

STUDIES ON THE NUTRITIONAL PROPERTIES OF KUNUNZAKI DRINK ENRICHED WITH COCOA POWDER

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Abstract

The effects of adding cocoa powder and different preservatives on the proximate composition, mineral, antioxidant and antinutrient contents of kununzaki – a fermented drink were investigated. The optimal processing conditions for the production of the drink enriched with cocoa powder was established. The most preferred sample (20%) in terms of cocoa powder addition was chosen with sensory evaluation after which preservatives were added. The drinks were stored at ambient and refrigerated temperatures for four weeks. The added cocoa powder increased the protein, ash, fat and moisture content from 1.40 to 3.12%, 0.16 to 0.38%, 0.38 to 0.93% and 84.95 to 91.20%, respectively and decreased the carbohydrate and energy content from 12.26 to 5.75% and 61.10 to 39.11 kJ, respectively. The pH decreased while the titratable acidity increased with storage time. The addition of cocoa powder increased the mineral content of the drinks significantly and increased the levels of some of the antinutrients but were within permissible level. There was an increase in the antioxidant activity. There were significant differences ($P < 0.05$) in the assessed sensory qualities. The study concluded that addition of 20% cocoa powder improved the protein, ash and fat content of Kununzaki and combination of lime and lemon not only improved the taste of the drink but also preserved the drinks longer than the commercial Kununzaki drinks for at least three weeks.

Keywords: Kununzaki, Cocoa Powder, Proximate, Mineral, Antioxidant, Preservatives

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1. INTRODUCTION

Kununzaki, also known as Kunu is a cereal-based non-alcoholic fermented beverage that is prepared from indigenous local cereals. It is a popular drink which is consumed throughout Nigeria but more predominant in the Northern part of the country (Adeyemi and Umar, 1994). It is a refreshing drink and it is less expensive because the materials are easily available and are cheap. It is nutritious and serves as a source of calorie supply, rich in vitamins especially vitamins B₁ and B₂ and essential mineral elements but low in protein (Bestshart, 1982). There is a need to enrich the drink to meet the dietary requirements of the consumer. Kununzaki has relatively short shelf-life of about 24-48 hours at ambient temperature. It therefore requires adequate preservation to make it shelf-stable. It has been reported that Kununzaki's shelf-life was extended to few days by pasteurization at 60 °C for 1 h and storage under refrigeration conditions (Adeyemi and Umar, 1994).

Studies have reported the health benefits of cocoa products (*Theobroma cacao*) (Abbe *et al.*, 2010). Introducing cocoa powder into kunu drink will enrich the product as well as expand the utilization of the cocoa powder.

Nigeria is a major cocoa producer and after the extraction of the oil, the cake is mainly used for cocoa drink or animal feed production. Polyphenol compounds present in cocoa powder could significantly contribute to its health promoting characteristics when consumed (Abbe *et al.*, 2010). Cocoa powder is also rich in flavonoids, which are compounds that reduce the risk of cardiovascular disease by decreasing blood pressure and improving blood vessel functionality (Francisco *et al.*, 2007). Consuming foods that are high in flavonoids reduces inflammation and help prevent insulin resistance, which can decrease the risk of type 2 diabetes. The antioxidant capacity of fibre rich cocoa powder and its physico-chemical properties make it suitable to be used in the preparation of low-calorie, high-fibre food (Arts *et al.*, 1999).

A functional food produced from addition of cocoa powder to a culturally acceptable drink like *kununzaki* will appeal to health-conscious consumers. This will be acceptable if such a drink will have a reasonable shelf-life engendered by addition of bio-preservatives like lemon and lime as against chemical preservative. The use of chemical preservatives in beverages is being questioned by many consumers in the world as a result of hidden toxicological implications (Kumar *et al.*, 2013). The objective of this study is to produce *kununzaki* drink, evaluate the proximate, mineral, antioxidant, antinutrient compositions and determine the physico-chemical and sensory characteristics during storage at ambient and refrigeration storage.

2. MATERIAL AND METHODS

Materials

Sorghum grains (*Sorghum bicolor*), black pepper, red pepper (*Capsicum annum*), ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum*), cocoa powder (industrial product), lime, lemon and packaged sugar were purchased from the University Central Market, Ile-Ife, Nigeria. These products were purchased in large quantities to avoid variation in samples.

Preparation of lime and lemon

A piece each of ripe and matured lime and lemon was washed and sliced into two equal halves and the juice squeezed out into two different beakers. With a pipette, 2.5 ml were taken from both beakers and added into a 500 ml of *Kununzaki* sample in a bottle (Fapohunda and Adeware, 2012).

