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Microbiological and Storage Properties of Spiced Tiger Nut *(Cyperus esculentus vassativa)* Drink

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Abstract: The microbiological and storage properties of spiced Tiger nutdrinkproduced from different ratios of fresh and roasted Tiger nut and date palm as sugar replacer were evaluated. Tiger nut drinks were prepared using three locally available spices namely, ginger (*Zingiberofficinale Rosc*), galic (*Allium sativa*) and ehuru *(Monodoramyristica*) spices, pasteurized at 72°C for 15mins and stored at room temperature (28°C) and refrigerated temperature (-4°C) for a period of three weeks. The drinks were evaluated for microbiological and storage properties such as pH, total titratable acidity (TTA), ºBrix and refractive index. Total bacterial count after three weeks was insignificant at refrigeration (4±2°C) temperature in fresh sample A, B, C and D. Thus, with the right proportion, spices have shown capacity to lower bacterial growth, hence prolonging the shelf life of Tiger nut drink.

Keywords: Storage, Microbiology, Properties, Spices, Tiger Nut, Drink

1. Introduction

The consumption and safety of non-carbonated drinks has become increasingly important and demand largely based on their value, flavour, aroma and colour [1]. However, despite the increasing popularity of drinks made from plant origin, the storage stability and microbiological safety calls for concern.

Tiger nut drinks are highly nutritious for human consumption, but limited by the short shelf life which may be as a result of the hygiene involved during preparation, packaging, storage and distribution which may expose it to microbial contamination. The drink has also shown to contain sufficient nutrients that can support microbial growth and subsequent spoilage.

Several factors encourage, prevent or limit the growth of microorganisms in drinks. The most important are water activity, low p^H, hygienic practices, storage temperature and concentration of preservatives [2].

The preservative effect of spices has been previously reported with particular reference to ginger and others [3-4]. The antioxidant properties of spices have also been recognized and demonstrated that spices effectively increased the antioxidant capacity of foods with their effects dependent on food matrices. The use of local spices to control the activities of micro-organisms in food has been reported [5-6].

Other spices like garlic (*Allium sativa)* has also been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and low density lipoprotein (LDL), increase antioxidant status, and inhibit angiotension-converting enzyme. Garlic reduces cholesterol, reduces blood pressure, and increases antioxidant status.[7]. While ehuru extract has also been studied and used as flavouring agent [8].

The use of date palm as sugar replacer is on the increase with its main component as carbohydrate (mainly sugars, sucrose, glucose, and fructose), which may constitute about 70%. The sugars in date are easily digested and can immediately be moved to the blood after consumption and can quickly be metabolised to release energy for cell

activities [9-10], thuswith the use of date palm, underutilized nature of Tiger nut for drink production and addition of various spices can be a good justification for the study. The objective of the study therefore is to determine the effect of different spices on the microbiological and storage properties of Tiger nut drink.

2. Materials and Methods

2.1. Materials

Tiger nut tubers (fresh and dried) were purchased from Rumuwoji market, Port-Harcourt. The spices (Ehuru, Garlic, Ginger) and date palm were purchased from fruit garden market, Port-Harcourt, Rivers State, Nigeria.

2.2. Chemicals

All chemicals and equipments used were of analytical grade and were obtained from the analytical laboratory, Department of Food Science and Technology, Rivers State University, Port-Harcourt.

