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## Microbiological evaluation and its impact on Indian traditional fermented foods

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### Abstract

Traditionally for several centuries man has adopted fermentation as a means of food preservation and also found them to have nutritional and therapeutic advantages. In developing and under developed countries fermented foods play a main socio-economic role and it contributes to fulfill the nutritional requirements of populations. Food is the major factor which affects the human health and cause food borne diseases. In this research, seven fermented food samples like idli, dhokla, chenna, kulcha, dosa, yogurt and probiotic drink were selected for microbiological analysis. Plate count analysis on the basis of CFU/g initial load in dosa was found to be  $8.5 \pm 1.5 - 0$  CFU/g, in kulcha initial load was found to be  $10 \pm 1 - 0$  CFU/g, in dhokla, idli, fermented yogurt the initial load was found to be  $56 \pm 6 - 0$ ,  $189.5 \pm 10.5 - 0$ ,  $23.5 \pm 1.5 - 0$  CFU/g. On the basis of  $\log_{10}$ CFU/g highest contamination was observed in dhokla followed by fermented yogurt and moderate risk of contamination was observed in idli. The results of most probable number (MPN) count technique used to estimate the bacterial counts on different media, which indicates the risk of contamination and involvement of pathogenic microorganisms in the fermented food products in less, moderate and high levels. Another test was performed for the detection of pathogenic bacteria *i.e.*, biochemical characterization of specific microorganism for their identification which came out to be negative. All fermented foods are related to with a unique group of microflora which increases the levels of proteins, vitamins, essential amino acids and fatty acids. Traditional fermented foods are made under primitive conditions, which result in low yield and poor hygienic quality. Hence, they serve as vehicles for the transmission of pathogenic microorganism associated with human diseases. This is an attempt to make a bridge between conventional and Non-conventional preparation methods by using advance approach from field of food microbiology.

**Keywords:** Food microbiology, Food safety, Food borne diseases, Fermented food, Nutritional value.

### Introduction

Fermentation is one of the oldest technologies used for food preservation and other nutritional benefits. Over the years it has evolved, refined and diversified. Today, a large variety of fermented foods are derived from this technology which is used in households, small scale food industries, semi local business, and large commercial enterprises (FAO/WHO 1995)<sup>[1]</sup>. Biochemically, fermentation is defined as the process that does not require any oxygen, the organic molecules is leveraged as electron acceptors and it is performed only by active living cells organisms (Mansi, 2003)<sup>[2]</sup>. Fermented foods are palatable and wholesome foods prepared from raw materials by microbial fermentation. Traditionally by trial and error skills have been developed to control technical parameters during fermentation inoculation of raw materials with a residue of a previous batch, *i.e.* a back slopping, accelerated the initial fermentation phase and controlled desirable changes (Holzapfel, 1997)<sup>[3]</sup>. Fermented foods, whether from plant or animal origin, are an integral part of the diet of people in all parts of the world. Basically, fermented foods are agricultural products which have been converted by enzymatic activities of microorganisms into desirable food products keeping nutritional value intact. The Indian population depends heavily on several cereals and pulse/legume based fermented products; that are made from combinations of cereal and legume grains like idli, dhokla, dosa which gives higher protein content and provides a better balance ratio of amino acids, overcoming the danger of lysine deficiency from cereals alone or sulphur containing amino acid deficiency from legumes alone. A large proportion of the world cereal production is processed by fermentation prior to consumption. It enhances and improves the flavor, texture, shelf life and digestibility of the food products (Nout, 2009)<sup>[4]</sup>. A survey was conducted on the consumption of fermented foods in Sikkim during 2003- 2005. The data show the per capita consumption of ethnic, fermented foods and beverages in Sikkim is 163.8 g/ day and the proportion of daily intake of fermented food and beverages to the consumption of total food is 12.56% (Tamang, 2010)<sup>[5]</sup>. There are different benefits which are associated to our health like increase in variety, used as ingredients, improves the nutritional quality of

foods, have a longer shelf life, improves the digestibility, and helps in detoxification of raw materials.

