

Microbiological Quality, Shelf Life and Sensorial Properties of Bread Preserved with Sorbic Acid and Calcium Propionate

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Abstract: This study focus on comparing the effect of sorbic acid, calcium propionate, sorbic acid+ calcium propionate (50:50) on the microbial load and sensory properties of bread samples stored at room temperature (28 ± 2 °C) for ten (10) days. Bread samples without preservative is the control. Microbiological analysis and sensory evaluation of the bread samples involved the use of Standard methods and 9-point Hedonic scale, respectively. During the period of storage, there was a steady increase in microbial load of all the bread samples with some exceptions. The total heterotrophic bacterial (THBC) and total fungal count (TFC) of all the bread samples with preservatives was within the range 2.85 - 4.46 and 0 - 4.25 \log_{10} CFU/g while the samples without preservative was 2.95 - 3.90 and 0 - 3.59 \log_{10} CFU/g, respectively. On average, bread samples preserved with sorbic acid had lower microbial load compared with other bread samples including the control. However, the bread samples preserved with sorbic acid were the least preferred by the sensory panelists whereas bread samples preserved with calcium propionate were the most preferred. Since wide acceptability of bread by consumers is largely influenced by sensory properties, the use of calcium propionate is recommended as a bread preservative.

Keywords: Food preservatives, bakery products, bread spoilage, food safety

1. INTRODUCTION

Bread is a convenient bakery product eaten as a staple food in almost all parts of the world [1, 2]. “Artos” is an ancient Greek word for bread which originated from the verb “ararisko” whose meaning is connect, put together while “artio” means brew and prepare. In modern times, the word ‘bread’ is obtained from a verb “psoo” used in time of old which means rub; a little part of the meaning of the word “psomys” referring to bread as ‘little bit, bite’ [3]. The history of bread, although a bit controversial, is traced to ancient Israelites living in Egypt about 12, 000 years ago or more [4, 5]. Baking of bread in Nigeria started in Lagos around 1900 when slaves freed from Brazil and West India established a bakery in the country [4, 6]. Presently, bread is the second most consumed non-indigenous food in Nigeria after rice [7, 8].

Bread is prepared by mixing yeast with leavened dough followed by baking. The major ingredients used in baking bread include wheat flour, yeast, water and salt. Other ingredients such as eggs, milk and other dairy products, nutritive sugar, carbohydrate sweeteners, lactic acid producing bacteria, enzyme, non-wheat flours, yeast nutrients, potassium bromate, calcium salts, potassium iodate, azodicarbonamide, dough strengtheners, colouring agents, spices, calcium iodate and/or calcium peroxide, among others are added in bread for the purpose of improving its nutritional value, flavour, texture and extending its shelf life [5, 8]. The process of producing bread involves mixing, kneading, proofing, shaping and baking [9]. A loaf of bread is a good source of carbohydrates, protein, fat, minerals and vitamins essential for nourishment of the body and vitality. Bread is rich in fiber and folic acid. Fortification of bread with micronutrients which include iron, calcium, thiamine and niacin is mandatory in the USA, UK and many developed countries [2, 8].

Freshly baked bread spoil easily as a result of changes in microbiological, physiological, biochemical and sensorial properties of the product [10]. Bread spoilage is a big financial loss to bakers and vendors. The health of the general public is at risk when unwholesome bread is sold by some vendors.

Staling, loss or gain of moisture and spoilage due to microbial activities are notable changes that occur in bread as a result of deterioration [11, 12]. Microorganisms commonly associated with bread spoilage include *Staphylococcus* spp., *Escherichia coli*, *Bacillus* sp., *Mucor*, *Rhizopus*, *Aspergillus* and *Fusarium* spp. [2021]. Demissie et al [1] reported that 10 minutes after baking of bread, the product was already contaminated with *Bacillus cereus* and *Staphylococcus aureus*. At 48 and 96 hr after the bread was baked, *Staphylococcus cohnii* and *Bacillus firmus* were encountered in the product, respectively. The study also reported that *Aspergillus flavus*, *Aspergillus niger* and *Penicillium citrinum* were encountered in bread 10 minutes after it was baked. *Mucor* sp. and *Rhizopus* sp. commonly called bread mould are usually the first fungal genera to appear in bread during spoilage followed by *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. [13].

