Journal of Food and Nutrition Sciences
2023; 11(4): 126-131
http://www.sciencepublishinggroup.com/j/jfns
doi: 10.11648/j.jfns.20231104.13
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Distribution of Mycoflora and Assessment of Moisture Content Measurement Methods of Indian Bakery Food Products

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To cite this article:
Sowmya K. L., Ramalingappa B. Distribution of Mycoflora and Assessment of Moisture Content Measurement Methods of Indian Bakery Food Products. *Journal of Food and Nutrition Sciences*. Vol. 11, No. 4, 2023, pp. 126-131. doi: 10.11648/j.jfns.20231104.13

Received: June 21, 2023; **Accepted**: July 7, 2023; **Published**: August 15, 2023

Abstract: Mycotoxins are toxic food pollutants that are created naturally by some fungus. By contaminating food, they have a harmful impact on human health. This study's major objective was to simulate food products from bakeries being contaminated. Separate bakery food samples were examined to see whether fungus and associated mycotoxins were present. One hundred fifty-nine (159) bakery food samples were collected randomly from different shops in rural sides and around Davangere city, during July to November 2022. Qualitatively, maximum number of molds were isolated and identified from bakery food samples. The most common species are *Trichoderma harzianum*, *A. niger, Rhizopus*, *A. flavus*, *Penicillium sp*, *Curvularia sp*, *A. parasiticus*, *Penicillium chrysogenum*, *Aspergillus sp*, *Fusarium sp*, *Trichoderma sp*, *Alternaria sp* and *Mucor sp*. The moisture content was found to be maximum in sweets made from dry fruits and milk followed by cakes, burfi, cookies, bread etc and minimum growth were observed in chips. Results regarding moisture content of bakery food products showed that, NS41-NS159 samples had high moisture content 60%, followed by NS1-NS11 (49.8%), NS12-NS20 (49.8%), NS21-NS35 (37.8%) and NS36-NS40 (13.6%) respectively. Results in moisture content determination in bakery food products reveals that high moisture content causes maximum growth of microorganisms on bakery food samples. Statical analysis showed that storage had a significant effect on moisture content of bakery food products.

Keywords: Fungal Spoilage, Moisture Content, *Aspergillus Flavus*, *Penicillium Sp*, *Curvularia Sp*, *A. Parasiticus*

1. Introduction

In the majority of the nation, bakery products are the most significant basic foods. The most common products are toast, bread, buns, cakes, cookies, and chips. We get the majority of our dietary calories and around half of our protein needs from the grains used in bakery goods, which are a significant source of nutrients. The nutrients found in baked goods include energy, carbs, proteins, lipids, vitamins, calcium, iron, and minerals [1]. Wheat flour, sugar, salt, eggs, lipids, yeast, baking soda, baking powder, maize starch, milk, butter, essence, cocoa powder, water, sweetener and others are frequently used in the manufacture of bakery food products. The term "spoiled bakery products" refers to baked goods that have suffered harm that makes them unfit for eating [2]. Mould spores are often destroyed during the baking process, but bakery products must be contaminated either during the chilling, slicing, or packaging processes after baking or via the air, a bakery surface, equipment, food handlers, or raw materials [3]. Due to inappropriate handling and sanitation, several filamentous fungi, including *Rhizopus, Mucor, Aspergillus, Penicillium, Fusarium,* and others, are responsible for the deterioration of bakery food products. Mycotoxins can cause a variety of adverse health effects and pose a serious health threat to both humans and livestock. The number of losses from these fungi depends on the season, the kind of product, and the processing methods used. In damaged baked products, these filamentous fungi create poisons. The phrase "mycotoxicosis," which was first used in 1955, is where the word "mycotoxin" originates. In the process of manufacturing or storage, fungi that develop on baked food products create mycotoxins [4]. Food contamination by