With this positive development, there are also some public health challenges for the urban population (Maxwell, 1999)<sup>[6]</sup>. WHO in year 2010, stated that millions of people fall sick or die as a result of eating unsafe food. Food safety has emerged as an important global issue with international trade and public health implications (WHO, 2010)<sup>[7]</sup>. According to the report on fermentation technology to improve food safety by joint collaboration with FAO/WHO workshop (1996) and the Dept. of Health, Republic of South Africa (1996), it was discovered that food-borne illness in industrialized countries has been estimated to be high as 10% of the population (WHO,2010)<sup>[7]</sup>.

This study was carried out to evaluate the presence of different pathogenic micro-organisms like *E.coli*, *salmonella* species, *bacillus cereus* in fermented food products purchased from markets in West Delhi and characterization of the isolated organism in terms of biochemical reaction.

### Materials and Methods

This study was conducted at Institute of Hotel Management, Catering & Nutrition, Pusa, New Delhi, India. Media and kits used during this study were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

### Sample Collection and Survey

A total of seven food samples were collected from randomly selected food shops. The samples investigated in this study were Dosa, Kulcha, Idli, Yogurt, Chenna, Probiotic Drink and Dhokla. The samples were aseptically collected in sterile polythene pouches and transported in ice packs to the laboratory for microbial analysis within an hour of procurement. A questionnaire was used to obtain preliminary information on the daily consumption of food items.

### Microbiological Analysis

These samples were collected from different sellers. The controls were fresh prepared food, sampled in the same manner as for the fermented products from the same shop. For the purpose of avoiding contamination from the working environment, all equipment, media test-tubes, Petri dishes were pre-sterilized. Equipment's and media were sterilized in an autoclave at 121°C for 20 min. Distilled water in test-tubes were also plugged with nonabsorbent cotton wool and sterilized by autoclaving. Inoculating loops and forceps were sterilized by heating to red-hot using Benson burner before and after use. Flaming was used to decontaminate the mouth of flasks, test-tubes and glass slides. All work was done in Laminar air flow chamber aseptically.

### Isolation and Enumeration of Microorganisms

Nutrient agar (NA), was generally used for the isolation of bacteria. This was prepared by 1000 ml of sterilized distilled water, 3 g of beef extract, 5 g of peptone, 15 g agar and 5 g NaCl. The pH was then adjusted to 7.4. In the isolation procedure, 10 g of each test sample was dissolved in 90 ml of peptone water. A homogeneous sample was prepared with 10 g of each food sample, aseptically dissolved into 90 ml to a test tube marked 10<sup>-1</sup>. This gives 1: 10 dilution of each sample. From dilutions, further dilutions were prepared in a series from 10<sup>-2</sup> to 10<sup>-6</sup>. This was done by adding 1ml of 1:10 into diluents tube marked 10<sup>-2</sup> shake vigorously. 1ml of 10<sup>-2</sup> (1:100) was then transferred to a tube marked 10<sup>-3</sup> (1:1000) and so on. After each transfer, suspension was shake

vigorously. Six dilution tubes were prepared for each sample. Nutrient agar plates were labeled from 10<sup>-1</sup> through 10<sup>-6</sup> and then 0.1 ml of dilution was transferred with the help of micropipette. The sample was spread on agar plate with the help of glass spreader. The following plating was done in the laminar flow to maintain aseptic conditions. Then plates were incubated at 37 °C for 24 hrs in an invert position for colony formation. Then plates was observed for colony count in Quebec colony count and determined the CFU/g of the sample by using only those plates that fit the criteria of the counting rules (*i.e.* colony count between 30 – 300).

Each colony formed on the plates originated from a single microbial cell and thus isolated in a pure form for further studies. A turbid suspension of each colony with distinct morphological properties was prepared. Pure colonies formed were subjected to identification to find which particular microorganism was present. Microorganisms growing on solid nutrient agar plate form colonies with distinctive morphology. Properties considered were colony form, elevation of colony and colony margin. Gram's staining was also carried out on a sample of each colony that was picked, smeared and fixed on a slide in order to divide the microorganisms into gram-negative and gram-positive microorganism (Atlas,1999)<sup>[9]</sup>. Four biochemical tests namely Methyl red test, Voges-proskauer test, H<sub>2</sub>S production and Citrate utilization were performed for identification of bacteria.

