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MYCOLOGICAL ASSESSMENT OF DIFFERENT TYPES OF CAKE SOLD IN PORT HARCOURT, NIGERIA

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ABSTRACT

Spoilage of cake during storage is commonly associated with fungi. Therefore, this study is aimed at assessing the quality of different types of cake (chocolate, fruit, vanilla, carrot and red velvet cake) sold in Port Harcourt metropolis. A total of fourteen cakes were sampled from three vendors and stored for twelve days at ambient temperature (29±2 ^{o}C). Monitoring of total fungal count (TFC) and identification of fungal species isolated from the cake samples were carried out at 24 hour intervals using standard microbiological methods. The result obtained showed that 48.21% of the cake samples met the standard recommended by the International Commission on Microbiological Specifications for Foods (ICMSF) and the Institute of Food Science and Technology (IFST) at the intervals they were monitored during the storage period. Non-viable fungal count were reported in the cake samples within the first 2 days; fruit cake samples till day 9. The TFC of the cake samples steadily increased during the storage period with few exceptions. Carrot cake samples had the highest TFC (7.02 log_{10}CFU/g). Fusarium spp. (32%), Aspergillus niger (27.33%), Penicillium notatum (17.33%), Saccharomyces cerevisiae (10.67%), A. flavus (9.33%), Cladosporium sphaerospermum (2.67%) and Candida albicans (0.67%) were encountered in the cake samples. During the period of storage, fungi was first observed in fruit cake on day 10 whereas, it was earlier reported in other types of cake. Therefore, freshly baked cake produced in commercial quantity and stored at ambient temperature should be consumed within few days to avoid individuals experiencing foodborne illnesses.

Keywords: Bakery, Shelf life, Fungi, Mycotoxin, Food spoilage

Running Title: Fungi in cake samples stored for twelve days at ambient temperature

INTRODUCTION

Bakery products such as cakes is an enjoyable snack rich in nutrients. It is well appreciated by everyone especially women and children (Alozie et al., 2020). Spoilage of cake is largely attributed to fungal species. Consumption of such products in large quantity unknowingly is a public health risk (Morassi et al., 2018). Ingredients rich in nutrients are mainly used for preparation of bakery products. Therefore, the product has a short shelf life unless preservatives are added (Saranraj and Geetha, 2012). It is extremely difficult to completely stop microbial

spoilage of bakery products despite the use of preservatives (Talukder et al., 2017). Bakeries are faced with the challenge of mould spoilage which often lead to huge economic losses (Ahmed and Hussein, 2012; Gonda et al., 2019; Patil and Kukade, 2020).

Cake is among the bakery products enjoyed by millions of people irrespective of their social status. Globally, it is estimated that market share of cake grows by 1.5 % annually (Fakhernia et al., 2015). In ancient times, some people living in different parts of the world offer cake to their gods and spirit. Till date, it is a tradition among the Chinese to use cake to celebrate Harvest moon festival. A deity in Russia known as Maslenitsa is offered sun cake known as blini as a sign of respect (Primavera et al., 2018; Redlich, 2020; Myachikova and Shamtsyan, 2022). In a bid to imitate solar movement on the first day of spring when Beltane festival is held, cakes are rolled down a hill by Ancient Celts (Ibejekwe and Nyam, 2018).

There are different types of cake available for the delight of consumers which vary in quality depending on the ingredients used, preparation methods, among other factors (Ben-Noun, 2018). Chocolate cake, cherry cake, fruit cake, candied-pineapple cake, banana cake, cream cake, sponge cake, ragi cake, plain cake and many others have unique sensory attributes (Ahmed and Hussein, 2012; Offia-Olua and Edide, 2013; Chaudhari et al., 2017; Qureshi et al., 2017; El-Kadi et al., 2018; Ibejekwe and Nyam, 2018; Morassi et al., 2018; Samsudin et al., 2019). According to Alozie *et al*. (2020), the number of cake recipes are literally above one million.

Chocolate cake is characterized by high moisture content, neutral pH and rich in nutrients (El-Fadaly et al., 2016; Nawawi et al., 2016). According to Gonda et al. (2019), sponge cake has a water activity within the range of 0.75 to 0.90. Different fillers such as fruit and chocolate are used to prepare different varieties of cake (Hengl et al., 2022). Key ingredients used in preparing cake include flour, fat, sugar, eggs, leavening agents and preservatives. Cake is ceremoniously presented during parties, birthdays, weddings and other ceremonies (Chaudhari et al., 2017; Ibejekwe and Nyam, 2018; Ben-Noun, 2018; Sudawa et al., 2022). Preparation of cake involves mixing, shaping, baking as well other suitable procedures. At least two ingredients, depending on individual choice, are used for preparing cake to achieve a desirable sensory characteristics (Hengel et al., 2022).

