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7. Comparative evaluation of the fungal-keeping properties of different cake preservatives

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Abstract

Cake as a perishable food is a rich substrate for moulds and yeasts. One of today's challenges for the food industry is to guarantee safe food throughout the supply chain whilst retaining the nutritional properties. Hence the application of preservatives in food to maintain wholesomeness and freshness. This research assessed the fungal keeping property of four different preservatives in wheat flour cakes. Cakes were prepared from wheat flour using potassium sorbate, brandy, lime juice, and pawpaw wine as preservatives. Fungal population was determined, at 3-day intervals, on each cake product using standard cultural methods. The isolates were identified using cultural, microscopic and biochemical characteristics. Organoleptic evaluation of cake samples was conducted with a 9-point hedonic scale using a 7-member panel. At day 12, the lowest fungal population (5.11 log cfu/g) was observed in potassium sorbate–preserved cake and the highest (6.38 log cfu/g) was observed in brandy-preserved cake. The acceptability of the cake products by the panelists decreased with storage duration. The most acceptable cake was the brandy-preserved cake with an overall acceptability of 8.85, followed by potassium sorbate-preserved cake (8.75). Among the isolated fungi (n= 28), *Aspergillus fumigatus* was predominant (35.7%), followed by *A. flavus* (28.6%). This study indicated that potassium sorbate was the most effective of the studied preservatives. However, the effective fungal keeping property of pawpaw wine indicated its potential to satisfy the need for natural preservatives in cake production.

Keywords: Preservative, fungi, keeping quality, potassium sorbate, lime juice, pawpaw wine.

Introduction

Cakes are sweet baked desserts prepared from flour (mostly wheat) and other ingredients, such as eggs, sugar, butter, and a leavening agent (Ubbor *et al*., 2022). They are popular, delicious ready-to-eat snacks and are constant ceremonial items on joyous occasions such as birthdays and weddings. Cakes are increasing in demand for their different tastes, design, and aesthetics, with a global market growth rate of 1.5% per annum (Wilderjans *et al*., 2013). It is, however, a perishable food that is prone to microbial spoilage particularly by fungi due to its rich content of carbohydrates, sugar and sometimes, condiments. The water activity (0.78 – 0.95) and acidity of cakes encourage fungal proliferation and the cake texture with its characteristic spongy nature is suitable for mycelia penetration and growth (Morassi *et al*., 2018; Garcia *et al*., 2021). Fungi that have been associated with contamination and spoilage of cakes include, *Penicillium citrinum*, *P. chrysogenum*, *P. crustosum*, *P. commune*, *P. brevicompactum*, *P. paxilli*, *P. glabrum*, *Aspergillus flavus* (Gonda, 2015; Morassi *et al*., 2018). Fungal activities in cakes may result in ropiness due to slime formation, discolouration by mycelial growth, off-flavour and mycotoxins formation (which may occur even without any visible change in the cake) (Abdelhameed and Khalifa, 2024). Fungal spoilage of cake usually results in consumer rejection which has occasioned great economic losses to the cake manufacturer.

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The general position is that microbial contamination of flour and flour mixture at pre-baking stage is usually eliminated by the baking process due to the enormous temperature of the oven (Morassi *et al*., 2018; Gonda *et al*., 2019). Baking, therefore, is a critical control point in ensuring the microbial stability of cakes. The major challenge to cake microbial stability is at the post-baking stage, due to product handling, exposure to the air, poorly sterilized equipment and faulty packaging (Morassi *et al*., 2018). One of today's challenges for the food industry is to guarantee safe food through the supply chain while retaining the nutritional attributes of food. In order to ensure the protection of the microbiological integrity of cakes, and even other bakery products, premium has been placed on the use of preservatives (Garcia *et al*., 2021). The preservatives that are commonly used in cakes include, potassium sorbate, calcium propionate, sodium bicarbonate, sodium benzoate, sodium metabisulphite and alcoholic drinks (e,g rum, and brandy) (Kikelomo, 2012; Okeke and Nwazuroko, 2014; Kumar *et al*., 2015; Garcia *et al*., 2021; Islam *et al*., 2023). However, the increasing demand for natural products by consumers has shifted research focus to the use of alternative and/or natural preservatives in baking products, including cakes. Nisin and essential oils are some of the biopreservatives currently being studied for their ability to preserve cakes (Cama-Curasi *et al*., 2022). The alternative preservatives of research interest in cake and bread preservation also include active packaging such as, oxygen scavengers and an ethanol emitter (Janjarasskul *et al*., 2016; Mugasundari and Anandakumar, 2022). Fermented pawpaw fruits have many ethnomedicinal uses, one of which is in the treatment of ulcers and gastroenteritis (Owoyele *et al*., 2013). Since it contains alcohol, it possible application in cake preservation as an alternative preservative, informed its selection among the preservatives in the present study. Preservatives differ in their ability to protect the microbiological integrity of food and thus the present study evaluated sorbic acid (potassium sorbate), lime juice, pawpaw wine, and brandy for their fungal keeping ability in wheat flour cakes.