### Test for Coliform detection in food samples

*Coliform* in foods may be enumerated by the solid medium method or by the most probable number (MPN) method (FSSAI, 2012)<sup>[10]</sup>.

### Determination for coliform detection in food samples by presumptive testing

All samples were tested for *coliform*, medium is dissolved in 1L of distilled water. Heat to boiling (22 mins), do not autoclave or dispense agar to plates. The homogenized food sample in a blender by mixing 10 g of food sample with 90 ml of peptone water or sterile water. This is 1:10 (10<sup>-1</sup>) dilution. Further serial dilutions were prepared *i.e.* 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> using 9 ml peptone water tubes. Label 9 tubes of LST broth and inoculate these with 1ml each of 10<sup>-2</sup>,10<sup>-3</sup>, and 10<sup>-4</sup> in triplicates. LST tube inoculated with 1 ml of *E. coli* is taken as a positive control. All tubes were incubated at 35°C for 48 hrs and observe for the gas production. Gently shake the tube to release the gas from the medium. The number of positive tubes was noted in each set and determines the MPN/gm of the food from the standard MPN table. Determine the dilution factor multiplier and multiply the tube values with it to convert the dilution. This will give the presumptive MPN/gm. Also transfer 1ml each of 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> dilutions to sterile petri plates. Pour the molten VRB agar and mixed the sample gently with the medium. *E. coli* culture is taken as a positive control. After solidification, the plates were incubated at 35°C for 24 hours. The presumptive *coliform* colonies were counted on sample's plates by matching the purple red colony colour surrounded by a halo of precipitate (ppt.) bile salt to those of positive control. The *coliform* CFU/gm of food samples were determined by considering the countable plates.

### Determination for Salmonella species in food samples

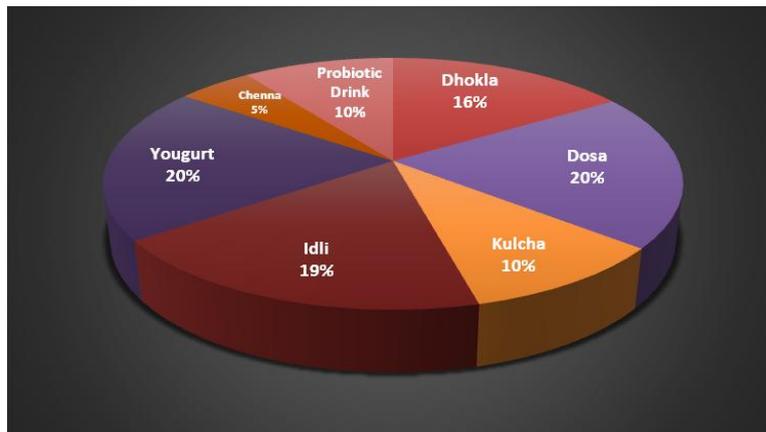
KB011 is a comprehensive test system that can be used for identification of gram- negative *Salmonella* species. "KB011 Hisalmonella™" identification kit can be used for screening

pathogenic organisms from feces, urine, blood and other relevant clinical specimen. It can also be used for validating known laboratory stains. Each KB011 kit is a standardized colorimetric identification system. The tests are based on the principle of pH change and substrate utilization. During incubation *salmonella* exhibit metabolic changes which are indicated by a color change in the media that can be either interpreted visually or after addition of the reagent. The kit was opened aseptically by peel of the sealing tape. Each of the samples was inoculated with 50 ml of the inoculum prepared. For Methyl Red Test 1-2 drops of Methyl Red reagent was added. For Voges Proskauer's Test 2-3 drops of Baritt reagent A and 1 drop of Baritt reagent B (R030) was added. For H<sub>2</sub>S test, triple sugar iron (TSI) agar is used to observe carbohydrate utilization pattern the medium contains 1% concentration of each of lactose and sucrose and 0.1% conc. of glucose. Acid base indicator is also added to detect the

production of acid during carbohydrate fermentation. TSI medium also contains sodium thiosulphate as substrate and ferrous sulphate for detection of H<sub>2</sub>S production by certain microorganisms through reduction of sulphur containing amino acids or inorganic sulphur compounds.

