

## Original Research Article

# Microbial Quality Analysis of Locally Packed and Commercially Available Bread in Chennai Region of Tamilnadu, India

### ABSTRACT

**Aims:** In today's world consumer preference towards packed products is increasing than locally available bakery shops due to unhygienic processing and improper handling. This reason became an interesting fact to analyse the microbial quality of Bread in Chennai region.

**Methodology:** Locally made bread samples and commercially available breads were procured in shops in Chennai region. The microbial quality was analysed so check the microbial load of bread samples using method of total bacterial count, total yeast/mould counts and total coliforms using standard microbial procedures. It was carried out with the help of Plate Count Agar, Sabouraud Dextrose Agar and Eosin Methylene Blue Agar through the serial dilution and plating techniques.

**Results:** The plates were incubated and results were collected through the colony counting method. Total aerobic bacterial count was found in all the bread samples while coliform count was absent in all samples. Yeast/mold/fungi was present in all commercially available breads and samples 3 of local bread.

**Conclusion:** It can be concluded that the local and commercially available breads were having the microbial load within the permissible limits.

**Keywords:** Bread, Bakery, Microbial profile, Plate Count Agar, Sabouraud Dextrose Agar, Eosin Methylene Blue Agar.

### 1. INTRODUCTION

In the past, bakery goods were thought to be a poor man's diet. The majority of the human population currently relies on them as daily nutritional staples [1]. With a yearly revenue of over 3000 crores, the bakery business in India is the largest of the food industries [2]. Bread is a common, high-energy food that is also convenient and low in calories, glycaemic index, vitamins E, B-vitamin, and minerals [3]. When paired with fillings made of meat or fish, veggies, and fat like margarine or mayonnaise, it is commonly used by people as a snack or a meal. Rich bread is made with sugar, eggs, milk, and flavouring added to the flour and raising agent; yeast bread is made with flour, liquid, fat, milk, yeast and salt [4].

Bread is a significant staple food created from baking dough comprised of flour and water [5]. In 2011, bread made from wheat provided 20% of the daily calories consumed worldwide, feeding the world's population [6]. One of the non-indigenous foods that is most commonly consumed in India is bread [7]. The demand for ready-to-eat food items has increased as a result of urbanisation [1].

In recent decades, there has been a growth in the sales of a range of breads and other bakery goods. Before adding grains to the making of bread, grains are ground to a powdery form to create flour. It has been revealed that flours give the final baked bread its primary structure. Worldwide, many flours derived from wheat, rye, barley, maize, and other types of grains are available. In both rural and urban populations, bread and wheat flour make up a sizable portion of the daily diet. The amount of minerals, lipids, and proteins in flour is reduced and the proportion of carbohydrates is higher [8].

The most frequent cause of bread product deterioration is water activity. Microbiological spoilage, particularly mould growth, is a major economic concern with regard to bakery products. For bakeries, mould deterioration is a severe and expensive issue [2]. Physical, chemical, and microbiological spoiling issues can affect bread and other bakery products. The latter is the most significant, notably bacterial (*Bacillus* sp.) and mould growth [9]. The main causes of microflora in bread are contamination in the baking environment, contamination from handlers, and contamination from market vendors [1]. The features of the product and how it is preserved have a big impact on the microbial flora that colonises a specific food. Intrinsic, extrinsic, and processing and preservation methods are among the factors that can affect the growth of microbes in food [10]. Due to the suggested endogenous disease and the fact that they have minimal water activity from a microbiological standpoint, flour and breads are typically considered to be safe foods [11].

This study was initiated to make a comparison for better quality with respect to microbial load of the commonly available breads so that consumers may get a nutritive, more hygienic and shelf stable product. Our objective is to assess the microbial load of selected bread samples.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The area selected for the collection of bread samples was Chennai, Tamilnadu, India. The popular shops nearby the laboratory were selected, so that the items were feasible to purchase. The number of shops selected were six. Among six, we purchased locally made breads from three shops and commercially available breads from three shops. Samples were labelled as per the table 1. The samples were collected aseptically in a sterile air lock bag to prevent the contact of any other source that can contaminate the sample. The collected samples are carried to laboratory further processing.