Preparation of enriched *kununzaki*

Figure 1 shows the flow chart of *kununzaki* production according to the method of Ayo *et al.* (2010).

One kilogram of sorghum grains were cleaned and steeped in twice its volume of clean water (1:2 w/v) for 24 h in a covered plastic bucket at ambient temperature. The steeped sorghum grains were washed and wet milled with the

spices (ginger, red pepper, black pepper, cloves) using a well cleaned disc attrition mill. The recipe is shown in Table 1. It was then wet sieved to remove the chaffs, the supernatant was decanted from the slurry. The slurry was divided into two unequal portions, 75% was added to boiling water, stirred for about two minutes and cooled to a temperature of $30 \pm 2^\circ\text{C}$ and subsequently added to the remaining one-quarter (25%) slurry. The mixture was mixed and sweetened with 10% granulated sugar and was left for about 8 h to ferment in a covered plastic bucket at room temperature.

Table 1: Recipe for the production of enriched *Kununzaki* drink

| Ingredients | <i>Kununzaki</i> | <i>Kununzaki</i> + cocoa |
|-----------------|------------------|-----------------------------|
| Sorghum(g) | 500 | 500 |
| Cloves(g) | 0.5 | 0.5 |
| Ginger(g) | 6.5 | 6.5 |
| Black pepper(g) | 0.5 | 0.5 |
| Red pepper(g) | 2.5 | 2.5 |
| Sugar(g) | 50 | 50 |
| Cocoa powder(g) | - | 12 |

(Crozier *et al.*, 2011; Obadina *et al.*, 2008)

Twelve grams of cocoa powder was dissolved in 240 ml of water to produce cocoa slurry using the method of Crozier *et al.* (2011). Different percentages (100, 80, 70, 60, and 50) of the *Kununzaki* were mixed with different percentages (0, 20, 30, 40, and 50) of cocoa slurry to obtain freshly processed enriched *Kununzaki* drink. 80/20% of *Kununzaki* /cocoa powder was chosen best and was used for further analysis.

Preservatives (sodium benzoate, lime and lemon) at 0.1% (gram) were added to different portions of the drinks. The product (*Kununzaki*) was packaged in plastic bottles, pasteurised at 60°C for 1 h and stored under ambient and refrigerated ($4 \pm 1^\circ\text{C}$) conditions for four weeks. Analyses were carried out on the drinks before, during and after storage for four weeks.