mycotoxins is a continuous issue that is both preventable and unpredictable [5]. The primary group of mycotoxins which represent a serious risk to both human and animal health include aflatoxin (AF), zearalenone (ZEA), patulin, and deoxynivalenol (DON) [6, 7]. As a result, our first goal was to screen for toxin-producing fungus and extract mycotoxin-producing strains from bakery food products. Food deterioration is largely caused by microbial growth that is influenced by moisture. Food that has been dried has less moisture to promote this microbial development. As a result, a major factor affecting the quality of preparation, storage, etc. is the moisture content of bakery food products [8, 9]. The rate of microbial development in bakery foods is influenced by a number of variables, including pH, temperature, water activity, and moisture content. Moisture content is a measurement of the total water contained in a food product usually expressed as a percentage by weight on a wet basis [10]. In developing countries, high outside temperatures and sometimes insufficient refrigeration and storage facilities make it more difficult to safely store food with a high moisture content. As a result, drying food goods is a widespread practice to prevent food deterioration. Food that has been dried has a longer shelf life because there is less moisture available to enable microbial development [11] both humans and live stocks.

2. Materials and Methods

2.1. Collection of Samples

A total of one fifty-nine (159) bakery food samples i. e., bread, bun, cake, chips, pizza base, cookies, chocolates, toasts, peda etc were collected randomly from different shops in rural sides and around Davangere city, during July to November 2022, specially collected from the local market which were decorated samples in open stall. The collected samples were brought in sterile polythene bags to the laboratory for further processing, until processing the samples were maintained in sterile condition.

2.2. Isolation of Fungi

Fungi were isolated from bakery food samples by spread plate method, serial dilution method and by direct plate method. Samples were inoculated on Potato dextrose agar (PDA), Rose Bengal agar (RBA) Czapec (dox) agar medium (CZA) and Sabouraud dextrose agar medium (SDA) plates and incubated at 37°C for 3-7 days. After incubation different fungi were observed and identified based on microscopic observation and morphology characteristics. The same procedure was carried out for all the samples.

2.3. Isolation of Mycotoxin Producing Fungi by Direct Plate Method

This is a good method for detecting, enumerating, and isolating fungi from bakery food products. In this method, about 1 gm of bakery sample was directly placed/ sprinkled on solidified agar media (PDA+RBA+CZA+SDA) and incubated at 30-37°C for 3-7 days. After incubation different fungi were observed and identified based on microscopic observation and morphology characteristics. The same procedure was carried out for all the samples.

2.4. Isolation of Mycotoxin Producing Fungi by Spread Plate Method

In this method, 1 gm of sample was mixed with 9 ml of distilled water and a homogenate (0.5-1 ml) was added on the surface of the media (PDA+RBA+CZA+SDA) and spreader evenly over the surface using sterile L- shaped spreader. Then, the plates were incubated at 30-37°C for 3-7 days. After incubation different fungi were observed and identified based on microscopic observation and morphology characteristics [12]. The same procedure was carried out for all the samples.

2.5. Isolation of Mycotoxin Producing Fungi by Serial Dilution Method

In this method, 1 gm of sample was mixed with 9 ml of sterile water and mixed well that gives 10^{-1} dilution and serially diluted up to 10^{-7}. From each dilution 0.1 ml of sample was inoculated on solidified agar media (PDA+RBA+CZA+SDA) by spread plate method. Then, the plates were incubated at 30-37°C for 3-7 days. After incubation different fungi were observed and identified based on microscopic observation and morphology characteristics [13, 14]. The same procedure was carried out for all the samples.

2.6. Characterization of Isolated Microorganisms

Fungi were identified based on morphological and microscopic observation, after staining with lactophenol cotton blue stain followed [15-19]. Different types of fungi were distributed in different bakery food products.

2.7. Determination of pH of Bakery Products

By combining 15 g of each bakery product sample with 100 ml of distilled water in a 250 ml Erlenmeyer flask and swirling on a magnetic stirrer for 15-20 minutes, the pH of each sample of baked products were calculated. A pH metre that had been calibrated was used to record the pH of the semi-liquid combination.