Moulds which include *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Monilia* spp., *Eurotium* spp. and *Mucor* spp. are commonly found in bakery products (Saranraj and Geetha, 2012). Spoilage microorganisms such as *Aspergillus* spp. could be found growing in cakes because the product has low water activity (Ahmed and Hussein, 2012). *Aspergillus* and *Penicillium* spp. are commonly associated with spoilage of bakery products (Nakhchian et al., 2014; El-Kadi et al., 2018). Some of the fungal species associated with bakery products are capable of releasing mycotoxins beyond permissible limits which could elicit health problems in humans after consumption (Adesina et al., 2017; El-Kadi et al., 2018).

Fungal assessment of bakery products which include cake sold in Port Harcourt metropolis was carried out by Williams et al. (2020). The minimum and maximum fungal count in the sample is 2.0 x 10^6 and 3.0 x10^6 CFU/g, respectively. The researchers did not take into consideration different types of cake consumed by residents of Port Harcourt. A lot of people store cake at room temperature for several days because preservatives have already been added to the product. Spoilage of cake by fungi could be influenced by the type of cake prepared using different ingredients and storage conditions. Therefore, this study is aimed at monitoring the fungal population and fungal species in five different types of cake obtained from three vendors and stored at ambient temperature for twelve days.

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MATERIALS AND METHODS

Sample Collection

Fourteen (14) samples of cakes were obtained from three (3) cake vendors 'A', 'B' and 'C' in Port Harcourt metropolis using a big sterile cellophane bag. The samples consists of five (5) types of cake which include carrot cake (n=2), vanilla cake (n=3), chocolate cake (n=3), red velvet (n=3) and fruit cake (n=3). All the samples were quickly transported to Microbiology laboratory, University of Port Harcourt within two hours for laboratory analysis.

Storage of cake samples

All the cake samples were properly labelled and stored inside a dry, clean shelf in the laboratory at ambient temperature (28±2 ^{o}C) for a period of 12 days. At 24 h interval, each of the cakes were observed and a portion removed for determination of fungal population and identification of fungal species.

Determination of total fungal count

The method described by Talukder et al. (2017) with a slight modification was adopted. One gram (1 g) of each sample of cake was dissolved in 9 ml sterile peptone water (diluent). This was followed by a 10-fold serial dilution up to 10^4 using sterile pipettes. One millilitre (1 ml) dilution 10^{-3} and 10^{-4} were plated in duplicates on freshly prepared potato dextrose agar (PDA). The inoculated Petri dishes were incubated at 25 - 30 ^{o}C for 7 days. Growth of fungal colonies in the Petri dishes were observed and noted. The colonies were subcultured on freshly prepared PDA to obtain pure colonies.

$$\text{CFU/ml} = \text{no. of colonies x } \frac{1}{dilution\ factor} \text{ x } \frac{1}{volume\ plated}$$

Characterization and identification of isolates

Identification of the fungal isolates was based on morphological characteristics which include shape, arrangement of spores, colour, motility of spores, structure of mycelium, texture, arrangement of conidia, presence or absence of septa, their characteristics hyphal and reproductive structures during incubation period (Mushimiyimana et al., 2016). A sterilized needle was used to scoop a little fragment of the fungus and placed on a clean grease-free slide. Three drops of lactophenol cotton blue were aseptically placed on the isolate on the slide and properly covered with a coverslip to avoid air bubbles. The preparation was viewed under a microscope using x40 and x100 objective lens. To observe yeast cells, a drop of distilled water was placed on a grease free slide. With the aid of a sterile wire loop, an inoculum was picked from the pure colonies and a smear was made on the drop of distilled water made earlier. The glass slide was then heat fixed and stained with crystal violet. The slide was allowed to dry viewed under a microscope using oil immersion lens.

