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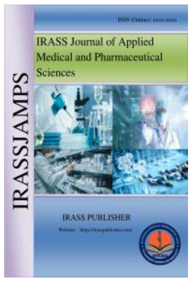

An Assessment of Potassium Bromide and Aspergillus Flavus Contamination in Bakery Products Sold in Bamenda

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Abstract: Bakery products especially bread are staple foods in the world and are recognized as semi- perishable foods. Usually, spoilage is due to improper storage. This work was aimed at isolating and identifying Potassium bromide and Aspergillus flavus contaminants in bakery products sold in Bamenda. The study was a cross-sectional study in which 25 samples of bakery products were conveniently purchased at random from different vendors and bakeries in Bamenda. The hypothesis of the study was prevalence of toxigenic fungi and potassium bromide in bakery products sold in Bamenda is high. The isolation and identification were done by their cultural and morphological characteristics by doing Lactophenol Cotton Blue staining of the various mould forms that grew on Saboraud Dextrose Agar and then examining under the microscope. The organisms found to be associated with spoilage of bakery products were strictly fungal organisms which included; Penicillium species (40%), Mucor species (26.67%), Aspergillus species (20%) and Fusarium species (13.33%). After analysing the samples, Penicillium species was found to be the most occurring in bakery products consistent with the study carried out by Legan J.D (1993), a clear prove that good hygienic handling of bakery products is essential, therefore, further investigation on isolation, identification and characterization with a larger sample size is very important.

Qualitative determination of Potassium bromide showed that 40% of the bread samples were positive for potassium bromide consistent with the study carried out by H.B Alhanashi et al (2020), a clear prove that Potasssium bromate is used as a dough improver in backery products in Bamenda, therefore further investigation with a quantitative analysis and on a larger sample size is important.

Keywords: Potassium bromide, bakery products, fungi contamination.

Introduction

1.1 BACKGROUND OF THE STUDY

In the majority of nations and civilizations, bakery goods are essential staple meals. Bread, buns, cupcakes, cookies, pizza bases, toasts, and many more are the most popular of these. The majority of our food calories and over half of our protein needs come from the grains used in baked goods, making them an important source of nutrients for our diet. Carbohydrates, proteins, fats, vitamins, calcium, iron, minerals, starch, and energy are the nutrients found in baked goods (V.S. Petil et al., 2020).

One of the most significant products of the food business is said to be baked foods. Bread is consumed every day all around the world and is a staple cuisine in many nations. Bread production is a relative mainstay in the European Union (E.U.) and has grown slowly in the majority of western nations. The UK and Ireland consume less than 50 kilograms of bread annually on average, whereas the Germans and Austrians consume the most, averaging 80 kg per person annually (The Federation of Bakers, 2012).

In contrast to Europe, the consumption of bakery products shows an increasing trend in most developing countries (Elsanhoty et al, 2013).

The majority of people in Cameroon rely heavily on baked goods to meet their nutritional needs. Because bakery products are ready to eat, whether they are hot or cold, they can be consumed right away at the point of sale without any additional processing. For this reason, products that are sent into the community to be sold should be carefully considered, and Hazard Analysis Critical Control Points (HACCP) handle this.Nowadays, this approach is regarded as a viable option for guaranteeing food safety. In order to prevent potential hazards, HACCP identifies Critical Control Points (CCPs) in the production process and puts monitoring procedures in place to stop them (Amaranta Carvajal Campos, 2019).

Consumer behavior in the baked goods industry has evolved during the last few decades. Much attention has been paid to consumers' concerns regarding food additives and safety in particular. Furthermore, there are now more consumers who are concerned about their health. As a result, there is a strong market demand for "natural" and "wholesome" foods free of chemical additives and preservatives. As a result, bread producers have been making more and more "clean label" goods to cater to consumers' better lifestyles. These goods' food labels make statements like "natural" and "no preservatives." Health-focused new product developments are actually being driven by marketing the lack of additives and preservatives (21% of new products introduced in Europe 2013/2014) and the presence of wholegrain. In turn, such minimally processed foods without chemical preservatives or other artificial additives should still be of high quality and have an extended shelf life (Mintel, 2014).

When Novozymes polled more than 4,000 bread consumers across Europe in 2011, they discovered that the primary reason bread was thrown out was because it had grown mold.Fungi are also in charge of producing mycotoxins and off-flavors, which may be created even before fungal proliferation is apparent, in addition to the unpleasant look of visible mold growth. Therefore, rotting bread poses a risk to the health of consumers (Magan et al, 2003b).

Spoiled bakery products may be defined as bakery products that have been damaged or injured so as to make it undesirable for human consumption (V. S Patil et al, 2020).

At several phases of bread preparation, including slicing and wrapping, the ingredients in bread promote the growth and multiplication of microbes. Moldiness and ropiness are the primary microbial deterioration kinds in bread that cause problems for bakers. A loaf of sliced bread is frequently the starting point for mold formation since the moisture content of the bread is higher than the surface, particularly in the creases (Salim-ur-Rehman et al, 2007).

When contaminated, other bread components, tools, and packaging materials may also serve as a specific entry point for unwanted and spoilage-related microbes into the baking environment. Following regulations such as Codex Alimentarius, which include putting in place systems like Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practices (GMPs), Good Hygienic Practices (GHPs), and Good Agricultural Practices (GAP), is crucial to ensuring food safety and quality throughout the food production chain (Reale et al., 2013).

Molds do not survive the bread-baking process in a typical bakery. On the other hand, because they are more heat stable, mycotoxins made by food-spoilage fungi and crop diseases pose a greater threat. According to Claudia Axel (2015), mycotxins have the potential to be immunosuppressive, nephrotoxic, neurotoxic, and/or carcinogenic, resulting in serious health issues.

While monitoring takes place in most countries, mycotoxin levels from fungal contamination in cereals and cereal products continue to be a major global concern. They are often found in processed cereal products (Aldana et al, 2014; Claudia Axel, 2015).

Physical techniques to eliminate post-baking pollutants in breads include ultraviolet light, infrared radiation, microwave heating, and ultra-high pressure treatment. Nevertheless, there haven't been many previous investigations using these techniques. Studies have increasingly established goals to replace conventional chemical preservatives with environmentally acceptable, "clean-label" alternatives in light of the ongoing consumer movement towards a healthier lifestyle. As a result, "bio preservation," which is the process of extending shelf life through the use of natural or regulated microbiota and/or their antimicrobial chemicals, has grown in importance as a research area (Stiles M.E, 1996).

Filamentous fungi including Alternaria, Aureobasidium, Cladasporium, Clavicceps, Epicoccum, Fusarium, and Helminthosporium are examples of common phytogenic diseases; infections with Fusarium are thought to pose a serious risk. According to Claudia Axel (2015), the most prevalent fungi linked to losses in baked goods are Aspergillus, Cladosporium, Endomyces, Fusarium, Monilia, Mucor, Pencillium, and Rhizopus.

The Use of Potassium Bromate in Baking

In baking, potassium bromated is commonly used as a flour enhancer in the United States. It helps the dough rise higher and strengthens it. As an oxidizing agent, it will entirely react to a form with a lower oxidation state when the bread is baked under the correct conditions. A flour ingredient called potassium bromated gives the dough strength and raises its altitude. Additionally, it adds a pleasing white hue to the final bread. However, this additive is associated with cancer, which is an issue (Viswanath Pilla, 2016).

In conclusion, it is important that all major players in the bakery industry should be aware of the risks posed by bakery products contamination as it relates to consumption of fungi toxins produced on contaminated products. Reports of loss of lives due to the activity of toxigenic fungus abound in the literatures and efforts should be made to reduce the risks. Therefore, there is need for systematic and universally applicable approach to food safety (Jay, 1996).

Also, it is important that the use of potassium bromate which is now regarded as an adulterant, which is an oxidising agent and a low- cost dough improver should be strictly monitored in the bakery industry since higher levels of the substance in humans can cause detrimental issues of the respiratory tract (Shanmugavel et al, 2020).

Statement of the Problem

The most common food pollutants worldwide are fungi. They are ubiquitous plant pathogens and major spoilage agents of food. Microbial contamination of bread may be as a result of unhygienic handling during production or the integrity of the raw material; unknown to the consumers, people that consume contaminated snacks may stand a risk of food borne illnesses and this may put a terrible economic burden on them, the society or country (WHO, 2000).

Snacks that are being sold may as well become compromised hygienically if safety regulations are not taken seriously during marketing, this exposes them to microbial contamination by fungi, bacteria, nematodes and many others (Johnathan et al, 2015, 2016).

