Original Research Article

Microbial Quality, Physicochemical and Sensory Properties of Freshly extracted Apple Juice and Cucumber Juice Subjected to Different Preservatives and Storage Conditions

ABSTRACT

Fruit juices are commonly consumed for their refreshing attribute, nutritive values and health benefits. Fruit juice samples were treated with 0.5 g/ml garlic, 0.5 g/ml ginger, 0.25 g/ml mix of ginger and garlic and 0.05% (w/v) of sodium benzoate respectively where the ginger, garlic and sodium benzoate serve as natural and artificial preservatives. Their effects on the fruit juices were evaluated during 8 days of storage at 4°C and ambient temperature (28±2°C). Juice treated with sodium benzoate had the least microbial contamination while the untreated juice had the highest. The bacterial isolated from the samples were *Staphylococcus sp, Bacillus sp. (*Most occurring bacteria isolated)*, Micrococcus sp,*and *lactobacillus sp*. The fungi isolated in the course of the study were *Aspergillus sp, Rhizopus sp* and *Penicillium sp. (*The highest occurred fungi isolated). There was a marginal decrease in pH values across treatments in stored juices with juice stored at 4°C showing the least change in pH compared to that stored at ambient temperature. The results obtained show that the preservatives exhibited an antimicrobial effect on the microbial load. The Total titratable acidity of all samples increased with time at ambient temperature and decreased at refrigerated temperature. The ascorbic acid content of both juices samples at storage temperatures decreased after the storage period due to the high sensitivity of vitamin C to oxygen, light, heat, etc. In conclusion, the findings of this study generally indicate that combination of chemical and natural preservation together with refrigeration is suitable for the preservation of fruit juice for a long time. Chemical preservatives can be recommended to be replaced with natural preservatives such as ginger and garlic, since the process is inexpensive and easy. However, preservation of fruit juice at ambient temperature for a long time should be discouraged to reduce microbial contamination.

Key words: apple juice, cucumber juice pH, microbial quality, sodium benzoate, temperature

INTRODUCTION

Fruit juices are frequently eaten for their reviving quality, nutritional quality, and health benefits. They have a several of significant therapeutic characteristics that could lower the risk of different diseases. They have a good taste and perfume, a lot of antioxidants, and vitamins C and E. [1]. Fruit juices can be made at home and are simple to process. Apple (Malus domestica) juice is one of the many widely consumed fruit juices. Both adults and kids frequently drink apple juice.

Apple juice is a non-alcoholic beverage, and its popularity is growing primarily as more people become aware of its health advantages. In the US, orange juice is the most popular fruit juice, with apple juice coming in second. Apple juice's nutritional advantages are sometimes underrated. For every 100 grams of whole fruit, there are 83.1% moisture, 9.2% sugar, 1.87% protein, 2.0 mg sodium, 8.7 mg potassium, 2.7 mg calcium, 0.22 mg iron, 0.11 mg copper, 5.1 mg phosphorous, and 4.5 mg vitamin C. The absence of fat, cholesterol, and sodium are just a few of the numerous factors that make apple juice and other apple products an essential component of a healthy diet when you closely examine their nutritional composition.

Cucumber (Cucumis sativus L.), a seasonal vegetable crop native to India and grown all over the world, is a member of the Cucurbitaceae family of plants [2]. Due to overproduction throughout the harvest season, a lot of cucumber spoils. By preserving the cucumber for use as a beverage or juice, this issue can be reduced. Cucumber juice is consumed in Central Asia on hot days to rehydrate individuals. In addition to helping people maintain a healthy weight and treat some renal and blood pressure conditions, cucumber juice has health advantages for the skin, nails, and hair. The high-water content of the cucumber makes it stand out and it fulfills all cravings [3]. One of the most widely utilized antibacterial agents for enhancing fruit juice preservation ability is benzoic acids and their salts. Due to their broad-spectrum effectiveness against bacteria, yeasts, and molds as well as their non-alteration of food flavor, they are widely used [4]. The development of new foods with great nutritional content and positive sensory appeal has been sparked by the food market. The food industry has pushed the creation of new goods that combine the nutritional benefits of two or more fruit juices with the benefit of a pleasant flavor. These products have been highly received by consumers [5]. Consumers are more concerned than ever about the negative consequences of chemical preservatives and the preference for natural additives. Researchers have focused on producing natural preservatives that exhibit antioxidant and microbial activity for use in food processing [6].[7] evaluated the microbial load during watermelon juice storage over three months in cool conditions. They found that pure watermelon juice was highly vulnerable to microbial spoilage because of the preservatives' absence, and a large volume of microbial loads was recorded, but when they mixed the juice with serendipity berry extract as a preservative, the load was highly reduced and the storage life extended.

In the past ten years, fruit juices drank in particular without thermal pasteurization have been linked to outbreaks of foodborne illness or spoiling issues. This study aims to determine the effect of preservatives and storage condition on the Microbial and physicochemical quality of freshly extracted apple (*Malus domestica*) and Cucumber (*Cucumis sativus L.)* juices

MATERIALS AND METHODS

Collection And Processing Of Fruits

Garlic bulbs, ginger rhizomes, and mature, ripe healthy apple fruits and cucumbers were purchased from different sales points in Port Harcourt metropolis, Rivers State, Nigeria and transported to EMADAVISTIC Medical & Research laboratory, where the research work was carried out. The apple and cucumber fruits were washed with distilled water to remove adhering soils, dirts and extraneous materials. The apples and cucumbers were stored, trimmed, peeled and cut into small pieces before the extraction of juice.

Preparation Of Fruit Additives/Preservatives

The ginger rhizomes and garlic bulbs were washed with potable water repeatedly. Their outer covering was peeled off with a sterile knife and then sliced into cutlets and dried using a hot air oven at 65°C for 48hrs. An electric blender was used to pulverize the dried ginger and garlic bulbs into powder

Production Of Apple and Cucumber Juice

The pieces of apple and cucumber were introduced into a juice extractor separately and their juices were extracted. The juices were then filtered using clean Muslim cloth into sterile conical flasks. **Treatment Of Apple and Cucumber Juices with Natural and Chemical Preservatives**

Natural Preservatives: exactly 0.5g of ginger powder was added into 100ml of apple juice and 0.5g of garlic powder was added into 100ml of apple juice. Similarly, a combination of 0.25g of ginger powder and 0.25g of garlic powder was measured and added to 100ml of apple juice.

Chemical Preservatives: 0.05% (w/v) sodium benzoate (sigma chemical company) was aseptically added to another 100ml of apple juice with another 100ml of apple juice container serving as a control (without preservative).

