; Muyanja et al., 2003). Among all the food items studied chhang was found to be most acidic having pH value 3.25 while seera was found to have higher pH value i.e., 4.20. In case of moisture, a wide variation in results was observed ranging from 9.0 to 75.0 % depending upon their physical state like solid, semi-solid and liquid form. Among the food items studied Lassi was found to have high moisture content i.e., 79.0 % while sepubari had lowest moisture content i.e., 9 %. It was observed from the table that in general babroo, jalebi batter, sepubari and bhatoru contains good amount of total protein i.e., 27.0, 24.0, 23.8 and 21.5 mg/g, respectively. Further, jalebi batter, dangalbari, babroo, sepubari and bhatoru were found to be good source of carbohydrates having 13.9, 13.9, 13.4, 13.3 and 13.2 mg/g of total carbohydrates. Total soluble solids were observed maximum in mashbari i.e., 16.0°B and minimum in jalebi batter i.e., 8°B.

The microbiological analysis of dough, seera, lassi, chhang, jalebi batter, dangalbari, sepubari and mashbari revealed that these had consortia of microorganisms which mainly consisted of lactic acid bacteria and yeast (Table 4). Total cfu count of all responsible to bring about characteristic fermentation in a specific food items were ranging between  $58 \times 10^6$  to  $67 \times 10^6$  cfu/g. Microbial isolates encountered in these food items were identified on the basis of morphological and biochemical characteristics as described in Table 3. The microflora of these fermented foods mainly dominated yeast (*Saccharomyces cerevisiae*), Lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*) and *Bacillus* spp (*Bacillus lentus*, *Bacillus mycoides*, *Bacillus subtilis* etc.). The above results are corroborated

by earlier findings which also encountered similar genera in fermented food products (Muyanja et al., 2003; Hesseltine, 1983; Adegoke and Oguntimein, 1995). *Saccharomyces cerevisiae* has been reported in predominance from various fermented foods and beverages such as bhalle, beer, burukutu, bourbon whiskey, coffee beans, cidar, Merissa, fufu, tape, ogi, puto, dosa, idli, papdam, kecap, laochao, warri, scotch whiskey, etc (Padmaja and George, 1999; Batra and Millner, 1974; Batra and Millner, 1976; Soni and Sandhu., 1990). Some species of *Bacillus* and other bacteria such as *Kocuriarhizophila*, *Pseudomonas synxantha* and *Microbacteriunsaperdae* have also been found. Further, Lactic acid bacteria and *bacillus* isolates were processed and biochemical characterization has been done (Table 3). They were round, smooth in texture, shiny and having variation in colour. All isolates were gram-positive and catalase negative, except DB<sub>1</sub>, B1, F<sub>7</sub> which are gram-negative and catalase positive (Figures 1, 2, 3 & 4). Maximum isolates have been shown to possess protease and amylase activity.

Lactic acid bacteria/*Bacillus* spp. was found to be predominant microflora in these fermented food items. Many LAB and *Bacillus* sp. were found to inhibit food borne/pathogenic bacteria as antagonistic potential of these microbes is of special interest to suppress the growth of many foodborne illness causing pathogens. The inhibitory action of this lactic acid bacteria and *bacilli* sp. was explored. The inhibitory action of LAB/*Bacillus* sp. was checked using bit/disc method. All the isolates were shown to possess wide range of inhibitory spectrum against pathogenic bacteria due to secretion of antimicrobial compounds viz. bacteriocins. Further, bacteriocin production was checked using well diffusion method. Potential bacteriocin producers were selected on the basis of their qualitative and quantitative antagonistic pattern against food borne/spoilage causing test indicators. The nomenclature of different bacteriocins that were isolated and purified is given in Table 6. The inhibitory action of LAB is mainly due to accumulation of main primary metabolites such as lactic and acetic acids, ethanol, carbon dioxide; or antimicrobial compounds such as formic, benzoic and acids, hydrogen peroxide, diacetyl and acetoin beside bacteriocin (Yuksekdag and Aslim, 2010). Bacteriocin is shown to possess inhibitory activities due to its strong bactericidal effect against pathogens (Jack et al., 1995). Hyper bacteriocinogenic bacteria isolated from different food items have been identified by using 16S rRNA gene technique and enlisted in Table 5.

Knowledge of microbial diversity and their sources of the traditional indigenous fermented foods like babroo, bhaturu, chhang etc. will help in establishing their genetic resources and preparing their database. The climate of Himachal Pradesh is generally cold and water is hard at places which is not good for digestion.

