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THE POTENTIALS OF GINGER AND SELECTED CHEMICALS AS PRESERVATIVES ON THE SHELF LIFE OF GROUND SEEDS OF MELON (*Colocynthis citrullus* L.)

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ABSTRACT

The research examined the potentials of ginger and selected chemicals as preservatives on the shelf life of ground seeds of melon (*Colocynthis citrullus* L.). Due to increased search for natural antimicrobials and preservatives, the preservative effect of ginger was compared with that of sodium chloride, sorbic acid and calcium propionate in extending the shelf life of ground melon seed (GMS) at concentrations of 0.1%, 1%, 3% and 5%. The treated and untreated samples were stored at room temperature for 12 weeks. The bacterial counts during the 12 week storage ranged from 0.2×10^5 to 1.69×10^6 cfu/ml while the control recorded the highest microbial counts. The sample treated with 5% ginger exhibited the highest preservative action at 5weeks while, the samples treated with calcium propionate at all concentrations exhibited the least preservative action. A marginal decrease in pH was observed for all samples except for those treated with sorbic acid. Phytochemicals isolated from ginger include tannins, glycosides, saponins, flavonoids, coumarins, phenols and terpenoids which might be responsible for its antimicrobial properties. In conclusion, 5% ginger gave the best preservative action followed by 5% sorbic acid and 5% Sodium chloride while all concentrations of calcium propionate showed the least preservative action.

Keywords: preservative, ginger, shelf life, phytochemical, antimicrobial, extract.

INTRODUCTION

Food is essential for survival. Since the beginning, people have been interested in preserving food for later consumption (Dharmadikari, 2015). In recent years, the use of chemical preservatives has increased due to the development in marketing and distribution of the food we consume and also because of the large variety of food offered for consumption (Dharmadikari, 2015).

A preservative is a substance that is added to products such as food, pharmaceuticals, paints, biological samples, wood, beverages etc. to prevent undesirable chemical changes and decomposition by microbial growth. Preservative food additives reduces the risk of food borne infections, decreases microbial spoilage and preserve fresh attributes and nutritional quality (Erich and Gert-Wolfhard, 2002).

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Since ancient times, spices and herbs have been added to foods as flavoring agents, folk medicine and food preservatives (Marija and Navena, 2009). As reported by Adesokan, (2014) many natural occurring compounds known as phytochemicals found in edible and medicinal plants, herbs and spices such as ginger have been shown to possess antimicrobial activities against food spoilage and food borne pathogen.

The genus *Zingiber* includes about 100 species of aromatic herbs from East Asia and tropical Australia. The name of the genus *Zingiber* is derived from a 'Sauskrit" word denoting horn-shaped in reference to the protrusion of the rhizome (Malu *et al*., 2009).

Previous studies have demonstrated that plant extracts and isolated compounds from *Z. officinales* possess strong antioxidant, antibacterial, antifungal, anticancer, and anti-inflammatory effects (Habib *et al*., 2008). Results of the study done by Saha *et al*. (2012) using different extracts and culture sensitivity against microbes namely *Bacillus cereus*, *Staphylococcus epidermis* and *Streptococcus viridans* evidenced the antimicrobial activity claimed for ginger extracts. The extract found effective were hexane and ethylacetate. The water extract was found ineffective against the above microbes. The inhibition of bacterial growth appeared to be dose dependent since no activity was observed at low concentrations (Malu, 2009).

Other scientific studies have shown that gingerol, one of the primary pungent components of ginger, helps counter liver toxicity by increasing bile secretions. It was recently discovered that the acetone and methanol extracts of ginger strongly inhibits gastric ulceration (Foster, 2009).

In addition, a study carried out by Dennis *et al*. (2013) on the preservative effects of ginger was compared with that of sodium benzoate in assessing the quality of locally prepared soursop juice. The result obtained showed that the treatment of freshly prepared soursop juices with sodium benzoate and a mixture of garlic and ginger improved the storage span and reduced health risks of infection or intoxication from their consumption. According to Nwokocha *et al*. (2012), the comparison of alligator pepper and ginger showed similarities which could be traced to similarities in its phytochemical constituents even though present in differing amounts.