Microbiological Analysis Of Fruit Juice

Sample Preparation

Samples will be stored for one week at different temperatures (Ambient temperature and the refrigeration temperature) and taken on different days; Day 0, Day 2, Day 4, Day 6 and Day 8.

Microbiological Analysis

A Stock solution was made with 25ml of the sample into 225ml of peptone and homogenized followed by a tenfold serial dilution of10^{-1}-10^{-7} .

Total viable counts - 0.1 ml of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} in duplicate were dispersed on already prepared Plate count Agar.

Staphylococcus Counts - 0.1 ml of 10^{-3}, 10^{-4}, and 10^{-5} were dispersed onto mannitol salt agar.incubated at 25-37°C for 24-48hrs. Counts obtained, cfu/g calculated, representing colonies were picked, subcultured and store in slants, perform series of biochemical tests for identification according to Cheesbrough,(2005).

Total fungi count- 0.1 ml of 10^{-4}, 10^{-5}, were dispersed onto already prepared Potato dextrose Agar incubated at 25°C for 3-7 days.Identification was done by staining the fungi lactophenol cotton blue, then viewing the specimen under the microscope using the x40 objective lens, and using a fungal atlas to identify the different pure fungi cultures.

Determination of pH

Ten milliliters of the juice was dispensed into a beaker and the pH was determined with a previously standardized pH meter. The pH meter was calibrated using phosphate buffer of 4.0 and 7.0 [9].

Determination of titratable acidity (%TA)

Determination of titratable acidity (%TA was done according to the procedures of [10]. .

Determination of vitamin C

The vitamin C (Ascorbic acid) was determined by redox titration with iodine according to the procedures of [10].

Sensory Evaluation of Apple and Cucumber Samples

Ready to serve drinks (apple and cucumber juices) were presented to a panel of judges for sensory evaluation of colour, taste, flavour and overall acceptability using an hedonic scale following the method described by [11], [35]. The panel members were selected based on their ability to discriminate and scale a broad range of different attributes of apple and cucumber products. An orientation program was organized for the panel members to brief them on the objective of the study. The drink samples were brought to the sensory analysis lab and were served to the panelists. The judges were provided with prescribed questionnaires to record their observation. The information contained in the performance was 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely. The panelists expectorated the drinks and rinsed their mouths using distilled water between samples. Sensory testing was made in the panel room completely free of food/chemical odour, unnecessary sound and mixing of daylight. The experiment was repeated twice and the values are presented as means.

Statistical Analysis- Microbiological data of Apple and Cucumber juice samples with different preservatives and storage conditions were obtained and entered into a Microsoft Excel spreadsheet before being evaluated with a one-way ANOVA using statistical software from the Statistical Package for Social Sciences (SPSS) version 16. Statistical significance was defined as a 95% confidence interval with a P value of 0.05 or lower

RESULT AND DISCUSSION

Microbial Counts of Juice Samples on Day0(first day)

The microbial count (total bacteria count [TBC] of the samples throughout the storage period of the analysis is shown in Figure 1-4 for apple juice at ambient temperature, apple juice at refrigerated temperature, cucumber juice at ambient and cucumber juice at refrigerated temperature respectively. Total fungal count [TFC] in Fig 9-12, total staphylococcus count [TSC]) in table 5-8. The TBC of apple juice (AJ) obtained was 4.6×10^3. While that of the cucumber juice (CJ) sample was 8.6×10^3. The total staphylococcus count (TSC) of the apple juice was recorded as 2.4×10^3, which was higher in comparison to that of cucumber juice with the count of 4.4×10^2. The total fungal count (TFC) was recorded at 3.2×10^3 for apple juice and 3.6×10^3 for cucumber juice extract.

Total Bacteria Count (TBC) of the Samples at Different Storage Conditions in Relation to Days of Monitoring

The total Bacterial count (TBC) of the samples at ambient temperature and refrigerated temperature is shown in figure 1and 2. The TBC of apple juice samples at refrigerated temperature (RAJC) decreased very slowly from 4.6×10^4 on the day2 to 3.8×10^3 on theday8 of the analysis compared to apple juice sample monitored at ambient temperature (RmAJC) in which there was an increase from 6.6×10^4 on day2 to 7.4×10^4 on day8. The cucumber juice sample (control) stored at refrigerated temperature (RCJC) had almost a static growth count of total bacteria from day2 having TBC of 1.02×10^4 to 1.62×10^4 on the day8 of the analysis. The ambient temperature cucumber juice (RmCJC) sample in comparison to refrigerated cucumber juice showed an exponential increase in growth count from 1.31×10^4 on the day2 to 2.6×10^6 on day8.

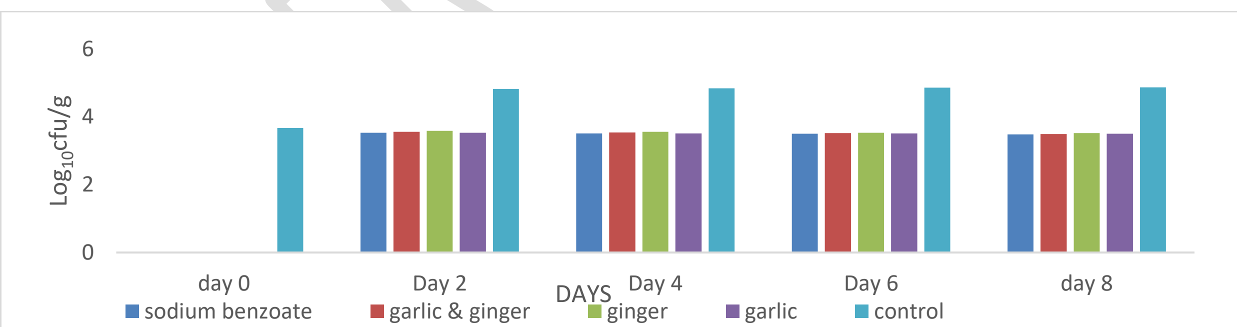

Fig 1: Bar Chart of Total bacteria count for different apple juice preserved with different preservatives stored at ambient temperature.

Fig 2: Bar Chart of Total bacteria count for different apple juice preserved with different preservatives stored at refrigerated temperature.