### Result and Discussion

Food borne diseases are a major global public health problem and developing countries bear the brunt of this majorly. Quality and safety of fermented foods depend on their microbiological flora. Microbiological content keeps on increasing from the point of improper handling of raw materials while fermentation process and due to poor hygiene aspects. The aim of this study was to investigate the food safety aspects and microbial quality of traditional fermented foods in West Delhi. In total seven samples were collected from local and semi-local shops of West Delhi.



**Fig 1.1:** Daily consumption priorities of traditional fermented foods by the consumers on the basis of consumer survey

Around 100 subjects were taken to know the consumption of most common fermented foods by them which were taken as the main samples for the microbial analysis. From the 100 subjects 20% subjects consumed fermented yogurt, 5% subjects consumed chenna, 10% subjects consumed probiotic drink, 20% subjects consumed dosa, 10% subjects consumed kulcha, 19% subjects consumed idli, and 16% subjects consumed dhokla. So, from the above figure 1.1 it can be concluded that idli, yogurt, dosa and dhokla were very famous and easily acceptable fermented foods among consumers whereas, chenna, kulcha, and probiotic drink were the foods which were less consumed by the subjects on the daily basis. After conduction of the consumer survey among the consumers/subjects, a food safety checklist was prepared to observe the food safety and hygiene aspects of local vendors from where the selected fermented food products were purchased. From that consumer survey, we had selected some famous and most consumed traditional fermented foods and these seven fermented food products were taken from West Delhi for microbiological analysis. The table 1.1 depicts the

number of samples analyzed.

### Microbiological analysis of some of the selected traditional fermented products

Each sample of fermented food products were analyzed by using different techniques such as qualitative and quantitative techniques were followed by using different microbiological tests.

### Total plate count

Total bacterial count was estimated in triplicates by using the standard plate count (SPC) technique. Plate with a total count less than 30 or greater than 300 were rejected as they lead to high degree of error and not acceptable for statistical analysis [8]. In current study, it was found that colonies were less than 300 (above the counting criteria limit) in all the samples analyzed.

Microbiological assessment of different fermented food products was performed as given in table 1.1 present the mean microbial load in samples analyzed.

**Table 1.1:** Mean microbial load in traditional fermented foods

Dilutions	MEAN + SE						
	Dosa	Kulcha	Dhokla	Idli	Yougurt	Chenna	Probiotic Drink
10 <sup>-1</sup>	TFTC	TFTC	56± 6	189.5± 10.5	42.5± 1.5	*	*
10 <sup>-2</sup>	TFTC	TFTC	23.5± 7.5	34.5± 5.5	11.5± 1.5	*	*
10 <sup>-3</sup>	TFTC	TFTC	6.5± 1.5	8± 1	6± 1	*	*
10 <sup>-4</sup>	TFTC	*	*	0.5± 0.5	5± 1	*	*
10 <sup>-5</sup>	*	*	*	*	*	*	*
10 <sup>-6</sup>	*	*	*	*	*	*	*

