

## Microbial Spoilage, Actions of Preservatives and Phytochemical Screening of Mango (*Mangifera indica*) Seed Powder

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### Abstract

The work was carried out to determine the organisms responsible for the microbial spoilage of kernels of *Mangifera indica*. A specialized kit was employed to confirm the Gram negative organisms present in the spoilt kernels of *M. indica*. The effects of chemical preservatives such as sodium benzoate, sodium acetate, citric acid and sodium chloride at different concentrations on the microbial counts and pH of mango seed powder stored at room temperature over a period of 12 weeks were studied. The mango seed kernel powder (MSK) was screened for phytochemicals. The bacteria isolated include: *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Enterobacter asburiae* and *Stenotrophomonas maltophilia*. The Gram negative organisms confirmed were *Enterobacter cloacae*, *Enterobacter asburiae* and *Stenotrophomonas maltophilia*. The isolated fungus was *Aspergillus niger*. In the analysis of different chemical preservatives on mango seed powder, the most effective preservative was 3.0% sodium benzoate followed by 5% sodium acetate and 5% common salt. Citric acid was the least effective of all the preservatives used at equal concentrations. Sodium benzoate at 3% had the least bacterial count of  $0.8 \times 10^3$  CFU/ml which was maintained from the 8<sup>th</sup> week to the last week of storage. Citric acid at 0.1% and 1.0% concentrations had bacterial counts of  $3.50 \times 10^3$  CFU/ml and  $2.0 \times 10^3$  CFU/ml respectively at the end of the 12 weeks of storage. The pH of the chemically preserved powdered kernels of *M. indica* from the 1<sup>st</sup> to the 12<sup>th</sup> week ranged from 2.70-6.01. The phytochemicals present in the mango seed powder included tannins, saponnins, polyphenol, alkaloids, flavonoids, cardiac glycosides and steroids.

**Keywords:** chemical preservatives, mango seed kernel, phytochemical screening

### Introduction

Mango (*Mangifera indica*) is a dicotyledonous plant of Sapindales order, family *Anacardiaceae*; it is juicy, with a single large kidney-shaped seed (Fowomola, 2010). The ripe fruit is variable in size and colour and may be yellow, orange red or green when ripened, depending on the cultivar. When ripened, the unpeeled fruit gives off a distinctive resinous sweet smell. In its center is a single flat oblong seed that can be fibrous or hairy on the surface (Fowomola, 2010).

Ripe mangoes are processed into frozen mango products, canned products, dehydrated products and ready to serve beverages (Ramteke and Eipeson, 1997). After consumption or industrial processing of the fruits, considerable amounts of mango seeds are discarded as waste (Table 1) (Puravankara *et al.*, 2000); seeds account for 35%-55% of the fruits, depending on the variety. Actual figures on the quantity of mango waste generated after commercial use of fruits are not readily available. Therefore, the utilization of mango secondary products, especially mango seeds, may be an economical way to reduce the problem of waste disposal from mango production.

During processing of mango, peel and kernel are

generated. Kernels take up about 17-22% of the fruit. The major components of mango seed are starch, fat and protein. The oil of mango seed kernel consists of about 44-88% saturated fatty acids. Mango seed kernels have a low content of protein but the most of the essential amino acids with highest values of leucine, valine and lysine. Mango seed kernel can be used as a potential source for functional food ingredients, animal feeds, antimicrobial compounds and cosmetic due to its high quality of fat and protein as wells as high levels of natural antioxidants.

For human, edible portion of a ripe mango fruit is about 70% by its weight. A typical composition of the mesocarp is water (84%), sugar (15%), protein (0.5%), fibers and skin (0.5%) (Pureseglove, 1991). The fruit is a rich source of vitamin A. It also contains vitamins B and C. The seed kernel contains 80% carbohydrate, 10% fat and 6% protein (Pureseglove, 1991).

Foods and microorganisms have an interesting association; foods are not only nutritious to consumers but are also excellent sources of nutrients for microbial growth, which may lead to food spoilage (Neeraj and Sharma, 2007). Although a large number of chemicals have been described that show potential as food preservatives, only a relatively

small number are allowed in food products. Those that have been approved and widely used in food are generally recognized as safe (GRAS) (Jay, 2005). Preservatives extend the shelf life of foods, making it possible to enjoy them long after harvest. They can also help foods retain flavor, color, texture and nutritional value. Preservation of food products containing chemical substances is usually based on the combined or synergistic activity of several additives, intrinsic product parameters (e.g. composition, acidity, water activity) and extrinsic factors (e.g. processing temperature, storage atmosphere and temperature) (Jay, 2005). The use of low temperature is another method of preservation. In the refrigerator or freezer, the low temperature reduces the rate of enzymatic activity in microbes and slows their rate of growth and reproduction while extending their shelf life (Edward, 2003).

Seeds of *Mangifera indica* contain phytochemicals such as tannins, polyphenol, alkaloid etc. (Kaphueakngam, 2009). These plant constituents are known to have health benefits for humans and great functions in plants. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs (Kaphueakngam, 2009). The medicinal value of plants lies in some chemical compounds that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Kaphueakngam, 2009).

## Materials and methods

### Collection of samples

Fresh mangoes (*Mangifera indica*) were plucked from tree at Tanke area, Ilorin. Collections were done using a sterile polythene bag to prevent contamination and fruits were taken to the laboratory for analysis. The flesh of the fruits was removed to get the seeds, which were properly washed.