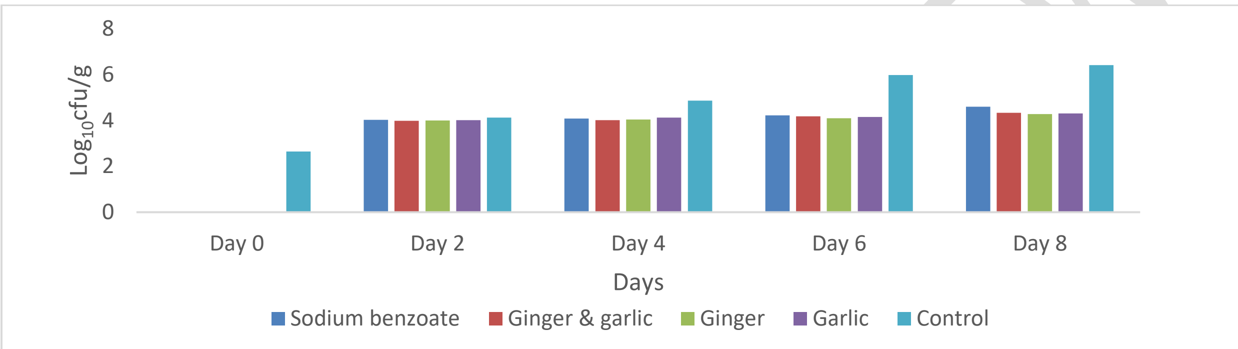

Fig 3: Bar Chart of Total bacteria count for different cucumber juice preserved with different preservatives stored at ambient temperature.

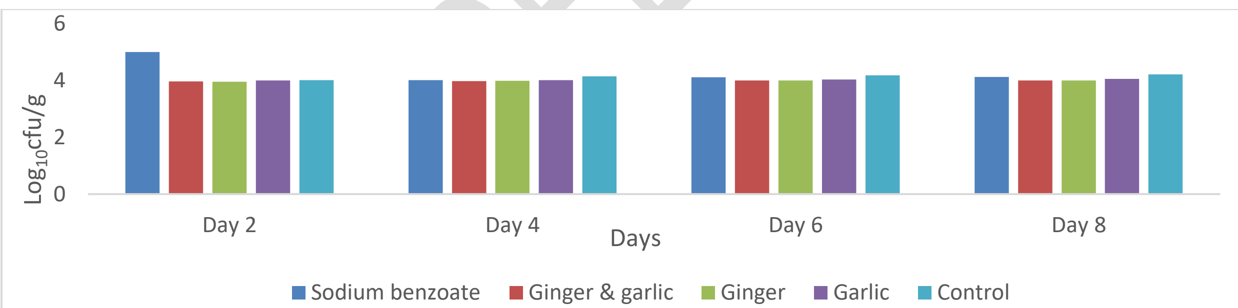

Fig 4: Bar Chart of Total bacteria count for different cucumber juice preserved with different preservatives stored at refrigerated temperature

Total Staphylococcal Count (TSC) of Samples at Different Storage Conditions in Relation to Days of Monitoring

The TSC of samples stored at two temperature condition of ambient temperature and refrigerated temperature as analyzed in relation to days is shown in fig:5 - fig .8. The TSC of RAJC sample and RCJC samples had increased count from 4.2×10^3 and 8.4×10^2 day2 to 4.9×10^3 and 3.9×10^3 respectively. The sample RmAJ and RmCJ stored at ambient temperature showed an increase in TSC from day2 to day8. RmAj sample increased from 4.4×10^3 to 5.0×10^3 on the day8 while the RmCJ sample increased from 9.6×10^2 on the day2 to 6.0×10^3 on the day8. Other samples followed the same trend.

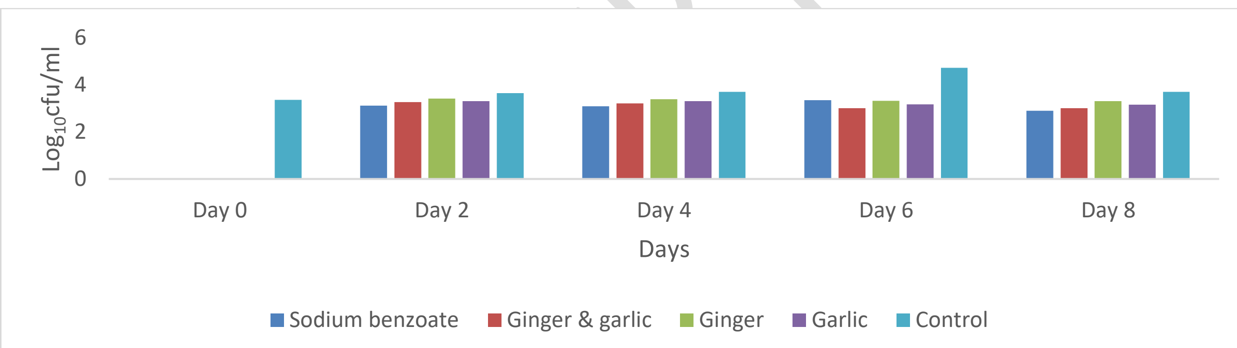

Fig 5: Bar Chart of Total staphylococcus count for different apple juice preserved with different preservatives stored at ambient temperature.

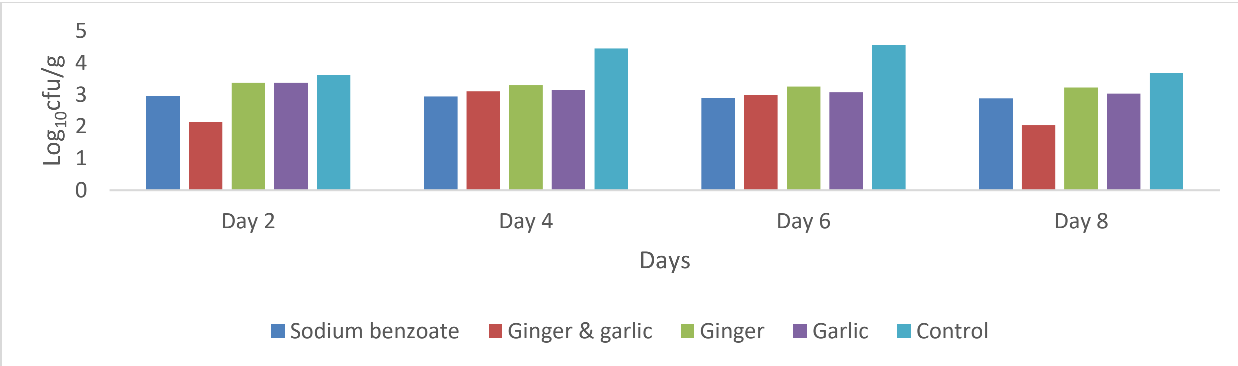

Fig 6: Bar Chart of Total staphylococcus count for different apple juice preserved with different preservatives stored at refrigerated temperature.