RESULTS

The result presented in Table 1 shows the microscopic and macroscopic morphology of fungal isolates encountered in five different types of cake obtained from vendor 'A', 'B' and 'C'. The percentage occurrence of fungal species encountered in the cake samples include *Fusarium* spp. (32%), *Aspergillus niger* (27.33%), *Penicillium notatum* (17.33%), *Saccharomyces cerevisiae* (10.67%), *A. flavus* (9.33%), *Cladosporium sphaerospermum* (2.67%) and *Candida albicans* (0.67%). Figure 1 shows the mean total fungal counts (TFC) of different types of cake stored for 12 days at ambient temperature (29± 2 ^{o}C). The mean total fungal count of chocolate cake, vanilla cake, red velvet cake, fruit cake and carrot cake were within the range of 0 - 6.68, 0 - 6.75, 0 - 6.75, 0 - 5.99 and 0 - 7.02 $\log_{10}$CFU/g, respectively. Figure 2, 3, 4, 5 and 6 shows the frequency of occurrence of fungi obtained at 24 h interval during the period of storage of chocolate, vanilla, red velvet, fruit and carrot cake from the three vendors, respectively.

Taken into consideration the TFC of vanilla and chocolate cake samples from vendors 'A', 'B' and 'C', the results show that each of the cake samples from vendor 'A' had higher fungal counts than vendor 'B' followed by vendor 'C'. The TFC of velvet cake samples from vendor 'C' were higher than vendor 'B' followed by vendor 'A'. With regards to TFC of fruit cakes from the three vendors, the samples from vendor 'C' were higher than vendor 'A' followed by vendor 'B'. Although vendor 'C' had no sample of carrot cake, the TFC of carrot cake samples from vendor 'A' was higher than vendor 'B'.

Among the different types of cake stored at ambient temperature, *Aspergillus niger* was the dominant fungal species whereas the least was *Canida albicans*. During the period of storage, visible signs of fungal growth in vanilla cake was first observed at Day 3; chocolate, red velvet and carrot cake at Day 4 and fruit cake at Day 10.

Table 1: Macroscopic and microscopic characteristics of fungal isolates

S/No.	Macroscopic morphology	Microscopic morphology	Probable isolate
1.	Creamy raised moist colony	Oval shaped, budding yeast cells	*Saccharomyces cerevisiae*
2.	Cream/white raised shiny colony	Oval shaped, budding yeast cells with branded hyphae	*Candida albicans*
3.	White cottonwood like colony ranging to light pink	Canoe shaped septate macro conidia and ovoid shaped micro conidia spread around randomly.	*Fusarium* spp.
4.	Dark brown to black spores, edge taped white or pale yellow	Smooth conidiophores protruding From a septate hyphae	*Aspergillus niger*
5.	Green spores, edge taped white and reverse cream coloured	Rough conidiophores unbranched which is non-septate	*Aspergillus flavus*
6.	Ash to dark velvety, edge taped white reverse cream and cracked	Dark, shady branched, septate conidiophores tree like structures	*Cladosporium sphaerospermum*
7.	Deep green velvety edge taped white, reverse is cream or pale yellow	Brush shaped Branched conidiophores septate mycelium	*Penicillium notatum*