### Preparation of mango seed kernel powder (MSK)

Mango seeds were washed with tap water to remove any contaminants, air dried and kernels were removed manually from seeds by cracking the seeds open. The kernels were grouped into three groups: A, B and C. The kernels in group A were allowed to spoil naturally and used to study the microbial spoilage. The kernels in groups B and C, to be used for the study of the effects of preservatives and screened for phytochemicals respectively, were chopped, spread thin in trays and air dried at room temperature. The dried materials were grinded in a blender into a powdery form and stored.

The different preservatives used in the study were sodium benzoate, sodium acetate, citric acid and sodium chloride at the following concentrations: 0.1%, 1.0%, 3.0% and 5.0%. The pH and bacterial count of the various samples were carried out at 7 days intervals for 12 weeks.

### Bacterial isolation

The bacterial isolation was carried out using the dilution plate method. This involved making a serial dilution of the sample and then isolating the bacteria from desired solutions. One gram of the group A sample of the mango seed kernel, which had been allowed to spoil naturally, was weighed into a sterile test tube containing 9 ml sterile distilled water. This

gave  $10^{-1}$  dilution; 1 ml aliquot was transferred from the  $10^{-1}$  dilution with the aid of sterile pipette into another test tube containing 9 ml of sterile distilled water, this gave  $10^{-2}$  dilution and was continued to dilution factor of  $10^{-6}$ . Work was done aseptically. After the preparation of dilutions, bacteria were isolated from the serial dilutions. 1 ml aliquot from the  $10^{-3}$  and  $10^{-5}$  dilutions was introduced into the middle of empty sterile petri dishes. 20 ml of molten nutrient agar which had cooled to about 45 °C was gently and aseptically poured on the inoculum. This was thoroughly mixed by swirling. The plates were incubated at 37 °C and examined after 24 hours. Sub culturing of the isolates were done until pure cultures of all the isolates were obtained. They were put into McCartney bottles and incubated at 37 °C for 24 hours. The pure cultures were then kept as stock culture in the refrigerator at 4 °C to avoid contamination.

### Characterization and identification of bacterial isolates

The characterization and identification of isolates were based on colonial, morphological, cultural and microscopic observation. The colonial morphology observed included the shape, colour, edge, elevation, optical characteristics and surface texture of the colonies; the traits were observed microscopically based on Fawole and Oso (2004) method. The cellular morphology of the bacteria isolates was determined through gram staining, capsule staining and spore staining. Different biochemical tests such as Gram staining, motility, fermentation of sugars, methyl red, vogues proskauer, indole, catalase, coagulase, citrate, oxidase and urease were carried out.

### Confirmatory test for the Gram negative organisms using the Microbact Gram-negative identification system 24E (12A (12E)+(12B) for Enterobacteriaceae

The Microbact Gram negative system is a standardized micro substrate system, designed to simulate conventional biochemical substrates used in the identification of Enterobacteriaceae and common miscellaneous Gram negative bacilli. Organism identification is based on pH change and substrate utilization. The Microbact Gram negative product consists of substrate strips which contains biochemical substrates. The kit also contains one holding tray, technical product insert, reagents, organism ID report forms and an interpretation chart. The Microbact Gram-negative identification system 24E (12A (12E)+(12B) for Enterobacteriaceae was further used to confirm the Gram-negative organisms isolated from the spoiled mango seed. The procedures followed were:

### Isolation

An 18 hour pure culture of the organisms to be identified was obtained using the appropriate agar media. Oxidase test was performed on the organisms to be identified.

### Preparation of inoculums

One to three isolated colonies were picked from an 18 hour culture and emulsified in 5 ml of sterile saline solution. It was mixed thoroughly to prepare a homogenous suspension.

### Inoculation

The wells of individual substrate sets were exposed by cutting the end tag of the sealing strip and slowly pulling it

back. The strip was placed in a holding tray and using a sterile Pasteur pipette, 4 drops of bacterial suspension were used to half fill each well in the set. Using a sterile pipette or dropper bottle, the substrates underlined on the holding tray were overlaid with sterile 2 drops of mineral oil to block the wells.

#### Incubation

The inoculated rows were re-sealed with the adhesive seal with the specimen identification number written on the end tag with marker pen. It was incubated at 35 °C for 18-24 hours. To determine the purity of the inoculums, a solid non selective medium was inoculated with the test suspension to act as a culture purity check.

#### Reading the test strip

The strips were removed from the incubator, the sealing tape was pulled back and all positive results were recorded. The reactions (Plates 1 and 2) were evaluated as positive or negative by comparing them with the color chart. The results were recorded under the appropriate heading on the report form with reference to the table of reactions.

#### Determination of effects of chemical preservatives on mango seed kernel

The group B of the mango seed kernel, which had been chopped, air dried at room temperature and grinded into powder was used for the chemical preservation. The MSK powder was weighed as samples of 100 g each, into 17 sterile containers, 16 of these for the chemical preservation and the 17<sup>th</sup> for the control, to which no preservative was added. The different preservatives used were sodium benzoate, sodium acetate, citric acid and sodium chloride at the following concentrations: 0.1%, 1.0%, 3.0% and 5.0% each. The different chemicals at the different concentrations were introduced into the MSK in the containers and labeled appropriately. The chemically preserved kernels were stored at room temperature for a period of 12 weeks. The microbial count and pH of the samples were determined at an interval of 7 days.

#### Determination of the total microbial counts of chemically preserved mango seed kernels

One gram of each of the samples was dispensed into 10 ml distilled water in a sterile test tube. 1 ml of this solution was transferred into 9 ml sterile distilled water to make a one in ten dilution. The serial dilution was repeated in six folds. One gram from the dilution of  $10^{-4}$  was used to seed the plates with nutrient agar using the pour plate method. The plate was incubated at 37 °C for 24 hours. The bacterial count was recorded after 24 hours incubation using a colony counter.