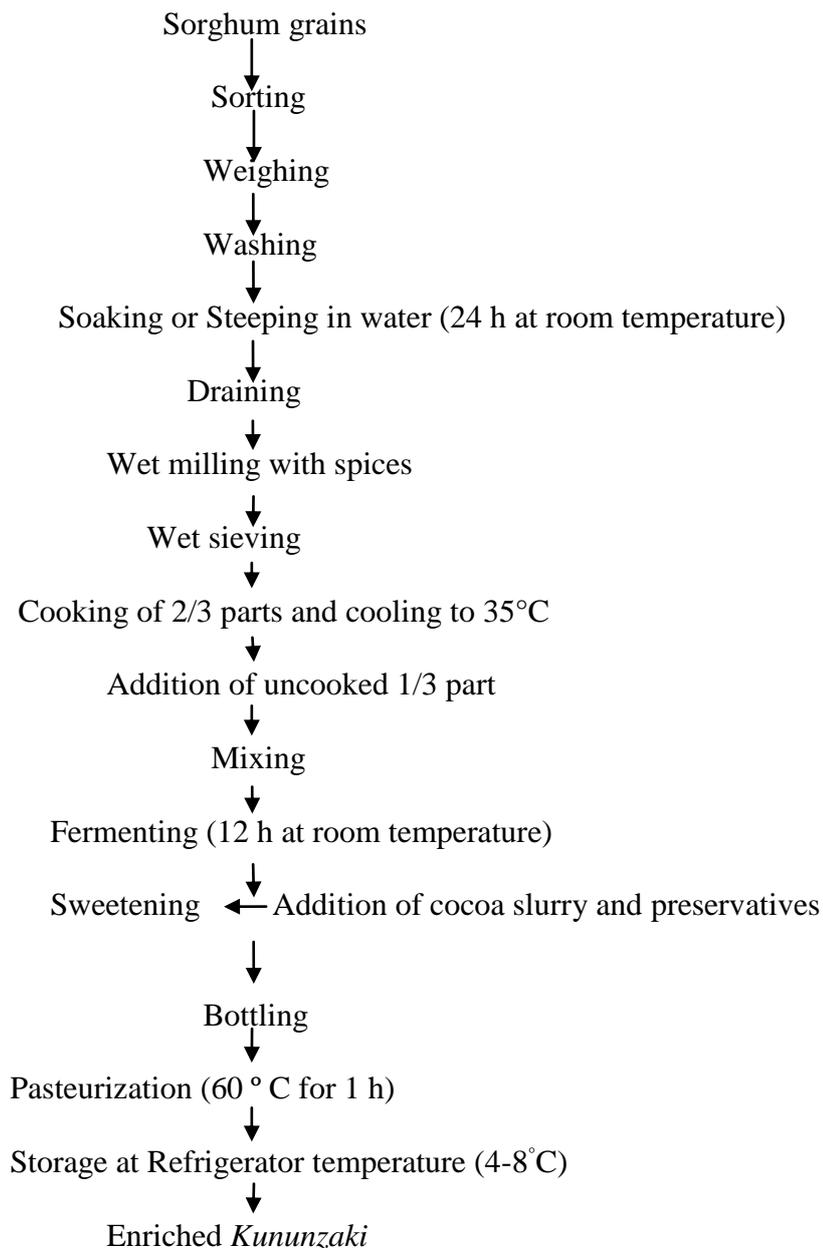


Figure 1: Modified process flow chart for the production of enriched *Kununzaki* drink
Source: Adapted from Ayo *et al.* (2010).

Proximate Composition of the Enriched *Kununzaki* Drinks

Proximate composition of the freshly prepared enriched *Kununzaki* samples and after storage for four weeks at ambient and refrigerated temperatures were determined for moisture, crude protein, ash, crude fat and carbohydrate contents based on the method of analysis of the Association of Official Analytical Chemists (AOAC, 2000).

Physicochemical Properties of the Enriched *Kununzaki* Drinks

These analyses were carried out after the production of the enriched *Kununzaki* and at a regular interval (weekly) for four weeks on the stored samples both at ambient and refrigerated temperatures. The method described by AOAC (2000) was used in the determination of pH, TTA and Total solids.

Determination of Minerals

The analyses for essential mineral elements was investigated using Atomic Absorption Spectrophotometric method (Fashakin *et al.*, 1991).

Determination of Antinutritional Contents of the Enriched *Kununzaki Drink*

The enriched *Kununzaki* samples were examined for the following antinutritional components: Tanins, Oxalates and Saponin. The modified vanillin – hydrochloric acid (MV – HCl) method of Price *et al.* (1978) was used for the determination of tannin. Oxalate was determined by the method of Falade *et al.*, (2005) while the spectrophotometric method of Brunner (1984) was used for saponin analysis.

Determination of Antioxidant Properties of Enriched *Kununzaki*

Extraction of antioxidant was carried out on dried *Kununzaki* following the method of Yurttas *et al.* (2000). The radical scavenging ability of the extract was determined using the stable radical DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) as described by (Pownall *et al.*, 2010).

The metal-chelating assay was carried out according to the method of Singh and Rajini, (2004) while the FRAP assay was measured with a spectrophotometer using the Benzie and Strain, (1999) method.

Sensory Evaluation

The 9-point Hedonic scale assessment as described by Larmond (1977) was used. The panelists were students from the Department of Food Science and Technology were selected based on their familiarity with *Kununzaki* beverage. The panelists were asked score the coded drinks in terms of degree of liking to taste, colour, texture and aroma. The coded samples were served in clean, transparent cups at room temperature 25°C. The panelists rinsed their mouth in between tasting of the samples. *Kununzaki* was bought from local producers in Ile-Ife central market, Nigeria and used as control along with samples produced in the laboratory. The results obtained were analyzed

using appropriate statistical methods of analysis.

Statistical analysis

The values obtained from each of the analyses were means of duplicate readings. The data obtained from physicochemical and sensory analysis were subjected to analysis of variance (ANOVA) and the means were separated by Duncan multiple range test (SPSS, version 16). Significance was determined at 5% level.

3. RESULTS AND DISCUSSION

Proximate Composition of Enriched *Kununzaki Drinks*

The proximate composition of the enriched *Kununzaki* samples, freshly prepared and after storage for four weeks at ambient and refrigerated temperatures is shown in Table 2.

The moisture content of the enriched *Kununzaki* samples ranged from 84.95 to 91.20% for freshly prepared samples with sample KHC (100% Commercial *Kununzaki*) having the highest value (91.20%) and sample KHN (100% *Kununzaki* with no preservative) having the lowest value (84.95%). After storage for four weeks, the values ranged from 85.7 to 91.25% and 85.7 to 91.20% at ambient and refrigerated temperatures respectively. There was an increase in the moisture content of the samples with the addition of cocoa powder. This could be due to the form in which the cocoa was added which was in solution. The high moisture content of the samples agreed with the findings of Enegbede (1999) that *Kununzaki* generally contains about 85.8% moisture. There was little or no change in moisture content during storage and effect of storage temperature was not significant ($P > 0.05$) on moisture content of samples.