Therefore, people generally prefer fermented food items for consumption. The development of starter culture will help to enhance the nutritive as well as digestive value of the food products. Further studies in particular their tolerance to low pH and high bile salt concentration and aggregation etc may reveal their importance as probiotics with relation to traditional foods used by the local people.

These probiotic properties and biopreservative aspects of the microflora isolated from these given fermented food items upon exploitation may lead to the development of healthy environment and healthy people. Some clinical research also available on health improvement from these fermented food items as fermentation has established in improving the nutritive value of the food viz.

Dough is rich in vitamin B especially thiamine, riboflavin and antioxidants due to increase in microflora especially yeast during fermentation. Antimicrobial natural bacteriocins are also helpful in eradicating harmful microflora and suppressing many kinds of serious food borne diseases which generally harm our health. Therefore, fermented foods which are more prevalent in Himachal as popular dishes are not only for taste but also serve as very important digestive aids to impart immense health benefits to human beings directly or indirectly.

## REFERENCES

1. Sharma, N., & Singh, A. (2012). An insight into traditional foods of North-western area of Himachal Pradesh. *Indian Journal of Traditional Knowledge*, 11(1), 58-65
2. Savitri, & Bhalla, T.C. (2007). Traditional Foods and beverages of Himachal Pradesh. *Indian Journal of Traditional Knowledge*, 6(1), 17-24
3. Borgstrom, G. (1968). *Principals of Food Science. Food Microbiology and Biochemistry*, Macmillan, New York
4. Wood, J.B. (1997). *Microbiology of Fermented Foods*, Blackie Academic Press, Glesgow, UK Rangana, S.: *Manual of analysis of fruits and vegetables products*. Tata McGrow-Hill pub. Com. Ltd., New Delhi, India. 2-95
5. Lowry, O.H., Rosebroyh, N.J., Fare, A.L., & Randall, R.J. (1951). *Journal of Biol Chem.* 193, 265
6. Dubois, M., Giller, K.A., Hamilton, J.K., Robers, P.A., & Smith, F. (1956). *Analytical Chemistry.* 26, 350
7. Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31: 426–428
8. Gram, H.C. (1884). *Uber die isoliertefarbung der Schizomuceten in Schnitt- und Trockenpreparaten (In German).* *Fortschritte der Medizin*, 2, 185-189
9. Aneja, K.R., (2003). In: *Experiments in microbiology, Plant pathology and Biotechnology, Biochemical activities of microorganisms*, 4th edition, New age International Publishers, New Delhi, 245-275pp.
10. Barefoot, S.F., & Klanhammer, T.R. (1983). Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, 45(6), 1808-1815
11. Kimura, H., Sashihara, T., Matsusaki, H., Sonomoto, K., & Ishizaki, A. (1998). Novel bacteriocin of *Pediococcus* sp. ISK-1 isolated from well – aged bed of fermented rice bran. *Annals New York Academic Science*, 864, 345-348
12. Bassapa, S.C. (2002). Investigations on Chhang from finger millet (*Eleusine Coracena* Gaertn.) and its commercial prospects. *Indian Food Ind.* 21(1), 46-53
13. Tamang, J.P., Sarkar, P.K., & Hesseltine, C.W. (1988). Traditional fermented foods and beverages of Darjelling and Sikkim- A review. *J Sci Food Agric*, 44, 375-385
14. Muyanja, B.K., Naruhus, J.A., & Langsrud, T. (2003). Isolation, characterization and identification of lactic acid bacteria from Bushera: A Ugandan traditional fermented beverage, *International Journal of Food Microbiology*, 80(3), 201-210
15. Hesseltine, C.W. (1983). Microbiology of oriental fermented foods, *Annual Reviews in Microbiology*, 37, 575-601
16. Adegoke, G.O., & Oguntimein, G.B. (1995). Microbiological and biochemical changes during the production of *Sekete*- a fermented beverage made from maize. *Journal of Food Science and Technology*, 32(6), 516-518
17. Padmaja, G., & George, M. (1999). Oriental fermented foods; biotechnological approaches. In: Marwaha SS, Arora JK (eds) *Food processing: biotechnological applications*, Asiatech Publishers Inc, New Delhi, pp.143–189

