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Paper Title: FOOD MICROBIOLOGY

Module -25: Bakery & Egg Product Spoilage

(Theory and Self-learning)

1. Details of Module and its Structure

Module Detail	
Subject Name	**Food Technology**
Paper Name	**Food Microbiology**
Module Name/Title	**Bakery & Egg Product Spoilage**
Module Id	**<Module Id>**
Pre-requisites	**Microbiology**
Objectives	After reading this module, we will be able to • Common microbes responsible for bakery and egg product spoilage • Illness caused by bakery and egg product spoilage
Keywords	Bakery and egg product spoilage, bacteria, fungus, mold

Structure of Module / Syllabus of a module (Define Topic / Sub-topic of module)	
Bakery and Egg product spoilage	General properties of bakery products, Microbial Spoilage of bakery and egg products, Mold and Yeast Spoilage, Bacterial Spoilage, Factors influencing microbial growth, Diseases commonly caused by bakery and egg product spoilage, Control of microbial Growth in bakery and egg products

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Bakery and Egg Product Spoilage

1. Introduction

A large assortment of bakery products can be found on market shelves including pizza, cakes, pies, biscuits, breads, savoury, quiches etc. In India, the bakery industry has achieved third position in generating revenue among the processed food sector. The market size for the industry is pegged at US$ 4.7 billion in 2010 and is expected reach US$ 7.6 billion by 2015 (IFH, 2010). Consequently it can be seen as an important part of Indian food sector. Apart from bakery products, egg consumption and thus its production has grown at a compounded annual growth rate (CAGR) of over 8% owing to rising economic prosperity amid the rising middle class together with growth in overall population and demographics, relative cost advantage, evolution of fast-food chains leading to broad egg acceptability and with eggs being a high nutrition value, affordable and quality source of animal protein.

Eggs are also an important ingredient in bakery products and contribute unique functional properties to them. However eggs are the most common ingredients which affect the spoilage of bakery products and mishandling can introduce serious food safety risks. In addition to the economic losses incurred because of bakery spoilage, possible foodborne illness cost millions of rupees to the industry every year.

The spoilage includes physical, chemical and microbial spoilage. Since the most common element of bakery products is water activity, microbiological spoilage, in particular mold growth is the major contributing factor to spoilage of bakery products. Mould spoilage is a serious and costly problem for bakeries. Commercially produced and properly handled bread generally lacks sufficient amounts of moisture to slow growth of any microorganisms except molds. As normal cooking temperature destroy fungal spores, post-process contamination from airborne spores and contact with contamination surfaces must be prevented. Filamentous fungi involved in spoilage of bread include *Rhizopus* sp., and *Mucor* sp., *Penicillium* sp., *Eurotium* sp., *Aspergillus* sp. and *Monilia sitophilia*. One of the most common is *Rhizopus stolonifer*, often referred to as the ‘bread mold’. Storage of bread under conditions of low humidity retards mold growth. In addition to the economic losses associated with bakery products, another concern is the possibility of mycotoxins production. Losses of bakery products due to mould spoilage vary between 1-5% depending on seasons, type of products and methods of processing. Members of the genus *Bacillus* bring about bacterial spoilage

of bread known as rope. Ropiness which is the most common spoilage of bread and is mainly caused by *Bacillus subtilis* but *Bacillus licheniformis, Bacillus magaterium* and *Bacillus cereus* have also been associated with ropy bread.

Alteration in functional properties of eggs due to changes in chemical composition and physicochemical properties, which might have occurred during shell egg storing, pasteurizing, drying and freezing is reflected in the loss of functionality of albumen (foaming powder) and yolk (emulsifying ability). This ultimately results in low quality bakery product. The main function of eggs in sponge cakes are foaming and coagulating. Sponge cakes are low in fat and belong to intermediate moisture food products. The water activity is within the range of 0.65-0.85. The loss of egg functionality considerably adds to the microbial spoilage in this moisture range.

Besides microbiological matters staling and chemical (rancidity) stability is also considered an important factor in shelf-life of bakery products. Proper storage conditions, use of fresh, healthy ingredients and use of appropriate preservatives improve shelf-life and prevent spoilage of bakery and egg products.

