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IMPACT OF MICROORGANISMS ON BAKERY EQUIPMENT SURFACES ON THE MICROBIAL QUALITY AND SPOILAGE OF BAKED PRODUCTS

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Abstract:

Food quality and safety are critical public health concerns, particularly in the context of globalization and the increasing complexity of the food supply chain. Consumers are increasingly prioritizing both the nutritional value and safety of food to avoid health risks. Food safety aims to mitigate hazards such as microbial contamination, pesticide residues, misuse of food additives, and chemical or biological toxins. This study examines microbial contamination in bakery equipment and finished products across five locations in Chennai. Forty samples were collected, including swabs from equipment (e.g., cutting knives, baking ovens, trays) and finished products. Microbial analysis identified 47 bacterial, 7 fungal, and 6 yeast isolates. The research evaluated the impact of physical factors like moisture and temperature on microbial growth, which can lead to spoilage and foodborne illnesses. Ten bakery products were analyzed for moisture content and microbial contamination, revealing that most had moisture levels below 50%, potentially fostering microbial growth. Temperature experiments showed that room temperature and 37°C supported optimal microbial and fungal growth, while 40°C inhibited it. The findings underscore the need for stringent control of environmental conditions in

bakeries to prevent contamination and reduce foodborne risks. This study highlights the importance of addressing microbial hazards to ensure food safety and protect public health.

Keywords: *Food Safety, Bakery Products, Microbial Contamination, Bakery Equipment, Environmental Factors.*

Introduction:

Bakery products are staple foods globally, providing essential nutrients like carbohydrates, proteins, lipids, vitamins, and minerals. The Indian bakery industry is a significant food sector, second only to the USA in biscuit production, with bread and biscuits dominating the market. The industry has grown substantially due to urbanization and demand for convenient, affordable foods, with a growing emphasis on whole grains and natural ingredients.

Bakery products, including flour, cocoa powder, sugar, eggs, oil, nuts, dried fruit, and spices, are susceptible to mold contamination (Levic *et al.,* 2004; Suhr *et al.,* 2004; Pitt and Hocking, 2009; Kocic-Tanackov and Dimic, 2012). Carbohydrates, primarily starch, are the main component. While baking destroys fungal spores, post-processing contamination from air and surfaces is a risk. Products with high moisture content (aw > 0.8), like cakes, muffins, waffles, and tortillas, are particularly vulnerable to mold spoilage, especially by *Penicillium* and *Aspergillus* species (Cook and Johnson, 2009). Airborne contamination in food production environments is a major source of microorganisms, including pathogens (Wray, 2011). Post-bake handling can introduce fungal surface contamination, influenced by the degree of contamination, moisture, and storage (Brown and Wray, 2014).

Food spoilage, a metabolic process rendering food undesirable due to sensory changes, may not always be unsafe if pathogens or toxins are absent (Doyle, 2009; Montville *et al.,* 2008). Contaminated equipment and improper handling can introduce pathogenic microorganisms. A study by Adesetan *et al.,* (2013) found kitchen equipment contaminated with *S. aureus*, *B. subtilis*, and *B. cereus*, highlighting the importance of

hygiene. Saranraj and Geetha (2011) noted that while baked goods are sterile after baking, post-bake handling can lead to contamination.

Spoilage organisms, such as heat-resistant bacterial spores like *Bacillus subtilis*, *B. licheniformis*, and *B. pumilus*, can survive the baking process (Ijah *et al.,* 2014). Microorganisms isolated from bread include *Bacillus*, *Staphylococcus*, *Micrococcus*, *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor*. Water activity (aw) is crucial in microbial spoilage. Low-moisture products (aw < 0.6) are less susceptible. Intermediate moisture products (aw 0.65-0.85) are prone to osmophilic yeasts and molds (Hammond, J. R., & Bamforth, C. W., 1993).

Bread stored at ambient temperature and 43% RH has a 9-12 day mold-free shelf life, with *Penicillium* and *Aspergillus* being common contaminants at lower RH (Legan and Voysey, 1981). Post-processing contamination during cooling and packaging is a major factor. Contamination can also originate from food handlers and raw ingredients.