It is recommended that freshly baked bread kept at room temperature should be consumed within 2-3 days. Bread kept inside a refrigerator without removing the original package from the bakery could last longer than 3 days [11]. In order to extend the shelf life of bread, chemical preservatives (organic acids) such as propionic acid, lactic acid, sorbic and phenylacetic acid in regulated quantities are added to the product [14]. Preservation of bread also entails the use of modified atmosphere packaging, ultraviolet irradiation, pasteurization, freezing, among others [12]. The effectiveness of chemical preservatives in bread is dependent on water activity, pH, composition and storage temperature of the product. Among the chemical preservatives for bread, calcium propionate and potassium sorbate are commonly used to inhibit the growth of moulds in bread. Both preservatives could also inhibit some bacterial contaminants of bread. Calcium propionate is effective against *Bacillus subtilis* responsible for rope spores which cause spoilage of bread. The optimum pH required for potassium sorbate and calcium sorbate to function as a chemical preservative is 6.0 and < 5.5, respectively [12]. In this study, a comparison between the effectiveness of sorbic acid and calcium sorbate added to bread as a preservative will be determined based on microbiological and sensory properties.

2. MATERIALS AND METHODS

2.1. Preparation of Bread

Ingredients used for the preparation of bread which include wheat flour, refined sugar, milk powder, iodized salt, butter, yeast, preservatives and bread improver were bought from Rumuomasi morning market, Stadium road, Port Harcourt. All the items were put inside a big sterile polythene bag. The dry ingredients (flour, sugar, milk powder, salt, yeast, calcium propionate and bread improver) were mixed together. Half cup of lukewarm potable water was added to the mixture and kneaded to the right texture and consistency. Thereafter, unsalted butter was added and the dough was allowed to rest in a bowl in warm condition for 1hr. The purpose is to allow the dough to rise as a result of activities of yeast. After the dough had risen, it was brought out of the bowl and cut into a desirable shape in readiness for baking. The shaped dough was put inside a 9 inch pan, covered and left to rise for 30 minutes after which it was put in the oven for 30 minutes at 180°C with the lid. After baking, the bread was allowed to cool completely on a wire rack, and then packaged in a sterile Ziploc bag. The above procedure was repeated using sorbic acid as a preservative while a combination of sorbic acid and calcium propionate in the ratio 50: 50 was also used. Bread prepared without addition of preservatives serve as control.

2.2. Storage of Bread Samples

The bread samples were stored at room temperature (28±2 °C) and observed for 10 days. Visual observations for mould growth were carried out on the bread samples.

2.3. Serial Dilution

One gram of bread sample was aseptically weighed and poured inside 9 ml of sterile peptone water and gently shaken. One millilitre (1 ml) of solution from the first tube containing the sample was transferred to the next tube containing 9 ml sterile peptone water using a sterile pipette. The step was repeated stepwise using a sterile pipette for each transfer until dilution 10⁻⁵ was reached.

2.4. Microbiological Analysis

2.4.1. Determination of Total Heterotrophic Bacterial Count

A sterile Petri dish containing molten sterilized nutrient agar (NA) was inoculated with 0.1 ml dilution 10^{-3} of each sample and this was done in duplicates. The inoculated Petri dishes were gently rocked anti-clockwise and allowed to solidify. Thereafter, the inoculated plates were incubated at 37 °C for 24 h. Upon incubation, the total number of colonies appearing on the plates were counted manually and the result was recorded. The bacterial population was calculated using the formula below and expressed as colony forming unit per millilitre (CFU/ml).

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

2.4.2. Determination of Total Fungal Count

A sterile Petri dish containing molten sterilized potato dextrose agar (PDA) was inoculated with 0.1 ml dilution 10^{-3} of each sample and this was done in duplicates. The inoculated Petri dishes were gently rocked anti-clockwise and allowed to solidify. Thereafter, the inoculated plates were incubated at 28 ± 2 °C for 5 days. Upon incubation, the total number of colonies appearing on the plates were counted manually and the result was recorded. The fungal population was calculated using the formula below and expressed as colony forming unit per millilitre (CFU/ml).

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

2.4.3. Determination of Pure Culture of the Isolates

Each bacterial and fungal isolate were repeatedly subcultured using freshly prepared NA and PDA, respectively until pure cultures were obtained. To achieve fungal growth, the streaked PDA plates were incubated at 28 ± 2 °C for 5 days whereas bacterial growth involved incubating the streaked NA plates at 37°C for 24 hours. McCartney bottles were used to store the pure cultures. PDA slants with pure fungal isolates were inoculated and incubated at 25°C for 48 hours, while NA slants with bacterial isolates were incubated at 37°C for 24 hours.