\*No growth

SE- Standard error, TNTC- Too numerous to count, TFTC- Too few to count

According to  $10^{-1}$  dilution the initial load of dosa was found to be  $8.5 \pm 1.5$  which was reduced to 0 in  $10^{-6}$  dilution. In  $10^{-1}$  dilution of kulcha the initial load was found to be  $10 \pm 1$  which was further reduced to 0 in  $10^{-6}$  dilution. Now in dilution  $10^{-1}$  of dhokla the initial load was found to be  $56 \pm 6$  which was reduced to  $23.5 \pm 7.5$  in  $10^{-2}$ ,  $6.5 \pm 1.5$  in  $10^{-3}$ , and 0 in  $10^{-4}$  to  $10^{-6}$  dilutions. In dilution  $10^{-1}$  of Idli the initial load was found to be  $189.5 \pm 10.5$  followed by other dilutions  $34.5 \pm 5.5$  in  $10^{-2}$ ,  $8 \pm 1$  in  $10^{-3}$ ,  $0.5 \pm 0.5$  in  $10^{-4}$  and reduced to 0 in  $10^{-5}$  to  $10^{-6}$  dilutions. Whereas, in fermented yogurt in dilution  $10^{-1}$  the initial microbial load was calculated to be  $23.5 \pm 1.5$ , which was reduced to 0 in  $10^{-6}$  dilution and it was observed that from the above table 1.1, that no growth were found in chenna and probiotic drink. Therefore probiotic drink and chenna were significantly healthy and safe fermented foods which were followed by dosa, kulcha and yogurt to be consumed by all age groups. The survival and growth of foodborne bacterial pathogens in fermenting batter of dhokla was observed during processing and fermentation of batter at around 12 to 14 hours. Pathogenic bacteria like *Bacillus cereus*, *Staphylococcus aureus* and *E. coli* can be present. At the time of fermentation,

the rise in volume of dhokla batter remained negatively correlated with its decline in pH. While steaming the batter for 15 minutes to prepare dhokla cakes, the count of pathogenic microorganisms went below the detection limit.

In past years various researcher conducted on a survival and growth of food borne bacterial pathogens. Roy and coworkers understood the fate of some selected food borne bacterial pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* during a chance contamination of black gram dough at the time of preparing wadi. During the first 10 h of fermentation, got contained by *Bacillus Cereus* and also the next 24 h of drying wadi. After 10 h of fermentation and 36 h of drying, the moisture content decreased from initial 61.9 to 33.5%, and the pH declined from 6.0 to 4.8. Fermenting dough of wadi may get contaminated by food borne bacterial pathogens due to unhygienic preparation practices. But their growth is inhibited during simultaneous drying and fermentation process (Roy et. al., 2010) [11].

The Figure 1.2 below depicts the microbial load of different fermented food samples in  $\text{Log}_{10}\text{CFU/g}$

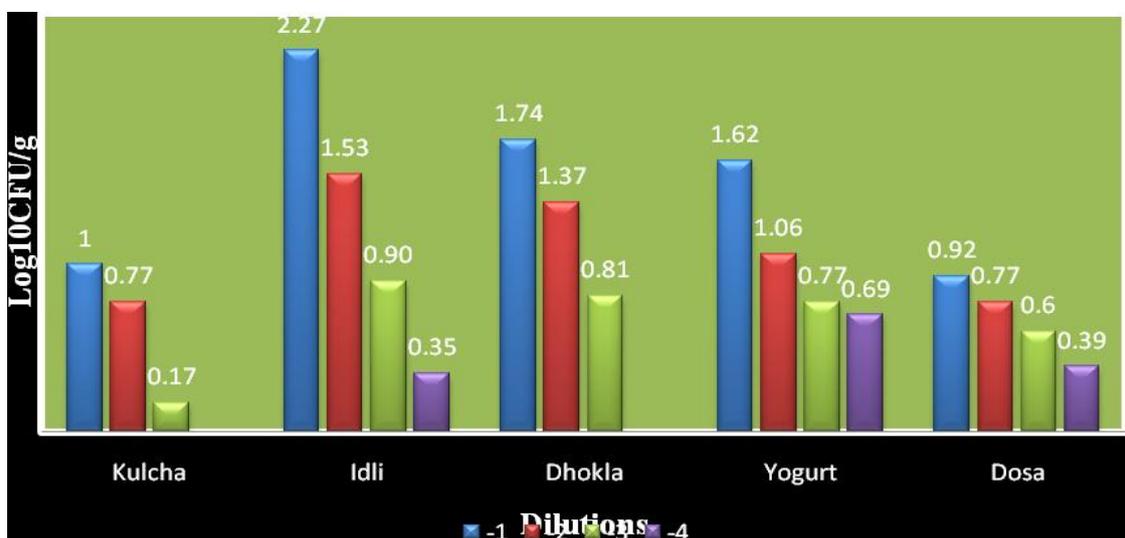


Fig 1.2: Microbial load of different fermented foods in  $\text{log}_{10}\text{CFU/g}$