Microbial spoilage significantly limits shelf life, causing economic losses. Spoilage can be bacterial, yeast, or mold-related (Needham *et al.,* 2004). Bacterial spoilage can manifest as "rope" in bread caused by *Bacillus* species, characterized by discoloration, odor, and a stringy crumb (Hansen *et al.,* 1995; Bailey and Holy, 1993). *Staphylococcus aureus* can contaminate fillings (Seiler, 1984), and *Salmonella* can be found in ingredients like chocolate and coconut (Dickson, 2001). Yeast spoilage, caused by organisms like *Trichosporon variable*, *Saccharomyces*, *Pichia*, and *Zygosaccharomyces*, can result in visible growth or fermentative spoilage (Legan and Voysey, 1991). Mold spoilage, a major concern, is caused by various species like *Rhizopus*, *Aspergillus*, *Penicillium*, *Monilia*, *Mucor*, and *Eurotium* (Knight, R. A., & Menlove, E. M. (1961). Mycotoxins produced by molds like *Aspergillus*, *Penicillium*, and *Fusarium* pose a food safety threat (Qazi and Fayyaz, 2006; Madigan and Martinko, 2005). Foodborne infections and intoxications can be caused by bacteria like *Staphylococcus*, *Bacillus*, *Escherichia*, *Shigella*, *Clostridium*, and *Salmonella* (Madigan and Martinko, 2005; Sousa, 2008). Therefore, analyzing the microflora of bakery products is crucial to ensuring consumer safety.

Methodology:

Collection of samples:

Totally thirty samples were collected from five different places in Chennai *viz;*. the samples include various sources viz; bakery processing equipment's (Cutting Knife, Baking Oven, Tray, Dough machine, Cake stand and Slicer) and finished bakery products.

Different sources of bakery processing equipment were used in various stages of product manufacturing like dough machine for flour mixing, base of tray for baking, knives for cutting dough, oven for baking, slicer for slicing finished products and cake stand for decorating products. Samples from the above-mentioned equipment that are more prone to get exposed to a wide range of microbial contaminants were collected by swab technique. Sterile cotton swabs soaked in nutrient broth (for bacteria) and saline solution (for fungi) were swabbed uniformly over the surfaces and then transported to the lab under aseptic conditions for further incubation and processing.

Isolation of microorganisms from different bakery equipment and products:

Processing of sample:

The finished bakery products were collected. One gram from the bakery product samples were weighed and taken in sterile Petri dishes. The product samples were grounded and minced properly in a sterile mortar and pestle in order to obtain a uniform extract further processed along with the swabs from the equipment's.

Isolation of Bacterial Isolates:

A loopful of inoculum from the incubated nutrient broth was streaked onto the sterile nutrient agar plates. At the same time, a loopful of the prepared extract (from the finished bakery products) was taken and streaked onto Nutrient agar plates. Both the plates were

incubated at 37°C for 24 hrs. After incubation, the plates were observed for their colony morphology. Next day colonies were picked up, gram staining, catalase and oxidase tests were performed and they were identified as per the standard protocols.

Isolation of Fungal isolates:

Swabs from the saline solutions were directly streaked onto the Potato Dextrose agar plates and incubated at room temperature for 3-7 days. Meanwhile, the finished bakery products were weighed(1gm) and serially diluted and inoculated onto the Potato Dextrose agar plates amended with chloramphenicol antibiotic. The plates were then incubated at room temperature for 3-7 days. After incubation, the plates were observed for their colony morphology and were identified as per the standard protocols.

Isolation of yeast from bakery equipments and finished bakery products:

One gram from each sample was inoculated in Glucose Yeast Extract Broth (GYEB) and incubated at 37°C for 24 h. A loopful from the previous inoculated broth was subcultured onto Sabouraud dextrose agar (Oxoid) plates and incubated for 48 h at 37°C. Separate colony from each of the suspected yeasts was picked up and streaked onto SDA plate, incubated at 37°C for 48 h to get a pure culture and stored at 4°C for further examination and were identified as per the standard protocols.

Physical factors influencing the microbial growth:

Different physical factors were said to be influencing the microbial growth on Bakery Equipments and finished products leading to spoilage of the finished goods and results in food borne illness and food borne intoxications. Totally, ten finished products were taken for the study and the parameters were assessed and recorded. From the results, it was observed that almost all the finished goods were said to possess less than 50% moisture content stating

1. **Moisture content.**
2. **pH**

1. **Moisture content of the finished bakery products: -**

The moisture content of the finished bakery products was determined by drying the sample (initial weight 5 g) in the Hot air oven at 105^0C for 12 hours. The moisture content present in the sample was calculated using the standard formulae given below:

Moisture content (%) = Initial weight – weight of the oven sample

Initial weight of the sample × 100

Results were expressed in percentage (%).