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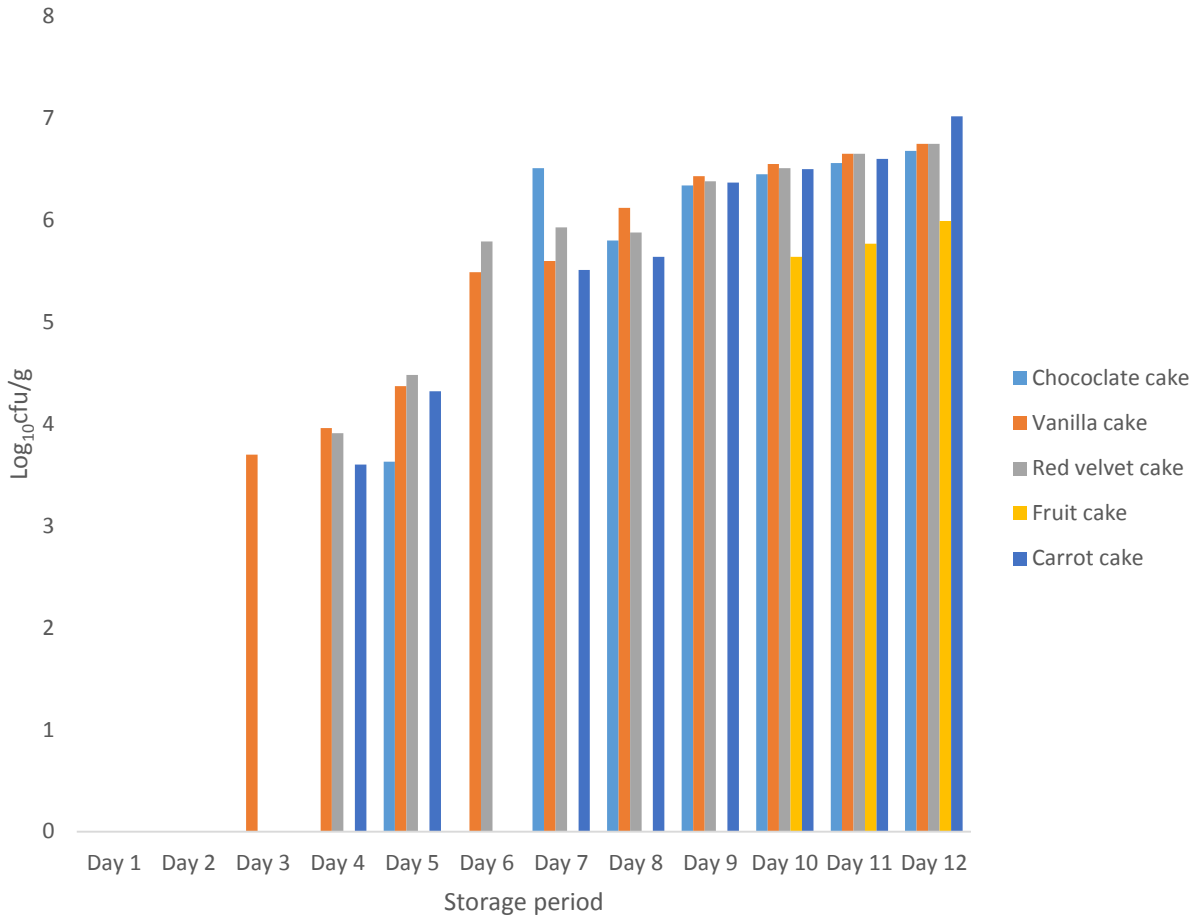

Figure 1: Mean total fungal count of different types of cake stored for 12 days at ambient temperature

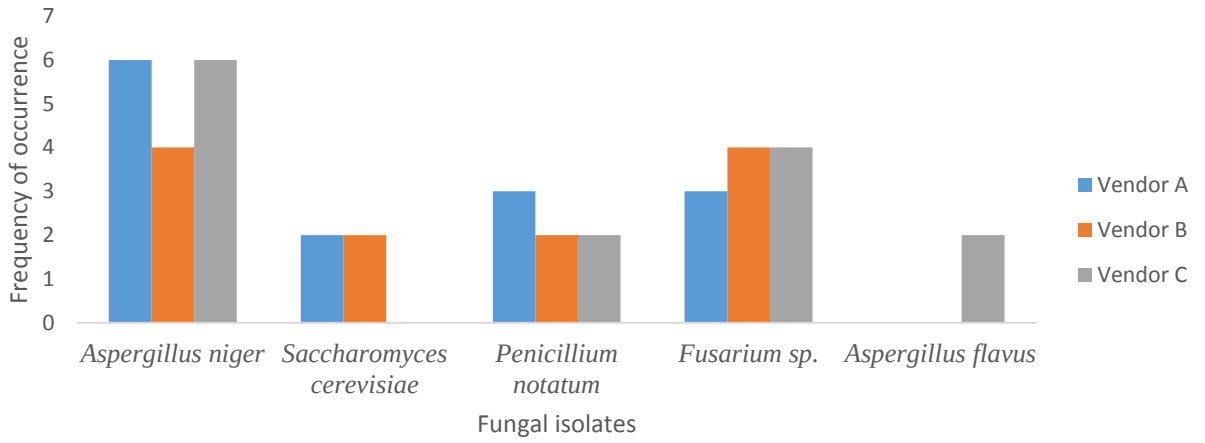

Figure 2: Frequency of occurrence of fungi isolated from chocolate cake samples obtained from three vendors and stored at ambient temperature for 12 days