#### Determination of pH of chemically preserved mango seed kernels

The pH of the mango kernel seeds being preserved was taken by weighing 1 g of the samples into a test tube containing 10 ml of distilled water, it was centrifuged and the pH determined using a pH meter (the instrument was standardized with a buffer solution).

#### Phytochemical screening assay

The group C, grounded mango seed kernel powder, was used for the phytochemical screening. 500 g of the MSK

powder was soaked in methanol for 3 days. Afterwards, it was filtered using a filter paper to get the extract, which was concentrated using a water bath. The concentrated filtrate was then used to carry out the various phytochemical tests. The method described by Trease and Evans (2002) was used to test for tannins, saponins, polyphenol, alkaloids, flavonoids, cardiac glycosides and steroids.

#### Results

The results of the bacterial culture, morphological and biochemical characteristics of spoilt mango seed kernel are presented in Table 1. The conditions of growth necessary for the isolates and the shape, size and appearance of the different isolates are highlighted. Altogether 5 bacteria were tentatively identified (Table 2): *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter cloacae*, *Enterobacter asburiae* and *Stenotrophomonas maltophilia*. The organisms confirmed using the Microbact kit includes: *Enterobacter cloacae*, *Enterobacter asburiae* and *Stenotrophomonas maltophilia* (Tables 3 and 4).

Table 1. Phytochemical compounds present in MSK

Phytochemical Compounds	Present (+)
Tannins	+
Saponnin	+
Polyphenol	+
Alkaloids	+
Flavonoids	+
Cardiac glycosides	+
Steroids	+

The change in the total bacterial counts of the mango seed powder preserved with different concentrations of sodium benzoate is graphically shown in Fig. 1. The total bacterial counts of the 0.1% concentration of sodium benzoate was reduced over the period of storage, as the count reduced from an initial  $1.8 \times 10^3$  CFU/ml in the 1<sup>st</sup> week to  $1.6 \times 10^3$  CFU/ml in the 12<sup>th</sup> week. The MSK preserved with 1.0% sodium benzoate had a slight decrease from  $2.3 \times 10^3$  CFU/ml in the 1<sup>st</sup> week to  $1.0 \times 10^3$  CFU/ml in the 12<sup>th</sup> week. The MSK preserved with 3% sodium benzoate gave the lowest bacterial count of  $0.8 \times 10^3$  CFU/ml at the end of the storage period. The MSK preserved with 5% sodium benzoate with a count of  $3.5 \times 10^3$  CFU/ml in the 1<sup>st</sup> week reduced to a count of  $1.2 \times 10^3$  CFU/ml in the 12<sup>th</sup> week.

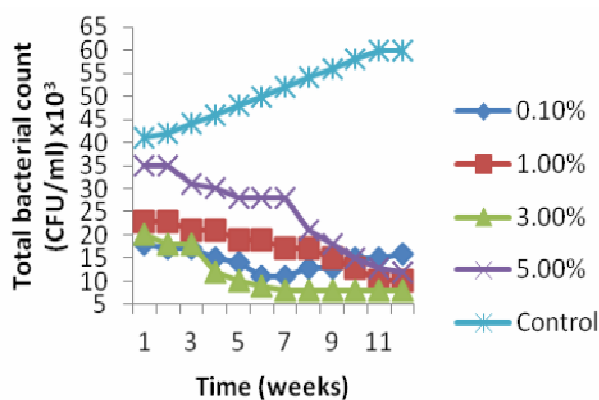


Fig. 1. Changes in the total bacterial counts of varying concentrations of sodium benzoate preserved powder of mango seed stored at room temperature for 12 weeks

Table 2. The cultural, morphological and biochemical characteristics of bacterial isolates

Isolate	Cultural characteristics	Morphological characteristics	Gram stain	Motility	Glucose	Sucrose	Lactose	Methyl red	Voges Proskauer	Indole	Coincubase	Citrate	Oxidase	Catalase	Urease	Tentative organism
A	Golden yellow <i>Staphylococcus aureus</i> smooth & raised	Spherical cocci, grape-like clusters			+		-	+	+	-	-	+	-	-	+	-
B	Hard and light pink <i>Staphylococcus subsp.</i>	Rod shaped			+	+	-	-	+	-	+	+	-	-	+	+
C	Yellow pigmentation <i>Enterobacter cloacae</i> with mucoid surface	Small rods			-	-	+	+	+	-	+	-	+	-	+	-
D	Pale yellow <i>Enterobacter asburiae</i> pigmentation with mucoid surfaces	Small to Medium rods			-	-	+	+	+	+	-	-	+	-	-	+
E	Creamy colonies <i>Serratia marcescens</i> with flat surfaces and <i>multophilic</i>				-	-	+	+	-	+	-	+	+	-	+	-

Legend: + = positive, - = negative

Table 3. Microbact biochemical identification of *Staphylococcus* spp. substrates and reactions 24E (12A (12E) + (12B))

S/No	Colour pre incubation	Colour post incubation	Sugars	2	Mannitol	Mannose	Sucrose	Trehalose	N-acetyl glucosamine	Arginine utilisation	Urease test	Beta-glucosidase	Alkaline phosphate	Beta-glucuronidase	Beta-galactosidase	Organism
1					+			+		+		+				+
2																
3																+

Legend: + = positive, - = negative

Table 4. MicroBact biochemical identification *Enterobacter* spp. substrates and reactions 24E (12A (12E) + (12B))