Moisture content (%) = × 100 45%

2. PH of the bakery products: -

The PH of the finished products were determined by mixing 15 g of a bakery product in 100 ml of distilled water in sterile 250 ml borosilicate flask and shaking on magnetic stirrer for 15 to 20 minutes. The PH of the semi liquid mixture was recorded by using calibrated PH meter.

Results:

Totally forty samples were collected from bakeries of five different places in Chennai. The sources of samples include Swabs from bakery processing equipment's Cutting Knife, Baking Oven, Tray, Dough machine, Cake stand and Slicer and finished bakery products. Out of which, forty-seven bacterial, seven fungal and six yeast isolates were obtained.

Table 1 Prevalence of bacterial isolates in bakery equipment and finished products (n=47)

Bacterial Isolates	Percentage of organisms
Bacillus species	4 (8.5%)

Bacillus amyliquilsacien	1(2.12%)
Bacillus cereus	2(4.25%)
Bacillus subtilis	1(2.12%)
Cirobacter freundii	3 (6.38%)
Clostridium species	1(2.12%)
Coagulase negative	2(4.25%)
Edwardseilla	1(2.12%)
Eschercia coli	3 (6.38%)
Klebseilla species	3 (6.38%)
Lactobacillus species	5 (10.6%)
Listeria monocytogenes	1(2.12%)
Morganella morgana	1(2.12%)
Salmonella species	3 (6.38%)
Shigella species	6(12.7%
Staphylococcus aureus	8 (17%)
Proteus species	2(4.25%)

Isolation and enumeration of Fungi and Yeast from Bakery equipments and products:

The seven fungal isolates and six yeast isolates thus obtained from bakery equipment and products varied in their colony morphology indicating diversified groups of fungi and yeast. LactoPhenol Cotton Blue staining was done to identify fungal isolates, the fungal isolates were identified and characterized based on the spores, they produced whether it is sporangiospores and conidiospores, nature of hyphae as *Aspergillus niger, Aspergillus flavus* and Rhizopus.

Identification of Yeast isolates:

Totally six yeast isolates from Bakery equipment and finished bakery products were identified based on macroscopic, microscopic and biochemical identification as *Candida albicans and Saccharomyces cerevisiae.*

Moisture content and pH of the finished bakery products:

Table 9 Showing the moisture content and pH of the bakery finished products

S.No	Bakery product	PH	Moisture content	Baterial Isolates
1	Bun	5.41	32%	*Klebsiella*
2	Bread	5.30	43%	*Pseudomonas*
3	Biscuit	5.90	13%	*Bacillus subtilis*
4	Pastries	5.33	40%	*Pseudomonas*
5	Cake	6.72	37%	*Bacillus sp*
6	Yellow cake	6.59	48%	*Escherichia coli*
7	Bun	5.39	30%	*Pseudomonas*
8	Bread	5.31	45%	*Klebsiella*
9	Biscuit	6.45	21%	*Pseudomonas*
10	Pastries	6.87	42%	*Bacillus sp*

The data shows a clear correlation between the moisture content of bakery products and the types of microorganisms present. Products with higher moisture content, such as cakes and bread, tend to support the growth of a wider range of microorganisms, including pathogenic bacteria like *Klebsiella*, *Pseudomonas*, and *Escherichia coli*. On the other hand, products with lower moisture, such as biscuits, generally support fewer microbial isolates.

pH values do influence microbial growth, with slightly acidic pH being common in most bakery products. However, moisture content remains a more critical factor in determining microbial activity and spoilage potential in these products.

2. **Effect of temperature: -**

To determine the effect of temperature favouring the growth of microbial and fungal isolates on Bakery equipment and finished products, the culture isolates which were considered to be the causal organisms of food borne illness and food borne intoxications

were chosen. These culture isolates were streaked onto sterile nutrient agar plates and incubated under different temperatures (Room temperature, 37 and 40). From the results it was observed that room temperature and 37°C which is the optimum temperature, favours the growth of maximum number of the microbes leading to spoilage. Whereas, the microbes did not exhibit better growth at 40°C inferring that at that temperature the growth of the food borne pathogens were inhibited.