The "egusi" (melon) *Colocynthis citrullus* L. is a member of the family Cucurbitaceae and belongs to the tribe Benicaseae (Ogbonna, 2013). The *Colocynthis* is a small genus of four to five species found in Africa, one of which is *C. citrullus*. There is confusion in the nomenclature of the crop. In some text, it is referred to as *Citrullus vulgaris*. In order to minimize this confusion, the use of the name 'egusi' was recommended to represent this crop (Ajibola *et al*., 1990).

The crop is cultivated for its seeds and prepared into condiments used especially for making soup, melon - ball snacks and "ogiri" (a fermented condiment). The egusi melon seed like soya-beans (*Glycine max*) is rich in oil and protein about 53.1% and 33.8% respectively. Melon seed is rich in unsaturated fatty acid, linoleic acid, which could lead to a possible hypocholestrolic effect. The oil expressed from the seed is used for edible purposes, while the residual cake is fried and consumed as snack. In some areas in the South-Eastern Nigeria, the seed is milled with ground pleurotus tuber regium and shaped into balls to substitute for meat in their diet (Ajibola *et al*., 1990).

The aims and objectives of this work were to isolate the spoilage microorganisms associated with ground melon; to determine the effectiveness of ginger powder and chemical preservatives on the microbial load of ground melon seeds when compared to the control; to determine which preservative showed the greatest antibacterial activity and to determine the phytochemicals present in dried ginger powder.

MATERIALS AND METHODS

Collection of samples

Fresh ginger rhizomes were obtained from Ipata market, in Ilorin, Kwara State. It was brought to the laboratory. It was identified at the herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria. The ginger rhizomes were peeled, washed and dried in the oven. It was pulverized into powder using sterile electric blender and was properly stored until needed.

Melon seeds were also purchased from Ipata market and identified at the herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria. They were picked and sorted to remove dirt and infected seeds. The melon was ground and divided into two. i.e. A and B. Milling was attained with a sterile blender, and the milled seeds was immediately stored in a sterile air-tight cellophane bag. It was kept in the laboratory cupboard for 6 weeks, after which caking of the ground melon, change in colour and odour were noticed.

The B part of the ground melon was used to study the effects of ginger powder and chemical preservatives on its shelf-life. The different chemical preservatives used in the study were Potassium sorbate, Sodium chloride and Calcium propionate. The ginger powder was used as a biological preservative. The following concentrations 0.1%, 1%, 3%, 5% were prepared for both biological and chemical preservatives. The pH and bacterial counts of the preserved samples were carried out at interval 7 days for 12 weeks.

ISOLATION OF MICROORGANISMS FROM SPOILT GROUND MELON

Bacteria and Fungi

Isolation of bacteria was carried out using the plate dilution method while that of fungi was carried out using the pour plate method as described by Fawole and Oso, 2007.

Determination of the Effects of Ginger and Chemical Preservatives on Ground Melon Seeds (GMS)

The group B of the ground melon was used to study the effects of the various preservatives used. Fifty grams of the ground melon was weighed into 17 sterile containers, 16 of these were used for the preservation and the 17th for the control in which no preservative was added. The different preservatives used were calcium propionate, potassium sorbate, sodium chloride and ginger powder at the following concentrations; 0.1%, 1%, 3%, 5% each. The different preservatives at different concentrations were introduced into the ground melon in the sterile containers and labeled appropriately. They were stored at room temperature for a period of 12 weeks. The bacterial counts and pH of samples were determined at an interval of 7 days.

Determination of Total Bacterial Counts of Ginger and Chemically Preserved Melon

One gram of each of the sample that had been preserved was dispensed into 9 ml of sterile distilled water in a sterile test tube. Serial dilutions were repeated in five folds. Dilution factors 10^{-2} and 10^{-4} were used to seed the plates with Nutrient agar, using the pour plate method. The same method was used to determine the bacterial counts of the control. The plates were incubated at 37°C for 24 hours. The bacterial counts were recorded after 24 hours incubation using a colony counter.

Determination of pH of Preserved Melon

The pH of the preserved melon was determined by weighing 1g of each sample into beaker containing 10 ml of distilled water, it was centrifuged and the pH was determined using phillip's PW 9418 pH meter, the instrument was standardized with a buffer solution.