Owing to huge economic as well as health burden the spoilage of bakery products have on Indian as well as global economy, it is important not only to understand the root causes of spoilage but also the measures that should be taken for its prevention and control.

2. Type of bakery products and general properties

A wide variety of bakery product exist, including leavened and unleavened breads, rolls, buns, croissants, English muffins, crumpets, cakes, pastries, waffles, pancackes, cupcackes and sweet rolls. Bakery products are an important source of nutrients *viz*., energy, protein, iron, calcium and several vitamins. Commercial bread and biscuits contain around 7.5 per cent to 7.8 per cent protein respectively. Bakery products are good targets for fiber enrichment. Fiber enrichment of several bakery products has recently been tested using an ingredient containing 95% short chain fructo-oligosaccharides. These soluble fibers are naturally found in many vegetables including wheat, rye, onion, Jerusalem artichoke and are structurally close to sucrose, therefore behaving like sugar regarding theology. These products are important constituent of a balanced healthy diet and convenience, taste and freshness determine to a great extent the appeal of bread products and are expected to remain the driving factors for purchases of bakery products.

These bakery products are typically baked at temperatures that are sufficient to destroy all bacteria, yeasts and molds in the product. Most baked products have a dry outer crust that prevents the

growth of bacteria that may recontaminate the products. However, typical pH values (between 5.4 and 7.5), high moisture content (water activity in the range of 0.75-0.98) and ambient storage temperatures act as favorable factors for the growth of spoilage microorganisms (Jay, 1996) and can result in substantial economic losses to wholesale bakeries.

3. Microbial Spoilage of bakery and egg products

Microbiological spoilage is often the major factor limiting the shelf life of bakery products. Spoilage from microbial growth causes economic loss for both manufacturers and consumer. These losses could be due to many individual cases such as, packaging, sanitary practice in manufacturing, storage conditions and product turnover. The microbial spoilage caused by bacteria, yeast and fungi and enzymic spoilage caused by lipoxygenase can be differentiated from one another and from unspoiled bread analogues after 48 hours using Cluster analysis, prior to signs of visible spoilage. Analysis of the bread analogues with gas chromatography mass spectrometry identifies volatiles produced by the different spoilage types and unspoiled bread analogues. Microbial analysis shows that the levels of each microorganism used increases with time (Rachel Needham *et al*. (2004)).

Table 1: Shelf life of bread and bakery food

Food Type	Room Temperature	Refrigerator	Freezer
Bread	5-7 days	1-2 weeks	3 months
Doughnuts	4-5 days	NA	3 months
Pasta	2 years	NA	NA
Pies and Pastries (baked)	NA	NA	1-2 months
Waffles	NA	4-5 days	1 month
Pizza	NA	3-4 days	1-2 months

3.1. Mold and Yeast Spoilage

Mold growth contributes far more to spoilage of bakery products than any other spoilage organism. Yeast is a much less prominent cause, but growth of either can cause surface spoilage. Mold spoilage is first evident as white, filamentous colonies which gradually turn from blue-green to black as spores are produced. On the other hand, surface yeast growth results in white or pink areas. Typically, untreated bread will become contaminated by mold within 5-6 days.

Mold spores are generally killed by the baking process in fresh bread and other baked products. Therefore, for bread to become moldy, it must be contaminated either from the air, bakery surfaces, equipment, food handlers or raw ingredients after baking during the cooling, slicing or wrapping operations. Moisture condensation on a product's surface, due to packaging prior to being completely cooled, may also be conductive to mold growth. Jarvis (2001) found that mold spoilage caused undesirable odors and is often found on the surface of the product. The most common molds found in bakery products are: *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Monilia* sp., *Mucor* sp. and *Eurotium* sp.

Mycotoxins are secondary metabolites produced during growth of many species of molds. Specific spoilage fungi of the genera *Aspergillus* and *Fusarium* produce mycotoxins when growing on food commodities. Aflatoxins produced by *Aspergillus flavus, A. parasiticus* and other closely related fungi are principal mycotoxins produced in grains and cereal products. Other fungal toxins including cyclopiazonic acid in peanuts, zearalenone from corn and ochratoxin from coffee and grains, can be of concern in particular ingredients (Robens and Richard, 1992). The health effects of these mycotoxins are compelex, some mycotoxins are vasoactive, others are carcinogenic, and some cause CNS damage (Sperber and Doyle, 2009).

