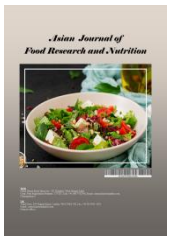

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Microbial Quality Analysis of Locally Packed and Commercially Available Bread in Chennai Region of Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study, performed the statistical analysis. Author SM wrote the protocol and wrote the first draft of the manuscript. Authors KB and KK managed the analyses of the study. Author SS managed the literature searches. All authors read and approved the final manuscript.

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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: This study was conducted to determine the microbial load of bread sold commercially and by the local in Chennai region. Locally made bread samples and commercially available breads were procured in shops from Chennai region. The microbial quality was analysed to check the microbial load of bread samples using standard microbial procedures such as total

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bacterial count, total yeast/mould counts and total coliforms. 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 except sample 1 & 2 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

Microbiological assay of the bacteria, yeast and mold counts were performed as per the methods outlines in the compendium of methods for microbiological examination of foods with few modifications [12].

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℃±2℃ for 48 hours while SDA plates were incubated at 28℃±2℃ for 72 hours. Following incubation, the number of colonies on each media were counted and the results were recorded in CFU/g [1]. Colony Forming Unit was calculated using the following formula:

CFU/g = (average no. of colonies x total dilution factor)/ volume plated

3. RESULTS AND DISCUSSION

'Table 2' shows the outcome of microbial analysis of local and commercial bread samples. The total bacterial counts of the bread samples ranged from $2.7x10^3$ to $8.3x10^3$ cfu/g with samples, 1 and 2;4 having the lowest and highest values respectively. Yeast/Mold/Fungi counts ranged from $3.9x10^1$ to $5.5x10^1$ cfu/g with the lowest and highest counts recorded for sample 4 and sample 3 respectively. There was no detection of Coliforms in the bread samples evaluated. The microbial counts were within the

Table 2. Microbial analysis of local and commercial bread

Sample	Total Bacterial Count PCA CFU/gm	Total Coliform Count EMB CFU/gm	Total Fungi Count SDA CFU/gm
Sample 1	$2.7x10^3$	ND	ND
Sample 2	$8.3 x10^3$	ND	ND
Sample 3	$5.5 x10^3$	ND	$5.5 x10^1$
Sample 4	$8.3 x10^3$	ND	$3.9 x10^1$
Sample 5	$4.1 x10^3$	ND	$4.1 x10^1$
Sample 6	$4.1 x10^3$	ND	$4.2 x10^1$

ND- Not Detected

permissible limit which is set by the Standard Organization of Nigeria, which states that the counts of aerobic bacterial must not exceed 10^3 cfu/g and coliform growth must not be detected in bread samples [13].

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'.

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.

The findings of our investigation indicate that the bread samples are safe for ingestion by humans because they do not present any significant health risks. The samples' microbial growth may have developed during processing from the raw materials (such as flour, sugar, and yeast) or possibly from the environment.

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 [14]. 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 [15].

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 [16]. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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