2.3. Methods

2.3.1. Tiger Nut Drink Extraction

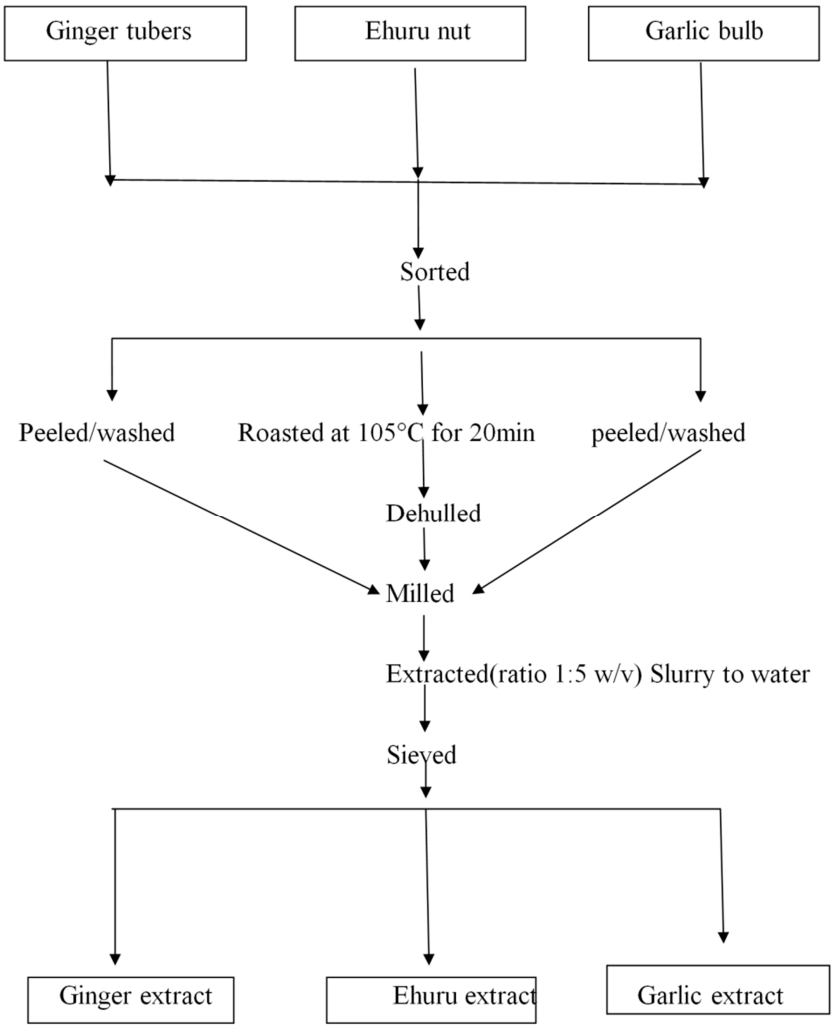
The method described by Udeozor Linda Oluchi [11] was modified for the extraction of Tiger nut drink from fresh and roasted Tiger nuts. Roasted and fresh Tiger -nut was manually sorted and cleaned to remove foreign particles and unwanted materials. The fresh Tiger nut was milled into slurry and extracted at a ratio of 1:3 of Tiger nut to water.

2.3.2. Spices

The dry spice (Ehuru) was sorted, washed and roastedin an electric oven at 105°C Tiger nut for 20mins before deshelling. The other spices (ginger and garlic) were all milled using a dry milling machine, model (GA-JBL 2002). Spices were extracted at ratio 1:5 w/v of spice to water.

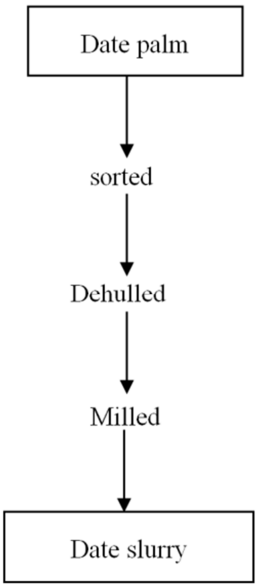
2.3.3. Formulation of Spiced Tiger Nut Drink

The formulation of spiced Tiger nut drink was done using 10ml each of the different spice extracts and 490ml of Tiger nut with varying quantities of date palm as sweetener ranging from 10-30g.