Invasion of food by various fungi may result in many results in remarkable rapid quality deterioration and consequently leading to food spoilage and sometimes production of secondary metabolites by the contaminants. Some moulds such as Aspergillus flavus,Aspergillus parasiticus and Penicilliium species have been associated with production of aflatoxins which have serious health effects (Johnathan et al,2016).
Aflatoxins are capable of causing acute and chronic effects in man and animals ranging from disorders of central nervous system, cardiovascular systems, pulmonary systems, intestinal systems and even death (Johnathan et al, 2016).

Also, not much attention has been given to fungi contamination in bakery foods like bread, cookies, cakes, etc as it has been done on other foods. Therefore, this study is to detect, isolate and identify the possible fungi that cause spoilage and contamination in bakery products. Due to nature of these food processing and also the ingredients needed in preparing the bakery products, microorganisms like fungi are paramount to breeding microorganisms in baked foods (Johnathan et al, 2015).

The Federal Ministry of Health prohibited potassium bromate in 1993 after a research into the health risks of the ingredient in bread revealed the following negative impacts on consumers. Potassium bromate has been shown to cause thyroid follicular cell tumors, renal cell tumors, and peritoneal mesotheliomas. Furthermore, studies designed to clarify the mechanism of carcinogenesis have demonstrated that potassium bromated is a full carcinogen, having the ability to both initiate and promote kidney tumorigenesis in rats. This implies that potassium bromide poisoning is a risk that consumers face. There is therefore the need for continuous surveillance and enforcement of the ban on the use of potassium bromated in the bakery industry (A. I Abubakar et al, 2017).

Research Questions

1. What are the possible fungal species in bakery products sold in Bamenda?
2. What is the prevalence of fungal species in bakery products sold in Bamenda?
3. What is the proportion of bakery products contain potassium bromide?

1.4.1 Main Objectives

To isolate and identify the different fungal contaminants and potassium bromide in bakery products sold in Bamenda.

1.4.2 Specific Objectives

1. To isolate and identify the prevalence of Aspergillus flavus contamination in bakery products sold in Bamenda.
2. To determine the prevalence of potassium bromide toxicity in bakery products sold in Bamenda.

1.5 Hypothesis/assumption

The bakery products in Bamenda contain potassium bromide and pathogenic fungi.

1.6 Significance of the Study

- This study is to ensure that bakery products free of fungal and potassium bromide contaminants should be sold to the population of Bamenda to reduce the prevalence of fungal food poisoning.
- Also, it is aimed at creating awareness of proper hygiene for bakers during preparation, packaging and storage and proper handling by the consumers after buying the products.

Scope of the Study (Delimitation)

The study was limited only to bakery products in Bamenda city which in Bamenda 1, 2 and 3 municilities.

Limitation of the Study

- The limitation to examination of end-product which is the bakery products and not the raw materials.
- Also limited was the time period which was the same time for preparation for exams.
- Also, as a limitation was the study area which was limited only to Bamenda 1, 2 1nd 3, this is not the whole region of the North West.

Literature Review

2.1 Composition of Bakery Products and its

Requirements

Water, sugar, salt, eggs, fats, yeasts, baking soda, baking powder, yogurt, essence, cocoa powder, chocolate slabs, fruits, jams, and sweeteners are all necessary materials for bakeries. Starch, sugar chains, and proteins—that is, amino acids—make up the majority of wheat flour. (V. S. Patil and others, 2020).

2.2 Requirements

Fresh whole grain four, sourdough, mixing bowl, bench knife, razor blade, bread knife, thermometer, scale, proofing basket, loaf pan (Diakonov,1999).

2.3 The Importance of Bakery Products

Since baked foods, particularly breads, are a widespread and significant food from an economic and cultural standpoint, baking is still a fundamental skill and has nutritional value.(Figoni Paula, 2011)
There are a vast array of varieties, shapes, sizes, and textures available worldwide as a result of the nearly endless combinations of various flours and varying ingredient proportions. Numerous techniques, ranging from the use of naturally occurring microorganisms to high pressure artificial aeration during preparation and baking, can be used to leaven (aerate) or leave it unleavened. Among the many additives that can be used are fruits, nuts, various lipids, and chemical additives that improve flavor, texture, color, and shelf life. Bread can be consumed as a snack, served in a variety of ways during meals throughout the day, or even utilized as a component in other recipes. Bread, a staple item around the world, has gained importance beyond its nutritional value and is now a part of religious ceremonies, secular culture, and language (Chevan et al., 1993).

2.4 Nutritional Values of Bakery Products

- ✓ **ENERGY**

Rida Safdar in 2019 indicated that Bakery products provide energy for daily leaving. One slice of Pat`s pan provides around 100kcal which is 5%of your recommended intake or RI (Reference intake) for a day which is 2000kcal).

✓ Protein

Bread is the third world largest contributor of proteins in our daily diet. Protein is essential for growth, development and repair of the body.

✓ Fats

Bread is naturally low in fats and form part of a healthy, balance diet.

✓ Carbohydrates

Bakery products produce complex carbohydrates which provide energy.

✓ Vitamins

Bakery products various B vitamins including Thiamine(B1) and Niacin(B3) which are essential for releasing energy from food.

✓ Iron

Iron is the key nutrient in wheat flour and essential for red blood cell formation which aids in oxygen transport around the body and is important for brain formation.

✓ Calcium

Flour is fortified with calcium in the form of calcium carbonate and calcium is known for its importance in formation of good teeth and strong bones, proper functioning of nerves, muscles, kidney and heart.

2.5 Nutritional Facts

The following nutrition information is provided by the United States Department of Agriculture (USDA) for one slice (32g) of whole wheat bread:

Calories________82g
Fat___________1.1g
Sodium________144g
Carbohydrates__13.8g
Fiber__________1.9g
Sugars_________1.4g
Protein________4g

Bread nutrition varies by type. Nutrition experts recommend increasing your intake of whole grains. For example, the nutritional value of bread doubles that of sandwich or toast. Commercially prepared white bread provides about 75 calories and 1g of fats per slice, 15g of carbohydrates or more, but with less than 2g of fibre. Rye bread may or may not be made from whole grains depending on the brand. A typical slice of rye bread provides 83 calories, about 1g of fats, 16g of carbohydrates, and 1.9g of fibre and 2.7g of proteins (J. N Morris, 1981).

2.6 Role of Essential Ingredients in Baking

✓ Yeast

The key ingredient in the bread-making process is yeast. It is the key component that causes the dough to rise and lends the scent and delicious flavor of homemade bread.Each packet of yeast contains thousands of active microorganisms that resemble plants. Tiny carbon dioxide gas bubbles are released by the yeast when it is fed sugar or starch and activated by a warm liquid. After baking, this gas gives the dough its light texture and causes it to rise.

✓ Flour

The most popular kind of flour for baking bread is wheat. It contains whole wheat flour, bread flour, and all-purpose flour. Gluten, a protein found in wheat, provides dough its strength and elasticity. The gluten stretches and develops into a network that captures the carbon dioxide bubbles that the yeast produces when flour and yeast are combined with liquid and then kneaded or pounded.

✓ Liquids

Water

It performs two vital functions, making it the most significant liquid.

- The yeast is dissolved and activated by it.
- It combines with the flour to form a dough that is elastic and sticky.

Juice, cream, buttermilk, or milk may be added to improve texture or flavor. In a recipe, only warm liquids should be put to dry ingredients because

- Yeast activity will be slowed down or stopped by an excessively cold liquid.
- A beverage that is too hot can kill the yeast and stop it from rising.

Sweetener

Sugar gives a bread's crust flavor and a deep brown hue. You can also use jams, molasses, honey, and brown sugar.

✓ Salt

When baking bread, salt is a crucial component since it slows the rising period, which develops the dough's flavor and enhances the flavor of the finished product.

✓ Eggs

Eggs give breads their color, flavor, and nutritional value. They also aid in making the crust soft and the crumb fine. Eggs provide protein and richness.

✓ Fats

Bread becomes soft and moist with the addition of butter, margarine, shortening, or oil. Bread stays fresher for longer because fat prevents moisture loss.

Potassium Bromate (KBrO3)

Potassium bromate has a major impact on food biomolecules like protein and starch because it changes the degree of gelatinization, viscosity, and swelling properties of both gluten and protein. It also eliminates the sulfhydryl group and causes disulfide linkages to form, which enhances the qualities of bread. Nonetheless, numerous investigations clarify its detrimental effects on human health. Because of its strong oxidizing qualities and mutageneity in vivo, it is categorized under the class 2B category and considered a possible human carcinogen. In vivo, it produces mild effects on the kidney, liver, and brain. In 2020, Shanmugaval et al.

In fact, the Food and Drug Administration states that there is insufficient proof of potassium bromate's harmful effects in humans, enabling additives to be used in bread baking at levels no higher than 75 parts per million (117). Therefore, regardless of whether potassium bromate has been used as a wheat processing additive, bromated levels in bread should be consistently and accurately checked (112).