	12A/24E	12B/24E
Colour pre incubation		
Colour post incubation		
Lysine		
Ornithine		
H <sub>2</sub> S		
Glucose		
Mannitol		
Xylose		
ONGP		
Indole		
Urease		
VP		
Citrate		
TDA		
Gelatin		
Malonate		
Inositol		
Sorbitol		
Rhamnose		
Sucrose		
Lactose		
Araninose		
Adonitol		
Raffinose		
Salicine		
Arginine		
Organism		

3 + *Enterobacter asburiae*

Legend: + = positive, - = negative, ONGP= O-nitrophenyl-β-d-galactopyranoside, VP= Voges-Proskauer reaction, TDA= Tryptophan deaminase

The changes in the total bacterial counts of MSK preserved with different concentrations of sodium acetate over the 12 week period are depicted in Fig. 2. At the end of the storage period, the kernels preserved with 3.0% sodium acetate, which had  $3.8 \times 10^3$  CFU/ml in the 1<sup>st</sup> week gave the lowest bacterial count of  $15.0 \times 10^3$  CFU/ml in the 12<sup>th</sup> week of preservation. In Fig. 3 is showing the total bacterial count of citric acid preserved MSK at different concentration; the 5% citric acid yielded  $2.6 \times 10^3$  CFU/ml in the 1<sup>st</sup> week and decreased to a count of  $1.6 \times 10^3$  CFU/ml at the end of the 12<sup>th</sup> week. The 0.1% citric acid preserved MSK with an initial bacterial count of  $5.0 \times 10^3$  CFU/ml in the 1<sup>st</sup> week had the highest bacterial count of  $3.5 \times 10^3$  CFU/ml in the 12<sup>th</sup> week.

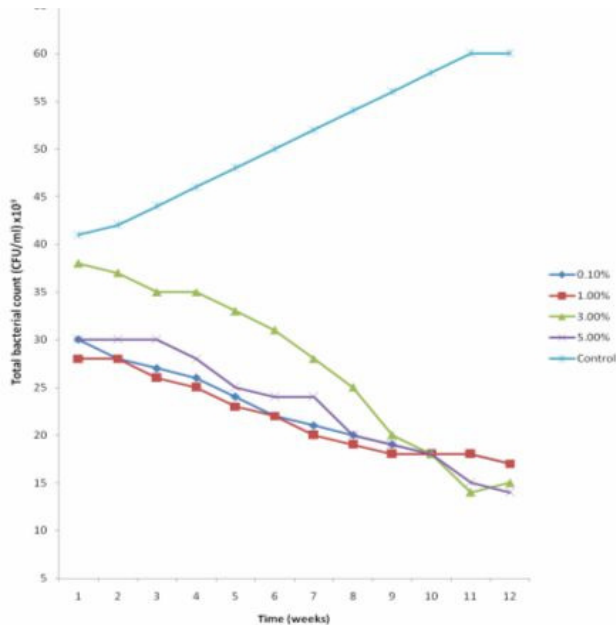


Fig. 2. Changes in the total bacterial counts of varying concentrations of sodium acetate preserved powder of mango seed kernels stored at room temperature for 12 weeks

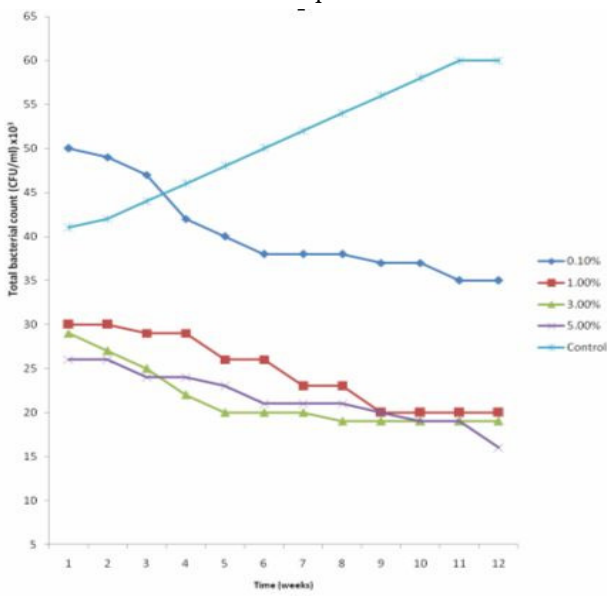


Fig. 3. Changes in the total bacterial counts of varying concentrations of sodium chloride preserved powder of mango seed kernels stored at room temperature for 12 weeks

The total bacterial count of MSK preserved with different concentrations of NaCl is shown in Fig. 4. The lowest count was for 5.0% NaCl, with an initial bacterial count of  $2.6 \times 10^3$  CFU/ml and final count of  $1.3 \times 10^3$  CFU/ml. 3% NaCl had the highest count with an initial count of  $3.5 \times 10^3$  CFU/ml and final count of  $1.8 \times 10^3$  CFU/ml in the 12<sup>th</sup> week.

The change in pH of sodium benzoate preserved MSK at different concentrations over a period of 12 weeks is depicted in Fig. 5. The highest pH value was recorded as 6.00 from the mango seed powder preserved with 3% sodium benzoate, from an initial pH of 4.9 in the 1<sup>st</sup> week. 1.0% sodium benzoate preserved MSK gave the lowest pH with an initial pH 4.89 in the 1<sup>st</sup> week and final pH of 5.49 in the 12<sup>th</sup> week.