In dilution  $10^{-1}$ – $10^{-3}$ , the value of kulcha varied from (1–0.17)  $\text{log}_{10}\text{CFU/g}$ . Minimal decrease were seen in every dilution, whereas in dilution  $10^{-1}$ – $10^{-4}$ , the values of idli reduced from (2.27–0.35)  $\text{log}_{10}\text{CFU/g}$ . In dhokla from dilution  $10^{-1}$ – $10^{-3}$ , values varied from (1.74–0.81)  $\text{log}_{10}\text{CFU/g}$ , whereas in further dilutions  $10^{-1}$ – $10^{-4}$  of yogurt and dosa, the values were varied from (1.62–0.69)  $\text{log}_{10}\text{CFU/g}$  and (0.92–0.39)  $\text{log}_{10}\text{CFU/g}$ . Now from the above fig 1.2 it had been seen that the moderate risk of contamination is found in dhokla, idli and yogurt which could involve any kind of hazard like physical, chemical and biological.

In the similar study, it was found that eight samples of yoghurt produced from Enugu State and Eastern Nigeria collected from various locations. Samples were incubated using various media for the isolation, identification and enumeration of the bacteria population within the yoghurt. Not all bacterial isolates were lactose fermenters. Members of the genus *Staphylococcus*, *Aeromonas*, *Klebsiella*, *Pseudomonas*, *Bacillus*, *Streptococcus* and *Lactobacillus* were isolated with 100, 25, 50, 33.3, 25, 12.5 and 25% frequency of occurrence, respectively. Viable counts of lactic acid bacteria were low indicating that probiotics effect

following consumption may be poor. Occurrence of pathogenic organisms indicates improper handling and inadequate sanitary measures. More care should be taken during yoghurt fermentation (Chike and Nwagu, 2010) [12].

#### Salmonella test

Another biochemical microbiological assessment of some selected fermented foods was done by using Hisalmonella™ identification kit to detect the presence of salmonella species in the selected fermented food products. To identify the biochemical characteristics specific to the microbial identification, process these biochemical test includes the following tests:

- Methyl red test
- Voges – proskauer test
- $\text{H}_2\text{S}$  production
- Citrate utilization

Each of the above tests was performed by using Hisalmonella™ identification kit and table 1.2 shows results of biochemical tests.

**Table 1.2:** Presence of *Salmonella species* in different fermented food products in four tests

No.	Test	Dosa	Idli	Dhokhla	Kulcha	Fermented yogurt	Chenna	Probiotic drink
1	Methyl Red	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Voges Prokauer's	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	H <sub>2</sub> S Production	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	Citrate Utilization	-ve	-ve	-ve	-ve	-ve	-ve	-ve

\* -ve – negative

*Salmonella* culture was taken as positive control from the laboratory of I.H.M, Pusa.

It had been observed in table 1.2, that all tests came out to be negative for all selected fermented food products therefore, it can be concluded that in all the selected fermented foods salmonella species was absent. The fermented foods were of good quality (table 1.2) and the beneficial microorganisms which were present in fermented foods naturally or added during the fermentation process inhibit the growth of pathogenic microorganisms like *salmonella species*.