Ideally therefore, the end product should contain no potassium bromate which has been broken down during the baking process into potassium bromide (KBr), a harmless byproduct (3).

2.7 Chemical Compisition of Bread

According to P. Saranraj (2011), bakery goods have a high starch and simple saccharide content, which makes them high in carbs. Later carbohydrate levels are influenced by the amount of sugar added during the production process as well as the chemical makeup of the flour. As a result, rye bread, which isn't enhanced with additional carbohydrates, typically has fewer sugars than wheat bakery goods. In addition to fruits and vegetables, bakery goods are a significant source of dietary fiber. Its amount ranges from 0.3 to 1.5g/100g of fresh bread, depending on the type of flour. As a result, whole meal bread has higher dietary fiber content. Although the latter is not broken down in the human digestive system, it has a beneficial effect on intestinal peristalsis and makes it possible to remove toxic metabolites. Cellulose and hemicelluloses are main fractions of dietary fibre in wheat bakery products while in rye bread, it embraces pectin, gums, and mucus substances.

Other nutrient concentration is also influenced by the types of flour and the amount of dietary fiber. Compared to white bread, whole grain and rye breads have two to five times the amount of iron, magnesium, manganese, copper, and zinc salts.

Fresh bread's protein content ranges from 4.5 to 8.0g/100g. Rye breads provide less protein than wheat bakery goods, however rye proteins are more useful due to their higher concentrations of key amino acids. The amount of fat in baked goods is minimal, ranging from 0.7% to 2.5%. Bread can consequently be stored for a considerable amount of time. B-complex vitamins, including thiamine (vitamin B1), niacin, riboflavin, folic acid, and vitamin E, are abundant in bakery products.

Wholemeal breads, especially yeast-fermented wheat bread, have higher concentrations of essential nutrients. Rye bread has a 50% humidity percentage, whereas wheat breads have a 45% humidity content (P. Saranraj, 2011).

Bakery goods range in energy value from 874 to 1924 KJ, or 208–459 kcal/100g of fresh goods, with the exception of pumpernickel and crisp breads, which have higher energy values and higher nutrient contents.

It is estimated that bakery products satisfy about 50% of nutritional needs of humans and around 30% of energy needs (P. Saranraj,2011).

2.8 Sources of Contamination

Ponte and Tsen, in 1978 realized that Although freshly baked products are free of viable vegetative moulds and mould spores, products soon become contaminated as a result of post baking contamination by mould spores from the air, bakery surfaces and equipment, food handlers and raw ingredients such as glazes, nuts, spices, and sugars. After baking, mold spores that are present in the air around loaves throughout cooling, slicing, packaging, and storage contaminate the bread. Because dry components, particularly flour, contain mold spores and flour dust travels rapidly through the air, the environment inside a bakery is not sterile.

The naturally high spore content of flour, old bread deposits, polluted air conditioning systems, etc., are the causes of extremely high levels of air contamination. Spores are dispersed throughout all manufacturing and storage areas, especially by air conditioning systems. As a result, the baked goods get significantly contaminated with fungus spores. Reiss (1981).

Ponte et al, in 1978 discovered that Industrially produced bakery products emerge from baking process with surface that is essentially sterile but post bake handling can quickly lead to fungal contamination as a result of exposure to airborne contaminants as well as equipment contact as follows;

- Slicing machine
- Bread coolers
- Conveyor belts and racks have been identified as potential sources.

2.9 Microbial Spoilage of Bakery Products

Within the European union (EU) the production of bread is relatively stable showing low growth in most western countries. (Elsanhoty et al, 2013).

However, bakery goods are susceptible to microbiological, chemical, and physical deterioration, just like a lot of processed meals. At several phases of bread preparation, including slicing and wrapping, the ingredients in bread promote the growth and multiplication of microbes. Moldiness and ropiness are the two primary microbial bread deterioration kinds that cause problems for bakers. Since moisture is more readily available within a loaf of sliced bread than on the outside, particularly in the crease, mold development frequently starts there. (Axel, Claudia, 2019).

The main cause of bakery items' short shelf lives is frequently microbial spoiling. Both manufacturers and customers suffer financial losses as a result of microbial growth-induced spoilage. These losses may result from a variety of specific factors, including product turnover, storage conditions, hygienic manufacturing practices, and packaging. (Needham Rachel et al., 2004).

Products from bakeries, particularly bread, have an intermediate moisture level and are highly perishable. Microbial spoiling and moisture loss are the two most prevalent types of bread deterioration. More than 90% of all microbial contamination occurs when fungus colonize and thrive on wheat bread and other bakery products (Arroyo et al., 2008).

Moreover, during bread preparation such as baking, only vegetative forms of micro-organisms are removed, but bacteria and fungi spores are to survive.

Due to its widespread consumption, bread quality is important. Due to unfavorable changes that start shortly after baking and degrade the crumb's texture and sensory qualities, bakery goods have a short shelf life. Reduced humidity, the development of filamentous fungus and yeast, and stalling are signs that bread is getting older (Claudia Axel, 2009).

Molds, yeasts, and bacteria can all lead to bread spoilage. However, fungal spores that are deposited from the baking environment are the main source of contamination after baking (Claudia Axel, 2015).

The most important variables influencing the growth of unwanted fungi on food items are water activity (aw), pH, temperature, and oxygen.At a pH of roughly 6, bread typically has a reasonably high moisture content and water activity between 0.94 and 0.97. Sliced, packaged, and wrapped bread are among the bakery goods most vulnerable to mold deterioration (Magan et al, 2003a, Claudia Axel, 2015).

2.9.1 Mould Spoilage

For bakers, mold deterioration is a major and expensive issue. Rhizopus species, Mucor species, Penicillium species, Eurotium species, Aspergillus species, and Monilia sitophilia are filamentous fungus that cause bread to deteriorate. Bread spoilage in low humidity conditions inhibits the growth of mold. However, the most frequent cause of spoiling in dried grains that may grow at 0.75aW is fungi such Eurotium species (Cisarova et al, 2018).

In fresh bread and other baked goods, the baking process usually eliminates mold spores. Bakery items must therefore be contaminated—either by the air, bakery surfaces, equipment, food handlers, or raw ingredients—or after baking, during the chilling, slicing, or packaging processes, in order to develop mold. This indicates that all mold-related spoiling issues arise after baking. Because of airborne contamination brought on by warmer temperatures and more humid storage conditions, fugal spore counts are higher in the summer than in the winter. The product's surface is frequently affected by fungal spoiling, which produces an unwanted odor (V. S. Patil et al, 2020).

Various mycotoxins, or poisons, are also produced by molds and fungus and have been scientifically connected to a wide range of illnesses, such as diabetes, cancer, damage to internal organs, etc. Large amounts of mycotoxins that cause illness and death can be produced by even tiny amounts of these fungus, which can grow rapidly inside the body. Water activity is therefore the most important element influencing the kind and rate of spoiling in various bread items (Ambreen Akhtar Saddozai and Samina Khalil, 2009).

Microorganisms (bacteria, yeast and moulds) can grow well when water activity and moisture content is high. Some strains cause a defect called ropiness, a soft sticky texture caused by starch degradation and slimy exopolysaccharide often accompanied by a fruity odour.

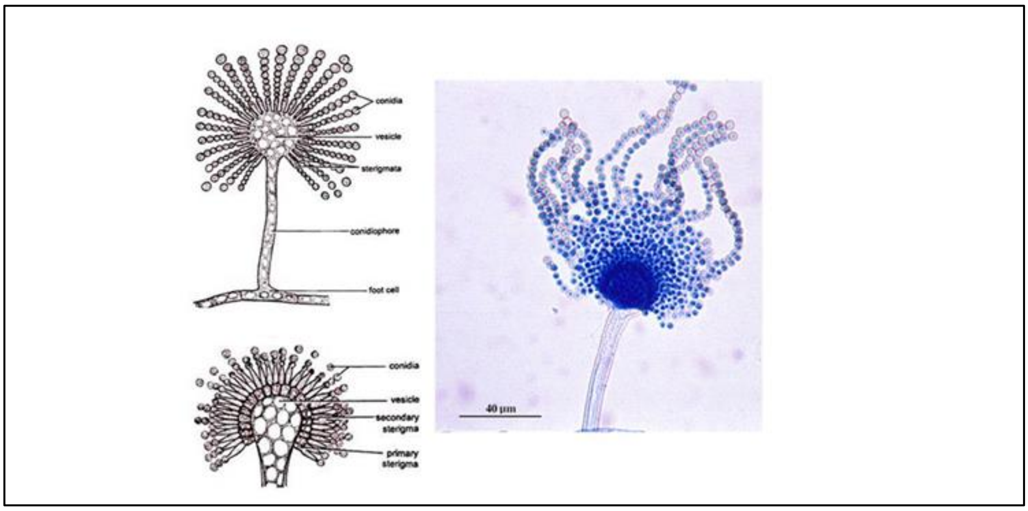
Furthermore, depending on the product type, season, and processing method, product losses from mold deterioration range from 1 to 5%. Hickey (1998) states that the bakery business loses an average of 200 million pounds of goods annually as a result of mold deterioration (P. Saranraj M. Geetha, 2012).