Materials and methods

Mama gold wheat flour and other ingredients for cake production were purchased at local retail stores in Ikotun, Lagos State. Potassium sorbate (prepared at 0.1%) and brandy (40% alc/vol) were bought respectively at Gannylab Scientific and a retail store in Lagos. Pawpaw wine (12.80 ± 0.02% alc/vol) and lime juice were produced in the Laboratory using the methods of Awe (2011) and Nwanekezi *et al*. (2014) for pawpaw wine and Jittanit *et al*., (2013) for lime juice.

Production of Cake

The method of Abegunde *et al*., (2019) was used with some modification. The margarine and sugar were thoroughly mixed in a bowl (bowl A) using a sterile locally made wooden stick. Milk, beaten eggs and preservatives were added to the mixture and thoroughly mixed with a whisk. In another but smaller bowl (bowl B), wheat flour was finely mixed with baking powder and salt. The content of bowl B was carefully transferred into bowl A and repeatedly kneaded to mix the mixture. The prepared mixture was measured out into three cake-baking pans that had been greased. The filled baking pans were then placed in an oven at 190^{o}C for 15 minutes. This procedure was done with each of the preservatives.

Enumeration of fungi in cake products

A 500g sample of each cake product was taken immediately after production and at 3day intervals and mashed

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using a sterile pestle and mortar. Ten (10) grams of mashed cake was weighed using a chemical balance and aseptically added to 90 ml sterile water and properly mixed. One milliliter of the dilution was aseptically pipetted and introduced into 9ml sterile water to make the 10^{-1} dilution and this was further diluted to the 10^{-4} dilution. Aliquot (1ml) of the 10^{-4} dilution was aseptically inoculated on sterile potato dextrose agar (PDA) using pour plate method. The plates were incubated at 28°C for 72h after which colonies were counted and the colony forming units per gram (cfu/g) was determined. Distinct colonies were subcultured to obtain pure cultures. The isolates were identified using plate morphology and microscopic characteristics (with the lactophenol cotton blue stain) (Samson *et al*., 2000)

Organoleptic evaluation of cake products

A 7-member sensory panel, comprising volunteer students in the Department of Microbiology, LASU was constituted for the organoleptic evaluation of samples of the cakes. Degree of acceptance or likeness based on appearance, texture, taste, flavour, and overall acceptability was expressed on a 9-point Hedonic scale (where;1= extremely dislike, 2= very much dislike, 3= moderately dislike, 4= slightly dislike, 5= neither like nor dislike, 6= slightly liked, 7= moderately liked, 8= very much liked, 9= extremely liked).

Statistical analysis

The statistical analysis was carried out using the analysis of variance (ANOVA) (of the SPSS software version 24.0) to compare the mean values of organoleptic properties across the different cake products. The ANOVA was chosen because it allows the determination of any significant difference in means among multiple groups.

Results

Fungal population of cake products in storage

At day 12 (end of storage period), the lowest fungal population was 5.11 log cfu/g recorded in potassium sorbate-preserved cake and the highest fungal population was 6.38 log cfu/g in brandy-preserved cake (Table 1).

Table 1: Fungal population ofcake containing different preservatives with days of storage

Storage days	Fungal population (Log cfu/g)				
	Control	Cake B	Cake LJ	Cake PW	Cake PS
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	3.84±0.12	2.54±0.04	2.47±0.03	2.45±0.01	2.36±0.00
6	4.73±0.15	4.70±0.01	4.50±0.01	3.80±0.12	3.55±0.02
9	6.35±0.01	5.45±0.01	5.28±0.01	5.23±0.03	5.04±0.03
12	7.43±0.01	6.38±0.01	5.88±0.01	5.87±0.03	5.11±0.03

Each value is the mean of 3 determinations

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NB: Cake B = brandy-preserved cake, Cake LJ = Lime juice-preserved cake, Cake PW = pawpaw wine-preserved cake, and Cake PS = potassium sorbate-preserved cake. All the cakes did not exhibit significant ($p > 0.05$) spatial variation over the storage days.

Percentage occurrence of fungi in the cakes.

Table 2 shows that *Aspergillus fumigatus* was predominant among the 28 fungal isolates with 35.7% occurrence, followed by *Aspergillus flavus* (28.6). The least occurring was *Fusarium* spp (7.1%).