18. Batra, L.R., & Millner, P.D. (1974). Some Asian fermented foods and beverages and associated fungi. *Mycology*, 66, 942–950
19. Batra, L.R., & Millner, P.D., (1976). Asian fermented foods and beverages, *Dev Ind Microbiol* 17, 117–128.
20. Soni, S.K., and Sandhu, D.K. (1990). Indian fermented foods: microbiological and biochemical aspects. *Indian Journal of Microbiology*, 30, 135–157
21. Yuksekdag, Z.N., & Aslim, B. (2010). Assessment of potential probiotic and starter properties of *Pediococcus spp.* isolated from Turkish-Type fermented sausages (Sucuk). *Journal of Microbiology and Biotechnology*, 20(1), 161-168
22. Jack, R.W., Tagg, J.R., & Ray, B. (1995). Bacteriocins of gram positive bacteria. *Microbiology Review*, 59, 171-200
23. Sharma, N., Attri, A., Gautam, N., & Gupta, R.K. (2012). Role of lenticin - a bacteriocin as biopreservative in different food products to enhance their safety. *Beverages and Food world*, 39(2), 23-27
24. Sharma, N., Kapoor, G., & Neopaney, B., (2006). Characterization of a new bacteriocin produced from a novel isolated strain of *Bacillus lentus* NG121. *Antonie Van Leeuwenhoek*, 89, 337-343
25. Gautam, N., & Sharma, N. (2009). Purification and characterization of purified bacteriocin of *Lactobacillus brevis* isolated from traditional fermented food of H.P. *Indian Journal of Biochemistry and Biophysics*, 46, 337-341
26. Sharma, N., Kapoor, R., Gautam, N., & Kumari, R. (2011). Purification and characterization of bacteriocin produced by *Lactobacillus sp. A75* isolated from fermented chunks of *Phaseolus radiate*. *Food Technology and Biotechnology*, 49(2), 169-176
27. Sharma, N., & Gautam, N. (2007). Use of bacteriocin as potential biopreservative in milk, cheese and apple juice. *Beverages and Food World*, 34, 44-47
28. Sharma, N., Gautam, N., & Sharma, H.K. (2009). Bacteriocin: Food preservative. *Food Science and Technology*, 137-167
29. Sharma, N., Kapoor, R., Gautam, N., & Neopaney, B. (2008). Purification and characterization of natural food biopreservative produced from *Lactobacillus sp. A75* isolated from traditional fermented food of India, presented in natural product discovery and production, Whistler BC Canada.

## APPENDICES



Figure 1: Bhaturu



Figure 2: Kadi





Figure 3: Seera



Figure 4: Sepubari



Figure 5: Mashbari



Figure 6: Dangalbari



Figure 7: Jalebi



Figure 8: Chhang

Table 1: Area Wise Distribution of Traditional Cereal, Pulse and Milk Based Foods

Item	Nature of Food	Ingredients	Importance of Food/Time of Consumption	Method of Preparation	Area
Babroo	Cereal based	Wheat/rice, salt, spices, water, ghee	Snack food	Fermentation followed by shallow frying on griddle (like dosa)	Kangra/Mandi
Gulgule	Cereal based	Wheat/rice, salt, spices, water, ghee	Snack food	Fermentation followed by deep frying	Kangra/Mandi
Bhaturu	Cereal based	Wheat, oil	Staple food	Fermentation followed by deep frying	Hamirpur, Mandi, Bilaspur
Kadi	Milk based	Buttermilk, spices, Gram flour	Accompaniment to the main diet	Slow heating	Kangra, Hamirpur, Bilaspur, Mandi
Jhol/khoru	Milk based	Buttermilk and spices	Accompaniment to the main diet	Slow heating	Mandi, Kangra, Hamirpur, Bilaspur
Seera	Cereal based	Wheat, sugar, ghee	Social ceremony snack food	Natural fermentation followed by deep frying in ghee	Kangra, Hamirpur, Bilaspur, Mandi



Table 1: Contd.,

Sepubari	Pulse based	Black gram, spices	Special dish in marriage feast	Steaming followed by deep frying	Mandi, Hamirpur, Bilaspur, Kangra
Mashbari	Pulse based	Black gram, spices	Staple food	Fermentation followed by sun drying	Mandi, Hamirpur, Bilaspur, Kangra
Dangalbari	Pulse based	Black gram, dangal, spices	Staple food	Fermentation followed by sun drying	Mandi, Hamirpur, Bilaspur, Kangra
Jalebi	Cereal based	Wheat, oil, water, sugar	Social ceremony snack food	Fermentation followed by deep frying	All over Himachal Pradesh
Chhang	Cereal based	Wheat/rice	Special occasions like ceremonies, marriages, get-together	Fermentation for 2-3 days at 23-35°C	Lahaul spiti, Kullu and Kinnaur
Lugri	Cereal based	Wheat/rice	Special occasions like ceremonies, marriages, get-together	Fermentation for 2-3 days at 23-35°C	Lahaul spiti, Kullu, Kinnaur, Mandi and Kangra