Table 2: Fungi involved in spoilage of cereal products

Fungi	Type of food spoiled	Type of spoilage
Aspergillus	Bread, Grains	Black mold, Black mold rot (aflatoxin)
Candida	Breads	Yeasty
Cladosporium	Breads	Brown/Black mold rot
Claviceps purpura	Breads, Corn, Grain	Ear rot (ergotism), Black rot
Fusarium	corn	Pink mold rot (fumonisins)
Penicillium	Breads	Blue-green mold
Rhizopus	Breads	Black mold
Sacchromyces	Breads and Pastas	Yeasty
Zygosacchromyces	Breads and Pastas	Yeasty

3.2. Bacterial Spoilage

Bacteria also have a potential to contaminate baked products although their growth is more restricted by low water activity and low pH. The spores of *Bacillus subtilis* for example are heat resistant, 55 per cent remain active in amylase after 20 minutes at 65°C. This microorganism, which is present in raw ingredients, e.g., flour, sugar, and yeast, causes rope in bread. Ropiness can develop very rapidly under warm and humid conditions. So, it is a common problem in the warm climates of Mediterranean countries, Africa and Australia. Ropey bread is caused mainly by *Bacillus subtilis* but other species of *bacillus* are capable of causing rope and these include *Bacillus licheniformis, Bacillus megaterium* and *Bacillus cereus.* Prevention of rope problems require strict sanitary as well as good manufacturing practices designed to control the spores of Bacillus species. Preservatives, such as propionate, can be usually used to eliminate this problem.

Staphylococcus aureus is one type of bacteria known to contaminate pie fillings. This microorganism has also been implicated in food poisoning outbreaks from cream filled bakery products. Other bakery ingredients, such as chocolate, desiccated coconut and cocoa powder have been found to be contaminated with *Salmonella*.

Table 3: Bacteria involved in spoilage of bakery products

Bacteria	Type of food spoiled	Type of spoilage
Bacillus	Breads, Cakes, Pastries	Slime
Clostridium	Breads	Ropy
Lactobacillus	Breads	Ropy
Leuconostoc	Breads, Cakes	Ear rot (ergotism), Black rot
Stasphylococcus Aureus	Pies	Slime
Salmonella	Frozen Pizzas	Ropy

Gram negative bacteria are primarily responsible for spoilage of egg products (Board and Tranter, 1995). The type of spoilage or rots can be characterized by the color of the spoiled eggs (Table 4).

Table 4: Bacteria associated with various types of egg spoilage or rot

Spoilage bacteria	Type of spoilage
Proteus spp.	Black
Aeromonas liquefaciens	Black
Serratia marcescens	Red
Pseudomonas maltophilia	Green
Pseudomonas fluorescens	Pink
Flavobacterium cytophaga	Yellow
Enterobacter spp.	Custard

4. Factors influencing microbial growth

Effects of temperature, pH and water activity Physical factors are the important factor governing mold free shelf life of bakery products. It plays a decisive role when molds compete with bacteria to spoil high moisture foods. Molds tend to be less fastidious in their relationships to pH than bacteria. Generally, molds are tolerant of acid conditions and favor an acidic pH (3.5 - 5.5). Therefore, foods with pH value <4.5 are not usually spoiled by bacteria but are more susceptible to mold spoilage.

Table 5: Factors affecting the development of microorganisms in foods

Intrinsic Factors	Extrinsic factors	Implicit factors	Processing factors
Nutrient content	Temperature	Synergism	Irradiation
pH	Relative humidity	Antagonism	Washing
Redox potential	Gaseous atmosphere	Commensalism	Slicing
Water activity	Growth rate		Pasteurization
Antimicrobial constituents & barriers			Packaging

4.1. Nutrient content

Microorganisms need water, a source of carbon, an energy source, a source of nitrogen, minerals, vitamins and growth factors in order to grow and function normally. In general, molds have the

lowest requirement, followed by yeasts, gram-negative bacteria, and gram-positive bacteria. *Penicillium verrucosum* and *Aspergillus ochraceus* are considered as nutritionally dominant over the other species.