In previous study, porridges inoculated with pathogenic bacteria (*Salmonella spp.*, *Shigella spp.*, *Staphylococcus Aureus*, *Yersinia Enterocolitica*, *Escherichia Coli*, *Citrobacter 3465/69*, *Enterobacter cloacae etc.*) showed that acidification, either by adding acids or by fermentation, prevented the bacterial growth. The most resistant *Salmonella* died at a rate of 1, 2 log cycle/h, the most resistant *Shigella* at 0.9 log cycle/h, the most resistant *Escherichia coli* at 0.6 log cycle/h. The fermentation was accelerated by inoculum recycling, which was necessary to obtain a pH low enough to prevent the growth of the pathogenic bacteria (Nout et. al., 1989) [13].

In another study, effect of lactic fermentation on survival of enteropathogens in cereal gruels.

Commonly used as weaning foods, were investigated by (Kingamkono et. al.,1996) [14] who added 28 enteropathogens to cereal gruels prepared from low tannin sorghum and inoculated them with a lactic acid starter culture. After 6 h of fermentation, *Campylobacter* strains were not detectable and after 12 h, *Salmonella*, *Shigella* and *Staphylococcus strains* were also not detectable. So, these researches strongly support that during fermentation growth of pathogenic bacteria was inhibited by lactic acid producing beneficial bacteria.

#### Coliform detection test

Another technique named Most Probable Number (MPN) was used to know the presence of *Coliform* in the food samples. This technique was based on probability statistics where in aliquots of decimal volumes/ dilutions of the samples were transferred to several 1 to 9 tubes of specific medium. Positive tubes were scored and the MPN estimate is directly calculated in selected food samples by using standard MPN table. The results showed that, the MPN/g results of dosa, kulcha showed low risk of contamination i.e., MPN/g value came out be 19 and 15 followed by fermented yogurt, dhokla were examined and in dhokla high risk of contamination were found but in fermented yogurt moderate risk of contamination were found, MPN/g values were calculated through standard MPN table given by "Food Safety and Standard Authority of India, 2012 [10] and found to be 53 and >1100 in Fermented Yogurt and Dhokla. On the other hand, in chenna and probiotic drink no growth on any of the media were found therefore, they are good in quality as well as can be consumed by the peoples for their health benefits also on regular basis. In dairy products like yogurt and probiotic drinks lactic acid bacteria (LAB) and other related microorganisms had been used as a starter culture and they helps in fermentation as well

also improve nutrition, increase shelf-life and prevent spoilage by anti microbial activity. Several researchers reveled that benefits of fermented foods are likely greater than the sum of their individual microbial, nutritive or bioactive compounds( Marco et. al., 2017 and Chilton *et al.*, 2015) [15, 16]. This study will further clarify the potential necessity of certain fermented foods in human diet and justification for inclusion into essential dietary guideline by Indian Government.

#### Conclusion

The current study concludes that the fermented foods should be consumed on regular basis and should be included in balanced diet for the better health. It helps in reducing malnutrition problem in children's as it contains many nutrients and has various beneficial bacteria which help in good gut functioning, enhancing the immunity, reducing colon cancer risk and provide different macro and micro nutrients like protein, vitamins, essential amino acids and fatty acids. In balanced amount or ratio, it provides variety of flavors and adds digestibility to the liver.

The present study had shown the growth of some pathogenic microorganisms which are harmful and can cause food brone illness even if consumed in lower amounts. However some of the foods like dosa, idli, chenna, kulcha, probiotic drink were found to be safe considering the hygiene aspects like locality of vendor shop, personal hygiene of vendor, storage and preparation of raw material, way of cooking etc. Vendor's should keep food items in different storage container to avoid cross contamination. Government should plan effective policies and measures to raise the awareness about consumption of fermented food products, food safety and hygiene aspects among local vendors. This will help in reducing the microbial load which is caused from different kinds of hazards like physical, chemical and biological, and keeping the nutrient value of food intact. There is a paramount need for a systematic training to educate people on avoiding food contamination. High priority research needs should be formulated within the areas of food safety, nutritional value, technology development, and technology transfer related socio- economic aspects. Health benefits of fermented food include new and desirable tastes, texture, reduce disease and obesity. Food is also having anti-diabetic ability. This will also modify food constitutes and synthesize metabolites; proteins etc. which provides live microorganisms to GI tract.

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