Table 2: Biochemical Analysis of Different Fermented Food Items

Food Item	pH	Moisture (%)	TSS(°B)	Total Proteins(mg/g)	Carbohydrates (mg/g)	Reducing Sugars(mg/g)
Babroo (dough)	4.17	53.0	10.0°B	27.7	13.4	36.4
Bhaturu (dough)	4.12	55.0	10.0°B	21.5	13.2	34.0
Seera	4.20	10.5	9.0°B	17.2	10.9	29.9
Kadi (Lassi)	3.50	79.3	9.0°B	18.5	10.3	25.0
Sepubari	3.75	9.0	14.0°B	23.8	13.3	34.1
Mashbari	3.31	9.2	16.0°B	20.4	09.4	28.0
Dangalbari	3.40	9.8	12.0°B	16.0	13.9	31.8
Jalebi (jalebi batter)	3.59	72.0	8.0°B	24.1	13.9	29.9
Chhang	3.25	75.0	12.0°B	18.3	13.0	10.9

Table 3: Morphological, Physiological and Biochemical Characteristics of Lactic Acid Bacteria/Bacilli from Fermented Food Items

Food Item	Isolate Number	Gram Reaction	Shape, Colour and Texture	Catalase Test	Carbohydrate Utilization	MRVP Test	Citrate Utilization	H <sub>2</sub> S Production	Protease Production	Amylase Production	Lipase Production	Testative Identification
Babroo (dough)	A <sub>1</sub>	Gram + rods	White, shiny	-ve	+G <sup>-</sup>	+	+	+	+	-	-	<i>Lactobacillus</i>
	A <sub>2</sub>	Gram + rods	Red, smooth	-ve	+G <sup>-</sup>	+	+	-	+	+	-	<i>Lactobacillus</i>
	A <sub>3</sub>	Gram + rods	Creamish, shiny	-ve	+G <sup>-</sup>	+	+	+	+	+	-	<i>Lactobacillus</i>
Bhaturu (dough)	F <sub>1</sub>	Gram + rods	Creamish, smooth, shiny	-ve	+G <sup>-</sup>	+	+	-	+	+	-	<i>Lactobacillus</i>
Kadi (Lassi)	F <sub>2</sub>	Gram + rods	White, smooth, flat	-ve	+G <sup>-</sup>	+	-	+	+	-	-	<i>Lactobacillus</i>
	F <sub>3</sub>	Gram + rods	Creamish, smooth, raised	-ve	+G <sup>-</sup>	+	-	-	+	-	-	<i>Lactobacillus</i>
	F <sub>4</sub>	Gram + rods	Transparent white, smooth, shiny	-ve	+G <sup>-</sup>	+	+	+	+	+	-	<i>Lactobacillus</i>
	B1	Gram - rods	Whitish, rough	+ve	+G <sup>-</sup>	+	+	-	+	+	-	<i>Bacillus</i>
	B2	Gram - rods	Creamish, smooth, raised	+ve	+G <sup>-</sup>	+	+	-	+	+	-	<i>Bacillus</i>
Seera	F <sub>1</sub>	Gram + coccus	Whitish, smooth, shiny	-ve	+G <sup>-</sup>	+	+	-	+	-	-	<i>Lactococcus</i>
Sepubari	SB <sub>1</sub>	Gram + coccus	Creamish, elevated, irregular margins	-ve	+G <sup>-</sup>	+	+	-	+	-	-	<i>Lactococcus</i>
Mashbari	MB <sub>1</sub>	Gram + coccus	White, elevated, circular	-ve	+G <sup>-</sup>	+	+	-	+	-	-	<i>Lactococcus</i>
Dangalbari	DB <sub>1</sub>	Gram - bacillus	White, shiny, elevated	+ve	-G <sup>-</sup>	+	+	+	+	+	-	<i>Bacillus</i>
	DB <sub>2</sub>	Gram + coccus	creamish, shiny, irregular margins	-ve	+G <sup>-</sup>	+	+	-	+	-	-	<i>Lactococcus</i>
Jalebi (jalebi batter)	F <sub>1</sub>	Gram - rods	White, raised, smooth	+ve	+G <sup>-</sup>	-	+	-	+	-	-	<i>Bacillus</i>
	F <sub>2</sub>	Gram + rods	Creamish, flat, smooth	-ve	+G <sup>-</sup>	+	+	-	+	+	-	<i>Lactobacillus</i>
Chhang	F <sub>1</sub>	Gram + rods	Creamish, raised, smooth	-ve	+G <sup>-</sup>	+	+	-	+	-	-	<i>Lactobacillus</i>
	F <sub>2</sub>	Gram + rods	Transparent white, smooth, flat	-ve	+G <sup>-</sup>	+	+	-	+	+	-	<i>Lactobacillus</i>