2.8. Determination of Moisture Content of Bakery Products

For moisture content determination of bakery food products, 5 gm of sample was weighed in aluminium dishes and dried overnight in an oven at 100°C. the moisture content of the sample was calculated as weight loss as percentage of initial weight [20].

The percentage of moisture content was calculated by using the formula,

$$\text{Moisture content (\%)} = \frac{\text{Initial weight of the sample}}{\text{Weight of the oven dried sample}} \times 100$$

2.9. Initial Weight of the Sample

Results regarding moisture content of bakery products

showed that storage had a significant effect on moisture content of bakery food products. Because of more amount of moisture content in bakery food products causes spoilage and diseases to the consumers.

3. Results and Discussion

It is well known that fungi may cause food to deteriorate in a variety of storage situations. In actuality, the bacteria, fungus, and other organisms that cause food spoiling live, develop, and sporulate on its surface. A total of 159 bakery samples, 11 different types of cakes, 9 different types of bread and bun, 15 different types of cookies, 5 different types of chips and 119 different types of sweets were used for our study. Out of 159 samples 83 fungal isolates were found to be grown on bakery food products. Further 83 fungal isolates were observed under microscope and identified based on the hyphae, conidia, conidiophore. A common technique for identifying fungi is to observe the key macro and micromorphological characteristics of cultures of diverse fungi on different media. In order to highlight the significance of such fundamental identification approaches for the quick screening of isolates in the majority of underdeveloped countries, where access to unusual instruments is a considerable issue, the morphological Characterization was carried out in this work. Consistent identification of fungus was made possible by paying attention to essential morphological traits. Isolates' physical traits, including colony colour, texture, and edges, were noted. The colonies often had velvety or unclear textures with a floccose centre. Klich's analysis of taxonomic descriptions revealed that the isolates in this investigation had colony morphology that was similar to several fungi. The prevalence of these fungus in bakery foods was various.

Six (6) bakery products were used for microbiological analysis according to Ogawa & Adachi. During this the fungal isolates obtained from different bakery food products were identified as *Trichoderma harzianum*, *A. niger*, *Rhizopus*, *A. flavus*, *Penicillium sp*, *Curvularia sp*, *A. parasiticus*, *Penicillium chrysogenum*, *Aspergillus sp*, *Fusarium sp*, *Trichoderma sp*, *Alternaria sp* and *Mucor sp.* Macroscopic features of isolates GS1, GS12 and GS 16 on PDA and RBA are shown in Table 1 and microscopic features of isolates GS 5, GS 7 and GS 2 are shown in Table 2. 23 bakery food samples were collected and identified four isolates out of 23 bakery food samples [1]. NS5 and NS14 (10.84%) showed high percentage of spoilage in bakery food products followed by NS6, NS4, NS7 and minimum occurrence was NS3 (3.61%) is shown in Figure 1.

The pH of bakery product samples was found to be acidic in the range between 3.10 to 5.90. which indicates that molds are responsible for the bakery spoilage as these are one of the favourite food choices of fungi is shown in Table 3. The pH of bakery product samples ranged from 4.94 to 6.00, which indicates that there is a higher probability of mould deterioration since moulds thrive in acidic environments [21]. pH was proposed as a key regulating factor in the development of rope deterioration [22]. A food's pH is also important since a low pH encourages the growth of mould and yeast. Foods with neutral or alkaline pH, including meats, are more susceptible to spoiling and decomposition due to bacteria. Thus, our results clearly substantiate the findings of earlier workers as spoiled bread which is acidic in nature was showed the presence and dominance of molds [23].