2.5. Identification of Bacterial Isolates

Each bacterial isolate was subjected to Gram staining, motility test, and biochemical assays which include catalase, oxidase, starch hydrolysis, sugar utilization and citrate tests.

2.6. Identification of Fungal Isolates

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation. Identification of the isolated fungi was also identified using lactophenol cotton blue stain. The test was performed by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was properly spread on the slide with the needle. A coverslip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with $\times 10$ and $\times 40$ objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with the taxonomic scheme[15].

2.7. Sensory Evaluation

The samples were coded and presented to ten (10) semi-trained panellist drawn from undergraduate students of University of Port Harcourt to evaluate the colour, flavour, odour, taste, appearance, after-mouth feel and general acceptability. The bread samples were evaluated on a 9-point Hedonic scale (1 stand for extremely dislike and 9 is extremely like). The panellists evaluated the bread samples independently and recorded the results in sensory evaluation forms issued to them.

2.8. Statistical Analysis

Meal values of sensory scores of the bread samples were compared using one way ANOVA. Significant differences among the samples were considered at 95 % confidence interval ($p < 0.05$) with the aid of SPSS software.

3. RESULTS

Plate 1 depicts the bread samples containing different preservatives and the control. Presented in Figure 1 is the total heterotrophic bacterial count (THBC) of bread samples containing different preservatives and the control. Figure 2 shows the total fungal count (TFC) of bread samples containing different preservatives and the control. During the period of storage, THBC of bread samples containing sorbic acid, calcium propionate, sorbic acid + calcium propionate 50:50 and the control was within the range of 2.85 - 4.31, 3.37 - 4.46, 3.0 - 4.44 and 2.95 - 3.90 log₁₀CFU/g while the TFC was 2.0 - 3.78, 0 - 4.18, 0 - 3.30 and 0 - 3.59 log₁₀CFU/g, respectively.



Plate1. Bread samples containing the different preservatives

Presented in Table 1 is the bacterial genera encountered in bread samples containing different preservatives and the control during storage. A total of seven (7) bacterial genera were identified based on Gram staining and biochemical assay. They include *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Corvnebacterium* sp., *Serratia* sp., *Brevibaccillus* sp. and *Proteus* sp.

Table1. Biochemical characteristics of bacterial isolates

Isolate code	Gram reaction	Oxidase	Catalase	Lactose	Glucose	Sucrose	Citrate	Motility	Indole	Butt	Slant	H ₂ S	Gas	Methyl red	Voges Proskauer	Probable organism
BCP1BCP6	+ rod	+	+	-	-	-	+	-	+	A	B	-	-	+	+	<i>Micrococcus</i> sp.
BSA1BCP9	+ rod	-	+	-	+	-	+	+	-	A	B	-	-	+	+	<i>Staphylococcus</i> sp.
BCP2BCP3	+ rod	+	+	-	+	-	+	+	+	A	A	-	-	-	-	<i>Bacillus</i> sp.
BSACP4BC P2	+ rod	-	+	-	-	-	+	+	-			-	-	+	-	<i>Bacillus</i> sp.
BSA1BCP3	+ rod	-	+	-	+	-	-	-	-	A	B	-	-	+	-	<i>Corynebacterium</i> sp.
BSA2BCP1	- rod	+	+	-	+	+	+	+	-	A	B	-	+	-	+	<i>Serratia marcescens</i>
BSA2BCP2	+ rod	-	+	-	-	-	-	+	-	A	B	-	-	-	+	<i>Bacillus</i> sp.

Microbiological Quality, Shelf Life and Sensorial Properties of Bread Preserved with Sorbic Acid and Calcium Propionate

BSACP2BC P6	+ rod	-	+	-	+	-	-	+	-	A	B	-	-	+	-	<i>Corynebacterium</i> sp.
BSACP1BC P8	+ rod	-	+	-	+	-	+	+	-	B	B	-	+	+	+	<i>Staphylococcus</i> sp.
BSA3BCP1	+ rod	-	+	-	-	-	+	+	+	B	B	-	-	+	-	<i>Bacillus</i> sp.
BSACP2BC P5	- rod	-	+	-	+	+	+	+	-	B	B	+	+	+	-	<i>Proteus</i> sp.
BSA1BCP1	+ cocci	+	+	-	-	-	+	+	-	B	B	-	-	+	-	<i>Micrococcus</i> sp.
BSACP1BC P9	+ rod	+	+	-	+	-	+	+	+	B	B	-	-	+	+	<i>Brevibacillus laterosporus</i>
BCP1BCP5	+ rod	+	+	+	+	+	+	+	+	B	B	+	-	+	-	<i>Bacillus</i> sp.
BSA1BCP2	+ rod	-	+	-	+	-	+	+	-	B	B	-	+	+	+	<i>Staphylococcus</i> sp.