**Table 4: Microbial Profile of the Traditional Fermented Food Items**

Food Item	Total Count cfu/g	Predominant Microorganism
Babroo (dough)	$41 \times 10^6$	<i>Saccharomyces cerevisiae</i> , <i>Bacillus</i> sp. A <sub>75</sub> , <i>Lactobacillus plantarum</i> , <i>Kocuria rhizophila</i> , <i>Leuconostoc</i> spp.
Bhaturu (dough)	$58 \times 10^6$	<i>Saccharomyces cerevisiae</i> , <i>Bacillus</i> sp. A <sub>71</sub> , <i>Lactobacillus fermentum</i> F <sub>3</sub> .
Seera	$52 \times 10^6$	<i>Lactobacillus fermentum</i> , <i>Lactobacillus</i> sp. F <sub>1</sub> .
Kadi, Jhol and Khoru (Lassi)	$45 \times 10^6$	<i>Lactobacillus fermentum</i> , <i>Lactobacillus</i> <i>acidophilus</i> F <sub>14</sub> , <i>Saccharomyces cerevisiae</i> <i>Bacillus</i> sp. A12, <i>B. mycooides</i> A12
Sepubari	$28 \times 10^6$	<i>Lactobacillus</i> spp., <i>Bacillus</i> sp. A <sub>31</sub> , <i>Saccharomyces cerevisiae</i>
Mashbari	$31 \times 10^6$	<i>Lactobacillus</i> spp., <i>Bacillus</i> sp. A <sub>94</sub> , <i>Saccharomyces cerevisiae</i>
Dangalbari	$24 \times 10^6$	<i>Lactobacillus</i> spp., <i>Bacillus</i> sp. A <sub>5</sub> , <i>Saccharomyces cerevisiae</i>
Table 4: contd.,		
Jalebi (jalebi batter)	$67 \times 10^6$	<i>Lactobacillus fermentum</i> , <i>Lactobacillus</i> <i>plantarum</i> , <i>Lactobacillus</i> sp. F <sub>8</sub> , <i>L. spicheri</i> G2
Chhang	$64 \times 10^6$	<i>Lactobacillus plantarum</i> F <sub>22</sub> , <i>Saccharomyces</i> <i>cerevisiae</i> , <i>Bacillus</i> sp., <i>B. amyloliquifaciens</i>

**Table 5: List of Microorganisms Isolated and Identified from Fermented Food Items of Himachal Pradesh**

Food Item	Microorganism Isolated and Identified (16S rRNA)	NCBI Accession Numbers
Dough	<i>L. fermentum</i>	KC242235
Kandal Vari	<i>L. brevis</i> UN	JX046150
Whey	<i>Bacillus</i> sp., <i>L. acidophilus</i> , <i>B. mycooides</i>	
Mung Bari	<i>B. subtilis</i>	JX129361.1
Jalebi batter	<i>L. spicheri</i> G2	JX481912
Chhang	<i>B. amyloliquifaciens</i>	JX129360.1

**Table 6: Potential Bacteriocinogenic Bacteria from Traditional Fermented Foods and their Sources**

Producer Isolate	Name of Bacteriocin	Source
<i>B. lentus</i> (Sharma et al., 2007; Sharma et al., 2006)	Lenticin	Dough
<i>L. brevis</i> (Gautam, N. and Sharma, ,2009)	Brevicin	Kandal Vari
<i>Bacillus</i> sp. A12 (Sharma et al., 2011)	Bacicin A12	Whey
<i>B. subtilis</i> (Sharma and Gautam, 2007)	Subtilin R75	Mung Bari
<i>B. mycooides</i> (Sharma et al., 2009)	Mycosin	Whey
<i>Lactobacillus</i> sp. A <sub>75</sub> (Sharma et al., 2008 )	Lacticin A <sub>75</sub>	Dal Bari