Phytochemical Screening of Ginger Powder

Phytochemical screening was carried out on the crude extracts of ginger powder, using standard procedures to identify the constituents such as steroids, glycosides, terpernoids, alkaloids, saponins, phenols, tannins, and flavonoids present as described by Trease and Evans, (1989) and Sofowora, (1993).

RESULTS

Identification of Microbial Isolates From Spoilt Ground Melon Seed

Identification of bacterial isolates

The results of the cultural, morphological and biochemical characteristics of bacteria isolated from spoilt ground melon seeds are presented in Table 1. Altogether 8 bacteria were identified, they were: *Lysinibacillus sphaericus, Bacillus cereus, Escherichia coli* strain 2009 EL-2071, *Escherichia coli* strain ST 540, *Enterobacter asburiae*, *Brevibacillus agri*, *Cronobacter sakazakii*, *Bacillus amyloliquefaciens* var *plantarum.*

The fungi isolates comprised of *Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizopus stolonifer*.

Table 1: The Cultural, morphological and biochemical characteristics of bacterial isolates

Isolate codes	Cultural characteristics	Morphological characteristics	Gram stain	Spore stain	Motility	Glucose	Sucrose	Lactose	Methyl-red	Vogues-Proskauer Indole	Catalase	Citrate	Oxidase	Urease	Starch hydrolysis	Tentative identity
A	Circular, white, raised, entire	Rod shaped	+	-	-	+	+	-	-	-	-	-	-	+	-	+	*Lysinibacillus sphaericus*
B	Circular, white, raised, entire	Rod in chains	+	+	+	+	-	-	-	+	-	+	+	+	-	+	*Bacillus cereus*
C	Circular, straw yellow, raised, entire	Rod shaped	-	-	+	+	-	+	+	-	+	+	-	-	-	-	*Escherichia coli*
D	Colonies had pale yellow pigmentation with mucoid surface	Rod shaped	-	-	+	+	+	-	+	-	-	-	+	-	+	-	*Enterobacter asburiae*
E	Circular, creamy, flat with wrinkled surface	Rod shaped	+	+	+	+	-	-	-	-	-	+	-	-	-	-	*Brevibacillus agri*
F	Circular, pink, flat, entire	Rod shaped	-	-	+	+	+	+	+	-	+	+	-	-	-	-	*Escherichia coli*
G	Irregular, cream, flat, glistening	Rod shaped	-	-	-	+	-	+	-	+	-	+	+	-	-	-	*Cronobacter sakazakii*
H	Irregular, cream, flat, opaque	Rods in chains	+	+	+	+	+	+	-	+	-	+	+	+	-	+	*Bacillus amyloliquefaciens* var *plantarum*

Key: +, positive; -, negative

Viable Bacterial Counts of Ground Melon Seed at Different Concentrations of Ginger

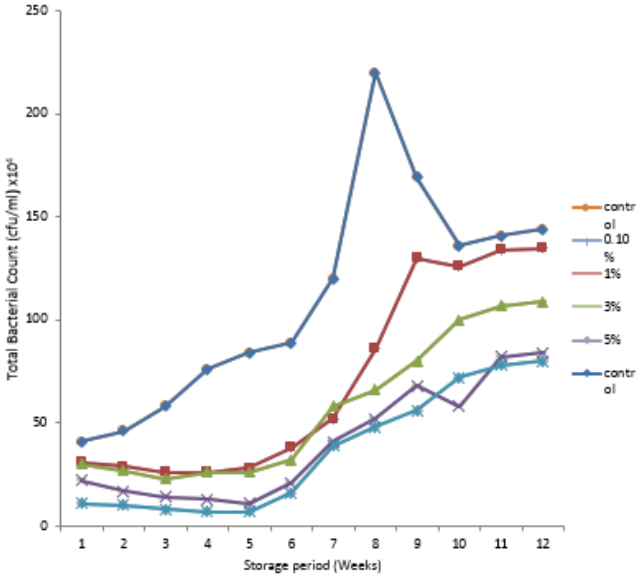

Figure 1: Changes in viable bacterial counts of ground melon seed stored at room temperature for 12 weeks at varying concentrations of ginger powder.