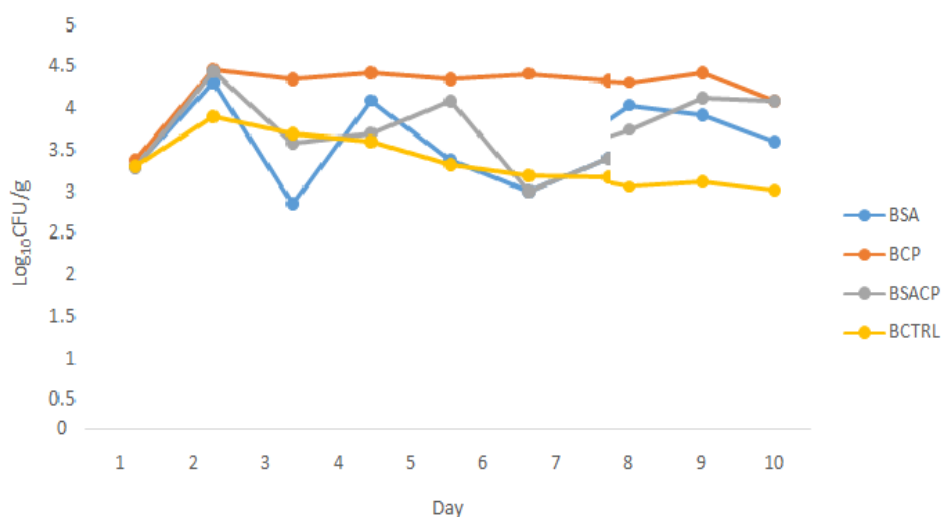


Figure1. Total heterotrophic bacterial count of bread samples containing different preservatives and the control

Key: BSA- Sorbic acid; BCP -Calcium propionate; BSACP - Sorbic acid + Calcium propionate (50:50) BCTRL= Control

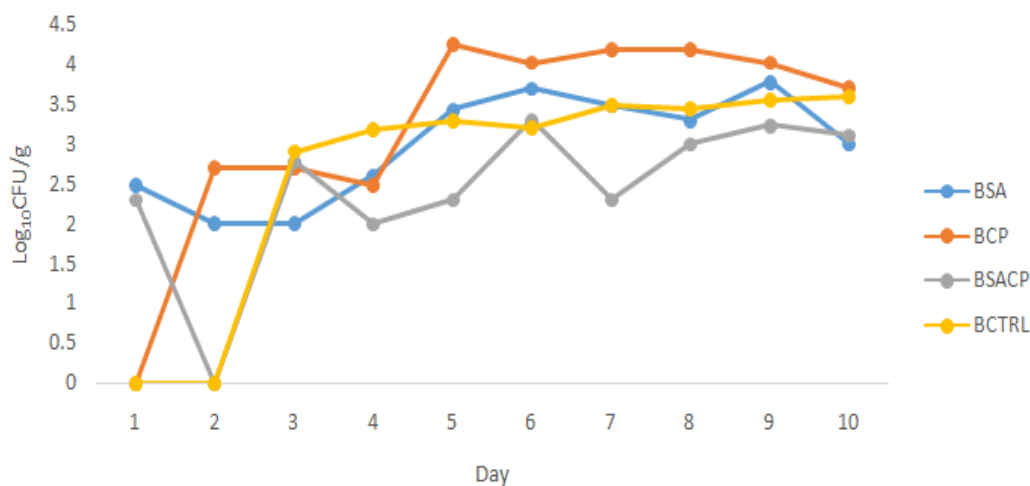


Figure2. Total fungal count of bread samples containing different preservatives and the control

Key: BSA- Sorbic acid; BCP -Calcium propionate; BSACP - Sorbic acid + Calcium propionate (50:50) BCTRL= Control

Microbiological Quality, Shelf Life and Sensorial Properties of Bread Preserved with Sorbic Acid and Calcium Propionate

A total of seven (7) fungal genera identified in this study include *Mucor* sp, *Rhizopus* sp, *Penicillium* sp, *Fusarium* sp, *Aspergillus* sp, *Neurospora* sp, and *Saccharomyces* sp. Depicted in Plate 2 - 4 are some of the fungal isolates from the bread samples containing preservatives and the control during storage.