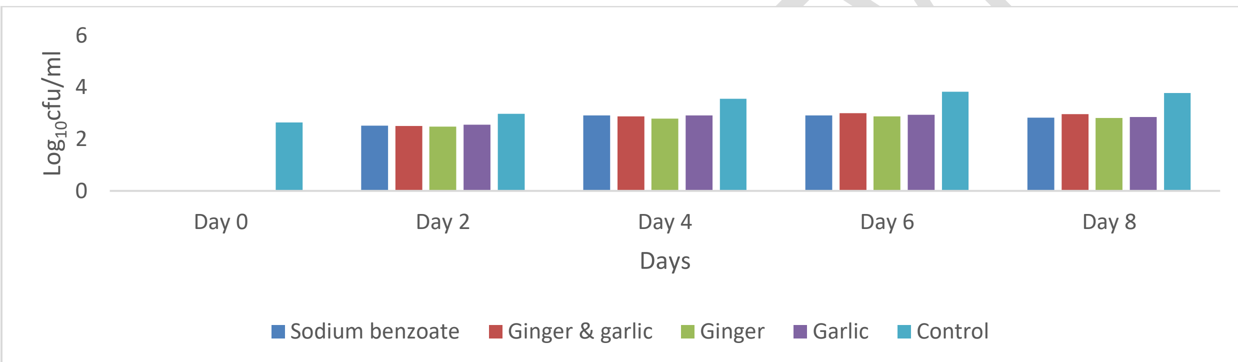

Fig 7: Bar Chart of Total staphylococcus count for different cucumber juice preserved with different preservatives stored at ambient temperature.

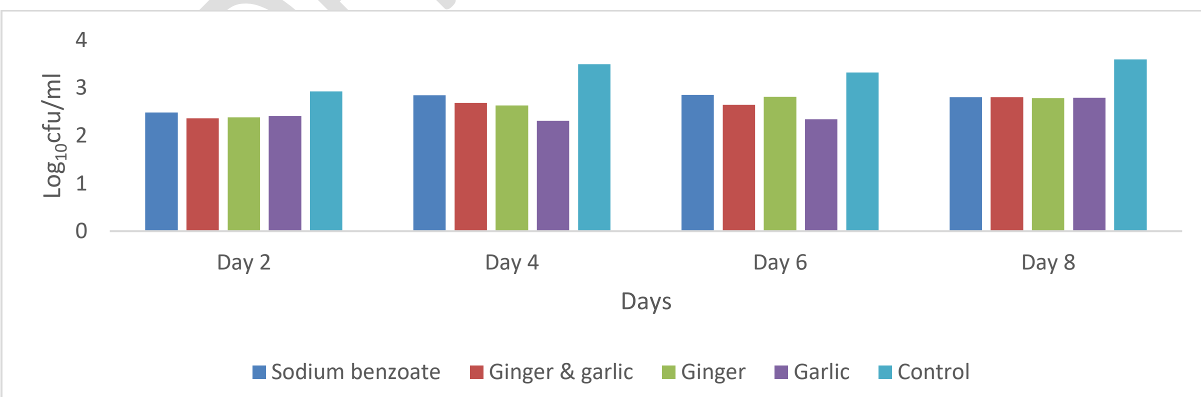

Fig 8: Bar Chart of Total staphylococcus count for different cucumber juice preserved with different preservatives stored at refrigerated temperature#

Total fungal count of the samples at different storage conditions in relation to days of monitoring

The TFC of the samples is shown in Figure:9 -.12.

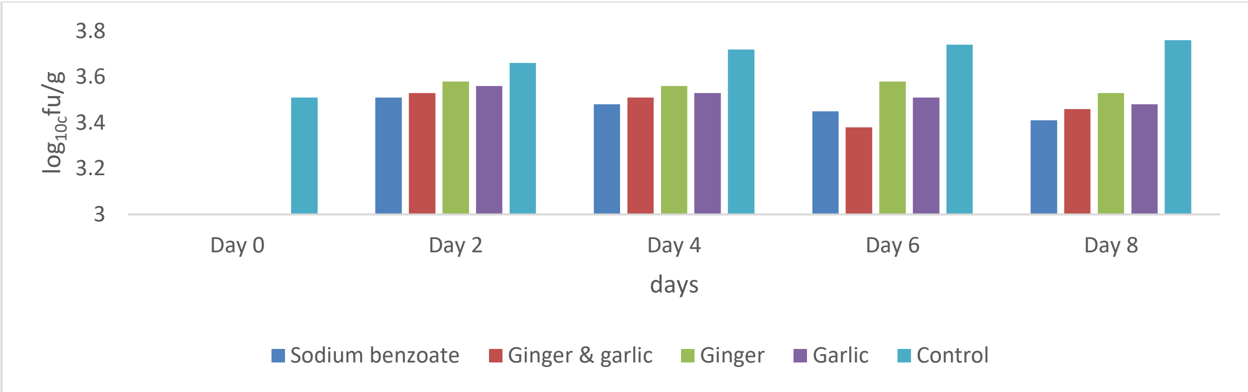

Fig 9: Bar Chart of Total fungi count for different apple juice preserved with different preservatives stored at ambient temperature

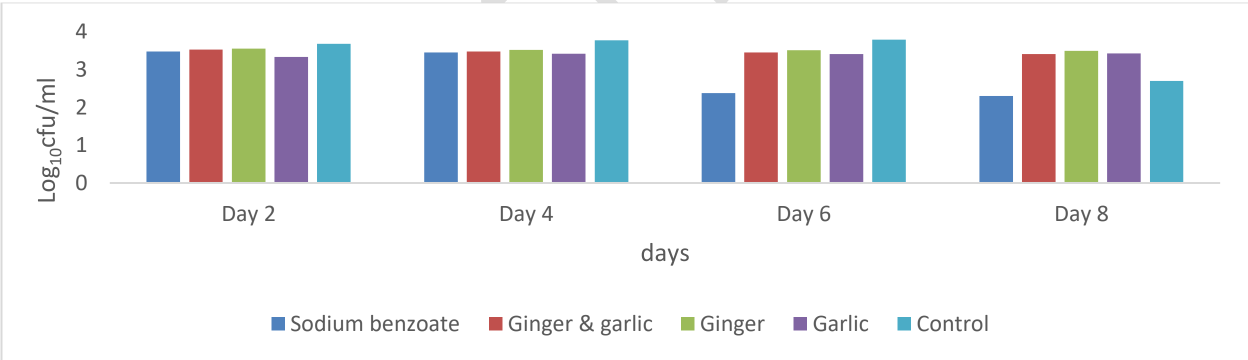

Fig 10: Bar Chart of Total fungi count for different apple juice preserved with different preservatives stored at ambient temperature at refrigerated temperature