4.2. Water Activity (a_W)

Water activity of a food is the ratio between the vapor pressure of the food, when in a completely undisturbed balance with the surrounding air, and the vapor pressure of pure water under identical conditions. Water activity, in practice, is measured as Equilibrium Relative Humidity (ERH) and is given by the formula:

Water Activity (a_W) = ERH / 100

Water is often the major constituent in foods. Even relatively 'dry' foods like bread and cheese usually contain more than 35% water. Various applications of water activity include:

- Maintaining the chemical stability of foods,
- Minimizing non enzymatic browning reactions
- Spontaneous autocatalytic lipid oxidation reactions
- Prolonging the desired activity of enzymes
- Optimizing the physical properties of foods such as texture.

Most foods have a water activity in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Based on regulations, if a food has a water activity value of 0.85 or below, it is generally considered as non-hazardous. This is because below a water activity of 0.91, most bacteria including the pathogens such as *Clostridium botulinum* cannot grow. Different microbial group have different water activity requirement as shown in table below.

Table 6: Minimum water activity values of spoilage microorganisms

Microbial group	Minimum a_w	Examples
Most bacteria	**0.91**	***Salmonella spp., Clostridium botulinum***
Most yeasts	**0.88**	***Torulopsis spp.***
Most molds	**0.80**	***Aspergillus flavus***
Halophilic bacteria	**0.75**	***Wallemia sebi***
Xerophilic molds	**0.65**	***Aspergillus echinulatas***
Osmophilic yeasts	**0.60**	***Saccharomyces bisporus***

Water acts as an essential solvent that is needed for most biochemical reactions by the microorganisms.

4.3. pH and Temperature

The range of pH over which an organism grows is defined by three cardinal points:

- The minimum pH, below which the organism cannot grow,
- The maximum pH, above which the organism cannot grow,
- The optimum pH, at which the organism grows the best.

In general, bacteria grow faster in the pH range of 6.0- 8.0, yeasts 4.5-6.5 and filamentous fungi 3.5-6.8, with the exception of *lactobacilli* and acetic acid bacteria with optima between pH 5.0 and 6.0. Temperature plays a dominant role in mold growth and in the germination of spores. The majority of molds grow within a temperature range of 18.3-29.4 °C. When the temperature of bakery product is reduced from that for optimum temperature. Chamberlain (1993) reported that the reduction in the storage temperature from 27 °C to 21 °C doubled the mold free shelf life of cake and emphasized the need for care during distribution and storage.

Table 7: Approximate pH ranges of different microbial groups

Minimum	Minimum	Optimum	Maximum
Most Bacteria	4.5	6.5 – 7.5	9.0
Yeasts	1.5 – 3.5	4.0 – 6.5	4.0 – 6.5
Molds	1.5 – 3.5	4.5 – 6.8	8.0 – 11.0

5. Diseases commonly caused by bakery and egg product spoilage

The exposure of foods and damaged cells to the environment attracts microorganisms (e.g. bacteria, molds and virus) and insects, which in turn accelerate the decomposition of the food or food spoilage. Illness form food can be mainly classified as:

- Food borne infection caused by pathogenic bacteria (disease-causing microorganisms, such as *Salmonella* bacteria, multiplying in victim's digestive tract causing diarrhea, vomiting and fever, etc.)
- Food borne intoxication (food poisoning resulting from toxin produced by pathogenic microorganisms, e.g. *Clostridium botulinum* and *Staphylococcus aureus*, in the digestive tract).

Some of the principal micro-organisms and diseases of concern with baked and egg products include:

Salmonella

Salmonella may be introduced into bakery products through a range of ingredients including eggs. Other ingredients that can be a source of the organism include flour and chocolate. Although the organism does not grow in these foods, it can survive for a substantial time. In most reported outbreaks of salmonellosis caused by eating contaminated bakery products, eggs have been the suspected vehicle of transmission. To get around this, pasteurized egg rather than raw shell eggs are now used in bakeries. The bakery foods implicated in salmonellosis include custard, pies, bread pudding, custard-filled cakes and pastries, quiche, meringue, puddings and cheesecake.