Similar values (83.98- 91.67%) were reported by Adelekan *et al.* (2013) in the production of *soy-kununzaki* drink. The moisture content of any food is an index of its water activity. High moisture content makes beverage suitable as a refreshing and quench-thirsting product which is characteristic of a good beverage. The commercial sample (KHC) had higher

(91.20%) moisture content than the laboratory prepared samples. The variations in the moisture content of the samples might be due to the methods of preparation and the type of cereal (millet) used (Adelekan *et al.*, 2013). There was no significant difference ($P > 0.05$) in the moisture content of samples stored under ambient and refrigerated temperatures. There was a slight variation in the moisture content with storage time up to four weeks but no consistent trend was discernable in the result. This suggests that the samples were relatively stable during storage. The protein content of the enriched *Kununzaki* samples ranged between 1.40 and 3.12% for freshly prepared samples with sample with cocoa and sodium benzoate (KES) having the highest value (3.12%) and the commercial sample (KHC) having the least protein value (1.40%). There was an increase (16.7 – 44.4%) in the protein content with the

addition of cocoa powder. This indicates that cocoa powder contributed to the increase in protein content of the drink. *Kununzaki* drink, as it is known, is not a good source of protein (Oluwalana and Adedeji, 2012). The low protein content of each sample may be attributed to the low protein content of sorghum (10.4 g/100 g) which was further lowered during processing which involved removal of bran and germ (Hamad and Field 1979; Ihekoronye and Ngoddy, 1985). Cocoa powder is noted to be a good source of protein (8.14% to 19.71%) (Elena *et al.*, 2007; Ndife *et al.*, 2013). The high protein content in cocoa powder gives it the potential of being used as a source of protein supplement in cereal based foods. This implies that addition of cocoa powder can be used to enhance the protein content of *Kununzaki*, thus increasing or boosting its nutritional content.

Table 2: Proximate Composition (%) of Enriched *Kununzaki* Drink Samples at different Storage Temperatures (Ambient and Refrigerated) and Time

| | KHN /100% | KEN 80/20% | KES 80/20% | KEL 80/20% | KHC 100% |
|------------------|-------------------------|--------------------------|--------------------------|-------------------------|-------------------------|
| Moisture (%) | | | | | |
| Wk 0 | 84.95±0.9 ^b | 90.30±2.6 ^a | 87.20±0.4 ^b | 87.20±0.0 ^b | 91.20±2.2 ^a |
| Wk 4(A) | 85.70±0.0 ^b | 86.00±0.6 ^b | 88.00±1.1 ^b | 87.80±2.2 ^b | 91.25±1.5 ^a |
| Wk 4(R) | 87.00±0.9 ^b | 87.60±1.6 ^b | 87.50±0.6 ^b | 85.70±0.00 ^b | 91.20±2.2 ^a |
| Protein (%) | | | | | |
| Wk0 | 2.16±0.04 ^{bc} | 2.64±0.02 ^b | 3.12±0.24 ^a | 2.52±0.04 ^b | 1.40±0.00 ^c |
| Wk4(A) | 0.20±0.00 ^b | 0.24±0.00 ^b | 0.48±0.04 ^a | 0.20±0.01 ^b | 0.28±0.06 ^b |
| Wk4(R) | 0.36±0.04 ^b | 0.28±0.04 ^{bc} | 0.54±0.04 ^a | 0.28±0.02 ^{bc} | 0.30±0.00 ^b |
| Fat (%) | | | | | |
| Wk 0 | 0.38±0.02 ^c | 0.93±0.03 ^a | 0.60±0.04 ^b | 0.54±0.06 ^b | 0.91±0.04 ^a |
| Wk4(A) | 0.28±0.00 ^c | 0.24±0.04 ^c | 0.33±0.06 ^b | 0.39±0.02 ^b | 0.90±0.02 ^a |
| Wk4(R) | 0.30±0.02 ^{bc} | 0.21±0.04 ^c | 0.36±0.00 ^b | 0.27±0.02 ^b | 0.97±0.06 ^a |
| Ash (%) | | | | | |
| Wk 0 | 0.25±0.06 ^b | 0.38±0.04 ^a | 0.28±0.00 ^b | 0.21±0.02 ^{bc} | 0.16±0.04 ^c |
| Wk4(A) | 0.16±0.02 ^c | 0.35±0.04 ^a | 0.20±0.06 ^b | 0.20±0.00 ^b | 0.13±0.05 ^c |
| Wk4(R) | 0.28±0.2 ^{bc} | 0.41±0.02 ^a | 0.30±0.06 ^b | 0.20±0.04 ^{bc} | 0.15±0.00 ^c |
| Carbohydrate (%) | | | | | |
| Wk 0 | 12.26±0.06 ^a | 5.75±0.02 ^d | 8.80±0.68 ^b | 9.53±0.00 ^b | 6.33±0.04 ^c |
| Wk4(A) | 13.66±0.04 ^a | 13.17±0.60 ^a | 10.99±1.06 ^{bc} | 11.41±2.28 ^b | 7.44±1.59 ^c |
| Wk4(R) | 12.06±1.18 ^b | 11.50±1.66 ^b | 11.30±0.62 ^b | 13.55±1.22 ^a | 7.38±0.04 ^c |
| Energy (kJ) | | | | | |
| Wk 0 | 61.10±2.00 ^a | 41.93±1.99 ^{bc} | 53.08±1.4 ^b | 53.06±0.04 ^b | 39.11±0.00 ^c |
| Wk4(A) | 57.96±0.08 ^a | 55.80±2.76 ^a | 48.85±2.86 ^b | 49.95±2.90 ^b | 38.98±0.00 ^c |
| Wk4(R) | 52.38±3.1 ^a | 49.01±2.10 ^b | 50.60±2.04 ^a | 57.75±2.18 ^a | 39.11±3.10 ^c |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same rows are not significantly different at 5 % probability level