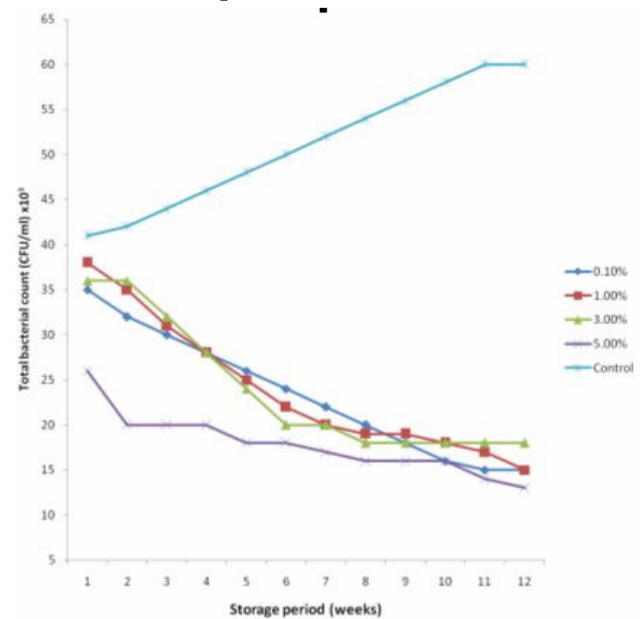


Fig. 4. Changes in the total bacterial counts of varying concentrations of citric acid preserved powder of mango seed kernels stored at room temperature for 12 week

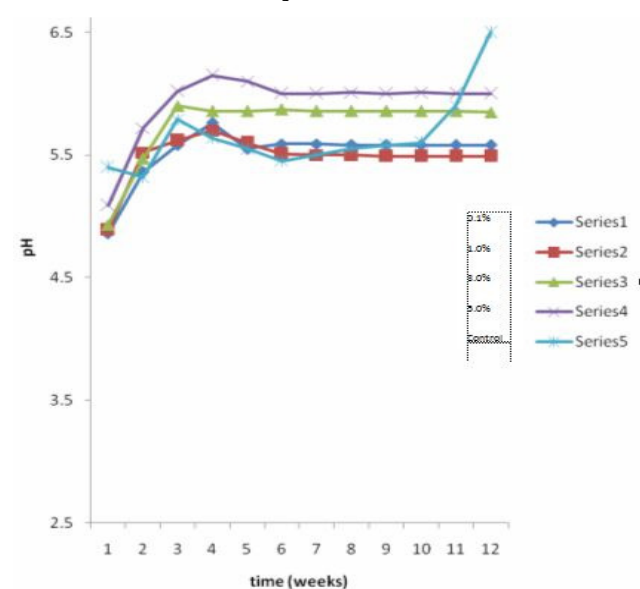


Fig. 5. Changes in the pH of varying concentrations of sodium benzoate preserved powder of mango seed kernels stored at room temperature for 12 weeks. Each reading represents an average of 3 determinates



In Fig. 6 the changes in pH of MSK preserved with sodium acetate at different concentrations are shown. At the end of 12 weeks, the mango powder preserved with 1.0% sodium acetate had the lowest pH value with an initial value of 4.80 in the 1<sup>st</sup> week and final value of 5.30 in the 12<sup>th</sup> week. The 5% sodium acetate preserved kernels had increased from 5.33 in the 1<sup>st</sup> week to 5.85 in the 12<sup>th</sup> week, which was the highest recorded pH value for this category.

The pH change of MSK preserved with citric acid over a period of 12 weeks is depicted in Fig. 7. The MSK preserved with 5% citric acid had the lowest pH recorded with an initial pH of 2.66 in the 1<sup>st</sup> week and final pH of 3.5 in the 12<sup>th</sup> week of preservation. The highest pH was recorded in the 1.0% citric acid preserved MSK with an initial pH of 2.7 in the 1<sup>st</sup> week and final pH of 5.6 in the 12<sup>th</sup> week of preservation.

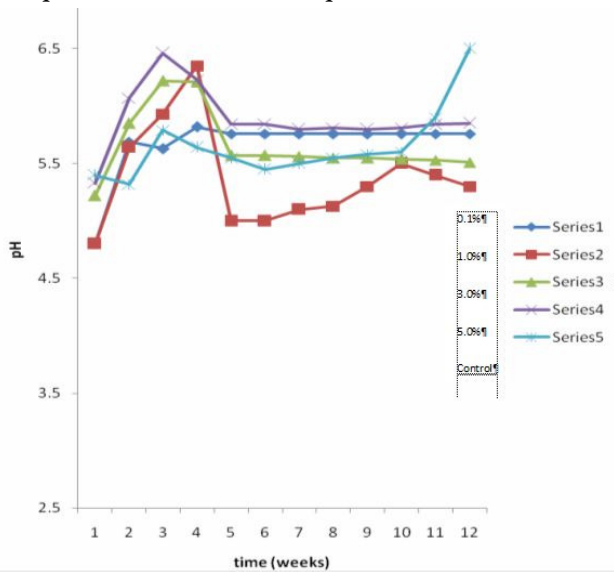


Fig. 6. Changes in the pH of varying concentrations of sodium acetate preserved powder of mango seed kernels stored at room temperature for 12 weeks. Each reading represents an average of 3 determinates

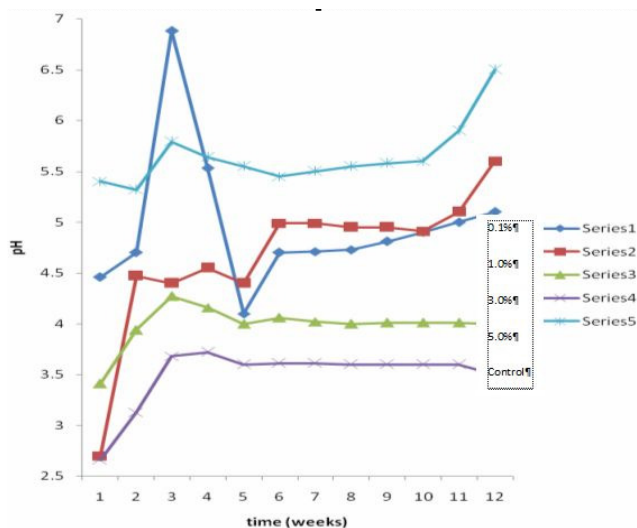


Fig. 7. Changes in the pH of varying concentrations of citric acid preserved powder of mango seed kernels stored at room temperature for 12 weeks. Each reading represents an average of 3 determinates