Table 10 Effect of temperature on bacterial isolates

Test bacteria	Room temperature	37°C	40°C
Eschercia coli	+	+	-
Staphylococcus aureus	+	+	-
Salmonella spp	+	+	-
Shigella spp	+	+	-
Listeria spp	+	+	-
Candida albicans	+	+	-

Effect of salt tolerance: -

The salt content is the initial nutrient for the growth of the microbes but at the same time, increase in concentration will inhibit their growth. Hence, different salt (NaCl) concentrations (0.5 %, 3%, 5%, 7%, 10%) were tested against the commonly isolated pathogens from bakery finished products. It was observed that, the majority of the isolates were able to tolerate the maximum salt concentration of 7%, but at the concentration of 10%, most of the isolates failed to grow, due to their intolerance except, *Staphylococcus aureus* and *yeast spp* .

Table 11 Effect of different salt concentration in bacterial isolates

Name of the isolate	0.5%	3%	5%	7%	10%

Escherichia coli	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	-
Salmonella spp	+	+	+	+	-
Shigella spp	+	+	+	+	-
Listeria spp	+	+	+	+	-
Candida albicans	+	+	+	+	-

Discussion:

Food quality and safety are paramount public health concerns, amplified by the globalization of food supply chains and their increasing complexity (Adams & Moss, 2000). Consumers prioritize both the nutritional and safety aspects of food, seeking products that pose no health risks. Food safety aims to eliminate health hazards, including microbial contamination, pesticide residues, food additive abuse, and chemical/biological toxins (Adams & Moss, 2000). Food quality, conversely, encompasses factors that enhance a product's value, from positive attributes like origin, flavor, and texture to negative ones such as spoilage and contamination. Microbial contamination of food products is a global concern. Bacterial growth and metabolism can cause foodborne infections and intoxications, leading to rapid spoilage.

Food products naturally harbor indigenous microflora, some of which may be spoilage-causing or pathogenic. These microbes can proliferate during manufacturing, affecting product shelf life. Most microbial contamination occurs during food processing and handling (Adams & Moss, 2000). Foodborne infections arise from consuming food contaminated with live microbes that then invade the body. Food can serve as a growth medium for microbes, and their metabolic byproducts can cause food poisoning. The

WHO reported 1.7 million deaths from diarrheal diseases linked to contaminated food in 2013. In developed countries, foodborne illnesses affect up to 30% of the population annually (Adams & Moss, 2000).

Foodborne intoxication results from consuming toxins produced by bacterial growth in food; in these cases, the toxins, not the microbes themselves, cause illness. These toxins may not alter the food's appearance, odor, or flavor (Adams & Moss, 2000). Bakery products are susceptible to microbial contamination, with bacteria being a significant concern. Spoilage-causing microbes depend on the product's initial microflora, and post-processing handling, packaging, and storage temperatures are critical factors (Aboloma, 2008). This has increased awareness of the need to assess bacterial populations associated with bakery product spoilage.

Bakery products are a significant part of a balanced diet, available in a wide variety, from unsweetened breads to sweet cookies. However, like other processed foods, they are subject to physical, chemical, and microbiological spoilage. While physical and chemical changes affect low- and intermediate-moisture products, microbiological spoilage by yeasts, bacteria, and molds is the primary concern in high-moisture products ($a_w > 0.85$). Food processing units can be sources of microbial contamination, leading to food poisoning, intoxication, and spoilage. Lapses in good manufacturing practices can drastically reduce product shelf life due to microbial contamination, causing significant economic losses (Bouarab *et al.*, 2018).

Bakery products have been implicated in foodborne illnesses involving *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus*, with *Clostridium* spp. being a concern in high-moisture products packaged under modified atmospheres. High-moisture bakery products are particularly vulnerable due to their ability to support the growth of diverse microbial populations. Several factors in bakeries increase the risk of contamination, including frequent food handling, the use of perishable ingredients and raw foods that may contain pathogens, and the potential for cross-contamination through equipment

reuse (e.g., piping bags) (Legan and Voysey, 1981). Many small independent bakeries may also lack sufficient knowledge of hygienic practices.