KHN 100% *Kununzaki* with no preservative

KEN 80/20% *Kununzaki*/cocoa with no preservative

KES 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL 80/20% *Kununzaki*/cocoa with lime and lemon

KHC 100% Commercial *kununzaki*

WK 0 Week 0, WK 4(A)-Week 4 at Ambient Temperature, WK4(R)- Week 4 at Refrigerated Temperature

After storage for four weeks, the protein values ranged from 0.20 to 0.48% and 0.28 to 0.54% at ambient and refrigerated temperatures respectively. There was a significant ($P < 0.05$) loss in the protein content during storage. The loss in protein may probably be due to enzymatic reactions. The higher decrease in protein content observed during storage at ambient temperature may be due to relatively higher microbial activities which was favoured under high temperature (28 ± 30 °C). Samples stored under refrigerated temperature showed better stability in terms of protein content than those stored at ambient temperature. At the end of the four weeks storage period, there was no significant difference ($P > 0.05$) in the protein content of all the samples at both ambient and refrigerated temperatures. Adedokun *et al.* (2012) reported a decrease in protein content (0.97 to 0.18%) of *Kununzaki* stored for 28 days which was due to the microbial activity. This suggests that the samples stored at refrigerated temperatures were more stable in terms of protein.

The fat content of the enriched *Kununzaki* samples ranged from 0.38 to 0.93%; 0.24 to 0.90% and 0.21 to 0.97% for freshly prepared samples and for samples stored for four weeks at ambient and refrigerated temperatures respectively. An increase of 42.1 to 59.1% was observed in the fat content of the samples with cocoa powder. Olosunde *et al.* (2014) reported that inclusion of *moringa* seed powder increased the fat content of *kununzaki*. Commercial sample (KHC) which was made from millet had a higher fat value (0.91%). Adedokun *et al.* (2012) reported that the fat content of *Kununzaki* from millet is of higher value than that from sorghum (0.39% from sorghum and 0.82 from millet). Ayo *et al.* (2010) also reported the fat content of *Kununzaki* from millet to be 1.03%. In the fresh cocoa- enriched samples, the fat content of samples with no preservative (KEN) was higher (0.94%) than the fat contents of samples containing sodium benzoate (KES) and the sample containing lime and lemon (KEL) (0.54 and 0.60 %) but at the end of four weeks of

storage, samples with preservatives had high fat content than those without preservatives. It is probable that the preservatives minimized the breakdown of fat to fatty acid and glycerol during storage through microbial activities. Samples containing sodium benzoate had higher fat content. Samples KHN (laboratory made *kununzaki*) and KHC (commercial sample) did not exhibit significant difference ($P > 0.05$) in fat contents with storage time. This is probably because the fat in the cocoa powder was broken down during storage (Ashaye *et al.*, 2006). Fat content of the samples stored for four weeks decreased (26.3 to 74.19%; 21.05 to 77.42%) with storage time under both ambient and refrigerated temperatures but a greater decrease was observed at ambient temperatures.