Results regarding moisture content of bakery food products showed that, NS41-NS159 samples had high moisture content 60%, followed by NS1-NS11 (49.8%), NS12-NS20 (49.8%), NS21-NS35 (37.8%) and NS36-NS40 (13.6%) respectively is shown in Figure 2. The results of determining the moisture content of bakery food products show that high moisture content promotes the most microbial development on bakery food samples. The moisture level of bakery food items was significantly impacted by storage, according to statistical study. Bread is a result of the numerous bakery products with a high moisture content that encourages the growth of all bacteria, yeasts, and moulds. High-microbial-count flour and/or the bakery environment, which may have contaminated the bread during the chilling process, are most likely to blame for the increased microbial counts of the bread samples [24].

Table 1. *Macroscopic features of isolated microorganisms.*

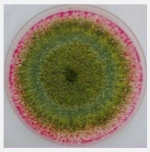
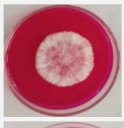
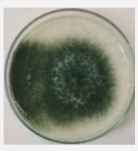
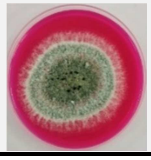
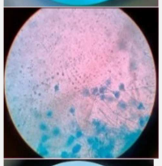
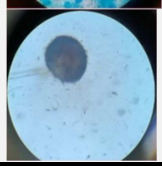
Isolates	Macroscopic features	Colony morphology on PDA	Colony morphology on RBA	Under micro lens (1.9X)
GS 1 (*Trichoderma harzianum*)	Colonies on PDA & RBA, are dark green with yellowish tint & cushion-shaped structure distributed.			
GS 12 (*Fusarium sp*)	Colonies on PDA and RBA are whitish, woolly to cottony, flat like appearance.			
GS 16 (*Trichoderma sp*)	Colonies on PDA & RBA, are dark green color with whitish tint & initially white in colour and later becoming green colour.			

Table 2. *Microscopic features of isolated microorganisms.*

Isolates	Microscopic features	Microscopic observation (40X)
GS 5 (*Rhizopus* sp)	Broad hyphae, sporangia are globose/spherical and directly connected to sporangiophores. Sporangiophores are branched.	
GS 7 (*Penicillium* sp)	Septate hyphae, Conidiophores are branched. Brush like clusters were observed at the end of the conidiophores. Conidia looks like round to ovoid.	
GS 2 *(A. niger*)	Filamentous hyphae, Conidial heads were radiated, walls were thick. Vesicles were globose. Conidia were brown.	