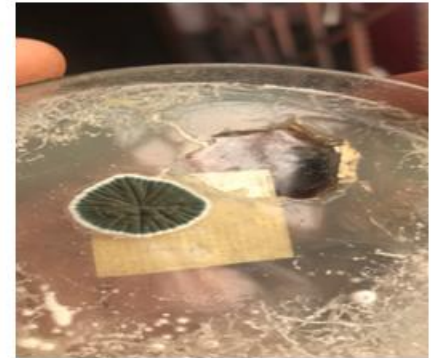
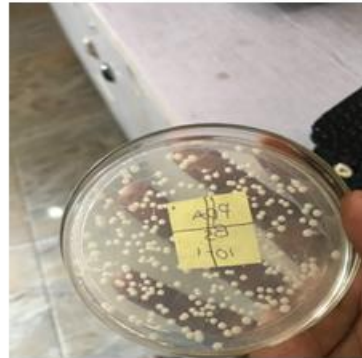


Plate2. *Aspergillus* sp. on

Plate3. Yeast colonies on PDA plate

Plate4. *Fusarium* sp. on PDA plate

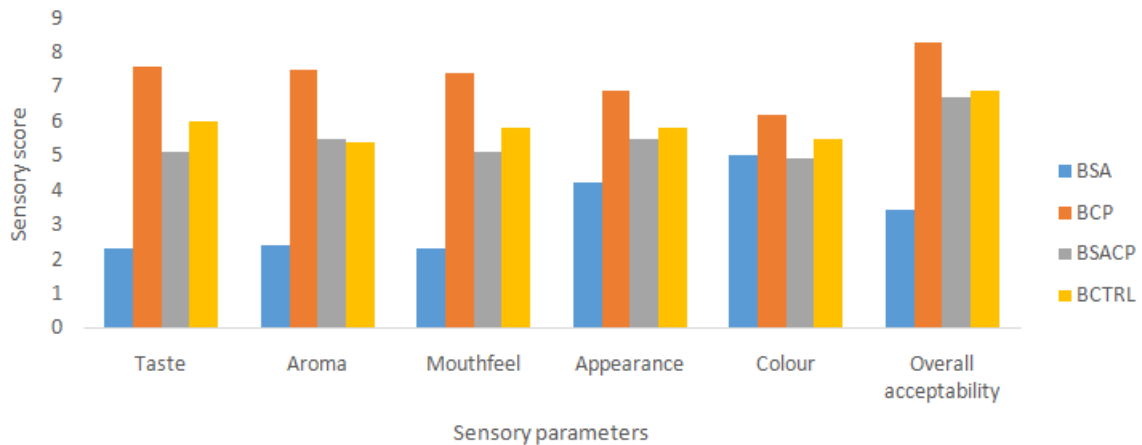


Figure3. Sensory scores assigned to sensory parameters of bread containing preservatives and the control

Key: BSA- Sorbic acid; BCP -Calcium propionate; BSACP - Sorbic acid + Calcium propionate (50:50)
BCTRL= Control

Hedonic scale: 9- like extremely; 8- like very much; 7- like moderately; 6- like slightly; 5- neither liked nor disliked; 4- disliked slightly; 3- disliked moderately; 2- disliked very much; 1- disliked extremely.



Plate5. First visible mould growth on the control sample at Day 5

4. DISCUSSION

The total heterotrophic bacterial count (THBC) of all the bread samples with preservatives and the control were within the range 2.85-4.46 and 2.95-3.90 \log_{10} CFU/g while the total fungal count (TFC) was 0 - 4.25 and 0-3.59 \log_{10} CFU/g, respectively. According to International Microbiological Standards, THBC of dry ready-to-eat (RTE) foods should be below 10^3 cfu/g. Aerobic bacterial count not exceeding 100 cfu/g is recommended by Standard Organization of Nigeria (SON) for bread [7, 16]. Almost all the bread samples evaluated in this study did not meet the recommended standard. Our result further shows that bread samples containing calcium propionate and/or sorbic acid as preservatives had higher bacterial and fungal count than the result reported for the control with some exceptions. This result is partly in agreement with research findings by Karaoglu *et al.* [17] which reported that total aerobic mesophilic bacterial count (TMBC), bacillus spore and yeast and mould count of white pan bread containing 0.2 % calcium propionate baked for 15 minutes is 2.47, 2.15, and 2.39 \log_{10} CFU/g while the result reported for the sample without the antimicrobial additive (0.2 % calcium propionate) is 2.00, 2.00 and 2.09 \log_{10} CFU/g, respectively. In contrast, TMBC, bacillus spore, yeast and mould of white pan bread without 0.2 % calcium propionate baked for 10, 20 and 25 minutes were higher than the result reported for white pan bread containing antimicrobial additive. A recent study carried out by Garcia *et al.* [18] reported that *Hyphopichia burtonii* (HB17) and *Paecilomyces variotii* (PV11) associated with bread spoilage demonstrated substantial level of resistance to calcium propionate and potassium sorbate commonly used as bread preservative.