Ash content of all the *Kununzaki* samples ranged from 0.16 to 0.38% for freshly prepared samples, 0.13 to 0.35% and 0.15 to 0.41% for samples after storage for four weeks at ambient and refrigerated temperatures respectively. There was an increase in the ash content of the cocoa enriched samples with no preservatives (KEN) and those containing sodium benzoate (KES) but a decrease was observed in samples containing lime and lemon (KEL). This might probably be because of the inclusion of cocoa powder. The ash content of commercial sample (KHC) was low (0.16%). Adedokun *et al.* (2012) reported that the ash content of *Kununzaki* produced from millet (0.98%) is lower than that produced from sorghum (1.12%). The ash content varied slightly with no significant ($P > 0.05$) variations among samples during storage period. The amount of ash content in the drinks in this work is lower than the values reported by Sopade and Kassum (1992), for *Kununzaki* 1.5% of ash content. Ash content is an index of inorganic mineral elements in the food (Onyeka, 2008). The mineral elements in the *Kununzaki* were more stable during storage irrespective of the preservatives used.

Carbohydrate content of the *Kununzaki* samples ranged from 5.75 to 12.26% for freshly prepared samples, 7.11 to 13.66% and

11.18 to 13.55% for samples after storage for four weeks at ambient and refrigerated temperatures. Sample KHN (Laboratory prepared sample, 100% cereal) had the highest carbohydrate content (12.31%). The addition of cocoa powder to the samples increased the protein content while the carbohydrate contents were reduced. The result in this work agrees with the findings of Ayo *et al.* (2010) that the carbohydrate content of *Kununzaki* enriched with beniseed is within the range of 7.23 to 10.21%. The results of this work are similar to the findings of Sopade and Kassum (1992) who reported that *Kununzaki* contains 12.2% of carbohydrate. There was a significant difference ($p > 0.05$) among the samples except for the cocoa enriched samples with sodium benzoate (KES) and samples with lime and lemon (KEL) which had no significant difference ($P < 0.05$). However, after storage for four weeks, there was an increase in the carbohydrate contents of all samples at both temperatures. This might probably be because of the decrease in the protein content of the samples with storage time at both temperatures and also since carbohydrate was obtained by difference. It indicated that, the added material (cocoa powder) which contains relatively lower carbohydrate could have affected the carbohydrate content in the *Kununzaki* and increased the protein of the enriched *Kununzaki*.

The energy values of all the *Kununzaki* samples ranged from 39.11 to 61.10 kJ for freshly prepared samples, 38.98 to 57.96 kJ and 39.11 to 57.75 kJ for samples after storage for four weeks at ambient and refrigerated temperatures. Laboratory prepared sample (KHN) had the highest energy value (61.10 kJ) while commercial sample (KHC) had the least value (39.11 kJ). The energy value decreased with addition of cocoa powder. The energy values were calculated from protein, fat and carbohydrate values. The energy values followed the trend of carbohydrate content for all samples. The result of this work is similar to the findings of Ayo *et al.* (2010) that the energy content of *Kununzaki* enriched with beniseed was within the range of 7.23 to

10.21%. After storage for four weeks at both ambient and refrigerated temperatures, there were variations in the energy values of all the samples. Laboratory prepared 100% cereal sample (KHN), cocoa enriched sample containing sodium benzoate (KES), commercial samples (KHC) decreased in energy values while cocoa enriched sample with no preservative (KEN) decreased in energy value with storage time at both temperatures. Enriched samples with lime and lemon (KEL) increased at refrigerated temperature and decreased at ambient temperature. The energy values of the drinks decreased significantly ($P < 0.05$); however, the product remained a good source of energy when consumed. This may be due to the percentage of cocoa powder added which is a good source of energy (USDA, Nutrient Database, 2011).

Physicochemical Properties of the Enriched *Kununzaki* Drinks during storage

Changes in pH of enriched *kununzaki* drinks during storage

Figures 2a and b show the pH values for all the samples when freshly prepared and during storage for four weeks at ambient and refrigerated temperatures. The pH of the samples was significantly different ($P < 0.05$) from each other.

The pH values obtained for fresh samples ranged from 3.75 to 5.0. The enriched sample containing sodium benzoate (KES) had the highest value (5.0) and commercial sample (KHC) had the lowest value (3.75). The pH values result of this work were comparable to pH 3.9 for *Kununzaki* reported by Oshoma *et al.* (2009). The values obtained in this work were higher than (3.70-3.90) reported by Makinde and Oyenike, (2012), when sesame seed was incorporated into *Kununzaki*. However, the results obtained was within the range of values (4.0-4.14) reported by Ayo *et al.* (2004) for *Kununzaki* from sorghum. The deviation from previous findings reported may be attributed to the method of preparation, type of cereal used and the preservation of the samples.