**Table 1: Sample Information and their codes.**

Sample Code	Bread Type
Sample 1	Locally made
Sample 2	Locally made
Sample 3	Locally made
Sample 4	Commercially available
Sample 5	Commercially available
Sample 6	Commercially available

### 2.2 MEDIA PREPARATION

#### 2.2.1 PLATE COUNT AGAR (PCA)

Plate Count Agar (PCA) media was used to detect the microorganisms. 2.35g of PCA and 2 gm of agar-agar was added to the conical flask containing 100 ml of distilled water and homogenized using heating mantle. The mouth of the conical flask is plugged with cotton wool and covered with aluminium foil and then autoclaved at 121°c temperature 15 minutes. Autoclaved media was poured on the sterile petri plate in laminar air flow chamber and solidified.

#### 2.2.2 SABOURAUD DEXTROSE AGAR (SDA)

Sabouraud dextrose agar (SDA) media was used to detect the yeast or mould or fungus. 6.5 gm of SDA and 2 gm of agar-agar was added to the conical flask containing 100 ml of distilled water and dissolved using heating mantle. The mouth of the conical flask is plugged with cotton wool and covered with aluminium foil. The media was autoclaved at 121°C temperature for 15 minutes. Autoclaved media is poured on the sterile petri plate in laminar air flow chamber and solidified.

### **2.2.3 EOSIN METHYLENE BLUE AGAR (EMB)**

Eosin methylene blue agar (EMB) media was used to detect the coliforms. 7.18g of EMB and 2 gm of agar-agar was added to the conical flask containing 100 ml distilled water. The media was dissolved using heating mantle. The mouth of the conical flask is plugged with cotton wool and covered with aluminium foil and then autoclaved at 121°C for 15 minutes. Autoclaved media is poured on the sterile petri plate in laminar air flow chamber and solidified.

### **2.3 Plating of samples on media**

Serial dilution method was used in this experiment. 1g of each sample was added into test tubes containing 9 ml sterile water and homogenized to prepare standard stock solutions. 1ml was removed from each of the stock solution and added to another set of test tubes containing 9ml of sterile water which makes  $10^{-1}$  dilution. The same procedure was repeated to make  $10^{-6}$  dilution. Like this sample solution was prepared for all the 6 samples. From  $10^{-6}$  dilution, 0.1ml of each sample solution was taken in micropipette and poured on solidified plates of PCA and EMB labelled accordingly with the sample code for each sample. Similarly for SDA plates of each sample, 0.1ml of  $10^{-3}$  dilution was taken in micropipette and spread on SDA plates. Each plate was gently swirled to mix the 0.1µl of diluted sample over the agar media using sterile L rod, clockwise and anticlockwise, to and for thrice and taking care that the contents do not touch the lid. The plates were left without moving for at least 15 minutes to allow the agar to set. PCA and EMB Plates were incubated at  $35^{\circ}\text{C}\pm 2^{\circ}\text{C}$  for 48 hours while SDA plates were incubated at  $28^{\circ}\text{C}\pm 2^{\circ}\text{C}$  for 72 hours. Following incubation, the number of colonies on each media were counted and the results were recorded in CFU/g [1].

## **3. RESULT AND DISCUSSION**

'Table 2' shows the outcome of microbial analysis of local and commercial bread samples. Total aerobic bacterial count was found in all the bread samples. Yeast/mold/fungi was present in all commercially available breads and samples 3 of local bread. Sample 1 & 2 did not show fungal presence. All bread samples breads maintained the absence of coliforms. In the present investigation the microbial profile of several packed local bread and commercial bread was analysed by comparing the colony forming units (CFU) as in 'Table 2'.

**TABLE 2: MICROBIAL ANALYSIS OF LOCAL AND COMMERCIAL BREAD**

Sample	Total Bacterial Count	Total Coliform Count	Total Fungi Count
	PCA CFU/gm	EMB CFU/gm	SDA CFU/gm
Sample 1	$2.7 \times 10^3$	ND	ND
Sample 2	$8.3 \times 10^3$	ND	ND
Sample 3	$5.5 \times 10^3$	ND	$5.5 \times 10^1$

<b>Sample 4</b>	8.3 x10 <sup>3</sup>	ND	3.9 x10 <sup>1</sup>
<b>Sample 5</b>	4.1 x10 <sup>3</sup>	ND	4.1 x10 <sup>1</sup>
<b>Sample 6</b>	4.1 x10 <sup>3</sup>	ND	4.2 x10 <sup>1</sup>

ND- NOT DETECTED

As per PCA microbiological data of packed local bread and commercial bread, Sample 1 showed the lowest values and sample 2 is showed highest values. In sample 5 and 6 probably got equal values. SDA (sabouraud dextrose agar) Among the studied, in local bread sample 1 and 2 no microbial count was found in all the samples, in sample 3 showed the highest values indicating high number of bacteria in the sample. When compared to commercial bread, local bread sample 3 had the highest microbial load. Among the studied coliform counting in EMB, no colony was determined in all the samples.