2.9.2 Mycotoxins

Because mycotoxin-contaminated staples have harmful effects on both humans and vertebrates, they pose a serious public health issue. Since their discovery, the main mycotoxins have been identified based on geographic locations, the lowest toxicity levels, the fungi that produce them, and the need to develop control measures to prevent negative effects on both human and animal health as well as to minimize financial losses. It is rarely advised to remove staples from the food chain after the mycotoxin content surpasses the threshold allowed by the laws (Amaranta Carnaval Campos, 2019).

One of the most commercially significant groupings of molds is Aspergillus section flavi; its harmful effects are a major public health concern, and the taxonomy's stability is a practical one.The species in this region are capable of producing a variety of mycotoxins, but aflatoxins are particularly harmful due to their detrimental effects on vertebrates (Amaranta Carnaval Campos, 2019).

Figure 1: Structure of Aspergillum

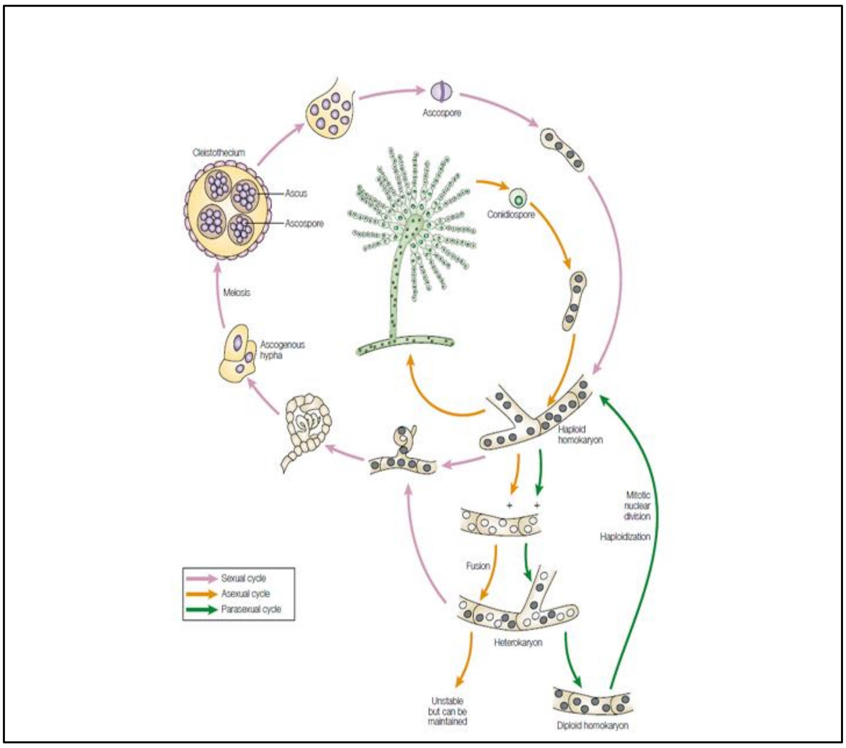


(*Amaranta Carnaval Campos*, 2019)

These species primarily flourish in tropical and subtropical climates across the world due to their physiological requirements. They are an issue in these regions because the conditions for harvest and storage are not always the best for the growth of mold and the creation of mycotoxin, and environmental factors also typically play a role in their production. resulting in two major problems: first, the health risks to humans and animals; second, contaminated staples cannot be exported, which has a detrimental impact on the economics of some nations that rely on exporting (Perrone et al, 2014).

Best known mycotoxins include aflatoxins, ergot alkaloids, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone.

Figure 2: Reproductive Life Cycle of Aspergillus Species



(Amaranta Carnaval Campos, 2019)

Table 1: Typical Sources, Impacts, and Principal Mycotoxin and Generating Species (AFSSA, 2009). The main Manufacturers of Mycotoxins are Indicated in Red.

Mycotoxin	Type	Main producer	Contaminated products	effects	Chemical nature
Aflatoxins	B1, B1, G1, G2	Flavus Apergillus A. parasiticus A. namius A. section flavi contains several species.	Cereals, rice, sorghum, wheat, corn, coconut, pistachios, almonds, spices, nuts, and dried fruit	Acute toxicity, hepatotoxic, carnogenic, immunogenic, and teratogenic	Polyketide
Trichotecenes	T-2 toxin, HT-2	F. langsethiae and F. triciniae F. F.sporotrichioides F. equiseti F. poae	Cereals, maize, wheat, rice, sorghum	Genetoxic, Imminotoxic, Reprotoxic, neurotoxic	Terpene
	deoxyrivalenol	Fusarilum tricinctum, F. langsethiae, F. culmarium, F.sporotrichoides, F. poae, F. equiseti, and F. solani	Sorghum, rice, wheat, and cereals	Haematopoietic, digestive, and imminotoxic	terpene
Fumonisis	B1, B2, B3	F. verticillioides, F. proliferation	Cereals, maize, rice, sorghum	Carcinogenic, neurotoxic	Poliketide
Ochratoxin A		A. ochraceus, P. nardicum,	Coffee, wine, grape juice, cereals,	Nephrotoxic,	polyketide

		A. carbonarius, and Penicillium verrocosum	chocolate, and spices	Immunotoxic, teratogenic	
Zearalenone	F-2 toxin	F. graminearum, F. culmarum, F. crookwellense	Cereals, wheat, rice, oats, maize, soy, and sorghum	Reprotoxic, immunotoxic	Polyketide
Patulin		P. expansum, Byssochlamys nivea	Juices from apples, pears, and derivatives	Neurotoxic, Genotoxic, Cytotoxic.	polyketide
Ergot alkaloids		Claviceps purpurea, C. paspali, C. africana, C. fusiformis	Rye, wheat, triticale	Vasoconstriction, digestive issues, and neurotoxicity	Alkaloids

Mycotoxins are categorized according to their chemical makeup, manner of action, and/or the fungus that produces them. Since the majority of them are heat stable and produce hazardous chemicals when degradation techniques are used, it is difficult to degrade them. With the exception of fumonis, these substances are often hydrophobic, which enables them to build up in plant and animal lipophylic tissues (Amaranta Caarnaval Campos, 2019).

The chemical composition of the mycotoxin, the length and dosage of exposure, the organism that consumes the microbe (species, sex, age, health, and nutrition), and the interaction of the mycotoxin's effects with other xenobiotics are some of the interrelated factors that affect mycotoxicosis symptoms. Mutagenic, teratogenic, carcinogenic, nephrotoxic, hepattoxic, immunotoxic, estrogenic, and chronic (low doses, long periods of time) or acute toxic (high doses, short periods of time) are some of the possible effects on vertebrates. Mycotoxins and the creatures that ingest them are essential to the neurological, digestive, endocrine, immunological, liver, lungs, and kidneys.

Antagonists, additives, or synergy are associated with the type of mycotoxin, the host species' decontamination process, exposure duration, and mycotoxin dosages and ratios (Amaranta Carnaval Campos, 2019).

Humans have been using mycotoxins for food preservation for as long as agriculture has been, possibly even before memory began to be formed. Mycotoxicosis occurrences can be traced back to folklore, literature, and the arts. For example, they may be seen in the Dead Sea Scroll or in the Bible as part of the seven plagues of Egypt (Richard, 2007).

Furthermore, fusariotoxins (toxin T2 and ZAE) may have contributed to the fall of the Etrusan Civilization in the fifth century B.C. Jouany and Yiannikouris (2002). However, the most well-known instances of mycotoxicosis in antiquity may be the hallucinations of "Saint Antony's fire" of ergotism (11th century) caused by the alkaloids of Claviceps purpurea on rye. Delirium, prostration, agonizing agony, limb abscess and gangrene, and even death are all signs of ergotism (Claudia Axel, 2015; Richard, 2007).

Similarly, another well-known instance of mycotoxicosis epidemic is "Shoshin-Kakke," also known as "yellow rice disease," which was documented in Japan and primarily affected the colder regions. It causes acute cardiac beri-beri. Exposure to Citrovirirdin, a mycotoxin produced by Penicillium citreonigrum, is the cause of this sickness. Due to inadequate circumstances and procedures, this fungus infiltrated rice while it was being stored (Claudia Axel, 2015).