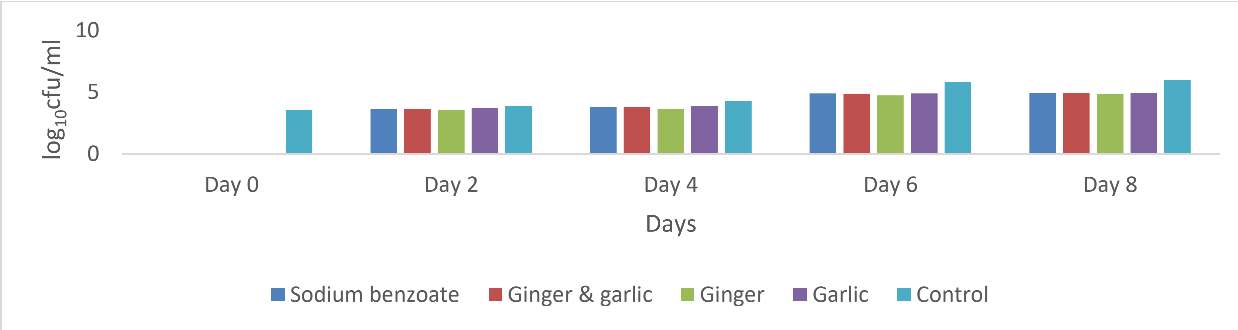

Fig 11: Bar Chart of Total fungi count for different cucumber juice preserved with different preservatives stored at ambient temperature

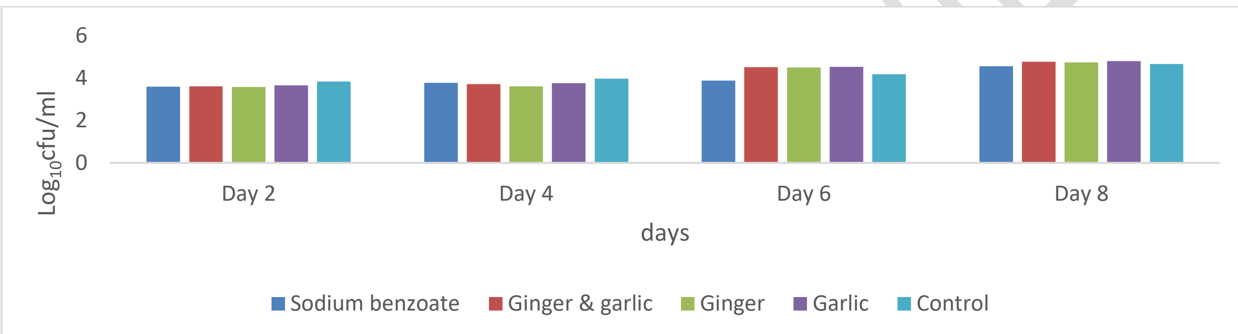

Fig 12: Bar Chart of Total fungi count for different cucumber juice preserved with different preservatives stored at refrigerated temperature

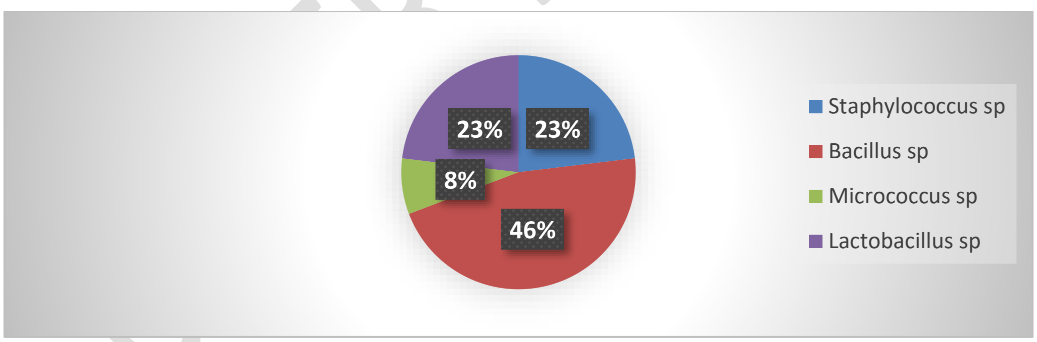

Fig 13: Percentage occurrence of the bacteria identified from the various samples

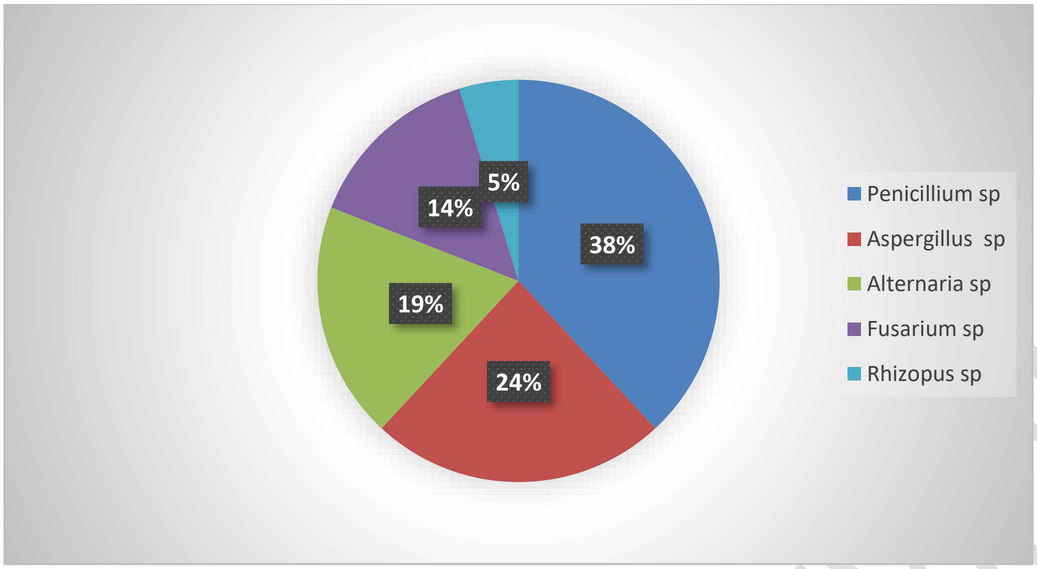

Fig 14: Percentage occurrence of the fungi identified from the various samples

Physicochemical Analysis of Fruit Juice Samples

The results showing the pH of apple fruit juice and cucumber fruit juice samples are represented in Tables 1 and 4, the TTA in Tables 2 and 5, and the ascorbic acid content in Tables 3 and 6 respectively.