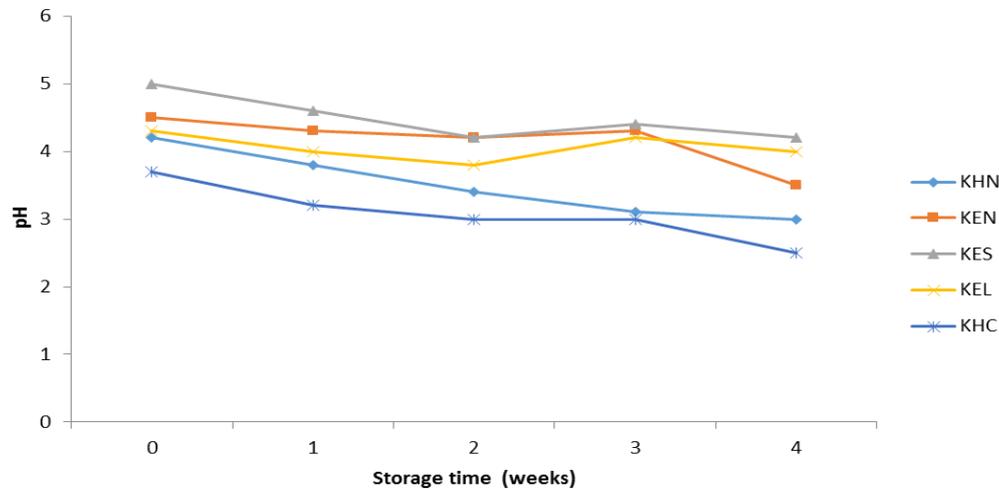


Fig 2a: pH of the Enriched *Kununzaki* Drink Samples during Storage at Ambient Temperature

KHN- 100% *Kununzaki* with no preservative; KEN- 80/20% *Kununzaki*/cocoa with no preservative; KES- 80/20% *Kununzaki*/cocoa with sodium benzoate; KEL- 80/20% *Kununzaki*/cocoa with lime and lemon; KHC-100% Commercial *Kununzaki*; Number represents the numbers of weeks of storage

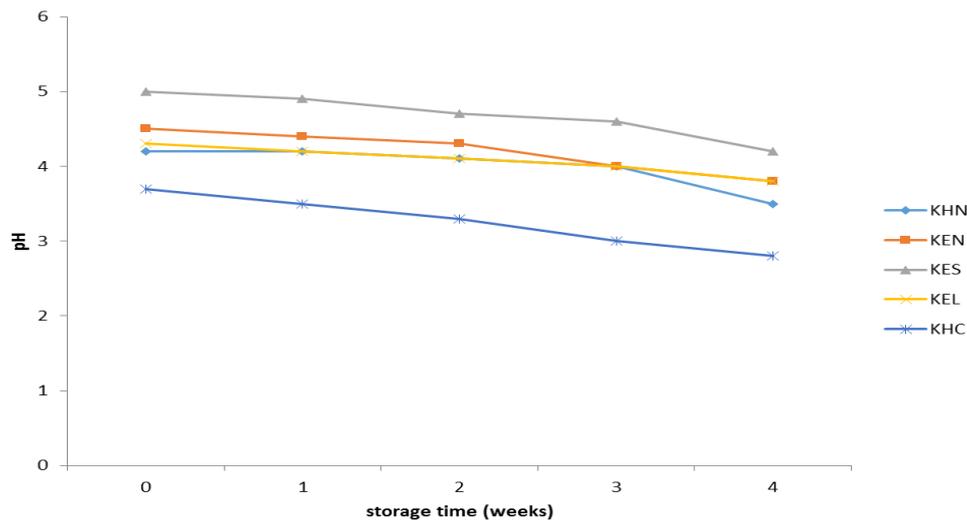


Fig 2b: pH of the Enriched *Kununzaki* Drink Samples during Storage at Refrigerated Temperature

KHN- 100% *Kununzaki* with no preservative
 KEN- 80/20% *Kununzaki*/cocoa with no preservative
 KES- 80/20% *Kununzaki*/cocoa with sodium benzoate
 KEL- 80/20% *Kununzaki*/cocoa with lime and lemon
 KHC-100% Commercial *Kununzaki*
 Number represents the numbers of weeks of storage