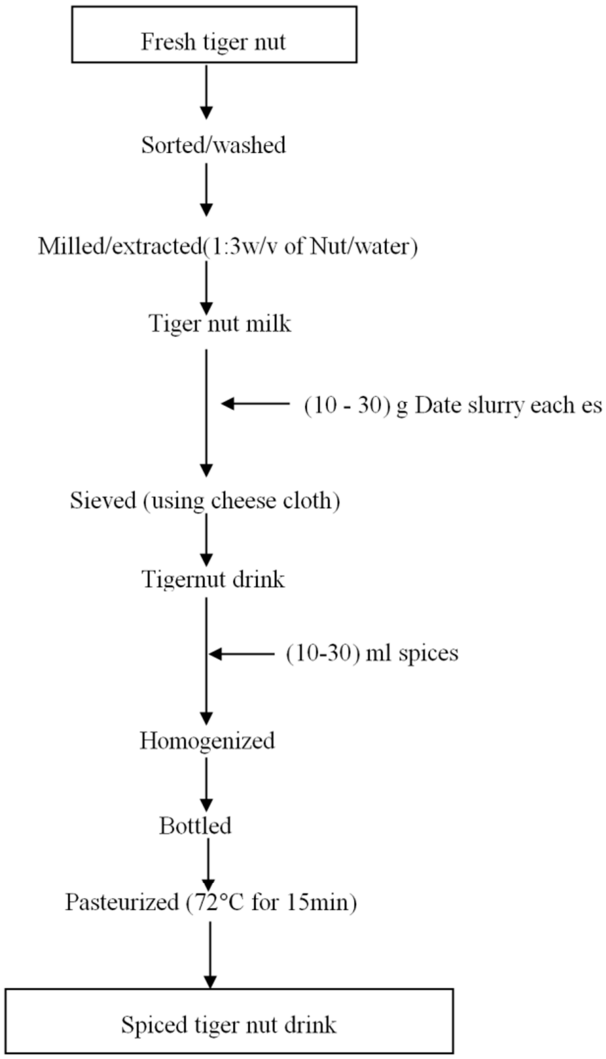
Source: [12]

Figure 1. *Flow chat for the extraction of different spices.*



Source [11]

Figure 2. *Flow chat for date slurry production.*



Source: [11]

Figure 3. *Flow chat for spiced Tiger nut formulation.*

2.4. Microbiological Assay (Total Bacterial Count)

2.4.1. Materials and Reagents

Peptone water, distilled water, nutrient agar, beakers, conical flask, measuring cylinder, autoclave, pipette, test tubes, refrigerator, incubator, foil paper, cotton wool.

2.4.2. Preparation of Materials

All glass wares used were sterilized by autoclaving at 121°C for 15min before use.

2.4.3. Preparation of Media

Nutrient agarwaspreparedaccording to the manufacturer's instruction,

2.4.4. Peptone Water

Peptone water was used as diluent. 15g was weighed and dissolve into 1000ml of distilled water in a sterile beaker. 9ml each was pipetted into a sterile test tube and was covered with cotton wool and foil before used.

2.4.5. Serial Dilution

Tenfold serial dilution method was used for the analysis. 1ml of sample was pipetted with sterile pipette into 9ml of diluent (10^{-1}) this dilution continue till 10^{-7} which provides a colony that can be counted.

2.4.6. Total Viable Bacteria Count

Bacteria plate count was done on the samples by spread plating method. 0.1ml of the appropriate dilution was pipetted into a sterile Petri dish containing nutrient agar for bacteria count. The plates were incubated at 37°C for 24-48hrs. After the incubated period, colonies were counted using a colony counter.

2.5. Storage Studies

The pasteurised and most preferred samples were stored at room temperature and refrigerated for weekly analysis of pH, ºbrix and total titratable acidity for a period of four weeks. The pH of the spiced Tiger nut drink was determined using a digital pH meter model (PHS-2F). The pH meter was first calibrated using buffered solution at pH7.

The degree brix (ºBrix) was determined using a digital refractometer (potable hand-held refractometer 0-30 ºbrix). The refractive index (RI) was measured using a digital refractometer (model: A77384), while total titratable acidity (TTA) was determined by theAOACmethod [13].

3. Results and Discussion

Table 1 and 2showthe microbiological results of the preferred samples at refrigeration and room temperature, over a period of three (3) weeks.

Total bacterial count for fresh samples ranged from 1.1×10^{10} -6.0×10^{9}CFU/ml and 1.1×10^{10}- 2.8×10^{10}CFU/ml at refrigeration and room temperature respectively. Total bacterial count for roasted samples ranged from 1.2×10^{10}–9.6×10^{9}CFU/ml and 1.1×10^{9} – 8.0×10^{8}CFU/ml at refrigeration and room temperature respectively.