Table 1: Apple juice (pH)

Ambient temperature	**Day 0**	**Day8**	**Refrigerated temperature**	**Day0**	**Day8**
Control	4.3	3.8	Control	4.3	3.8
Nabenzoate	4.8	4.4	Nabenzoate	4.8	4.7
Garlic	4.4	4.0	Garlic	4.4	4.2
Ginger	4.3	4.0	Ginger	4.3	4.0
Ginger+garlic	4.4	4.0	Ginger+garlic	4.4	4.1

Table 2: Apple Juice (Total Titratable acidity [g/100ml])

Ambient temperature	Day 0	Day8	Refrigerated temperature	Day8
Control	1.085	1.614	Control	0.806
Nabenzoate	0.65	1.060	Nabenzoate	0.90
Garlic	0.750	0.962	Garlic	0.960
Ginger	0.70	1.110	Ginger	0.805
Ginger+garlic	0.80	1.184	Ginger+garlic	0.721

Table 3: Ascorbic Acid Content (Apple Juice)

Ambient temperature	Day 0	Day8	Refrigerated temperature	Day8
Control	**16.0**	**28.51**	**Control**	**24.45**
Nabenzoate	**15.0**	**26.10**	**Nabenzoate**	**21.60**
Garlic	**15.0**	**21.00**	**Garlic**	**21.2**
Ginger	**13.0**	**24.25**	**Ginger**	**21.5**
Ginger+garlic	**14.5**	**22.25**	**Ginger+garlic**	**21.3**

Table 4: Cucumber (pH)

Ambient temperature	Day 0	Day8	Refrigerated temperature	Day8
Control	5.8	5.0	Control	5.0
Nabenzoate	5.9	4.6	Nabenzoate	5.2
Garlic	5.9	3.9	Garlic	5.0
Ginger	5.8	4.1	Ginger	4.1
Ginger+garlic	5.9	3.8	Ginger+garlic	5.1

Table 5: Total Titratable acidity (TTA_g/1000ml) (cucumber)

Ambient temperature	Day 0	Day8	Refrigerated temperature	Day8
Control	0.698	0.768	Control	0.304
Nabenzoate	0.526	0.763	Nabenzoate	0.606
Garlic	0.664	0.736	Garlic	0.345
Ginger	0.321	0.522	Ginger	0.300
Ginger+garlic	0.664	0.692	Ginger+garlic	0.540

Table 6: Ascorbic Acid Content (Cucumber)

Ambient temperature	Day 0	Day8	Refrigerated temperature	Day8
Control	**4.09**	**3.90**	**Control**	**4.05**
Nabenzoate	**3.9**	**3.80**	**Nabenzoate**	**4.3**
Garlic	**4.01**	**3.65**	**Garlic**	**4.0**
Ginger	**4.06**	**3.74**	**Ginger**	**4.1**
Ginger+garlic	**4.00**	**3.60**	**Ginger+garlic**	**4.0**

Table 7 : Sensory Evaluation of Apple and Cucumber juice

Sensory Quality	Color		Taste		Flavour		Overall acceptability	
	Ambient	Cold	Ambient	Cold	Ambient	Cold	Ambient	Cold
Day0								
A-Control	8.0		9.5		9.2		8.9	
C-Control	8.3		8.7		9.8		8.93	

Day2								
A-Control	6.4	6.6	7.3	7.6	7.5	7.7	7.1	7.3
A-Sodium benzoate	6.6	6.7	7.6	7.7	7.5	7.6	7.2	7.3
A-Garlic	6.5	6.7	7.5	7.7	7.6	7.9	7.2	7.4
A-Ginger	6.5	6.6	7.4	7.6	7.5	7.5	7.1	7.2
A-Ginger/garlic	6.4	6.5	7.3	7.5	7.4	7.6	7.0	7.2
C-Control	6.1	6.3	7.0	7.4	7.1	7.3	6.7	7.0
C-Sodium benzoate	6.3	6.5	7.2	7.5	7.3	7.4	6.9	7.1
C-Garlic	6.2	6.4	7.1	7.4	7.3	7.5	6.9	7.1
C-Ginger	6.3	6.4	7.0	7.3	7.2	7.4	6.8	7.0
C-Ginger/garlic	6.2	6.3	6.9	7.2	7.2	7.5	6.8	7.0
Day8								
A-Control	3.0	3.5	4.1	4.4	4.2	4.3	3.8	4.1
A-Sodium benzoate	3.2	3.6	4.2	4.6	4.4	4.4	3.9	4.2
A-Garlic	3.1	3.4	3.9	4.3	4.2	4.3	3.7	4.0
A-Ginger	3.0	3.3	4.0	4.4	4.3	4.5	3.8	4..1
A-Ginger/garlic	3.0	3.4	3.8	4.3	4.1	4.3	3.6	4.0
C-control	2.9	3.3	4.2	4.5	4.2	4.4	3.7	4.1
C-Sodium benzoate	3.0	3.4	4.4	4.7	4.3	4.5	3.9	4.2
C-Garlic	3.1	3.4	4.3	4.5	4.2	4.3	3.9	4.1
C-Ginger	3.2	3.5	4.4	4.6	4.2	4.5	3.9	4.2
C-Ginger/garlic	3.1	3.5	4.3	4.6	4.2	4.4	3.9	4.2

9 point hendonic scale: 10= strongly like; 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.