The bacterial genera isolated from the bread samples containing preservatives and the control include *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Corynebacterium* sp., *Serratia* sp., *Brevibacillus* sp. and *Proteus* sp. Most of the bacterial genera encountered in vended bread samples in different locations reported by [19] and [20] is in agreement with the findings from this study. A possible source of contamination of the bread samples with *Bacillus* sp. is the environment where the bacterium survive mainly as spores. *Bacillus* sp. is widely distributed in many habitats mostly as saprophytes. The organism is found in soil, water, air and vegetation. *Bacillus* sp. is capable of withstanding heating which is not favourable to many bacterial genera. The bacterium is known to release toxins in food which could cause food poisoning [16, 20]. *Brevibacillus* species isolated from the bread samples are known as spore formers which are natural inhabitants of the soil. In that habitat, they could exist as saprophytes while some species could cause illness in humans.

Staphylococcus sp. encountered in the bread samples could be from the skin particularly *S. aureus* which is part of the normal flora. Other possible sources of *S. aureus* include soil, water, air and raw ingredients such as flour, yeast and sugar used for bread making. The possibility of *S. aureus* to produce enterotoxins in food is when the population of the bacterium is within the range of $10^5 - 10^6$ cfu/ml. Enterotoxin B released by *S. aureus* is responsible for food poisoning. According to Khanom *et al.* [16], *S. aureus* was found at different stages of baking bread which include mixing, milling, before and after baking.

The gastrointestinal tract is inhabited by *Proteus* sp. and *Corynebacterium* sp. Some strains of *Proteus* are responsible for enteric infections that occur in humans [20]. *Serratia marcescens* is known to be present in food, soil, water and other environments. *S. marcescens* is associated with food borne illness when food contaminated with large population of the bacterium is consumed [21].

The source of *Mucor* sp, *Rhizopus* sp, *Penicillium* sp, *Fusarium* sp. And *Aspergillus* sp. in the bread samples could be from the environment where the fungal genera exist in large numbers as spores. Through dust particles, the spores in the environment could spread and settle on food materials which will germinate into vegetative cells under favourable conditions. A recent study carried out by Onifade and Akande [22] reported the presence of *Rhizopus* sp., *Fusarium*, *Penicillium* sp., *Aspergillus*, *Saccharomyces cerevisiae*, *S. saprophyticus*, *Mucor* and *Cladosporium* sp. in vended bread samples in Akure metropolis.

Sensory evaluation report indicate that bread samples preserved with calcium propionate was most preferred by the panellist whereas the least was bread samples preserved with sorbic acid. The taste, aroma and mouthfeel of bread samples preserved with calcium propionate was liked moderately by the sensory panellist. In terms of overall acceptability, the sensory panellist very much liked the bread samples preserved with calcium propionate. This result is substantially in agreement with a related study carried out by Shahnawaz *et al.* [12] which involved sensory evaluation of bread preserved with

0.8g of calcium propionate. The panellist disliked very much the taste, aroma and mouthfeel of bread preserved with sorbic acid while the overall acceptability was disliked moderately.

5. CONCLUSION

The bread samples containing calcium propionate, sorbic acid, sorbic acid + calcium propionate in the ratio 50:50, and the control were contaminated with bacterial and fungal species. During the period of storage at room temperature, the population of the microorganisms steadily increased with some exceptions. On average, the microbial load of the bread samples preserved with sorbic acid was lower than what was reported for bread samples preserved with calcium propionate, a combination of both preservatives and the control. According to the sensory report, the most preferred bread samples were preserved with calcium propionate whereas the bread samples preserved with sorbic acid was the least.

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