Microbial growth in food can lead to food intoxication, with symptoms appearing soon after consumption due to pre-formed toxins (Madigan & Martinko, 2005; Sousa, 2008). Key bacterial genera causing foodborne infections and intoxications include *Staphylococcus*, *Bacillus*, *Escherichia*, *Shigella*, *Clostridium*, and *Salmonella*. Studies have shown that bacterial isolates from bread are mainly *Bacillus* spp. (e.g., *B. subtilis*, *B. psychrophilus*, *B. megaterium*, *B. sphaericus*, *B. polymyxa*) and *Staphylococcus aureus* (Pundir & Jain, 2011). These findings are consistent with other studies that have isolated *B. subtilis*, *B. megaterium*, and *S. aureus* from bread (Ogundare & Adetuyi, 2003). *Bacillus* spp. (e.g., *B. subtilis*, *B. megaterium*, *B. licheniformis*) are known to cause rope spoilage in bread (Smith 1993; Guynot *et al.*, 2005). *Bacillus* spp. and *S. aureus* are major contaminants in bakery products during post-preparation handling, which can lead to food poisoning outbreaks due to enterotoxin production.

Bacillus spp. have also been isolated from flour and ropy bread, with raw materials and bakery equipment identified as contamination sources (Sorokoluva *et al.*, 2003). Toxigenic *B. cereus* has been isolated from Nigerian flour-based foods (Yusuf *et al.*, 1992). *S. aureus* is frequently isolated from equipment (66.7% occurrence), followed by *B. subtilis* (14.1%) and *B. cereus* (7.7%). *Bacillus* spp. (84.62%) and *Staphylococcus* spp. (76.92%) are commonly found in bakery products and equipment. *S. aureus* contamination often increases after baking, likely due to recontamination by food handlers, who are a major source of food contamination, although equipment surfaces can also be a source.

Staphylococci are found in various environments, and humans and animals are primary reservoirs. *S. aureus* is present in the nasal passages, throats, hair, and skin of a significant portion of healthy individuals. Poor hygiene practices and inadequate cleaning of equipment contribute to contamination. Ingredients can also be a source of *S. aureus*. *E.*

coli contamination in bakery products suggests unsanitary conditions and potential fecal contamination (Adams & Moss, 2000).

Fungal flora in bakery products varies. Breads often have the highest mold counts (Banwart, 2004), followed by cakes (Aneja, 2007), with lower counts in other products like biscuits and pastries (Pundir & Jain, 2011). *Aspergillus luchuensis* is found in various bakery products, indicating its ability to grow in diverse conditions. *Rhizopus stolonifer*, *Penicillium oxalicum*, *Alternaria tenuissima*, and *Mucor* spp. are also common. *Aspergillus flavus*, *A. terreus*, and *Scopulariopsis* have been isolated from bread. The higher mold count in bread may be due to its higher water activity, which favors microbial growth. Flour and the bakery environment can also contribute to contamination (Ogundare & Adetuyi, 2003). The acidic pH of bakery products (4.94-6.00) favors mold growth (Patriarca *et al.*, 2011). pH and moisture content are key factors influencing microbial growth (Patriarca *et al.*, 2011).

While baking destroys many microorganisms, post-baking contamination from air, equipment, and handlers is a risk (Anon., 2000). Good hygiene practices are crucial to prevent staphylococcal food poisoning, given the high prevalence of *S. aureus* carriage by humans. This study emphasizes that food handlers are a major source of *S. aureus* contamination, although equipment surfaces can also be a source. *S. aureus* and *Bacillus* spp. can be controlled through proper hygiene. Common microbial genera isolated from bakery products include *Staphylococcus*, *Escherichia*, *Bacillus*, *Aspergillus*, *Penicillium*, *Rhizopus*, and yeasts. These microorganisms can produce toxins that are harmful to humans and animals.

Conclusion:

Foodborne illnesses and pathogens are increasingly prevalent due to contamination during handling, harvesting, processing, and transportation. Spoilage significantly reduces the shelf life of many bakery products, causing substantial economic losses. Controlling microbial growth and extending shelf life are crucial for the baking industry, especially given increasing global demand. This study identified *Bacillus cereus*, *Bacillus*

subtilis, and *Staphylococcus aureus* as rapid spoilage agents in bakery equipment and products, with *S. aureus* being the most common. Other bacteria like *Klebsiella* and *Proteus*, along with molds such as *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus*, and some yeasts were also found.

Effective hygiene practices, cleaning and sterilization of equipment, pest control, and proper temperature management during storage and transport (including retail display at -18°C) are essential to minimize bacterial load and spoilage, thus reducing consumer health risks. Further research is needed to fully understand spoilage mechanisms and develop strategies to minimize product loss and health risks. Exploring natural preservatives is a promising avenue for controlling microbial growth in bakery products.

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