The high total bacterial count observed for fresh and roasted spiced Tiger nut drinks may be attributed to the cells of lactic acid bacteria that might have survived through the processing of pasteurization treatments done on the spiced samples..

Table 1. *Total bacterial count in CFU/ml of fresh spiced Tiger nut drink at refrigeration (4°C) temperature, over a period of three weeks.*

Samples	Day0	Week 1	Week2	Week3
A^{-7}(control)	NG	1.2×10^{10}	2.4×10^{10}	TNTC
B^{-7}	INSIG	6.0×10^{9}	1.2×10^{10}	TNTC
C^{-7}	4.2×10^{9}	1.1×10^{10}	TNTC	TNTC
D^{-7}	INSIG	INSIG	INSIG	5.3×10^{9}
E^{-7}	NG	INSIG	2.1×10^{10}	TNTC
F^{-7}	INSIG	INSIG	INSIG	INSIG

KEY:
A^{-7}100% Tiger nut (control), B^{-7}(490ml Tiger nut: 10ml ginger:10gdatepalm), C^{-7}(490ml Tiger nut: 10ml ginger:20g datepalm), D^{-7}(490ml Tiger nut: 10ml ginger: 30g datepalm), E^{-7} (490ml Tiger nut: 10mlEhuru: 20g datepalm), F^{-7} (490ml Tiger nut: 10ml Ehuru: 30g datepalm), SP = spoiled, TNTC = Too numerous to count, NG = No growth, INSIG=Insignificant

Table 2. *Total bacterial count in CFU/ml offresh spiced Tiger nut drink at room (28±2°C) temperature, over a period of three weeks.*

Samples	Week 0	Week1	Week2	Week3
A^{-7}(control)	1.6×10^{9}	2.0×10^{10}	SP	SP
B^{-7}	NG	2.0×10^{10}	SP	SP
C^{-7}	2.4×10^{10}	TNTC	SP	SP
D^{-7}	NG	2.8×10^{10}	SP	SP
E^{-7}	TNTC	1.7×10^{10}	TNTC	TNTC
F^{-7}	1.1×10^{10}	2.3×10^{10}	1.1×10^{10}	SP

KEY:
A^{-7}100% Tiger nut (control)
B^{-7}(490ml Tiger nut: 10ml ginger: 10g datepalm)
C^{-7}(490ml Tiger nut: 10ml ginger: 20g datepalm)
D^{-7}(490ml Tiger nut: 10ml ginger: 30g datepalm)
E^{-7}(490ml Tiger nut: 10ml Ehuru: 20g datepalm)
F^{-7}(490ml Tiger nut: 10ml Ehuru: 30g datepalm)
SP= spoilt
TNTC = Too numerous to count
NG = No growth
INSIG =Insignificant

Table 3. *Total bacterial count in CFU/ml ofroasted spiced Tiger nut drink at refrigerated (4°C) temperature, over a period of three weeks.*

Samples	Day0	Week 1	Week2	Week3
A^{-7}(control)	2.0×10^{9}	4.8×10^{9}	TNTC	SP
B^{-7}	1.7×10^{9}	3.5×10^{8}	4.3×10^{9}	5.0×10^{9}
C^{-7}	NG	1.0×10^{10}	1.5×10^{10}	1.6×10^{9}
D^{-7}	NG	4.0×10^{9}	4.3×10^{9}	8.0×10^{8}
E^{-7}	3.1×10^{9}	3.9×10^{9}	4.0×10^{9}	5.1×10^{8}
F^{-7}	1.8×10^{10}	2.4×10^{9}	3.1×10^{9}	9.9×10^{9}

KEY:
A^{-7}100% Tiger nut (control)
B^{-7}(490ml Tiger nut: 10ml ginger: 10g datepalm)
C^{-7}(490ml Tiger nut: 10ml ginger: 20g datepalm)
D^{-7}(490ml Tiger nut: 10ml ginger: 30g datepalm)
E^{-7}(490ml Tiger nut: 10ml Ehuru: 20g datepalm)
F^{-7} (490ml Tiger nut: 10ml Ehuru: 30g datepalm)
SP=Spoilt
TNTC =Too numerous to count
NG = No growth
INSIG =Insignificant