The changes in pH of MSK preserved with different concentrations of NaCl for 12 weeks are shown in Fig. 8. The lowest pH at the end of 12 weeks was 5.5, which was recorded in the 1.0% NaCl preserved MSK, while the highest pH at the end of 12 weeks was recorded as 5.60 in the 3% NaCl preserved MSK.

The entire chemically treated mango seed powder showed a relative decrease in the bacterial counts at the end of the 12 weeks period of preservation. The pH values also showed an increase in all the recorded values at the end of storage.

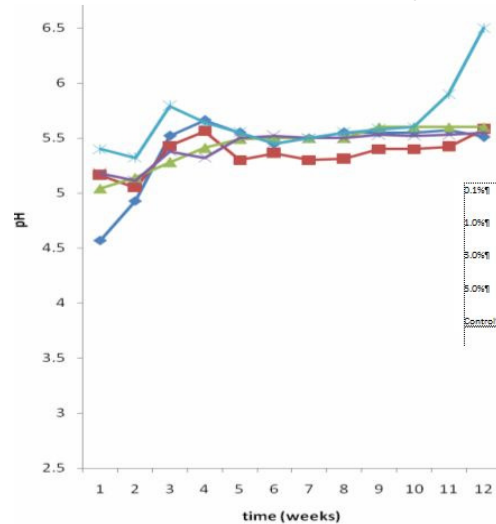


Fig. 8. Changes in the pH of varying concentrations of sodium chloride preserved powder of mango seed kernels stored at room temperature for 12 weeks. Each reading represents an average of 3 determinates



Plate 1. Microbact wells before and after inoculation with organisms



Plate 2. Color change in the Microbact wells after 24 hours incubation

## Discussions

Microbial growth is the degradation of food quality which leads to visible changes in color, odor and texture, due to the actions of microorganisms. Microbial growth in foods is controlled by intrinsic factors such as pH and moisture content, but also by extrinsic factors. The microbiology of mango seed powder spoilage was studied during the course of this project, altogether five bacteria were isolated. Two of the bacteria were Gram positive. They include *Bacillus subtilis* and *Staphylococcus aureus*. The three Gram negative isolates were *Enterobacter cloacae*, *Enterobacter asburiae* and *Stenotrophomonas maltophilia*. The only fungus isolated was *Aspergillus niger*, a primary pathogen and was observed early on the mango seed kernel during the spoilage process spreading rapidly throughout the length of the kernel.

The data obtained is in accordance with result of Jay (2005), who reported that due to the extremely high fat and low water content of products such as walnuts and pecans, these products are quite prone to spoilage by bacteria. This result also confirms the work of King et al. (1990) who reported that microorganisms associated with commercially shelled nuts and kernels are numerous and varied. King et al. (1990) reported that the genera of bacteria isolated from almonds include *Bacillus* sp., *Staphylococcus* sp. and *Enterobacteria* sp., but also molds such as *Aspergillus niger* and *Aspergillus flavus* were found associated with commercially shelled nuts.

Because of the extensive use of MSK as human food and livestock feeds, the microbiology and safety of the seeds is very important. The sources of contamination are numerous, but all are traceable to the environment in which the kernels are grown, handled and processed. The source of these organisms could probably be from the soil, the seeds themselves, the handlers, birds, animals, storage, air and dust (Jay et al., 2005). *Aspergillus* spp. was a primary pathogen and was responsible for black mould rot of mango seed. They are naturally found on fruits and other substrates that can provide nutrient, they can tolerate high concentration of sugar; they have been found to be an important pathogen of many crops and cause serious economic losses (Jay et al., 2005).

The chemically preserved kernels of *Mangifera indica* were found to undergo series of changes. These changes include modifications in total bacterial counts and pH. These changes are as a result of the effects caused by the various chemical preservatives used on the microorganisms present in the preserved powder of *M. indica*.

In Fig. 1, the differences in bacterial counts of sodium benzoate preserved powder of *M. indica* at 3.0% and 5.0% concentration from week 1 to week 12 dropped consistently at a slow rate over the period of preservation. Besides, the 0.1% sodium benzoate preserved mango seed powder showed a relative decrease in bacterial counts throughout the period of observation. The bacterial counts of the 5% sodium benzoate preserved mango seed powder remained high at  $3.5 \times 10^3$  CFU/ml within the first 2 weeks of preservation. This could be as a result of the stable condition of the mango seed powder, but which over time became unfavourable as a result of exhaustion of nutrient in the medium, after which death of the organisms set in, leading to a decrease in the number of microorganisms present. From Fig. 1, it can be deduced that

the most effective concentration of sodium benzoate on powdered seed of *M. indica* was 3%. The bacterial counts at 3% sodium benzoate was the lowest from the 6<sup>th</sup> week up to the 12<sup>th</sup> week and it maintained a bacterial counts of  $0.8 \times 10^3$  CFU/ml from the 7<sup>th</sup> week to the 12<sup>th</sup> week. The control sample showed an increase in total bacterial counts over the period of observation with the highest count of  $6.0 \times 10^3$  CFU/ml.