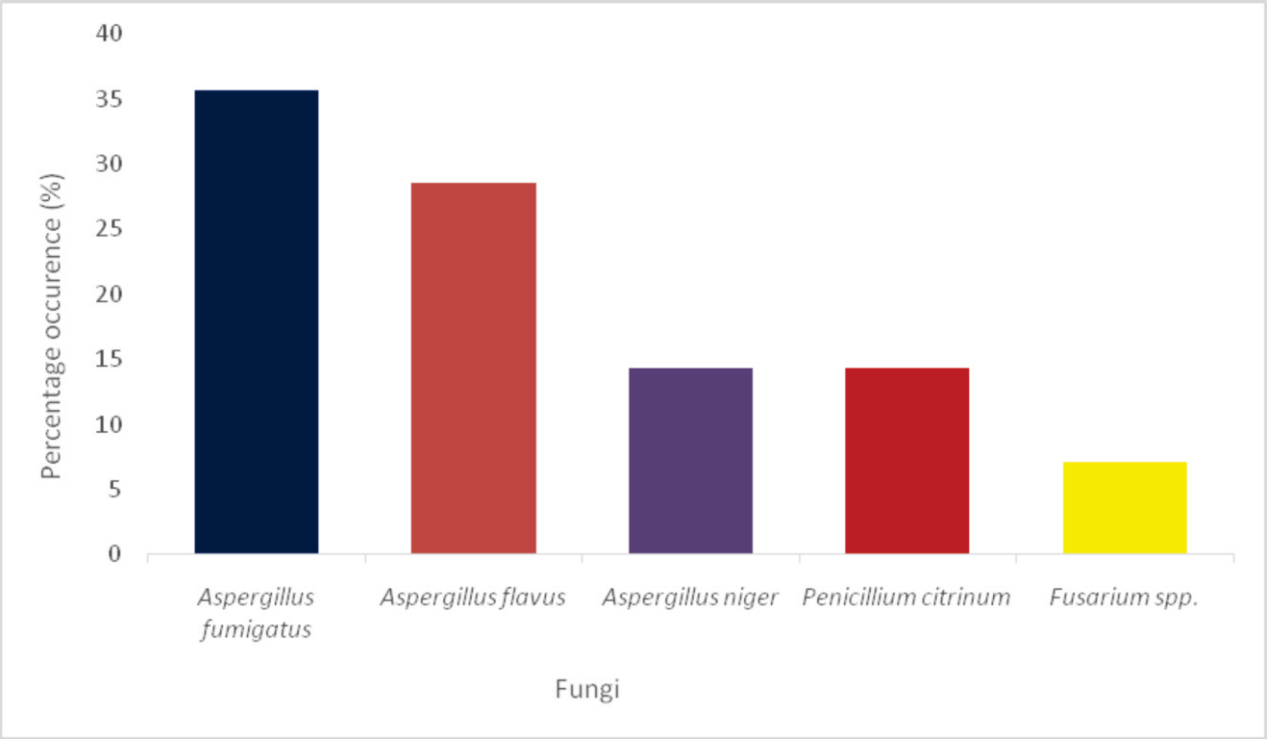

Figure 2: Percentage occurrence of fungi in the cake products.

Organoleptic evaluation of the cake products

The results in Table 3 show that the Brandy-preserved cake was the most acceptable with an 8.85 overall acceptability value, followed by potassium sorbate-preserved and pawpaw wine-preserved cakes with 8.75 and 8.30 respectively.

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Table 3: Organoleptic quality of wheat flour cake with different preservatives

Parameters	Control	Cake B	Cake LJ	Cake PW	Cake PS	Mean Difference	Sig.
			Day 0				
Appearance	9	9	9	9	9		
Texture	9	9	9	9	9		
Sweetness	8	9	8	8	9	8.50000	0.0000
Flavour	8	9	8	8	9		
			Day 3				
Appearance	9	9	9	9	9		
Texture	9	9	9	9	9		
Sweetness	8	9	8	8	9	8.50000	0.0000
Flavour	8	9	8	8	9		
			Day6				
Appearance	7	9	9	9	9		
Texture	7	9	9	9	9		
Sweetness	7	9	8	8	8	8.75000	0.0000
Flavour	7	9	8	8	9		
			Day 9				
Appearance	6	9	9	8	9		
Texture	5	9	9	9	9		
Sweetness	6	9	8	8	8	8.25000	0.0000
Flavour	5	9	8	8	9		
			Day 12				
Appearance	5	7	7	7	8		
Texture	4	8	8	7	9		
Sweetness	4	9	9	8	7	7.75000	0.0000
Flavour	4	9	9	9	9		
Overall acceptability	**6.75**	**8.85**	**8.45**	**8.30**	**8.75**		

Each value is the mean of 7 determinations

Cake B = brandy-preserved cake, Cake LJ = Lime juice-preserved cake, Cake PW = pawpaw wine-preserved cake, and Cake PS = potassium sorbate-preserved cake.