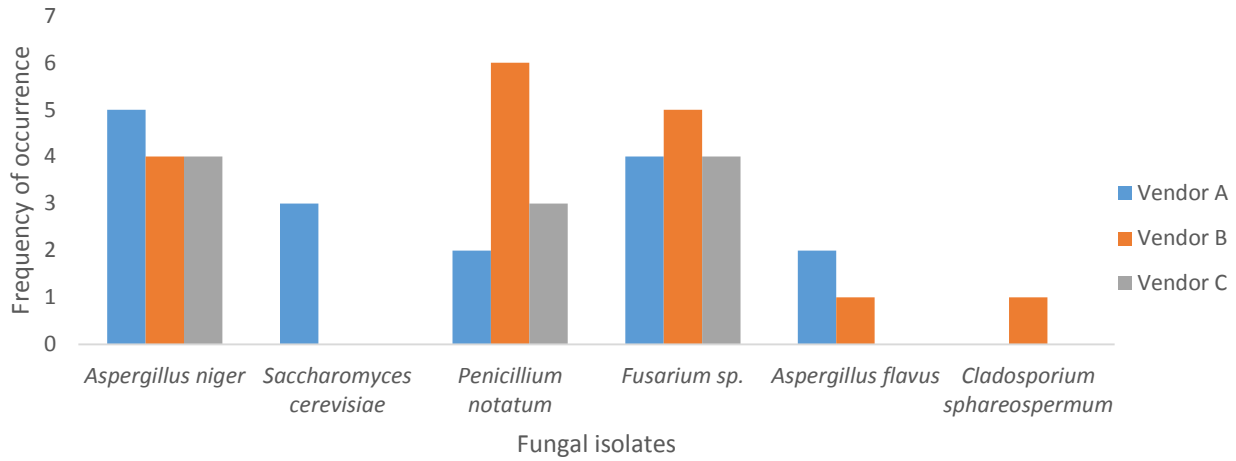

Figure 3: Frequency of occurrence of fungal isolates from vanilla cake samples obtained from three vendors and stored for 12 days

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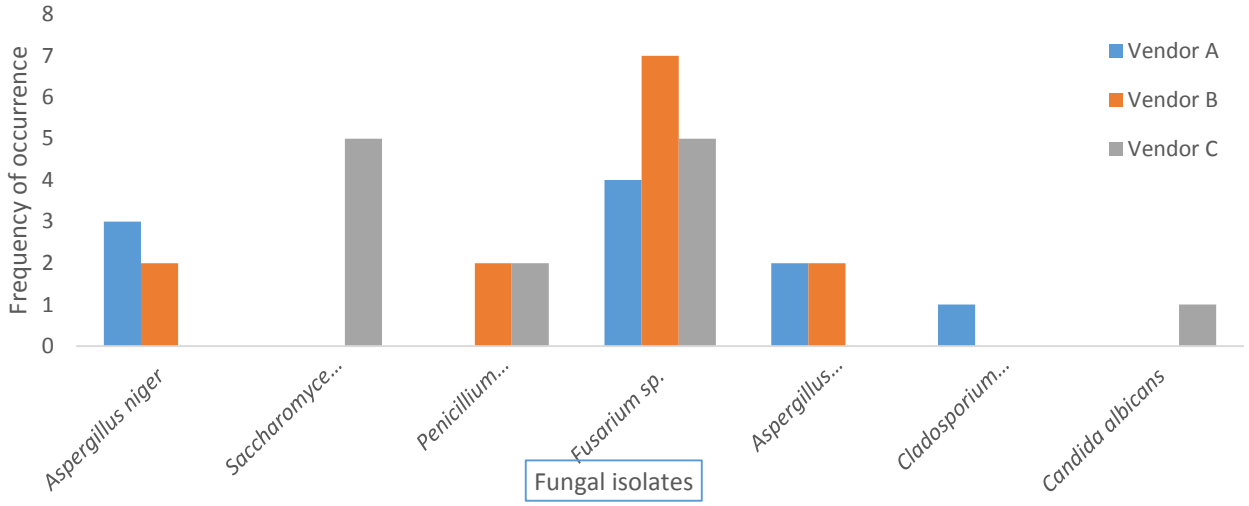

Figure 4: Frequency of occurrence of fungal isolates from red velvet cake obtained from three vendors and stored for 12 days