The mechanism of action of sodium benzoate starts with the absorption of benzoic acid into the cell. If the intracellular pH falls to 5 or lower as indicated in Fig. 6, the anaerobic fermentation of glucose through phosphofructokinase decreases sharply (Krebs et al., 2004) inhibiting the growth and survival of microorganisms that cause food spoilage.

From Fig. 3, the bacterial counts decreased slightly at 0.1% and 1.0% concentration from week 1 to week 12. The 3.0% and 5.0% sodium acetate preserved kernels had a steady but slow decline in the bacterial counts from the 1<sup>st</sup> week to the 12<sup>th</sup> week, with the lowest bacterial count being recorded as  $1.4 \times 10^3$  CFU/ml in the 12<sup>th</sup> week of the 5.0% concentration sodium acetate preserved mango powder. The control sample showed a steady increase in total bacterial counts over the period of study, with the highest count of  $6.0 \times 10^3$  CFU/ml. In the food industry, sodium acetate is used as a preservative because it helps the food maintain a specific pH, thereby prohibiting the growth of unwanted bacteria.

In Fig. 4, the citric acid preserved mango seed had a high bacterial count in the first four weeks of preservation. The bacterial counts reduced slightly from week 5 to week 12 with the 0.1% citric acid preserved mango seed powder having the highest bacterial count of  $3.5 \times 10^3$  CFU/ml. The 1.0% and 3.0% citric acid preserved mango seed powder had bacterial counts of  $2.0 \times 10^3$  CFU/ml and  $1.9 \times 10^3$  CFU/ml in the 12<sup>th</sup> week. The 5.0% concentration of preserved mango seed powder yielded the lowest bacterial counts in this category at the end of 12 weeks, with a final bacterial count of  $1.6 \times 10^3$  CFU/ml. The control sample showed a slow increase in the bacterial count over the period of study with the highest count of  $6.0 \times 10^3$  CFU/ml. Citric acid shows an unexpected ability to enhance the antimicrobial power of a wide range of antibiotic agents to kill or inhibit a wide range of bacterial species. Citric acid prevents bacterial growth by enhancing the antimicrobial properties of polyphenols of plant origin, which have been found to be present in mango seed. The main effect of citric acid as a preservative in food is due to the prevention of absorption of essential nutrients by the microorganisms, due to disruption of the protein motive force which provides energy for active absorption of nutrients (EFSA, 2012). It alters the permeability of cell wall causing damage and hence cell death especially in Gram negative bacteria. It is also capable of chelating metal ions present in the cell wall thereby causing cell damage (EFSA, 2012).

Moreover, Fig. 5 shows the varying concentrations of NaCl used in preserving the mango seed. The total bacteria counts reduced slightly from week 1 to week 6 and maintained almost the same range of values up to the 12<sup>th</sup> week with slight fluctuations in values. The 0.1% and 1.0% concentrations of preserved mango seed powder had initial bacterial counts of  $3.5 \times 10^3$  CFU/ml and  $3.8 \times 10^3$  CFU/ml respectively in week 1 and reduced gradually to  $1.5 \times 10^3$  CFU/ml and  $1.4 \times 10^3$  CFU/ml respectively in week 12. The 5.0% concentration of preserved mango seed powder had the lowest bacterial counts

in this category with the value of  $1.3 \times 10^3$  CFU/ml at the end of 12 weeks. The mechanism through which NaCl works is through the dehydration of water from cell membrane. Hence, salt is effective as a preservative, because it reduces the water activity of foods. The water activity of food is the amount of unbound water available for microbial growth and chemical reactions (Potter and Hotchkiss, 1996). This ability to reduce the water activity of foods is thought to be due to the ability of Sodium and Chloride ions to associate with water molecules (Potter and Hotchkiss, 1996). It is possible that the quantity of water present in the samples and cells' membrane in the first few weeks were higher, which resulted in a high increase in microbial activities and bacterial counts than those of the subsequent weeks. It has been suggested that for some microorganisms, salt may limit oxygen solubility, interfere with cellular enzymes or force cells to expend energy to exclude sodium ions from the cell, reducing the rate of microbial growth (Shelef and Seiter, 2005).

Hashmi *et al.* (2007) observed that potassium sorbate, potassium metabisulphite, sodium benzoate, separately or in combination with other chemical preservatives used for the improvement of sensory characteristics of mango puree, control microbes and retain overall acceptability. Sofos *et al.* (1981) and Sonia *et al.* (2003) studied the physico-chemical and sensory characteristics of stored mango pulp. They proposed from their study that pulp can be stored with the application of sodium benzoate at the concentration of 600 mg and 1200 mg of potassium metabisulphite for 360 days at room temperature without severe effect on the sensory characteristics of mango pulp. They also found that the effect of these preservatives on the taste of pulp is variable.

In Figs. 6, 7, 8 and 9, the pH values for the chemically preserved samples ranged from 2.70-6.0, while the values recorded by Arekemase *et al.* (2012) attained a pH stability of 6.50 for chemically preserved *Irvingia gabonensis*. The variation in values could be as a result of the technique of preservation. The slight increase in the pH of preserved powdered mango seed might be due to the chemical preservatives used. These preservatives inhibited microbial growth resulting in a low population. The rate of release of metabolic products was slow and the amount was small causing little or no production of acid in the medium, thereby having little or no effect on the reduction of the pH, but rather a slight increase. Also, the nature of microorganisms present could influence the increase in pH; for example, the presence of acid producing bacteria such as lactic acid bacteria in the medium will cause decrease of pH in the medium. Thus samples with higher pH suggests low acidity and high alkalinity and inhibition of growth of other microorganisms that are acid tolerant.