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Discussion

Food safety entails the elongation of food shelf life and the delivery of safe food to consumers. To achieve these objectives, preservatives are added to food products and in recent times, the use of natural products as preservatives is gaining increasing attention. Thus, research studied the ability of two common cake preservatives (potassium sorbate, and brandy) and two natural products (lime juice, and pawpaw wine) to control fungal proliferation in wheat flour cakes.

Potassium sorbate was the most effective among the test preservatives in inhibiting fungal growth in the studied cake products, followed by pawpaw wine since the cake preserved with potassium sorbate (cake PS) had the lowest fungal population at the end of the storage duration. Also, the fungal population in the cake PS and cake preserved with pawpaw wine (cake PW) remained within the satisfactory limit of 4Log cfu/g [stipulated by the Centre for Food Safety, (2014)] on the 6^{th} day of storage and remained within acceptable limits of 4 to 6Log cfu/g throughout the studied duration of 12 days. Also, there was no significant ($p > 0.05$) spatial variation in the cake fungal populations over the storage days.

This outcome is in agreement with previous reports indicating that potassium sorbate is effective in inhibiting the growth of spoilage fungi of cakes and other bakery products (Kumar *et al*., 2015; Garcia *et al*., 2021). The inhibitory effect of pawpaw wine on the cake spoilage fungi may be due to its alcoholic content since alcohol (ethanol) has been reported to effectively reduce the fungal population in rice cakes (Oh and Kim, 2021). Brandy and lime juice were not as effective as potassium sorbate, and the pawpaw wine in limiting the fungal population in the cake products, since the fungal population of the lime juice and brandy preserved cakes (cakes LJ and Cakes B respectively) only remained satisfactory till the 3 days of storage and while the fungal population of the Cakes LJ remained within acceptable limits even at day 12, the fungal population of the cakes B exceeded the acceptable limit at day 12. The more effective fungal-keeping property of cake PW than cake B, suggests that ethanol may not be the sole antifungal constituent of the pawpaw wine, since *Carica papaya* fruit extracts have been reported to have antifungal activity (Aljuhani *et al*., 2024).

In the present study, the isolated fungi, in order of prevalence were *Aspergillus fumigatus*, *A. flavus, A. niger, Penicillium citrinum, and Fusarium* sp. This result is similar to that of Morassi *et al* (2018), who reported *A. flavus*, *P. citrinum*, *P.paxilli,* and *A. niger* from wheat cakes. However, the authors reported *A. flavus* as the predominant fungus. The differences in the results of the studies may be attributed to differences in the environment of production and storage, exposure, and handling of the cake products. Similarly, Williams *et al*., (2020) reported the genera; *Aspergillus*, *Fusarium*, and *Penicillium* among others, as contaminants of bakery products which included cake. However, Sudawa *et al*., (2022) reported the presence of *Mucor* spp and *Rhizopus* spp (which were not found in the present study) in addition to *Aspergillus* spp and *Penicillium* spp in cakes sold in Kano, Nigeria. The differences in the mycobiota of cakes in the present study and that reported by Sudawa *et al*., (2022) may be attributed to different conditions of exposure and handling of the cake products. This is because microbial contamination of cakes occurs post-baking due to improper exposure and handling (Morassi *et al*., 2018).

All the preserved cakes were generally acceptable throughout the study period since their acceptability scores were 7 and above and there was no significant ($p > 0.05$) spatial variation in the organoleptic qualities of the

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cakes. However, the control cake was no longer acceptable on day 9 and its overall acceptability fell short of the acceptable score of 7. One implication of the outcome of the sensory analysis is that, preservatives is important in ensuring the microbiological stability of cake, and also that preservatives do not negatively affect the organoleptic properties of cake (Okeke and Nwazuroko, 2014; Islam *et al*., 2023), irrespective of the increasing consumer preference for natural preservatives which is based on health concerns.

The Brandy-preserved cake was the most acceptable based on the sensory analysis. This may be due to the likely flavour enhancement of the cakes by brandy. It has been reported that, some natural preservatives do not only possess antimicrobial, or antioxidant properties but also positively impact the flavour of the product (Erseda *et al*., 2023).

Conclusion

This study indicated that potassium sorbate was the most effective of the studied preservatives. However, the effective fungal-keeping property of pawpaw wine indicated its potential to satisfy the need for natural preservatives in cake production. Thus, pawpaw wine is recommended for application as a natural preservative in cakes.

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