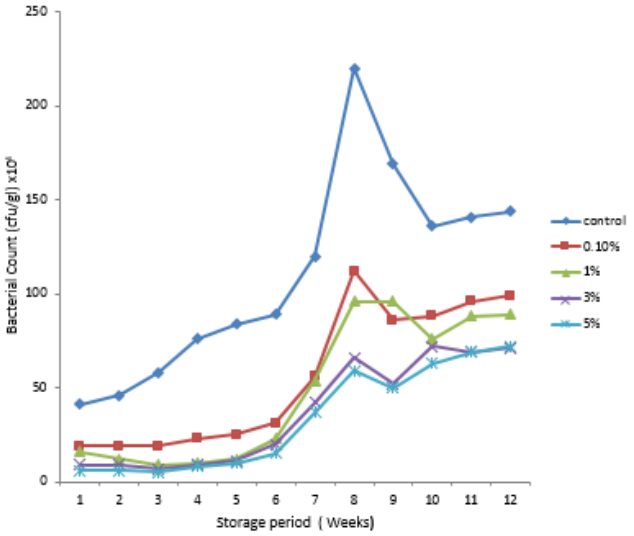

Figure 2: Changes in viable bacterial counts of ground melon seed stored at room temperature for 12 weeks at different concentrations of Sodium chloride

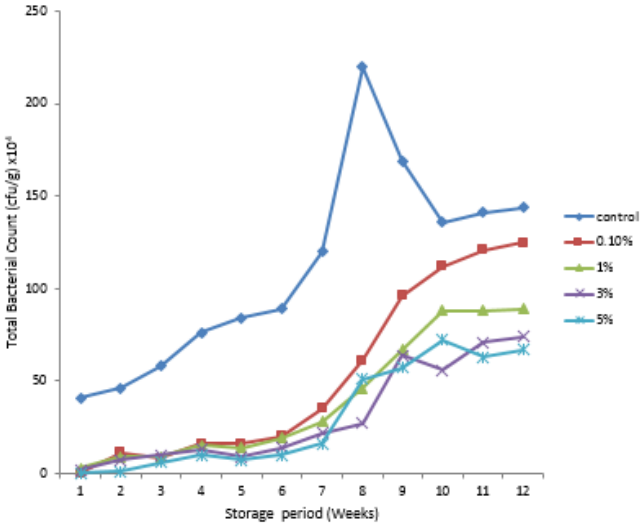

Figure 3: Changes in viable bacterial counts of ground melon seed stored at room temperature for 12 weeks at different concentrations of Calcium Propionate

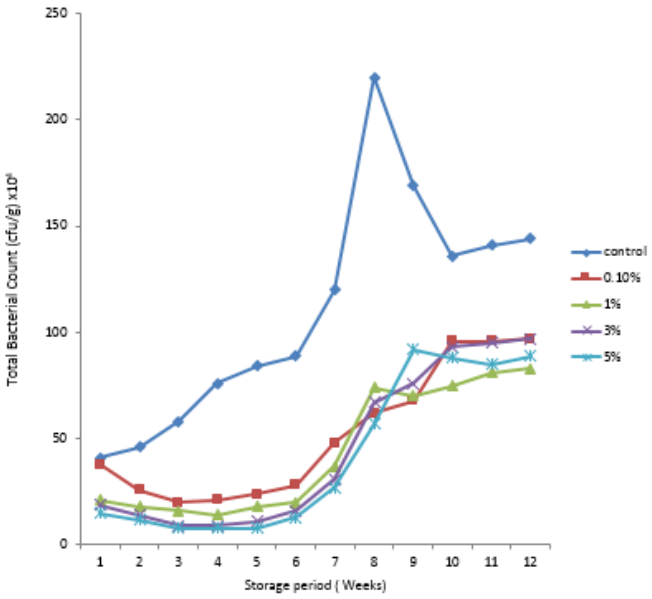

Figure 4: Changes in viable bacterial counts of ground melon seed stored at room temperature for 12 weeks at different concentrations of sorbic acid