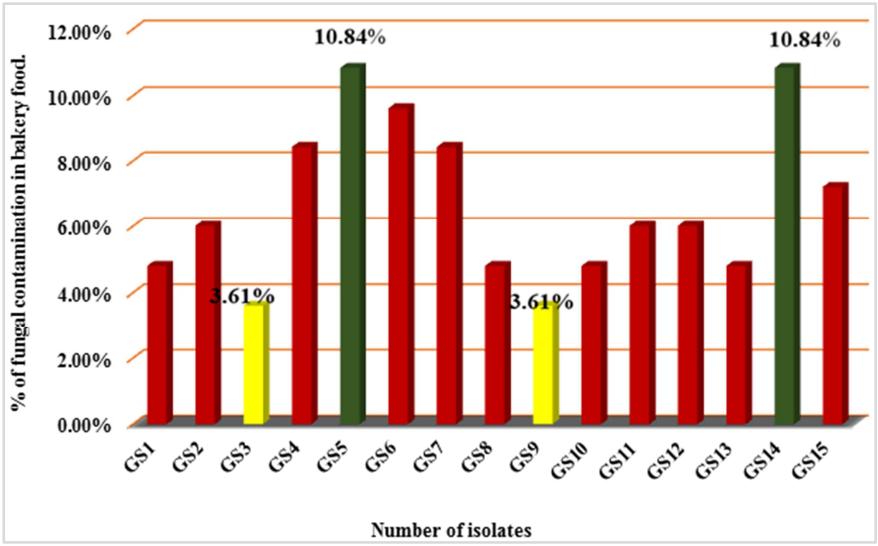


Figure 1. *Distribution of mycoflora in bakery food products.*

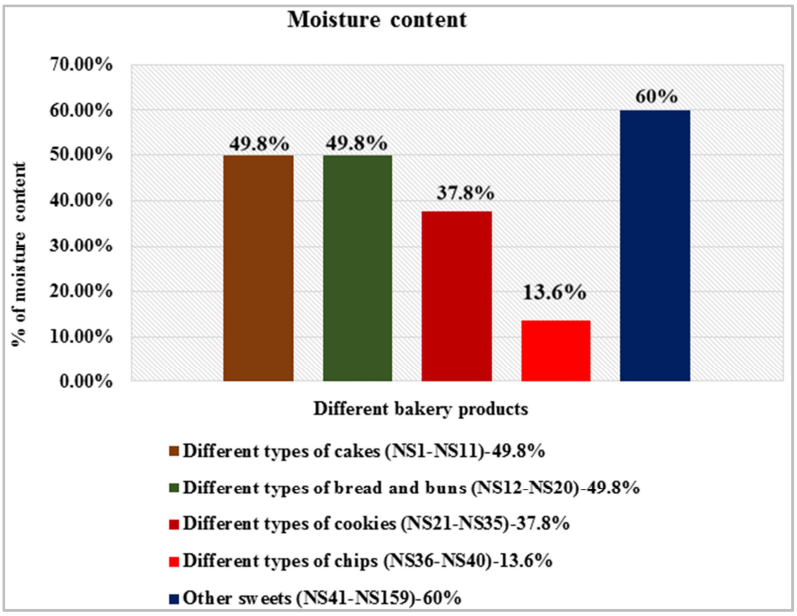


Figure 2. *Distribution of mycoflora in bakery food products.*

Table 3. *pH of Bakery samples.*

Sl. no.	Bakery products	pH
1	Different types of cakes (NS1-NS11)	5.25-5.50
2	Different types of bread and buns (NS12-NS20)	4.95-5.50
3	Different types of cookies (NS21-NS35)	3.10-4.30
4	Different types of chips (NS36-NS40)	4.50-5.50
5	Other sweets (NS41-NS159)	5.00-5.90

4. Conclusion

Fungi, however, have the ability to ruin the majority of baked products. It is unfortunate that fungus spoiling bread products has drawn increasing attention. The main elements influencing the fungi that harm bread products have been discovered, along with their sources in bakeries. Many high and intermediate moisture bread products' shelf lives are still severely impacted by mould deterioration. The baking companies have been losing money as a result of losses from mould deterioration.

Therefore, strategies to prevent mould growth and lengthen the shelf life of baked goods are crucial for the baking company, where there is a rising need for worldwide consumption. The best choices include additional efforts like maintaining high cleanliness in bakeries and, if necessary, further post-packing heat treatments or modified environment packaging.

The present study that *Trichoderma harzianum*, *A. niger*, *Rhizopus*, *A. flavus*, *Penicillium sp*, *Curvularia sp*, *A. parasiticus*, *Penicillium chrysogenum*, *Aspergillus sp*, *Fusarium sp*, *Trichoderma sp*, *Alternaria sp* and *Mucor sp* are the common genera of molds generally isolated from the bakery food products during the present investigation.

Author Contributions

Sowmya KL., collected the samples and conducted all the experiments in the Bioprocess and Fermentation Technology Department of Studies in Microbiology, Davangere University, Davangere under the guidance of Dr. Ramalingappa. B. Professor & Dean of studies in Science & Technology who critically reviewed the study and summarised the manuscript.

Conflict of Interest

The authors declare that there is no Conflict of Interest.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Acknowledgements

The work was carried out in the Bioprocess and Fermentation Technology Laboratory, Department of Studies in Microbiology, Davangere University, Davangere, Karnataka. Author is greatful to research supervisor, Department of Studies in Microbiology, Davangere University, Davangere, Karnataka, India for providing well equipped laboratory facility to carry out the present research work.

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