DISCUSSION

Effect of Preservatives on Juices During Storage

Despite the potential benefits offered by fruit juices, people are developing concerns about their safety and quality, the processed fruit juices that were investigated showed high microbial loads. The microbial analysis of the fresh sample revealed the total heterotrophic bacteria count of apple juice was 4.6×10^3 (3.67 log cfu/ml) and cucumber juice, 8.6×10^3 (3.93 log10cfu/ml) with apple fruit juice having the lowest count and cucumber fruit juice samples having significant high heterotrophic bacteria count ($p<0.05$). The staphylococcal counts of 2.4×10^3 (3.36 log cfu/ml) for apple fruit juice sample and 4.4×10^2 (2.64 log10cfu/ml) for cucumber juice samples were also obtained from the fresh juice samples. The Microbiological limits in fruit juices and nectars according to [12] is a maximum of 10^3 CFU/g Total Plate Count and 30 CFU/g maximum for yeasts and moulds. In another development, the Good Manufacturing Practices (GMP) standard limit for yeasts in fruit juices is $<10^3$ CFU/ml for unpasteurized fruit juices and <10 CFU/ml for pasteurized fruit juices though the maximum acceptable level is 10^6 CFU/ml. Based on this standard limit, it can be deduced that the microbial load of the fruit juice samples analyzed are significantly high ($p<0.05$). [3] had reported on a number of factors responsible for contamination of fruit juices. Before processing, most fruit contains bacterial counts of 1×10^5 CFU/ml on their surface. These microorganisms may reflect in the final fruit juice products. Improper washing of fruits adds these bacteria to juices leading to contamination. In addition, the lack of appreciation of basic safety issues by fruit processors contribute to augmentation of the microbial loads. These include the use of unsterilized extractors, homogenizers and other equipment used in the process line, unavailability of treated running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust [14]. The period of storage is shown in Figures 1--12. The addition of the preservatives helps to reduce the bacterial load in all juice sample treatments stored at refrigerated and ambient temperature (28±2°C) with juice having sodium benzoate showing the highest reduction from 3.0×10^3 to 2.3×10^3 cfu/ml and 3.4×10^3 to 3.0×10^3 cfu/ml for apple juice stored at refrigerated and ambient temperature respectively. There was a decrease in the bacterial load for control samples stored at refrigerated temperatures of 4.6×10^4 to 3.8×10^3 cfu/ml which is in contrast to the findings of [15], in which he had no increase in bacteria count. There was increase an sample stored at an ambient temperature 6.6×10^4 to 7.4×10^4 cfu/ml. The same trend was seen in the fungal load with juice treated with sodium benzoate having the highest reduction of 3.0×10^3 to 2.0×10^2cfu/ml and 3.2×10^3 to 2.6×10^3cfu/ml for samples stored at refrigerated and ambient temperature respectively as compared to other treatments with the control sample having an increase in the fungal load 4.6×10^3 to 5.7×10^3cfu/ml for the period of storage at ambient temperature. For the cucumber fruit juice, there was an increase in bacteria count during storage period. The juice with ginger had the lowest bacteria count of 9.2×10^3 to 9.9×10^3cfu/ml and 9.9×10^3 to 1.90×10^4cfu/ml cucumber juice stored at refrigerated and ambient temperature respectively. There was an exponential increase in cucumber juice without preservative (control) at ambient temperature and a slow growth of bacteria in the cucumber juice kept at refrigerated temperature. This trend was also noticed in the total fungal count of the cucumber juice with ranges 3.6×10^3 to 7.5×10^4cfu/ml and 3.49×10^3 to 5.6×10^4cfu/ml for ambient and refrigerated temperature respectively.

The bacterial isolated from the samples are *Staphylococcus sp,Bacillus sp, Micrococcus sp* and *Lactobacillus sp,* of which *Bacillus sp* was the most occurring bacteria isolated (46.1%). These bacteria are similar to those from [16],[40] from the microbial analysis of juice. *Bacillus* and *Lactobacillus* occurred predominantly up to the day 8 when other bacteria were reduced or absent in both refrigerated and ambient temperature samples. The presence of species of *Bacillus* across all the fruit juice samples investigated maybe due to the presence of their endospores. These structures are extraordinarily resistant to environment stresses such as heat, ultraviolet radiation, chemical disinfectants and desiccation [17]. Spoilage organisms are heat-resistant spores of bacteria belonging to the *Bacillus* genera, which survive the storage process.

The fungi isolated in the course of the study were *Aspergillus sp, Rhizopus sp, penicillium sp, Alternaria sp,* and *Fusarium sp. Penicillium sp* had the highest percentage (38.1%) of occurrence among other fungi isolated. This is in line with the report of [16][39]. Moulds growth even occurred in refrigerated temperature samples, because molds are much more tolerant to cold than heat. Molds, such as *Aspergillus sp, Rhizopus sp and penicillium sp,* are responsible for the spoilage of fruit juices [18]. Some species of *Rhizopus* and *Penicillium* have been reported to produce potent mycotoxins responsible for various mycotoxicosis in humans [18].

Effect of Preservatives on pH During Storage

Figures 1 and 4 shows the effect of the different treatments on the pH of apple and cucumber juice respectively. The pH of the apple juice sample stored at refrigerated temperature with ginger ranges between 4.0 to 4.3 and juice with garlic ranges between 4.0 to 4.40. The juice with combination of ginger/garlic ranges between 4.10 to 4.40, juice with sodium benzoate ranges between 4.7 to 4.8 and juice alone (control) ranges between 3.8 to 4.3. While cucumber juice samples stored at refrigerated temperature with a ginger range between 4.1 to 5.8, juice with garlic ranges between 5.0 to 5.9. The juice with a combination of ginger/garlic ranges between 5.10 to 5.90, juice with sodium benzoate ranges between 5.2 to 5.9 and juice alone (control) ranges between 5.0 to 5.8. For juice samples stored at ambient temperature (28±2oC), the pH ranges between 3.8 to 4.3, 4.0 to 4.3, 4.0 to 4.4, 4.0 to 4.4 and 4.4 to 4.8 for control sample, juice and ginger, juice and garlic, juice and ginger/garlic, juice and sodium benzoate respectively. That of cucumber at ambient temperature ranges between 5.0 to 5.8, 4.1 to 5.8, 3.9 to 5.9, 3.8 to 5.9 and 4.6 to 5.9 for control sample, juice and ginger, juice and garlic, juice and ginger/garlic, juice and sodium benzoate respectively.

The chemical composition of apple and cucumber fruit juice samples were determined as represented in Tables 1 and 4 respectively, and showed that apple juice had decrease in the pH of control sample from day0 to day8 (4.3 to 3.8) at both storage temperatures and other samples as well were observed to have a decrease in the pH. A decrease of 1.085 to 0.806g/100ml TTA of the apple juice without preservative was observed and that of other samples except samples with garlic/ginger and control showed an increase in TTA in both storage temperatures. While cucumber juice had pH of 5.8 to 5.0 during storage at both temperatures for control, decrease in pH of juice with sodium benzoate from 5.9 to 4.6 and 5.2 at ambient and refrigerated temperature respectively. A pH of cucumber juice with garlic at ambient temperature reduced from 5.9 to 3.9 and refrigerated temperature reduced to 5.0 from day0 to 8. Cucumber juice with ginger and that of ginger/garlic also had a decrease in pH. The TTA of the cucumber juice samples showed an increase after eight days of storage at ambient temperature and a decrease at refrigerated temperature.[37]