Mycotoxins and their harmful consequences were first recognized in London, England, in 1962, despite the fact that fungal contamination did occur and that they were thought to be related to certain disorders. A unusual ailment known as "Turkey X syndrome" was found in poultry, killing at least 10,000 birds. Curiously, upon investigating the cause of the disease, it was found that peanuts used to feed chickens were tainted with aflatoxins, which are secondary metabolites of Aspergillus flavus. It was discovered years later that cyclopiazonic acid also contributed to this outbreak.The most well-known mycotoxins are Trichothecenes, Patulin, Zearalanone, Ergot alkaloids, Fumonisis, Aflatoxins, and Ochratoxins (Claudia Axel, 2015).

2.9.2.1 Aflatoxins

Aspergillus species create a class of closely related and well studied mycotoxins known as aflatoxins. Before harvest, aflatoxins can be identified in the field, and contamination can rise during post-harvest processes such crop drying or storage. But even when there is no field contamination, aflatoxins can nevertheless contaminate products that have been stored. Aflatoxin production is limited to a small number of fungal species, all of which are members of the Aspergillus section flavi. Aspergillus flavus, Aspergillus paraciticus, Aspergillus nomius, Aspergillus psuedotamarii, Aspergillus bombycis, and Aspergillus ochraceoroseus are the species in question. While some of them are less common, Aspergillus flavus and Aspergillus paraciticus are economically significant and occupy overlapping habitats. Aspergillus flavus is one of the most prevalent and widespread soil-borne molds in the natural world. Nonetheless, within each aflatoxin species, the toxigenic capacities of distinct strains vary both qualitatively and quantitatively. Only half of the A. Flavus stains are reportedly capable of producing more than 106 µg/kg aflatoxins. While Aspergillus paraciticus produces both B and G aflatoxins (AFG1 and AFG2), Aspergillus flavus typically only produces B aflatoxins (AFB1 and AFB2). Conidia, asexual spores, and scleriotia, an asexual fruiting body with a resistant structure that allows the strains to endure in hostile environments, are all produced by Aspergillus flavus. In people and animals, Aspergillus flavus also produces mycoses, which are fungal infections as opposed to mycotoxicoses, which are illnesses brought on by ingesting fungal toxins. Aspergillus flavus is unique in that it is an opportunistic pathogen of both plants and animals (Yogendrarajah P., 2015).

2.9.2.2 Ochratoxins

A dihydroisocoumarin bound to phenlalanine makes up this class of pentaketide metabolites. The primary producer of the most prevalent congener, ochratoxin A (OTA), is Aspergillus ochraceus.

Ochratoxins A, B, and C are known to be produced by Aspergillus species, including Penicillium verrucosu. Grain, coffee, chocolate, spices, wine, beer, and pigs are frequently contaminated with ochratoxins. It is a strong nephrotoxin that is implicated in the development of Balkan endemic nephropathy, a disease that affects humans. It has also been demonstrated to be immunotoxic, teratogenic, and hepathotoxic.

2.9.2.3 Trichothecenes

Based on the differences in the functional hydroxyl and acetoxyl side groups, there are over 170 species that have been found and are divided into four kinds (A-D). Fusarium species create a number of naturally occurring trichothecene mycotoxins in food and feed. Although the most prevalent kind in grains is not considered carcinogenic to humans, it can have negative health effects such as immunological changes, endocrine dysfunction, weight loss, anorexia, and malnutrition (Pestka, 2010).

2.9.2.4 Fumonisins

They are made up of a collection of compounds that have two tricarballytic acid groups esterified at positions C14 and C15 on the carbon chain backbone. Fusarium proliferatum and Fusarium verticilloides are the primary producers of them. It is categorized as a group 2B potential human carcinogen and is nephrotoxic and hepatotoxic. In high-risk groups, it has been linked to oesophageal and liver malignancies (Persson et al, 2012).

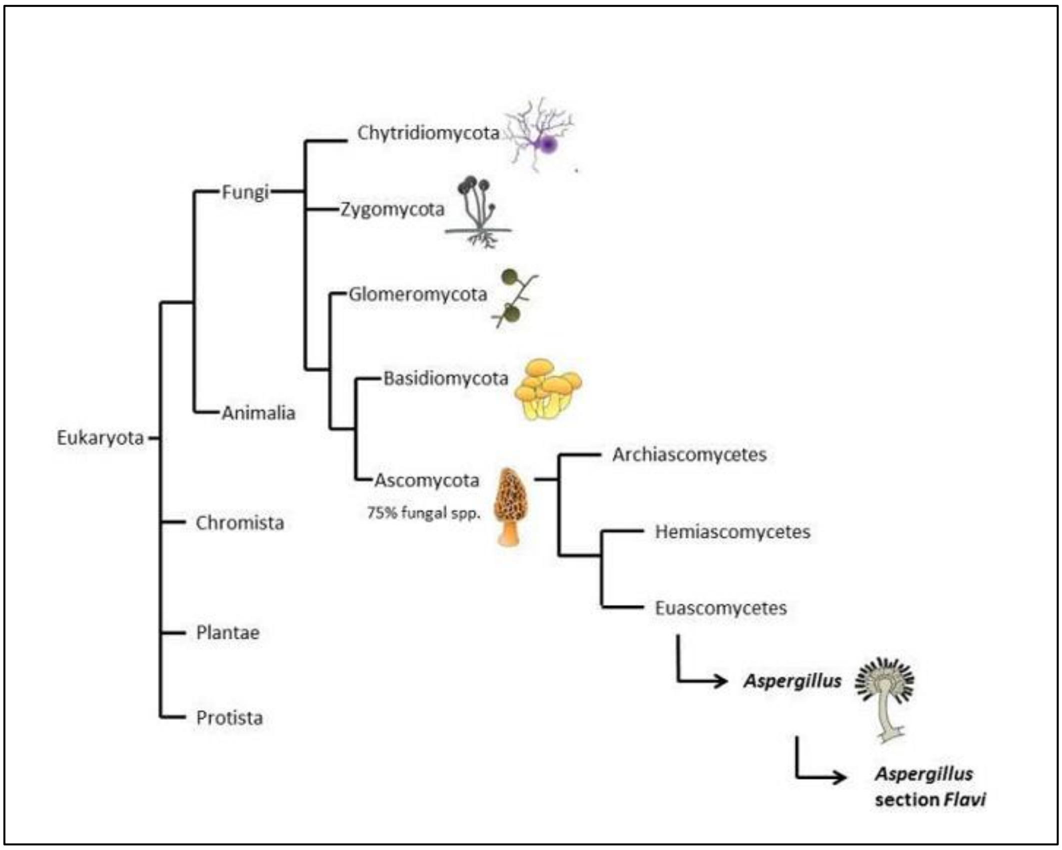
2.9.2.5 Zearalenone

This estrogenic mycotoxin, which is mostly produced by Fusarium graminearum and Fusarium culmoru, is present in high moisture corn, wheat, barley, and sorghum. It has also been discovered in moldy hay and palletized feeds. Although it is not considered harmful to humans, its estrogenic activity makes it powerful. It binds to the estrogen receptors in mammalian target cells because it closely resembles 17-β-estradiol, the main hormone generated in human ovaries. Accordingly, it may cause girls to reach puberty too soon (Yogendrarajah P., 2015).

2.9.3 Fungi

An estimated 3.5 to 5.1 million creatures, ranging from unicellular to tiny multicellular, make up the complex eukaryotic kingdom of fungi, which occupy a variety of ecological niches across the globe. These species, which include saprophages, symbionts, and pathogens, are important decomposers in the nutrient cycle. As heterotrophs, fungus produce enzymes that help them break down and absorb nutrients from the extracellular digestion of other species. In order to develop, fungi need specific elements that are utilized in their primary and secondary metabolisms. These elements are primarily sources of carbon and nitrogen, with trace amounts of potassium, phosphorus, and magnesium also being needed. Furthermore, their development depends on environmental elements including pH, light, temperature, and water availability (Amaranta Carnajal Campos, 2019).

Figure 3: Fungi Classification



(Pitt and Hocking, 2009)

Because of their varied life cycle tactics, a number of fungal species are studied in great detail and utilized in biotechnological businesses to generate enzymes, drugs, biocontrol agents, natural fertilizers, natural pigments, cosmetics, alcoholic drinks, and food (Amaranta Carnaval Campos, 2019).

Ascomycetes, Basidiomycetes, Zygomycetes, and Chytrids are the different groups of fungi. The majority of species, including the most significant to humanity, are found in the Ascomycota and Basidiomycota. About 33,000 species have been described in the Ascomycota, which includes 90% of harmful fungi and the majority of known lichens. Ascomycota fungi are distinguished by their ascus reproductive organs, albeit the majority of species also generate asexual spores.