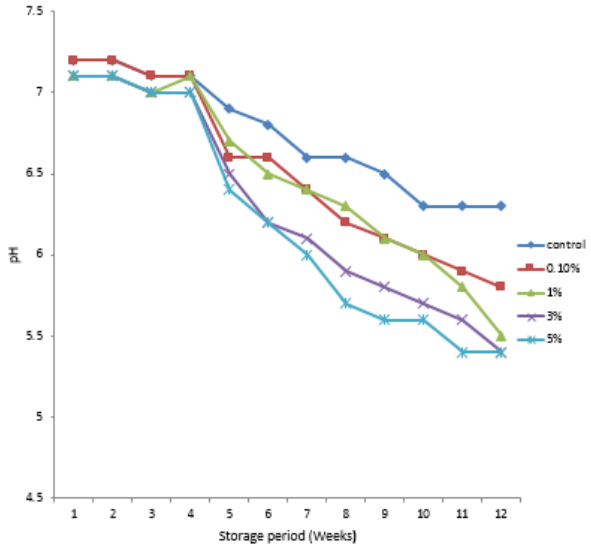

Figure 5: Change in pH of ground melon seed stored at room temperature for 12 weeks at different concentrations of ginger powder.

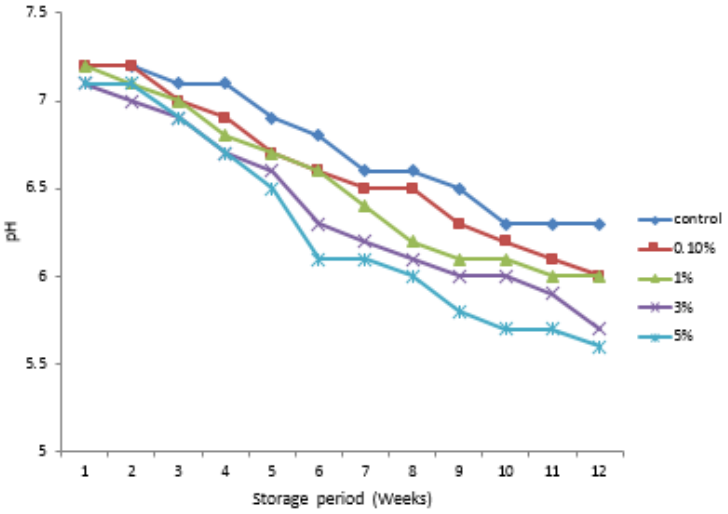

Figure 6: Changes in pH of ground melon seed stored at room temperature for 12 weeks at different concentrations of Sodium chloride

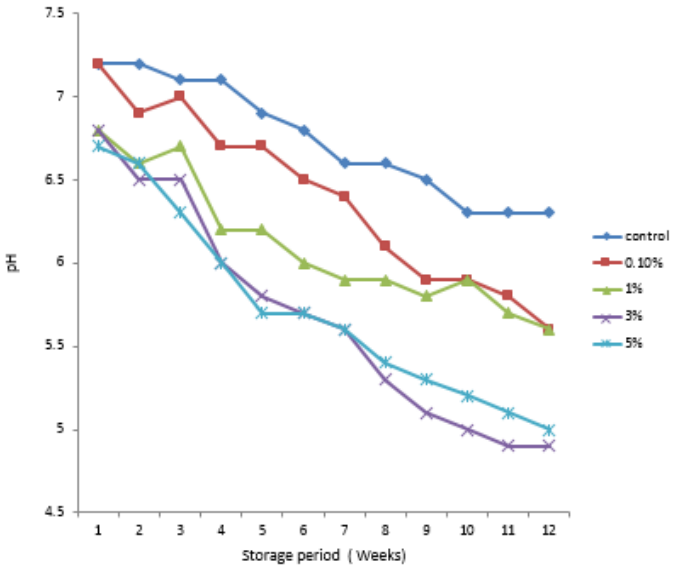

Figure 7: Changes in pH of ground melon seed stored at room temperature for 12 weeks at different concentrations of calcium propionate