It is clearly observed from the samples used as control, which were not chemically preserved, that the total bacterial counts were higher when compared to those that were chemically preserved at various concentrations and stored at room temperature, and the rate of spoilage was greater. The bacterial counts of the mango seed powder used as the control increased steadily from the 1<sup>st</sup> week to the 12<sup>th</sup> week of preservation with an overall high counts than the kernels preserved with the various chemical preservatives used during the course of this study. The slow increase in the microbial count of the control samples can be said to be due to the dried form in which the MSK were kept, because the moisture content of the powder had been reduced by drying before

storage, thereby limiting the activities of microorganisms. The samples that were chemically preserved showed a decline in microbial counts over the period of preservation as shown in previous figures when compared to the non-chemically preserved samples. The effective preservation of the added chemicals on the preserved mango seed powder was found to have retarding effects on the microorganisms present, with the sodium benzoate preserved powdered seed having the best effects at a concentration of 3.0%. The rate of spoilage was minimized due to the chemical method of preservation used.

In general, fruits and vegetables contain many antioxidant compounds. Mango seed has also been confirmed to contain various phenolic compounds and can also be a source of natural antioxidants. During the course of this project the mango seed kernels was subjected to various phytochemical tests to ascertain the presence of antioxidants. The phytochemical study indicated the presence of an array of phytochemicals which are saponnin, polyphenol, alkaloids, flavonoids, cardiac glycosides and steroids. This result agrees with Jasminder *et al.* (2010). They also isolated saponnin, flavonoid, glycosides and alkaloids in the preliminary investigation of the antibacterial activity of mango (*Mangifera indica*) seed.

## Conclusions

In conclusion, mango seed kernel is prone to microbial spoilage and the use of chemical preserving measures is very important to keep the kernels and foods generally in their natural state and prolong their shelf life, especially seasonal foods such as the mango seed kernel, in order to make them available all year round. The various chemical preservatives used in this study are generally regarded as safe and should be encouraged in foods. Also, natural antioxidants, some of which were isolated in the course of this study, are preferred in cosmetic and food processing industry. The limitation of using a plant extract as a natural antioxidant is the availability and cost. However, the availability and cost factor appear to be negligible when it is possible to generate a plant based natural antioxidant from a waste material such as mango seed kernel.

## References

- Arekemase MO, Omokafe D, Ajiboye EA, Adedayo MR (2012). Studies on some preservatives and phytochemical screening of *Irvingia gabonensis*. World Journal of Biological Research 5 45-60.
- Edward IA (2003). An introduction to microbiology. Jones and Bartlett Publishers, Inc. 238 p.
- European Food Safety Authority (EFSA) (2012). Food safety 10(2):2571.
- Fawole MO, Oso BA (2004). Laboratory Manual of Microbiology. Spectrum 3<sup>rd</sup> edition. Academic Press Ibada, p. 21-51.
- Fowomola MA (2010). Some nutrients and anti nutrients contents of mango seed (*Mangifera indica*). African Journal of Food Science 4(8):472-476.
- Hashmi MSS, Alam A, Shah AS (2007). Studies on Microbial and Sensory Quality of Mango Pulp Storage with Chemical Preservatives. Pakistan Journal of Nutrition 6(1):85-88.



- Jay MJ, Martin J, Loessner I, David AG (2005). Modern food microbiology, 7<sup>th</sup> Ed. p. 301.
- Jay MJ, Martin J, Loessner I, David AG (2005). Modern Food Microbiology, 7<sup>th</sup> edition. Chapman and Hall, New York, p. 301.
- Kaphueakngam P, Flood A, Sonwani S (2009). Production of cocoa butter equivalent from mango seed almond fat and palm oil-mid fraction. Asian Journal of Food and Agro-industry 2(4):441-447.
- Krebs HA, Wiggins D, Stubbbs M, Sols A, Bedoya F (2004). Studies on the mechanism of the antifungal action of benzoate. Biochemical Journal 214(3):657-663.
- Neeraj D, Sharma S (2007). Food spoilage, food infections and intoxications caused by microorganisms and methods of their detection. Food and Industrial Microbiology. <http://nsdl.niscair.res.in/jspui/bitstream/123456789/386/2/FoodSpoilage.pdf>.
- Potter NN, Hotchkiss JH (1995). Food science. Texts series. 5<sup>th</sup> edition. New York; Chapman and Hall.
- Puravankara D, Bohgra V, Sharma RS (2000). Effect of antioxidant principles isolated from mango (*Mangifera indica*) seed kernels on oxidative stability of buffalo ghee (butter fat). Journal of the Science of Food and Agriculture 80(4):522-526.
- Purseglove JW (1991). Tropical crops; Dicotyledons. Harlow, Longman Scientific and Technical Publishers, Harlow, p. 29.
- Ramteke RS, Elieson WE (1997). Effect of additives on the stability of mango aroma concentrate during storage. Journal of Food Science and Technology 34(3):195-199.
- Shelef AJ, Selter J (2005). Indirect and miscellaneous antimicrobials in food. 3<sup>rd</sup> ed. Taylor and Francis. Boca Raton FL, p. 573-598.
- Sofos JN, Busta FF (1981). Antimicrobial activity of sorbate. Journal of Food Protection 44:614-622.
- Sonia SDS, Sogi A, Bawa S (2003). Shelf-life studies on chemically preserved sand pear pulp. Journal of Food Science Technology 40:230-232.
- Trease GE, Evans WC (2002). Pharmacognosy. 15<sup>th</sup> Ed. London; Saunders Publishers; p. 42-44, 221-229, 246-249, 304-306, 331-332 and 391-393.