The microbial load data obtained from the apple and cucumber fruit juices suggest that both the natural preservatives (ginger, garlic and their mixture) and the synthetic sodium benzoate preservative were effective in prolonging the shelf-life of the apple and cucumber fruit juices with the sodium benzoate showing a better bacteriostatic and fungistatic performance at both temperatures of storage. The reason for this observation may be attributed to the insolubility of natural preservatives used for the study. The use of some chemical preservatives such as sorbate and benzoate to improve the shelf life of drinks has been reported [19], [20]. According to [21], Sodium benzoate is a bacteriostatic and fungistatic preservative in acidic environment and is mostly used in acidic foods like salad dressings (vinegar), carbonated munchies (carbonic acid) and condiments. The inability of the apple juice to stay for a long period without preservatives could synergize the nutrient status of the fruit juices creating the appropriate food base for the colonization by microorganisms [22],[23]. Ginger (*Zingiber officinale*) and garlic (*Allium sativum)* are traditionally used as spices in food preparation but also have both antioxidant and antimicrobial properties [24]. Ginger was reported to have bactericidal effect against *Escherichia coli* and *Streptococcus* [25]. Benzoates are preferable in the preservation of fruit juices due to the solubility of their salts. Benzoates are used at low temperatures to extend the shelf-life of minimally processed juices [26]. Their wide usage is due to their broad-spectrum activity against some microorganisms, as well as their non-alteration of food flavor. The reduction in the microbial loads over the storage period in juice treated with both natural preservatives and the synthetic preservative may be attributed to the antimicrobial and phytochemical properties of the preservatives and apple/cucumber juice respectively as antimicrobials can effectively extend the shelf-life of fruit products.[27],[28]. The results of this investigation also revealed that untreated fruit juices (control) had a higher-level microbial load during the period of storage. This could probably be due to the microbial activities favored by the absence of refrigeration in the juice stored at ambient temperature and preservatives.

This finding, therefore, suggests that the addition of these preservatives to apple and cucumber juice might prolong the shelf-life of the juice. The fruit preservation performance of these phylogenes showed no difference in the reduction of the microbial loads in fruit juices treated with ginger, garlic and mixture of both respectively. The pH results (figures 7-10) showed a gradual acidic inclination for the period of storage in samples stored at ambient temperature and refrigerated temperature except for juice treated with ginger. This aligns with the reports of [29], [4].that the addition of ginger and garlic moves the pH towards acidity and also reduces the microbial load profile in the fruit juice. Environmental factors and activities of microorganisms together with the incomplete dissolution of the natural preservatives may also alter the pH of the juice during storage [28]. The excellent keeping quality of fruits and soft drinks is influenced by low pH [30]. The near anaerobic condition of the study may have favored the early appearance of bacteria species and the late emergence of fungi isolates as equally observed in the microbiological results from the work of [31] on a homemade soursop (Annona muricata L.) fruit juice. This is contrary to what was reported by [34] those natural preservatives (garlic, ginger and their mixture) were less effective in prolonging the shelf life of juices.

Titratable Acidity

Results in Tables 2and5 shows how the titratable acidity of all the samples increased with time on storage at ambient temperature. The titratable acidity ranged between 1.085-1.614 for pure apple juice, 0.698-0.768 for pure cucumber juice through the storage period, which is very low compared to the ones reported for other fruits blends by[31]. From the results obtained on day 8 from Table 2, the pure juice of apple and cucumber (control) showed the highest titratable acidity (1.614 and 0.768 respectively). Apple juice with garlic recorded the lowest titratable acidity (0.962) and cucumber juice with ginger also recorded the lowest TA (0.522) at ambient temperature. The TA of apple and cucumber juice samples was found to decrease significantly at refrigerated temperature (Table 2 and Table 5). Decreased acidity might be due to acidic hydrolysis of polysaccharides where acid is utilized for converting non-RS into RS [36].

Vitamin C (Ascorbic acid)

The vitamin C levels in the apple juice sample without preservation (control), with sodium benzoate, garlic, ginger and that of garlic and ginger on the day0 are 16.0 mg/100ml, 15.0mg/100ml and 15.0 mg/100ml 13.0mg/100ml and 14.45mg/100ml respectively. cucumber juice samples at room temperature in day0 for control, juice+ sodium benzoate, juice+ garlic, juice+ginger, juice+garlic and ginger are 4.0g/100ml, 3.9g/100ml, 4.01g/100ml, 4.06g/100ml, 4.09g/100ml respectively. The ascorbic acid content of both apple juice and cucumber juice samples at both temperatures decreased after the storage period as seen in table 3 and table 6. This observed diminution in vitamin C content is in agreement with what was reported in the literature as vitamin C content is known to be an important parameter for assessing the nutritional quality of fruits blends as it degrades during storage [32]. This degradation is perhaps due to the high sensitivity of vitamin C to oxygen, light and heat - and consequential oxidation in the presence of oxygen by both enzymatic and non-enzymatic catalysts [33]. Apple juice without preservation recorded the highest vitamin C level (28.51g/100ml for ambient temperature and 24.45g/100ml for refrigerated temperature) sodium benzoate recorded the second highest vitamin

C level (26.10 mg/100ml and 21.60g/100ml for ambient and refrigerated temperature respectively.) than other preservatives due to slow rate of oxidation. This implies that sodium benzoate preserved vitamin C more than lime during the storage period. Consequently, vitamin C contents of fresh fruit juices can be enriched using sodium benzoate as preservative, although, the abuse (excess addition) of such preservative has been linked to food poisoning/toxicity, immune depression and cancer in human [34][38].

Sensory Evaluation

Organoleptic parameters like colour, flavour, and taste of the apple and cucumber juices were acceptable throughout the period of storage (excluding day8) except for juice stored at ambient temperature which showed deterioration in taste, flavour and overall acceptance after the 3rd day of storage. There was variation in color and flavor in prepared juice with the four different preservatives which shows that temperature a has great effect the on storage quality of food products. Sensory evaluation showed that there was no significant difference among the juices with four different additives considering color and overall acceptability.

Conclusion

Home-made non-commercial juices that are processed by using a mechanical or electrical juice extractor, squeezing, macerating or aqueous/solvent extractions, with or without the application of heat have a short shelf life and low organoleptic property as found in the present study. The findings of this study generally indicate that a combination of chemical and natural preservation together with refrigeration is suitable for the preservation of fruit juice for a long time. These methods considerably decreased the microbial count.

Recommendation

Since these methods are simple, inexpensive and convenient, they can be adopted for industrial use in the processing and preservation of fruit juice. Thus, preservation of fruit juice at ambient temperature for a long time should be discouraged to reduce microbial contamination. The utilization of the preserved juice should be encouraged as health/therapeutic drink. Above all, the preservation of apple juice is important because of the seasonality of its production which makes it abundantly available during its season and scarce during the off season. Chemical preservatives can be replaced with natural preservatives such as ginger and garlic.

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