Staphylococcus aureus

Dairy ingredients apart from human nasal passage, throat and skin can also be sources of this organism. The number of cases of *S. aureus* food poisoning associated with the poor handling and storage of custard or cream-filled bakery products, is seen as a problem in temperate countries where refrigeration is a problem. A period of growth is needed before toxin is produced, typically when the population reaches 100,000cfu/g. Other foods where *Staph. aureus* has been implicated include oatmeal raisin cookies, apple muffins, cream puffs and pizza. Important attributes of this bacterium which make it an issue with baked problems include its ability to grow at low (≤0.83) water activities and the toxin that is produced by it is heat stable.

Bacillus cereus

The spore former *Bacillus cereus* has been implicated in several outbreaks of foodborne illnesses involving bakery products. There is also some evidence that *B. subtilis* and *B. licheniformis* (responsible for 'rope' spoilage of bread) can cause foodborne illness. Species of Bacillus are commonly found in the environment. From there, they contaminate ingredients such as flour, milk, cream, spices, dried egg, yeast and improvers, dried fruits and cocoa. As *with S. aureus, B. cereus* is a toxin producer. It produces two toxin types (an 'emetic' type which is associated with cereal based foods and a 'diarrhoeal' type which is associated with proteinaceous foods). The emetic type is heat stable and so can survive a baking process, as can the spores of the organism. Outbreaks of Bacillus food poisoning have been associated with naan bread, crumpets, and cream and custard-filled pastries. *B. cereus* has also been isolated from meat pies, bread and pastry.

Clostridium botulinum

C. botulinum is another spore forming, toxin producing bacterium. It is able to grow in the absence of oxygen. The bacterium has caused food poisoning outbreaks where mortality rate has been high. Therefore it is regarded as an important pathogen. It has been found associated with agricultural and animal products, including dairy products such as cheese, fruits and vegetables, and honey. Two forms of this pathogen are known. A cold-tolerant ('psychrotrophic') form, which has been associated with chilled foods, and a 'mesophilic' (literally ambient temperatureliking) form, which has a potential for causing problems with MAP breads.

Listeria monocytogenes.

L. monocytogenes is a pathogen which is readily found in the environment, and is occasionally associated with bakery ingredients such as flour and dairy products.

Mycotoxigenic moulds

Mycotoxins are toxins which can be excreted into foods by moulds. Some of these can be carcinogenic, and many are very heat resistant. If moulds are prevented from growing on baked products and in ingredients, then mycotoxins are not an issue.

6. Control of microbial Growth in bakery and egg products

Several methods can be used to control mold growth on bakery products including reformulation, freezing, and most commonly, the use of preservatives.

6.1. Reformulation to reduce product a_w (available water)

Reformulation involves a reduction of available water e.g., a_w in bakery products to obtain a longer shelf life. Reduction in product a_w can be achieved by

- **By the addition of solutes or hydrophilic colloids.**
- **By Cooking**
- **By Drying and dehydration: (e.g., egg powder, pasta)**
- **By concentration (e.g. condensed milk) or freeze-drying**

The degree of a_w reduction is of practical significance in making a food non-perishable. The response to a given degree of aw varies greatly among microorganisms in different environments

6.2. Freezing

Freezing has been used for long term preservation of bakery products particularly, cream filled products. Quick freezing is important in controlling the formation of ice crystals. Large ice crystals are formed when the rate of freezing is slower; the large crystals can disrupt membranes and

internal cellular structures. Cakes, cookies, short cake, and pancakes are commonly frozen and marketed in the frozen form. Bread has been held fresh for many months by storage at -22°C. In contrast to fresh bread, which stales in less than a week, frozen bread stales very slowly. Therefore, the lower the temperature, the more slowly it stales.

6.3. Preservatives

Preservatives are most commonly used to control mold growth in baked goods. The Code of Federal Regulations (CFR) defines preservatives "as an antimicrobial agent used to preserve food by preventing growth of microorganisms and subsequent spoilage".