Table 4. *Total bacterial count in CFU/ml ofroasted/spiced Tiger nut drink at room (28±2°C) temperature, over a period of three weeks.*

Samples	Week 0	Week1	Week2	Week3
A^{-7}(control)	NG	TNTC	SP	SP
B^{-7}	1.1×10^{9}	4.1×10^{9}	SP	SP
C^{-7}	2.0×10^{10}	TNTC	SP	SP
D^{-7}	1.9×10^{9}	5.8×10^{8}	6.9×10^{9}	8.0×10^{9}
E^{-7}	1.2×10^{10}	1.4×10^{10}	1.6×10^{9}	TNTC
F^{-7}	1.5×10^{9}	4.9×10^{10}	SP	SP

KEY:
A^{-7}100% Tiger nut (control),
B^{-7}(490ml Tiger nut: 10ml ginger: 10g datepalm)
C^{-7}(490ml Tiger nut: 10ml ginger: 20g datepalm),
D^{-7}(490ml Tiger nut: 10ml ginger: 30g datepalm)
E^{-7}(490ml Tiger nut: 10ml Ehuru: 20g datepalm)
F^{-7} (490ml Tiger nut: 10ml Ehuru: 30g datepalm)
SP = Spoilt
TNTC = Too numerous to count
NG =No growth
INSIG =Insignificant

Figures 4-15 shows the storage properties namely pH, °brix and total titratable acidity of spiced Tiger nut drink, prepared with same levels of different spice extract.

pH ranged from 3.08-4.71 and 3.19-6.01 for fresh and roasted samples respectively as shown in figure 4 to 5. The pH of all samples reduced with storage at room and refrigerated temperatures. Spice treatment showed that samples E (490ml Tiger nut: 10ml Ehuru: 20g datepalm) and F (490ml Tiger nut: 10ml Ehuru: 30g datepalm) which had Ehuru spice and date palm were more stable at room temperature. The pH range in the present study falls below the value reported byAdesokanet al. [14] for spiced*zobo* drink. Roasted Tiger nut drinks as shown in figures 6 to 7 gave better stability and consistency in pH. Tiger nut drink samples showed a consistent decrease in value from week one (1) through to week three (3). The decrease in pH may be due to the high organic acid content of the drink which may have encouraged the growth of acid tolerant bacteria that are sensitive to pasteurization. pH controls microbial growth in foods by directly inhibiting microbial growth and reducing the heat resistance of the micro-organism [15] and also define the properties of the product in terms of flavour, consistency and shelf-life.

pH of preferred sample (Fresh) at room and refrigerated temperature.