In actuality, they are most frequently observed in their conidial state, and sexual reproduction appears to have been lost in a number of species. Aspergillus and Penicillium conidial states are typically seen in phialides, and these configurations are typically employed as diagnostic states. Aspergillus is characterized by enlarged conidiophore tips that form a visicle, and either a palisade of sterile cells and metulae precedes the pjialides (biseriate) or the phialides begin immediately on the surface of the visicle (uniserate).

A straight monoverticillate arrangement or a sequence of metulae followed by phialides characterizes the tip of the conidiophore; the degree of ramification may vary from one to numerous series of metulae. Vesicles are absent from penicillium.

The cellular wall Chitin and glucans make up the majority of ascomycetes, and their generally defective septum creates a central pore that leads to coenocytic mycella. Ascomycota, which are classified into three major groups: Archiascomycetes, Hemiascomycetes (yeast), and the gigantic Euascomycetes (molds), most likely originated between 500 and 900 million years ago.

Table 2: Fungi Involved in Spoilage of Cereal Products

Fungi	Type of Food Spoiled	Type of Spoilage
Apergilus	Bread, grains	Black mold, black mold rot(aflatoxin)
Candida	Breads	Yeasty
Cladosporium	Breads	Brown/black mold rot
Claviceps purpura	Breads, corn, grains	Ear rot(ergotism), black rot
Fusarium	Corn	Pink mold rot, fumonisins
Peniccillium	Breads	Blue-green mold
Rhizopus	Breads	Black mold
Sacchomyces	Breads, pasta	Yeasty
Zygosaccromyces	Breads, pasta	Yeasty

Generally speaking, mycotoxigenic fungi can be divided into two groups: Aspergillus and Penicillium, which are more likely to colonize staples and produce mycotoxins in crops, and Fusarium species, which are more likely to do so during storage. Apergillus flavus is one of the fungi that can colonize at both stages, albeit (Amaranta Caranjal Campos, 2019).

2.9.4 Bacterial Spoilage

Although they can only develop in environments with low pH and water activity, bacteria can potentially taint baked goods. For instance, 55% of Baillus subtilis spores remain active in amylase after 20 minutes at 65°C, demonstrating their heat resistance. Rope in bread is caused by this microbe that is found in raw components like flour, yeast, and sugar. Ropy bread is distinguished by its brown to black discoloration, putrid odor, and incredibly moist, sparse bread crumb. The warm and muggy summer months are when this issue typically arises. The first sign of ropy spoiling in bread is an odor resembling that of pineapple. Later, the bread becomes inedible due to the crumb becoming discolored, mushy, and sticky to the touch. Slime formation is caused by the combined action of the proteolytic and amylolytic enzymes generated by some Bacillus strains, which deteriorates the texture of bread. Strict hygienic measures and Good Manufacturing Practices that limit Bacillus species spores are necessary to prevent rope issues. This issue may typically be resolved by using preservatives such propionate (P. Sanranraj et al, 2011).

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(egotism). Black rot
Staphylococcus aureus	Pies	Slime
Salmonella	Frozen pizzas	Ropy

2.9.5 Yeast Spoilage

Yeast problems equally occur in bakery products. Wild yeast includes Trichosporon variable, Saccharomyces, Pichia and Zygosaccharomyces. Saccharomyces speciewhite spots in bread that can be leading to the term 'chalk bread'. Legan and Voysey (1981) studied that yeast problem in bakery products can be divided into two types:

a) Visible yeast which grows on the surface of the bread in white or pinkish patches and

b) Fermentative spoilage associated with alcoholic or essence odours and hence osmophilic yeasts.

The majority of the yeasts responsible for bread surface spoiling are Pichia burtonnii, or chalk mold. Osmophilic yeasts typically contaminate products due to dirty tools and equipment. Thus, osmophilic yeast contamination will be reduced by adhering to Good Manufacturing Practices (P. Saranraj et al, 2011).

2.10 The Critical Control Point of Hazard Analysis (HACCP)

The societal effects of contaminated bakery items might result in gastrointestinal disorders, such as epidermics, whereby the environment in which the contaminated bakery products are consumed is impacted, causing unsettled stomachs. Because of this, bakery goods that are distributed to the public for sale should be carefully considered, and HACCP does just that. Nowadays, most people think of this approach as a way to guarantee food safety (Jay, 1996).

HACCP for Bakeries

It is a HACCP output document that outlines methods to be used in baked goods manufacturing to ensure control of physical, chemical, and biological risks based on the seven HACCP principles. The HACCP plan is unique to pan bakery goods and the procedures used in their production, including.

Production line;

1. A trained HACCP team.
2. Production description.
3. Intended use.
4. Process flow diagram.
5. Hazard analysis chart.
6. Critical control point identification.
7. HACCP control chart.

Product Description: includes all of the details about the ingredients, formulation, and processing methods used to make baked goods.

- Formulation (e.g., allergenic components, raw materials derived from plants or animals).
- Compositional factors (such as aw, pH, preservatives, acidity, nutrition labeling, and allergen declaration) that influence microbial development.
- Technologies used in production, such as ambient, sterile, pasteurization, freezing, and baking.

Intended use:
discusses the target populations that will buy and use the products; for example, children, hospital patients, persons with compromised immune systems, and people with allergies may be more or less at risk (Wallace et al, 2011).

2.11 Effects of Residual Bromate in Bakery Products

According to H.B. Alhanash et al. (2020), the baking sector has been using potassium bromate, a common food enhancer, for more than a century. Assuming that potassium bromate is entirely broken down into less hazardous compounds during baking, many scientists asserted in the early 1990s that its usage as a bread additive is probably safe. However, there was a ban in the UK after the WHO declared in 1993 that substantial levels of residual bromate were found in 75% of the loaves tested in the country.

Potassium bromate appears as a white crystalline solid with no taste and colour and is readily soluble in water. It makes the bread stronger, increases its volume and improves it appearance and equally gives bulkiness to the dough development, resulting in more bread loaves. It acts as a slow oxidizing agent affecting the structure the rhetorical properties of the dough.

It causes flour maturation and strengthens the gluten network via the oxidization of the sulfhydrl groups in the protein chains forming disulphide bonds, hence it helps the gas retention and product volume.

Potassium bromate together with gluten present on bread results in soft bread, when closely investigated has small uniform air cells. Whereas, bread with low gluten will result in big air holes, dense and thin bread. It is commonly used by flour miller worldwide because it is cheap and reduces the age cost.

Although potassium bromate is widely used in the baking business worldwide, research has shown that it is harmful to health and ought to be prohibited. It causes severe inflammation and damage to the kidney tissues and central nervous system, as well as cancer, sore throats, kidney failure, abdominal pain, and diarrhea. Because of this, potassium bromate has been prohibited globally since 1993 in the majority of European nations after the WHO declared it to be a potential human carcinogen. In addition to being carcinogenic, potassium bromate has been shown to have an impact on bread's nutritional value by degrading the major vitamins it contains, including niacin, A1, B1, and B2. In the United States, potassium bromate is still used, but with limitations. The Food and Drug Administration has set a maximum permissible quantity of 50 mg/kg of flour, whereas in Japan, it is only 10 mg/kg of flour mass. In the bakery sector, potassium bromated is acknowledged as one of the top dough enhancers. Potassium bromate is transformed into potassium bromide, which is regarded as safe for consumer use, under carefully monitored circumstances (oven temperature, exposure time, and amount of potassium bromate utilized). Potassium bromate has been taken off the list of approved flour treatment additives due to scientific research that suggests it may be carcinogenic. The industry's ability to reduce the bromate residues to less than five parts per million of finished bakery products will determine if potassium bromate will be included again in future lists because there are chances for some residues to be left in the finished baked products. Consequently, a fast, reliable and easy means of detecting low levels of bromated residues is required (Kadure and Serret, 2010).

2.12 Potassium Bromate Alternatives

Ascorbic acid (vitamin C), azodicarbonamide (ADA), potassium and calcium iodide, the amino acids cysteine and glutathione, enzymes, and yeast derivatives are among the bromate substitutes that are currently available on the market. Other oxidizing chemicals that are employed as dough conditioners have the major drawback of acting more quickly than bromate, which predominantly acts during baking and proving. This significantly reduces the replacement's tolerance for changes in formulation or constituent types (Lynn Kuntz, 1994).

2.13 Preservatives

Preservatives are typically used to stop mold from growing on baked foods. The Code of Federal Regulation classifies shelf life preservatives as "an antimicrobial agent used to preserve food by preventing growth of microorganisms and subsequent spoilage" (CFR). Chemical and natural preservatives that are authorized as mold inhibitors in baked goods include acetic acid, sorbic acid, propionic acid, and their salts. Natural food preservatives such as vinegar, raisins, and cultured foods are specified on the ingredient list by their common name (P. Saranraj and M. Geetha, 2011).