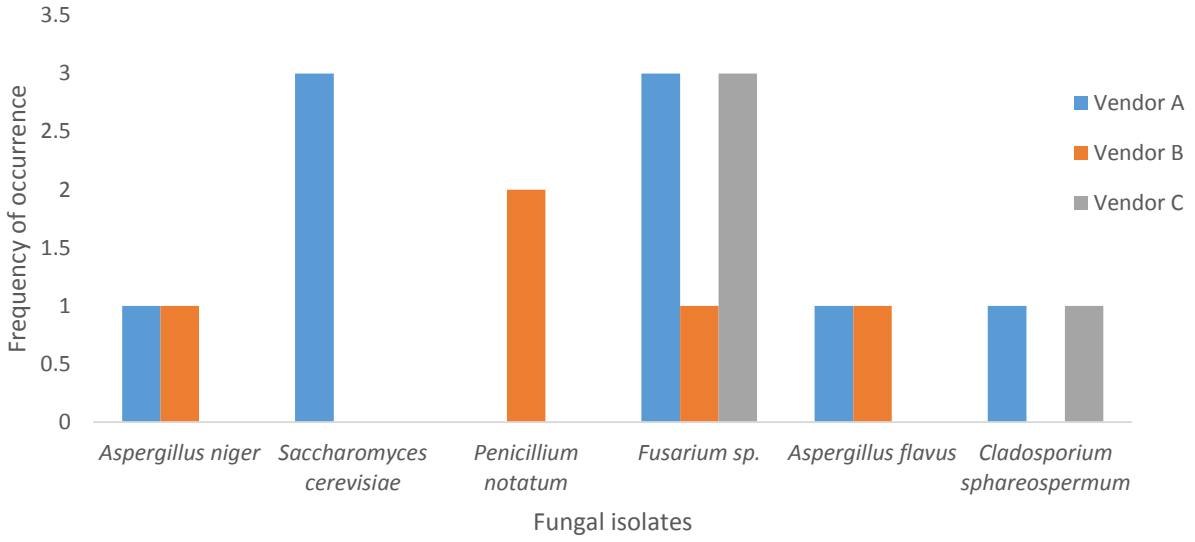

Figure 5: Frequency of occurrence of fungal isolates from fruit cake obtained from three vendors and stored for 12 days

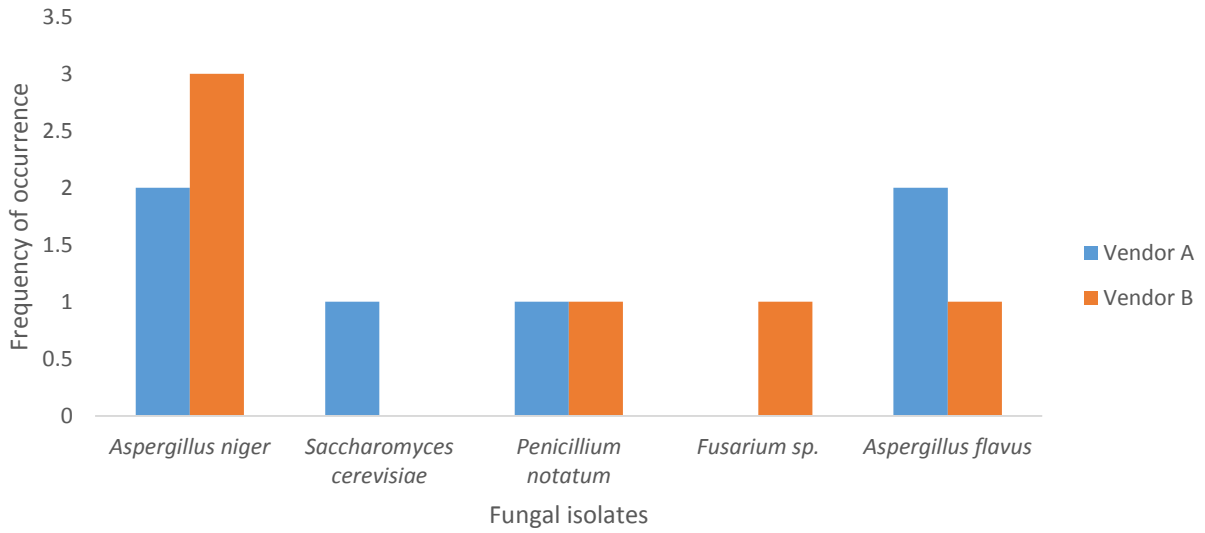

Figure 6: Frequency of occurrence of fungal isolates from carrot cake obtained from two vendors and stored for 12 days