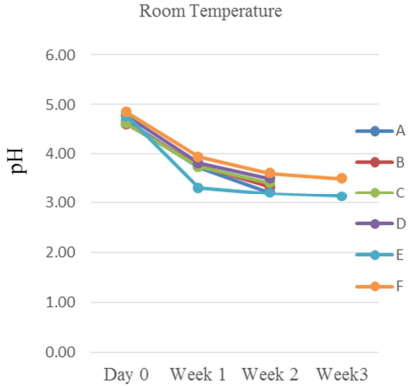


Figure 4. *pH of preferred sample (Fresh) at room temperature.*

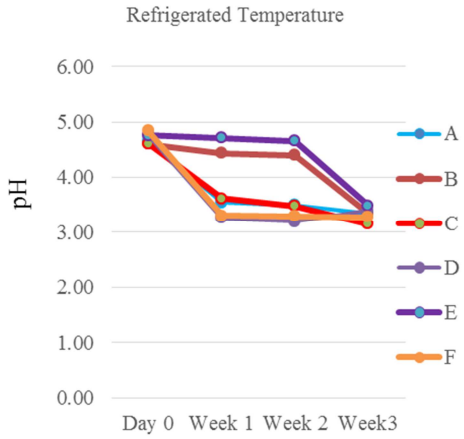


Figure 5. *pH of preferred sample (Fresh) at refrigerated temperature.*

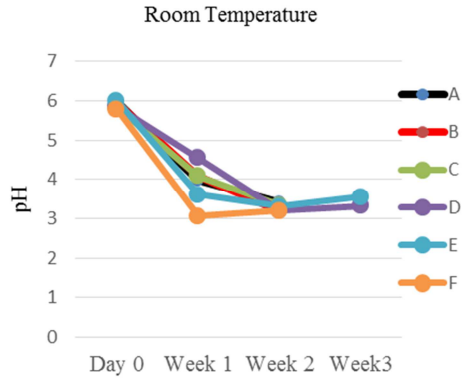


Figure 6. *pH of preferred sample (Roasted) at room temperature.*

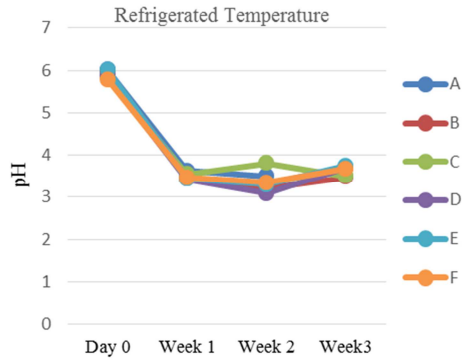


Figure 7. *pH of preferred sample (Roasted) at refrigerated temperature.*

Degree Brix is the sugar content of an aqueous solution, which expresses one degree Brix as 1gram of sucrose in 100grams of solution. ºBrix ranged from 1.9%-11% and 4.0%-16.0% for fresh and roasted samples respectively, with sample D having the highest value of brix while sample A (control) the least for fresh Tiger nut drink. The result of the present study showed a decrease in ºBrix with storage at both room and refrigerated temperature. Result also showed that at room temperature, the samples (A, B, C, D) for fresh and (A,B,C,F) for roasted could not store satisfactorily beyond week two despite the inclusion of different spices.

ºBrix of preferred sample (Fresh) at room and refrigerated temperature

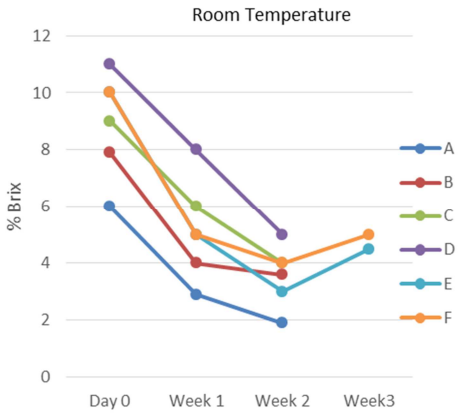


Figure 8. *Percentage (%) Brix of preferred sample (Fresh) at room temperature.*

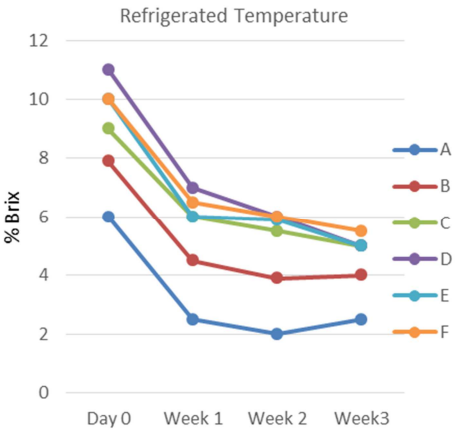


Figure 9. *Percentage (%) Brix of preferred sample (Fresh) at refrigerated temperature.*