2.14 Effects of Chemical Preservatives

Marin et al. (2002) used the barrier technology technique to prevent the growth of common fungi that contaminate bread goods, such as isolates from the genera Eurotium, Aspergillus, and Penicillium. Calcium propionate, potassium sorbate, and sodium benzoate were tested at different concentrations (0.003%, 0.03%, and 0.3%) on a model agar system using a full-factorial experimental design. Aw (0.80, 0.85, 0.90, and 0.95) and pH (4,5, 6, and 5.5) were the other variables that were examined. It was discovered that the best preservative (in terms of preventing fungal spoiling) to use in conjunction with the standard pH level was potassium sorbate.

In order to prevent common mold species (Eurotium, Aspergillus, and Penecillium) from growing on a fermented bakery product analog (FBPA) and causing intermediate moisture bread goods to expire, Guynot et al. (2004) also employed a hurdle technology method. The variables that were investigated included a range of weak acid preservative concentrations, including pH (4.5-5.5), aw (0.80-0.90), and potassium sorbate, calcium propanate, and sodium benzoate (0-0.3%). Potassium sorbate proved to be the most efficacious at the highest concentration tested (0.3%), regardless of water activity. Calcium propionate and sodium benzoate at the same concentration only worked at low water activity levels. Calcium propionate and sodium benzoate at the same concentration only worked at low water activity levels. Nevertheless, potassium sorbate was substantially broken down at pH 5.5, and the 0.3% was only effective at 0.80 aw. Francesca et al. (2009) used different REP-PCR patterns to identify 17 strains and assessed their

3.3 Study Population

antifungal properties. Fps0 were tested against endomyces, Penicillium reqeforti, and Aspergillus niger, three fungal species that commonly contaminate baked goods. A flour-based medium that resembled a real food system was used to nurture them. The study's findings showed a high inhibitory activity of fibuliger that was similar to that of calcium propionate, a popular preservative (0.3%w/v).It is known that sorbic acid and its potassium salts as effective anti-mould agent and have been considered historically safe for food, sorbic acid and potassium sorbate are ‘’ generally regarded as safe’’ (GRAS), for their use in other foods (P. Saranraj et al, 2011).

Materials and Methods

3.1 Description of the Study Area

From February 18 to March 27, 2021, this study was conducted at the SCIENCE FOR LIFE FOUNDATION LABORATORY BAMENDA.The study's focus is the North West Region of Cameroon's Bamenda urban-rural continuum. As illustrated in figure 1, Bamenda is administratively classified as an urban community comprising three subdivisions (Bamenda 1, Bamenda 2, and Bamenda 3) as a result of the recent decentralization process prompted by law No 2004/019 of 22 July 2004, whose implementation started in 2008. There is a distinct municipal council for every subdivision. Since Bamenda became the major metropolitan center in Cameroon's Western Highlands, its population has grown, more than quadrupling between the 1976 and 1987 censuses (Achio-chi, 1998).Bamenda's population increased from 48111 in 1976 to 488883 people/km39, more than 10% year, with an average of 14700 people annually. The city's urbanization rate is 42% (Bucrep, 2010).

The rural-urban continuum of Bamenda is located in the agro economic zone of Cameroon Western Highlands which benefit from a tropical mountain climate characterized by two fairly regular seasons:

Humid and rainy seasons (7months), and a dry season (5 months). Bamenda and its regions receive average rainfall between 1700 and 3500mm/year in a unimodal rainfall pattern with mean temperatures ranging from 17 to 24 annually (Acho-Chi, 1998; Nzembayie,2007 and Tchindjang,2012).

3.2 Research Design

This study was a cross-sectional study in which 25 bakery products were purchased randomly from different vendors and bakeries in Bamenda 1, Bamenda 2 and Bamenda 3.

Figure4: Bamenda rural urban continum seperimposed in google setelite map (Source, Kamga, 2010).

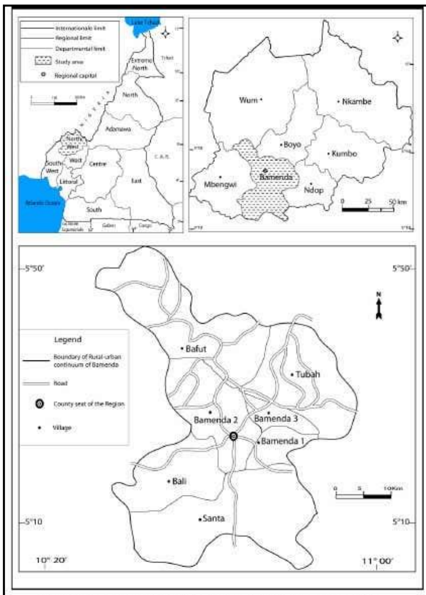


Figure 1: Location of the study area (source: Kamga, 2011)

3.4 Sample and Sampling Technique

The study population comprises of products samples sold by various vendors and bakeries in Bamenda.

A convenient random sampling technique was used in which 25 bakery products were purchased from the different vendors and bakeries.

3.5 Data Collection Tools

The tools are register, pen, stickers, permanent markers, focus group discussion, plastic bags.

3.6 Validation of Data Collection Tools

The tools were examined and checked if they are in good working conditions, for example, making sure that there was no water in the plastic papers and that the samples were not yet bad before analysis to avoid faulty results. Also, a proper registration of the sample was done and a serial number given to each one.

3.7 Ethical Consideration

An authorisation document was obtained from the North West Regional Delegation of Public Health. All information obtained from this study will be kept with strict confidentiality and details given to authorities so they realize the benefits of this study.

3.8 Materials and Methods

- Sterile polythene bag
- Petri dishes
- Sabouraud Dextrose Agar (SDA)
- Glass wares
- Autoclave
- Scale balance
- Sterile normal saline
- Pipettes
- Cotton wool
- Bunsen burner
- Incubator
- Water bath
- Sterile slides and cover sips
- Microscope
- 70% alcohol
- Test tubes
- Test tube racks
- Match
- Chloramphenicol.

3.9 Preparation of Media

- Sabouraud Dextrose Agar (SDA) was prepared following the manufacturer's instruction and chloramphenicol added to inhibit bacteria growth.
- 65g of SDA powder was weighed on a weighing balance and dissolved in 1000ml of distilled water, it was mixed well to dissolve.
- It was autoclaved at 121oC for 15 minuites. It was allowed to cool to approximately 45-50 (to at least cheek temperature).
- Before pouring the media into the petri dishes, a Bunsen burner was lighted to keep the working area sterile and free from unwanted microorganisms.
- Chloramphenicol was added to the media just before pouring into the petri dishes to inhibit bacteria growth.
- Sterile petri dishes were aligned and about 20ml of SDA media accurately poured into each of the plates and allowed to gel.
- Plates were allowed to dry in an incubator in an inverted position with lid facing upward and the media facing downward.

3.10 Isolation and Identifcation Method

3.10.1 Isolation Method

The methods used in this study were carried out according to the standards recommended by Dabey et al, (2004).

Nine grams of distilled water were used to dissolve one gram of each sample, and the mixture was left to stand for forty-five minutes to fully dissolve. Then, using the spread plate technique, 1 milliliter of each prepared sample was aspirated aseptically, poured in the middle of the plate, and spread out across the whole surface. For 14 days, the plates were incubated at room temperature, and their growth was checked every day. Any growth that resembled yeast was subjected to the Germ Tube method.

3.10.2 Germ Tube Test (GT)

Germ tube test is a screening test which is used to differentiate Candida albicans from other yeast.

Germ tube formation was first reported by Reynolds and Braude in 1956. When Candida albicans is grown in human or sheep serum at 37 for 3 hours, they form germ tubes which are detected as filamentous outgrowth extending from yeast cells.

Approximately, 95-97% of Candida albicans develop germ tubes when in a proteinaceous media.

Principle

Increased ribonucleic acid and protein synthesis is linked to germ tube formation. Tryptic soy broth and fetal bovine serum, which are necessary for protein synthesis, are present in germ tube solution. For stability, it is lyophilized.The germ tube is a quick test for the probable identification of Candida albicans and one of its virulence factors.

Procedure

A test tube was filled with 0.5 milliliters of human serum. Using a wire loop, a colony of yeast was carefully emulsified in the serum.
NB Too much innoculum will inhibit germ tube formation.
It was incubated at 37 for 3 hours, after which a drop the mixture was transferred into a slide, cover slip put Son and observed microscopically under the 10X and then the 40X objectives.

3.11 Determination of Potassium Bromide Content

This was done following the procedure carried out by Ahmad Abu-Obald et al, 2016).