Two classifications of preservatives:

- Chemical
- Natural

6.3.1. Chemical Preservative

Sorbic acid (CH3-CH=CH-CH=CH-COOH) and its potassium salt, are recognized as effective antimold agents, and have been considered historically safe for food use. Sorbic acid and potassium sorbate are "Generally Regarded As Safe" (GRAS) for their use in foods. This acid or its potassium salts, has been used to retard microbial degradation in a large variety of food items. The levels of sorbate used in bakery products ranges from 0.001-0.3%. Major groups of foods in which sorbate has been used commercially because of its antimicrobial activity include bakery products such as cakes, cake mixes, pies, pie filling, doughnuts, etc (Marin et al., 2003).

Propionic acid, an aminocarbolic acid (CH_3CH_2-COOH), is a naturally occurring organic acid. This acid or its salt can be used to prevent the bacterial spoilage of bread known as rope caused by certain Bacillus sp. Concentrations of propionate ranging from 8 to 12 per cent are effective in controlling mold growth on the surface of bakery products. However, not all molds are equally sensitive to the inhibitory effect of propionate. For example, at 0.3 per cent calcium propionate, growth of *Monilia sitophila* and *Pencillium virdiicatum* in bread are inhibited for 2 days and 0.5 day respectively (Doores, 1993).

6.3.2. Biopreservatives

In recent years, bio-preservative (The use of microorganisms and their metabolites to prevent spoilage and to extend the shelf life of foods) has gained increasing interest due to consumer's demands. Lactic acid bacteria (LAB) as bio-preservation organisms are of particular interest due to

their antifungal (Hassan and Bullerman, 2008) as well as antibacterial activity. Effect of sourdoughs produced with *Lactobacillus plantarum, Lactobacillus alimentarus, Lactobacillus reuteri, Lactobacillus brevis* has been observed with antimicrobial activity and inhibition of rope forming bacillus strains and spore forming fungi (*Aspergillus niger, Fusarium culmorum* or *Penicillium expansum*) with different efficacy has been observed.

Use of good manufacturing practices and excellent sanitation programs are important to control spoilage of bakery and egg products. These programs minimize microbial contamination and growth for ingredients, components and finished product. Raw materials are carefully assessed as these can also be a source of contamination.

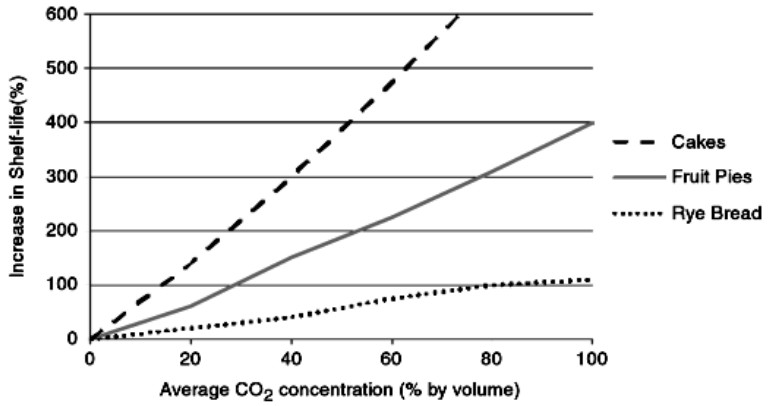

Fig. 1. Increase in mold free shelf life of various bakery products packaged in differing concentration of carbon dioxide. (Source Seiler, 2000)

Other manufacturing process such as modified atmosphere packaging (MAP) are also aimed at preventing contamination of finished products. Special, heat-resistant laminated films, packaging of products in an atmosphere rich in co_2, combining ethanol (for reducing water activity of the product) and co_2 during packaging, Vacuum packaging utilizing nitrogen and co_2 are being used in bakery industries.

7. Conclusion

Bakery products are influential contributors to country's industrial economy, moreover high nutritional value of these product is resulting in heightened consumer interest and more consumption. Microbial spoilage of these bakery products is still a major problem limiting the shelf life of many high and intermediate moisture bakery products. Losses due to microbial spoilage have been resulting in lost revenue to the baking industries. However these can be minimized by adopting strategies to prevent, destroy and also to control post baking contamination. Measures

such as good hygiene in the bakeries and if necessary complementary post packaging heat treatments or modified atmosphere packaging are some of the best alternatives.

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