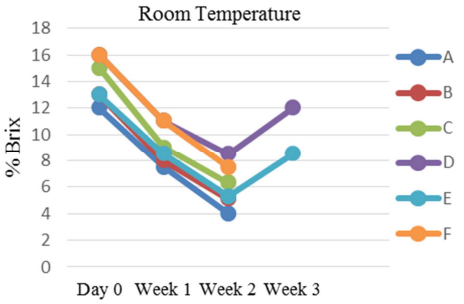


Figure 10*. % Brix of preferred sample (Roasted) at room temperature.*

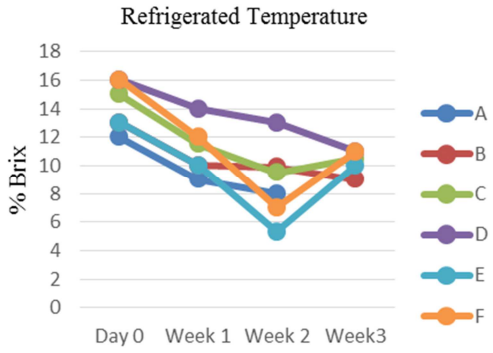


Figure 11. *% Brix of preferred sample (Roasted) at refrigerated temperature.*

Total titratable acidity ranged from 0.2- 1.6 and 0.15-1.49 for fresh and roasted Tiger nut drink at room temperature respectively. While total titratable acidity ranged from 0.11-1.60 and 0.15-2.04 for fresh and roasted samples at refrigerated temperatures respectively. Sample D for roasted Tiger nut showed a gradual increase in value during storage from week one (1) to week three (3). Increase in acidity might also be due to formation of acids by degradation of polysaccharides and oxidation of reducing sugar [16].

Total Titratable Acidity of preferred sample (Fresh) at room and refrigerated temperature

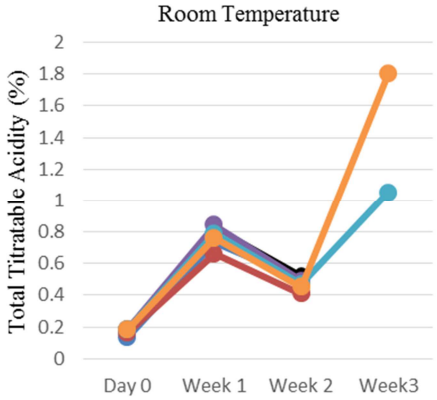


Figure 12. *Total Titratable Acidity of preferred sample (Fresh) at room temperature.*

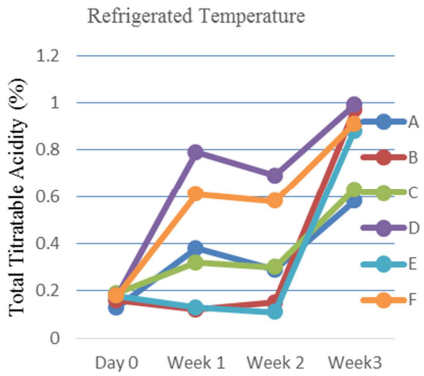


Figure 13. *Total Titratable Acidity of preferred sample (Fresh) at refrigerated temperature.*

Total Titratable Acidity of preferred sample (Roasted) at room and refrigerated temperature

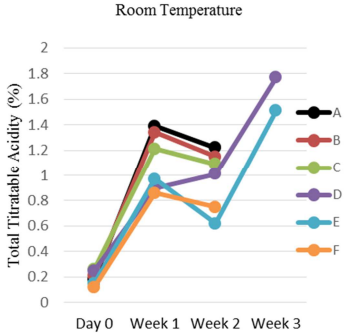


Figure 14. *Total Titratable Acidity of preferred sample (Roasted) at room temperature.*

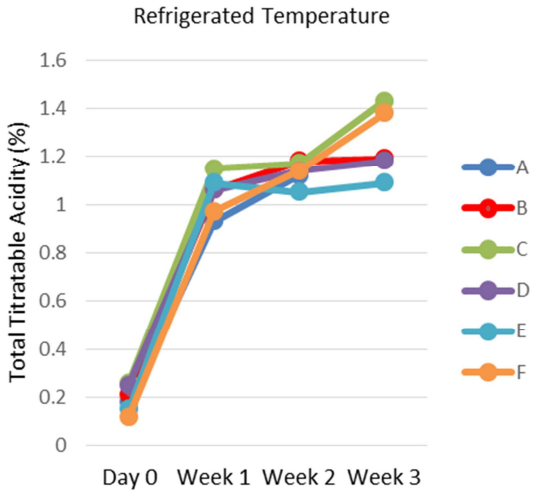


Figure 15. *Total Titratable Acidity of preferred sample (Roasted) at refrigerated temperature.*

4. Conclusion

The microbiological and storage properties of the fresh and roasted Tiger nut drinks treated with different spices revealed that the shelf life of the Tiger nut drinks were differently affected by the various treatments and storage conditions. Therefore the study concluded that the Tiger nut treated with ehuru and stored at refrigerated temperature proved to be the best.

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