Materials and Methods

- Hydrochloric acid (SDFCL)
- Glycerine (riedel)
- Potassium iodide (Frutarium)
- Potassium bromated (Frutarom)
- Distilled water

Apparatus

- Hydrogen iodide (0.5%)
- Micropipette(500µl)
- Transparent glass test tubes
- Hydrochloric acid
- Bread samples

Sample Treatment

A stirrer was used to cut, smash, and filter 1.0g of dough into 10ml of distilled water. Two milliliters of the filtrate were then added to each test tube. 500µl of 0.5%HI + HCl was added to it using a micropipette. It was well mixed, and there was a discernible color shift. If the color changed from orange to purple, it meant that potassium bromide was present in the bread sample.

3.12 Lactophenol Cotton Blue Staining

This is in accordance with Acharga Tankeshwar's (2014) methodology. Lactophenol is used as a mounting fluid in the formulation of LPCB stain, together with cotton blue. The presence of phenol kills organisms floating in the stain. The chitin found in fungal cell walls is stained by the acid dye cotton blue. The elements of fungi are heavily dyed blue.
White rhizopus.
Candida: pristine white colonies.
The fungal components are preserved with the aid of lactic acid.
Phenol is a disinfecting agent.
Fungal components and intestinal parasites (cysts, eggs, and oocysts) are stained by cotton blue.
Glycerol is a hygroscopic substance that keeps things from drying out.

Principle

Lactic acid, one of the LPCB stain's ingredients, serves as a cleaning agent and helps to maintain the fungal structures. Similarly, glycerol stops drying, whereas phenol fixes and kills the organism. The chitin in fungal cell walls is stained by cotton blue, and filamentous fungi are identified by their microscopic morphology, which includes the size, shape, and arrangement of their spores and hyphae, which give the structure color. It can be used either by itself or in combination with K0H.

Requirements

LPCB stain, compound microscopy, a clean, grease-free slide, a dropper or bamboo stick, and the development of fungi in a medium.

Procedure

- Take a spotless, oil-free slide.
- Use a Pasteur pipette to add a big drop of LPCB stain.
- Add a tiny amount of culture to the drop.
- Use a teasing needle to gently tease the culture (if it's a mold). in order to obtain a consistent spread.
- Gently put on a cover slip to prevent air bubbles from becoming trapped.
- Analyze with a high-power (40x) and low-power (10x) objective.

Observation

- Fungal spores, hyphae, and fruiting structures all take on a blue hue, and fungi are visible as dark blue-stained mycelium.
- Pale blue background stains

Limitations of LPCB Stain

- It is limited to presumptive fungal identification techniques.
- The components of the LPCB solution have the potential to alter the fungi's natural shape.
- Only adult mushrooms and their structures may be identified with the stain; juvenile vegetative forms of fungi cannot.
- In parasitology, a wet mount preparation shouldn't be excessively thick or thin.
- It is not recommended to use LPCB stain in parasitology since it destroys Entamoeba and Trichomonas trophozoites.
- Because this LPCB stain has an expiration date, it can only be used prior to that date.

4.0 Results:

After incubation at room temperature for 14 days, the following results were obtained;

Table 4: Microscopic and Biochemical Charactristics of Isolation

Sample Number	Size	Shape	Colour	Consistency	Hyphae	Germ Tube	LFCB staining	KBrO3
1	Small/medium	Raised	Yellow/white	Mucoid	Black	Negative	Penicillium	Positive
2	Small	Flat	White	Non mucoid	Absent	Positive	Mucor	Negative
3	Small/ Medium	Flat/ Raised	White	Mucoid	Black/ White	Negative	Penicillium	Negative
4	Large	Flat	White	Non mucoid	Absent	Negative	Negative	Positive
5	Medium	Flat	White/ Yellow	Mucoid	White/black	positive	Penicillium	Negative
6	Small	Raised	White	Mucoid	Absent	Negative	Aspergillus	Negative
7	Small/ Medium	Raised	Yellow/ White	Mucoid	Black	Negative	Aspergillus	Positive
8	Medium	Raised	White	Mucoid	White/ Black	Negative	Penicillium	Negative
9	Small/ Medium	Raised	Yellow/ White	Mucoid	White/ Black	positive	Fusarium	Negative
10	Small	Raised	White	mucoid	White/ Black	Negative	Aspergillus	Positive
11	Small/ Medium	Flat	Yellow/ White	Mucoid	White	Negative	Fusarium	Negative
12	Small/ Medium	raised	White	Non Mucoid	Absent	Negative	Negative	Negative
13	Medium	Flat	White	Non Mucoid	White/ Yellow	Negative	Negative	Negative
14	Small/ Medium	Flat	White	Mucoid	White	Negative	Mucor	Positive
15	Small	Flat	White	Mucoid	White	Negative	Negative	Negative
16	Small/ Medium	Flat	Yellow/ White	Mucoid	White	Penicillium	Negative	Positive
17	Small/ Medium	Raised	Yellow/ White	Mucoid	Yellow/ White	Negative	Negative	Negative

18	Medium	Raised	Yellow/ White	Mucoid	Yellow	Negative	Negative	Positive
19	Medium	Raised	White	Non Mucoid	Green	Positive	Negative	Positive
20	Medium	Raised	White/ Yellow	Mucoid	Green/ White	Penicillium	Negative	Negative
21	Medium	Raised	White	Mucoid	Absent	Negative	Negaive	Negative
22	Medium	Raised	Yellow/ White	Mucoid	White	Negative	Mucor	Positive
23	Large	Flat	White	Mucoid	Absent	Negative	Negative	Negative
24	Small/ Medium	Raised	Yellow/ White	Non Mucoid	White/ Blue	Negative	Mucor	Positive
25	Small	Raised	White	Non Mucoid	Absent	Negative	Negative	Positive

Table 5: Distribution Species in Bakery Products Sold in Bamenda of Fungal

Fungal species	Frequency	Percentage
Aspergilus	3	20%
Mucor	4	26.67%
Penicillium	6	40%
Fusarium	2	13.33%
Total	15	60%

From the above table, 60% of the samples was positive for different fungal species from which 20% 0f fungal species was Aspergillus, 26.67% was Mucor species, 40% was Penicillium species and 13.33% was Fusarium species.

5.1 Discussion

The prevalence of fungi in a majority of the samples may be due to the fact that these microorganisms are widely spread in the environment. This work gives a description of the isolation and identification of four different fungal species which were able to grow on SDA. They belong to the genera:

Penicillium, Aspergillus, Fusarium and Mucor speies. The main contaminating fungus was from the genus Penicillium with a percentage of 40%, this finding as consistent with (Legan, 1993) who found out that Penicillium is the most common species.

Potassium bromide was also present in most of the samples. This confirms that potassium bromate is still used as a bread improver in some bakeries in Bamenda.

Germ tube technique showed four positive samples giving a percentage of 16% indicating the presence of Candida species in some bread samples, this finding consistent with Reynolds and Braude (1956).

5.2 Conclusion

In conclusion, the study's findings of a 40% prevalence of Penicillium, 26.67% mucor, 20% aspergillus, and 13.33% Fusarium clearly demonstrate the importance of hygienic handling of bakery products. As such, bioaerosols in the bakery production environment should be managed with the use of suitable filters in conjunction with production procedures that reduce aerosols, as is typically done by HACCP. The health of employees depends on the control of airborne illnesses. The overall quantity of molds has increased as a result of poor worker, equipment, and air hygiene (working environment).

Since most fungal species are common and found in the air, they readily settle on products when exposed, which is why the high prevalence of fungal species found on bakery products at roadside locations is a reflection of the lack of health policies to ensure adequate coverage of these products to prevent contamination.

The high prevalence of potassium bromide identified in bakery products sold in Bamenda is an indication that high quantities of potassium bromated is used in most bakeries in Bamenda during bread baking process.

These findings agree with the hypothesis that the prevalence of toxigenic fungal and potassium bromide in bakery products sold in Bamenda is high.

5.3 Recommendation

From the results obtained, the following recommendations were made:

To the Government

- Strategies for routine sanitary inspection in bakeries, roadside vendors and bakery product retailing stations should be put in place to reduce fungal contamination.
- Government should implement the control in the use of potassium bromate as a rising agent in

bakery products and ensure that bakeries always comply with the safety guidelines.

- Government should also adopt sensitisation strategies, for example, radio and television talks, health education on the prevention of fungal contamination of bakery products.

To the Population

- The population should adopt healthy attitudes in handling bakery products by properly covering them and avoid eating bakery products with visible mould.
- Bread makers should use alternative flour improvers that are much more safe to humans.

To Health Sector/Students

- Students can research on the quantitative aspect of potassium bromide contamination in bakery products.
- Students can equally research on isolation and determination of the total number of mould types in each bakery products.

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