Typically, bread rotting is caused by moulds such *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium*, and *Fusarium*. While the products are manually cut, packaged and delivered, bacteria including *Bacillus*, *Escherichia* and *Salmonella*, *Streptococcus aureus*, etc. may also infect and cause ropiness of the bakery items [12].

Any bread item that is kept open or partially sealed can become contaminated and degraded by airborne fungus, moulds, and bacteria because relatively high moisture content promotes the growth and development of mould and bacteria on the bread [13].

It was discovered that correct vending practises, such as cleanliness of the retailer or storekeeper, were related to the microbiological status of various food products; this relationship is also firmly thought to be related to the level of education of the vendors [14]. In the current study it was found that fungal count was high in commercially available breads than locally prepared breads. Whereas the coliforms were absent in all samples which is safe for consumers.

#### 4. CONCLUSION

From this study, it was observed that the microbial load was within the permissible limit for human consumption. It is preferable to consume bread within the expiry date as mentioned by the suppliers. The determination of micro flora carried out in this study is necessary in safeguarding public health. This study therefore provides basic information about the micro flora in bread likely to cause food-borne disease when present in bread which is a ready-to-eat type of food.

#### REFERENCES:

1. Nirmala R. Study on microbial profile of bread during storage, *International Journal of Advanced Research in Biological Sciences*. 2016;3(9): 60-63.
2. Saranraj P, Geetha M. Microbial Spoilage of Bakery Products and Its Control by Preservatives. *International Journal of Pharmaceutical and Biological Archives*. 2012;3 (1): 38-48.
3. Balamurugan V, Sivabalan S, Thamizhselvan I. Effect of traditionally treated wheat on bread making qualities. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*. 2018;4(5), Page No.425.
4. Isong NB, Akpan MM, Udota HI, Barber LZ. Antibacterial and microbial assessment of breads in Abak local government area, Nigeria, West Africa. *Journal of Microbiology and Biotechnology Research*. 2017;3, 155-159.
5. Haruna M, Udobi C, Ndife J. Effect of added brewers' dry grain on the physico-chemical, microbial and sensory quality of wheat bread. 2011;1(1): 39-43. DOI:10.5251/AJFN.2011.1.1.39.43.
6. Shiferaw B, Smale M, Braun HJ. et al. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Sec*. 2013;5, 291–317. <https://doi.org/10.1007/s12571-013-0263-y>.
7. Das LU, Raychaudhri RC. Effects of baking conditions on the physical properties of herb bread using RSM. *Int. J. Food Agri. Vet. Sci*. 2012;2:106-114.

8. Batool SA, Rauf N, Tahir SS, Kalsoom R. Microbial and Physico-Chemical Contamination in the Wheat Flour of the Twin Cities of Pakistan. *International Journal of Food Safety*. 2012;14, 75-82.
9. Saranraj P, Sivasakthivelan P. Microorganisms involved in spoilage of bread and its control measures. *Bread and Its Fortification: Nutrition and Health Benefits*. Taylor and Francis Group, LLC; 2015;132-149.
10. Mossel DAA, Corry JEL, Struijk CB Baird RM. *Essentials of the microbiology of foods: a textbook for advanced studies*. John Wiley and Sons, Chichester, UK; 1995.
11. ICMSF (International Commission on Microbiological Specifications for Foods), 2002. *Microorganisms in foods: microbiological testing in food safety management*. Kluwer Academic/Plenum Publishers, New York, USA; 2002.
12. Al-Defiery ME and Merjan AF. Mycoflora of mold contamination in wheat flour and storage wheat flour. *Mesopotamia Environmental Journal*. 2015;1(2) pp. 18-25.
13. Ponte JG and Tsen CC. Bakery products in *Food and Beverage Mycology* (ed, L. R. Beuchat). 1978;191-223.
14. Abdulkareem L, Garba D and Abubakar A. A study of cleanliness and sanitary practices of street food vendors in Northern Nigeria, *Advances in Food Science and Technology*. 2014;2 (5):209-215.

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