DISCUSSION

The result obtained from this study shows that a steady increase in total fungal count (TFC) of the cake samples occurred during the period of storage at ambient temperature (29±2 ^{o}C) with few exceptions. The mean total fungal count encountered in chocolate cake, vanilla cake, red velvet cake, fruit cake and carrot cake samples were within the range of 0 - 6.68, 0 - 6.75, 0 - 6.75, 0 - 5.99 and 0 -7.02 $\log_{10}$CFU/g, respectively. According to the guideline published by the International Commission on Microbiological Specifications for Foods (ICMSF), total fungal count of cake within the range of 0 - 10^3 ICMSF standard per gram is regarded to be safe for human consumption (Sudawa et al., 2022). The Institute of Food Science and Technology (IFST) stipulate that pastries and cakes should have a maximum permissible yeast and mould population of 5 $\log_{10}$CFU and 4 $\log_{10}$CFU, respectively (Chaudhari et al., 2017). There was no viable fungal count reported in all the cake samples within the first 2 days of storage. According to Samsudin et al. (2019), baked goods such as cake reported to have yeast and mould count below 2 $\log_{10}$CFU/g is satisfactory. Borderline microbial counts of cake within the range of 4 - 6 $\log_{10}$CFU/g is acceptable whereas > 6 $\log_{10}$CFU/g is unsatisfactory and pose a health hazard to consumers. On day 3, all the cake samples had no viable fungal count with the exception of vanilla cake (3.7 $\log_{10}$CFU/g). On day 4, no viable fungal count was also reported in fruit and chocolate cake samples whereas other cake samples recorded 3.96 $\log_{10}$CFU/g as the highest TFC. The highest TFC of cake samples on day 5 was 4.48 $\log_{10}$CFU/g whereas no viable fungal count was reported in fruit cake samples. On day 6, no viable fungal count was reported in carrot and chocolate cake samples. A similar result was reported in fruit cake samples until day 9. The TFC of the cake samples exceeded 6 $\log_{10}$CFU/g between day 9 - 12 with the exception of fruit cakes. Non-viable fungal count reported in 35.11% of the cake samples at the intervals they were monitored during the storage period is satisfactory based on the ICMSF standard. Total fungal count (TFC) < 5 $\log_{10}$CFU/g reported in 13.10% of the cake samples at the intervals they were monitored during the storage period was within the limit stipulated by the IFST while few of them were at the borderline based on the ICMSF standard. The TFC > 6 $\log_{10}$CFU/g which involved 51.79% of the cake samples at the intervals they were monitored during the

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storage period is unsatisfactory. Consumption of such cakes could be hazardous to human health.

Storage temperature of cakes which are usually between 4.4 - 60 ^{o}C is a danger zone because it encourage the growth of many food spoilage organisms (Nawawi et al., 2016). A study carried out by Nawawi et al. (2016) reported that growth of moulds in banana cake stored at room temperature was noticed on the 6th day of storage. Das et al. (2020) reported that TFC of cake samples obtained from different markets early in the morning after receiving fresh supply from bakeries were within the range of 1.5 x10^2 to 2.4 x 10^3 CFU/g. Low fungal count of the cake samples could be attributed to the fact that the samples were freshly baked cakes unlike the cake samples evaluated in this study. Williams et al. (2020) reported that TFC of cake samples sold in Port Harcourt range from 2.0 x 10^6 to 3.0 x 10^6 CFU/g. The report is in agreement with findings from this study.

The fungal species encountered in the cake samples and their percentage occurrence include *Fusarium* spp. (32%), *Aspergillus niger* (27.33%), *Penicillium notatum* (17.33%), *Saccharomyces cerevisiae* (10.67%), *A. flavus* (9.33%), *Cladosporium sphaerospermum* (2.67%) and *Candida albicans* (0.67%). These fungal species are associated with spoilage of cake samples. In a related study, Morassi et al. (2018) isolated *Fusarium* species, *Aspergillus flavus*, *A. niger*, *Cladosporium* spp., among other fungal species in the raw materials and environment where chocolate and orange cakes were prepared. A study carried out by Sudawa et al. (2022) in Kano reported that cake sold to the residents were contaminated with *Aspergillus* spp., *Mucor* spp., and *Rhizopus* spp. Nakhchian et al. (2014) also reported the presence of *Aspergillus* spp. *Penicillium* spp., *Cladosporium* spp., among other fungal species in cake samples obtained from different locations not too far from the bakery. In Croatia, Hengl et al. (2022) reported that fruit cake and other types of cake (chocolates, creams or toppings) were contaminated with moulds. The presence of yeasts in curd cake was reported by Kačániová and Juhaniaková (2011). Patil and Kukade (2020) reported that fungal genera which include *Penicillium, Fusarium* and *Aspergillus* were seen growing in cupcake which resulted in spoilage of the product. A study carried out by Ibejekwe and Nyam (2018) reported that samples of plain cake obtained from eateries and parties in Jos were contaminated with *Aspergillus fumigatus, A. niger, A. flavus, Rhizopus stolonifer, Penicillium citrinum* and *P. chrysogenum*.