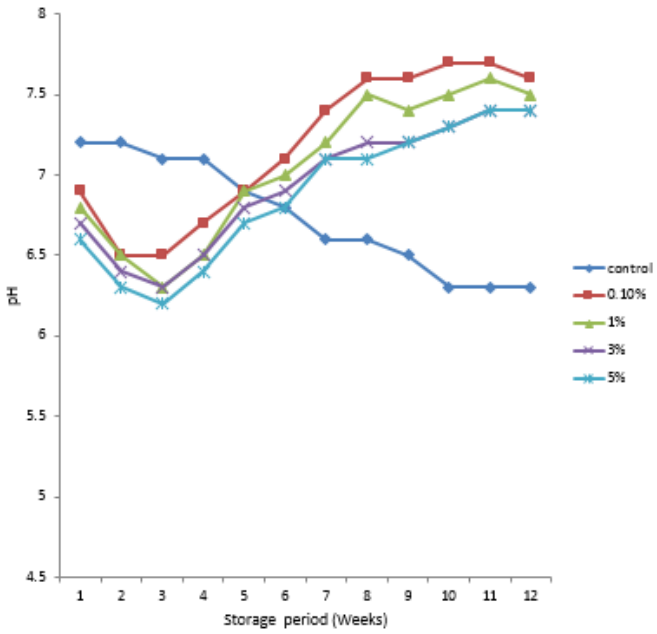

Figure 8: Changes in pH of ground melon seed stored at room temperature for 12 weeks at different concentrations of sorbic acid

Table 2: Phytochemical analysis of Ginger Powder.

Glycosides	++
Terpenoids	+
Steroids	-
Saponnins	++
Phenol	+
Flavonoids	++
Coumarins	+
Tannins	+

KEY:
++ = Heavily present
+ = Moderately present
- = Absent

DISCUSSION

The spoilage of ground melon seeds was observed during the course of this research. Altogether eight bacteria were isolated, four of which were gram positive and they include: *Lysinibacillus sphaericus, Bacillus cereus, Brevibacillus agri, Bacillus amyloliquefaciens* var *plantarum.* While the remaining four were gram negative organisms and they consist of *Cronobacter sakazakii*, 2 strains of *Escherichia coli* and *Enterobacter asburiae.* The bacterial counts during the 12 weeks ranged from 0.2×10^5-1.69×10^6cfu/ml. The control sample had the highest bacterial counts ranging from 4.1×10^5-1.69×10^6cfu/ml; an increase in bacterial counts was observed in the control sample weekly. This result is in accordance with work of Jay (2005) who reported that due to extremely high fat and low moisture content of products such as pecans and groundnut, these products were quite prone to bacterial spoilage. This result also conforms to the work of King *et al.* (1990), who reported that the bacteria associated with shelled nuts and kernels were numerous and include: *Bacillus* species, *Enterobacter* species and *Staphylococcus* species.

Due to the extensive use of ground melon seeds as human food, the microbiology and safety is very important. The sources of microbial contamination of GMS included the environment where these seeds was grown, handled and processed. The sources of these organisms might be from the soil, the handlers, animals, method of storage, air and dust (Jay, 2005).

The fungus isolated from the spoilt GMS include: *Aspergillus flavus, A. fumigatus* and *Rhizopus stolonifer*. This conforms to the work of Adegoke and Ndife (1993) who reported that the dominant fungal flora associated with the spoilage of stored ground melon seeds included the genera *Penicillium, Botryodiplodia, Rhizopus* and *Aspergillus.* These organisms have been found to be of medical and economic importance.

The ginger and chemically preserved ground melon seeds were found to undergo changes in viable bacterial counts and pH. The ground melon seeds preserved with 5% ginger was found to show the best preservative effect, when compared with other concentrations of ginger powder used in this study. This result is in accordance with the work of Edward and Ohaegbu, (2012) who reported that the magnitude of effectiveness of ginger as an organic preservative was concentration dependent. The preservative ability of ginger decreased with increase in length of storage, this might be due to the volatile nature of the bioactive compounds of ginger limiting the antimicrobial activity (Roy *et al*., 2006). Similar findings had also been reported by Efunwevere and Akoma, (1999) during 'kunun-zaki" preservation using inorganic preservatives.