Among the fungal species encountered in chocolate cake, *Aspergillus niger* had the highest frequency of occurrence followed by *Fusarium* spp. In a related study, El-Fadaly et al. (2016) reported the presence of *Aspergillus niger* in chocolate cake samples obtained from different markets located in Damietta and Dakahlia Governorates, Egypt. *Aspergillus niger* is widely distributed in nature and often isolated from foods. Since the fungus can withstand salt and sugar in high concentration, it is among the contaminants in bakery products. According to Ibejekwe and Nyam (2018), many species of *Aspergillus* are capable of resisting heat associated with bakery products. The dominance of *Aspergillus* spp. among the fugal species encountered in cake samples was reported by Nakhchian et al. (2014) and Sudawa *et al*. (2022). In a related study, Williams et al. (2020) reported the presence of *Aspergillus niger* in bakery products which include cake sold in Port Harcourt metropolis. A similar result was reported by El-Fadaly et al. (2016) after carrying out microbiological assessment of some chocolate cake samples. The presence of *Aspergillus niger* in cakes is a threat to human health because the fungus is capable of releasing mycotoxins into the product. Aspergillosis is an infection caused by *Aspergillus* spp. El-Kadi et al. (2018) also reported the presence of *Aspergillus niger* in cakes produced by different companies which include chocolate cake and cream cake.

Mycological assessment of all the cake samples showed that the product from vendor 'A' had the highest level of fungal contamination, followed by vendor 'B' and then vendor 'C'. The variation in quality of the stored cake samples could be attributed to product handling by the cake producers and vendors, differences in cake preparation methods, the type of preservatives used, quality of cake ingredients used, level of hygiene of personnel and environment where the cakes were produced (Voysey and Legan, 1999).

All the fungal species reported in this study were encountered in vanilla cake with the exception of *Candida albicans.* The total fungal count of stored vanilla cake was within the range of 0 - 6.75 $\log_{10}$CFU/g. According to Romero-Cortes et al. (2019), vanilla juice could inhibit (fungistatic effect) the growth of fungi which include *Alternaria alternate*. All the fungal species reported in this study were encountered in red velvet cake. This result is an indication that red velvet cake is more susceptible to fungal spoilage than other types of cakes. The result obtained from this study shows that fruit cake is least susceptible to fungal spoilage compare with other types of cake. Fungal growth was first observed in fruit cake samples on the 10th day of storage at ambient temperature. The total fungal count of fruit cake was within the range of 0 - 5.99 $\log_{10}$CFU/g. In a related study, Qureshi et al. (2017) reported that yeast and mould count of fruit cake prepared using grape fruit albedo powder and stored for 30 days is within the range of 1.87±1.36 to 2.02±1.42 $\log_{10}$CFU/g. The difference in both results could be as a result of the type of fruit and other ingredients used in preparing fruit cake, among other factors.

The ubiquitous nature of *Pencillium* spp. and its ability to cause food spoilage has been well established. The infection known as penicilliosis which is associated with immunocompromised individuals or hosts is caused by *Penicillium* species. They are capable of producing mycotoxins which are highly poisonous to humans (Williams et al., 2020). According to Nakhchian et al. (2014), *Aspergillus* spp., *Penicillium* spp. and *Cladosporium* spp. were the dominant airborne fungi found in the bakery located in Bucharest Romania. According to Ire et al. (2020), *Fusarium* spp. produce toxins known as moniliformin. Kidney disorder and Kodua poisoning is the result effect of consuming products contaminated with citrinin and cyclopiazonic acid which are *Penicillium* toxins, respectively. *Saccharomyces cerevisiae* is capable of causing infection in individuals whose immune system has been compromised. *Fusarium* spp. also had the highest frequency of occurrence in fruit cake compare with other fungal species encountered in the product.

CONCLUSION

A total of seven fungal genera which include *Aspergillus niger, A. flavus, Saccharomyces cerevisiae, Cladosporium sphareospemum, Candida albicans, Penicillium notatum* and *Fusarium* spp. were encountered in five different types of cakes stored for 12 days at ambient temperature. *Fusarium* spp. had the highest frequency of occurrence in majority of the cakes during storage. Among all the cakes monitored during storage, fruit cake was least susceptible to fungal growth whereas red velvet cake was the most susceptible.

Competing Interests

The authors have declared that no competing interests exist.

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