The ground melon seeds preserved with sodium chloride at all concentrations showed a decrease in bacterial counts in the first few weeks and increased

with length of storage, this reduction, might be due to the mechanism of action of salt which is through dehydration of water from food substances and cell membrane. Salt is effective as a preservative because it reduces the water activity of food, thereby decreasing microbial growth and chemical reaction. The increase in bacterial counts subsequently might be due to increase in moisture content and temperature. These two parameters are likely to increase during storage due to metabolic activities of microorganism such as respiration (Foster, 1990).

All ground melon seeds treated with calcium propionate at all concentrations recorded the least preservative effect. This might be due to the high pH of the GMS observed during the first few weeks, as the efficacy of calcium propionate is at pH below 5.0 (Prem-Jose *et al*., 2015).

The GMS preserved with 3% and 5% sorbic acid were the most effective for this category. At these concentrations, the decrease in bacterial counts observed in the first few weeks might be due to the initial pH of GMS which was below 6.5 after the 1st week. This initial low pH enhanced the preservative activity of sorbates. Dharmdiakri (2015) reported that the optimal pH for the antimicrobial activity of sorbates is below a pH of 6.5. The antimicrobial action of sorbic acid was due to its inhibitory influence on various enzymes in the microbial cell such as enolase and lactate dehydrogenase, involved in carbohydrate metabolism, malate dehydrogenase, isocitrate dehydrogenase, which are enzymes of the citric acid cycle, and several enzymes containing the sulphahydryl (SH) group and other enzymes such as catalase and peroxidase. A subsequent increase in bacterial counts with length of storage might be due to the increase in pH above 6.5 thereby rendering the preservative ineffective.

The control sample had highest counts when compared with the ginger and chemically preserved GMS at various concentrations. The bacterial counts of the control increased steadily from the 1st week to the 12th week, recording the highest bacterial counts all through the study. The rate of spoilage was greater and faster for the control when compared with preserved samples at all concentrations. The ginger and the chemically preserved ground melon seeds exhibited a bacteriostatic effect over a period of time when compared with the control that was not preserved in any form.

The reduction in pH observed for all samples treated with ginger, Sodium chloride and Calcium propionate and the control samples might be due to the fact that room temperature favoured the growth of many bacteria which released metabolic products into the medium, thereby resulting in lowering of the pH. This result is supported by the work of Arekemase *et al*. (2007) who reported a reduction in pH with length of storage of *Irviniaga bonensis* stored at room temperature.

The pH of GMS treated with sorbic acid reduced initially and increased with length of storage. This might be as a result of release of metabolic products which are alkaline in nature by the microorganisms thereby causing an increase in pH. This finding is similar to what was reported by Akinwande *et al*. (2012) in the comparison of picolinic acid with common chemical preservatives. The best preservative effect on ground melon seeds was exhibited by 5% ginger; it recorded the lowest microbial counts and extended the shelf life of the ground melon seed by 5 weeks.

Phytochemical constituent such as terpenoids and several other aromatic compounds are secondary metabolites of plants that serve as defence mechanism against microorganisms, insects and herbivores (Bonjar *et al*., 2004). Flavonoids exhibit antimicrobial activity by its ability to form complexes with extracellular and soluble proteins, which then bind to bacterial cell wall, while tannins bind to proline - rich protein and interfere with the protein synthesis. The antimicrobial properties of saponins are due to its ability to cause leakages of enzymes and protein from cell wall (De Boer *et al*., 2005). The antimicrobial activities of ginger might be due to the presence of these phytochemicals. This result agrees with what was reported by Okezie *et al*. (1997) who also isolated glycosides, terpenoids, flavonoids and coumarins, during the comparison of ginger and alligator pepper extracts,

and that these compounds were responsible for their antimicrobial activities.

CONCLUSION

In view of the potential of ginger powder and other selected chemicals used as preservative on microorganisms and for testing for the effectiveness on microbial loads/counts, it has been found out by this work that ginger is very effective in reducing microbial load as compared to other chemicals used. So, if it is further purified in terms of carrying out toxicological studies to know the best concentration (s) which can be used safely on food for human consumption, as it has a great potential as a preservative agent which might prolong the shelf life of melon.

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