The addition of cocoa powder reduced the pH content of the *Kununzaki*. Samples with cocoa powder had lower pH values than samples without cocoa powder. This agreed with the report of Ndife *et al.* (2013) that addition of cocoa powder decreased the pH of the *Kununzaki* and therefore could positively affect the storability of the *Kununzaki*. The pH values for all samples decreased with storage time but

slightly more in samples containing preservatives. It is expected that an environment became more acidic and so the microbes would not be very active. Samples with preservatives showed stable pH values within the storage period of four weeks. The decrease in pH values during storage could be attributed to increased acidity resulting from organic acids produced during microbial

fermentation (Efiuvwevwere and Akoma, 1995). The pH of samples stored at refrigerated temperature decreased slowly compared to those stored at ambient temperature. The pH of the Kununzaki samples stored at ambient temperature tends more towards acidity as the days increased compared to the refrigerated samples. Microbial activities are slowed down at lower temperatures, thereby affecting rate of substrate metabolism (Prescott *et al.*, 1999).

Titratable acidity of the enriched *kununzaki* drinks

Changes in the titratable acidity of the enriched *Kununzaki* samples in storage both at ambient and refrigerated temperatures are shown in Figures 3a and b respectively. The values of the titratable acidity of the samples ranged from 0.42 to 0.72% when freshly prepared. Commercial sample (KHC) had the highest value of titratable acidity. Abel *et al.* (2011) reported the titratable acidity of *Kununzaki* preserved with sodium benzoate and sodium metabisulphite to be in the range of 0.011 to 0.048%, these values were lower compared to those obtained from the samples in this study but lower than values (0.98 to 1.13%) reported by Adedokun *et al.* (2012) for *Kununzaki* samples flavoured with *Aframomum danelli* extracts. Acidity increased slightly with the

addition of cocoa powder. Addition of lime and lemon further increased the acidity. The increase in acidity may be attributed to the acidic substances in lime and lemon. The titratable acidity (TTA) showed an increasing trend with storage time in all the samples and this correspond with the fall in pH as the storage period progressed. The increase in the TTA of the samples preserved by sodium benzoate and stored at refrigerated temperature was steady unlike those at ambient temperature. The stability observed in the % TTA of the refrigerated samples may be due to the effect of reduced microbial activity or chemical reactions (Ojimekwe *et al.*, 2013). A steady increase was observed in titratable acidity values of all samples between week two to week four in refrigerated samples, but rather a sharp increase was noted in samples stored at ambient temperature. Lower rate of metabolism in organisms at cold temperatures may account for the difference in TTA values of samples at the two temperatures (Adedokun *et al.*, 2012). Low pH and TTA values obtained for all the samples suggested that the drink contained lactic acid bacteria which may inhibit the activity of spoilage microorganisms thereby improving the shelf life of the products (Ojimekwe *et al.*, 2013).

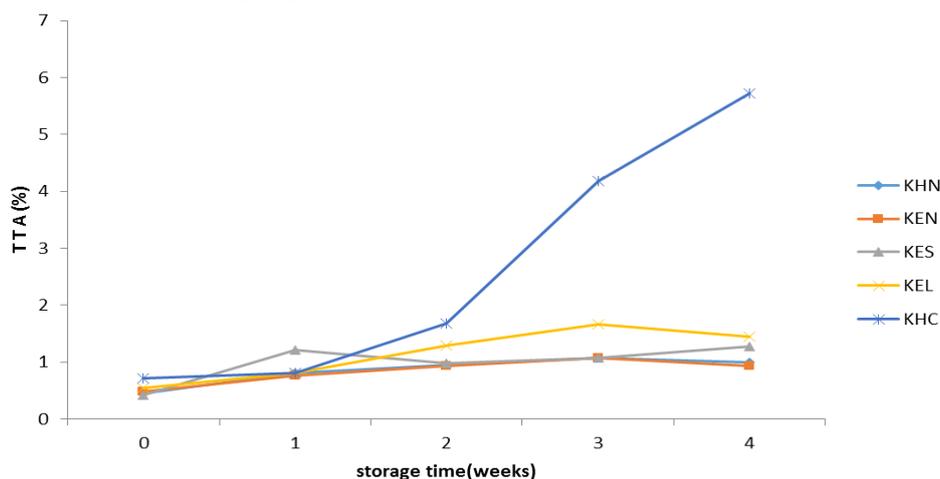


Fig 3a. TTA of the Enriched *Kununzaki* Drink Samples during Storage at Ambient temperature

KHN- 100% *Kununzaki* with no preservative
KEN- 80/20% *Kununzaki*/cocoa with no preservative
KES- 80/20% *Kununzaki*/cocoa with sodium benzoate
KEL- 80/20% *Kununzaki*/cocoa with lime and lemon
KHC - 100% Commercial *Kununzaki*
Number represents the numbers of weeks of storage.

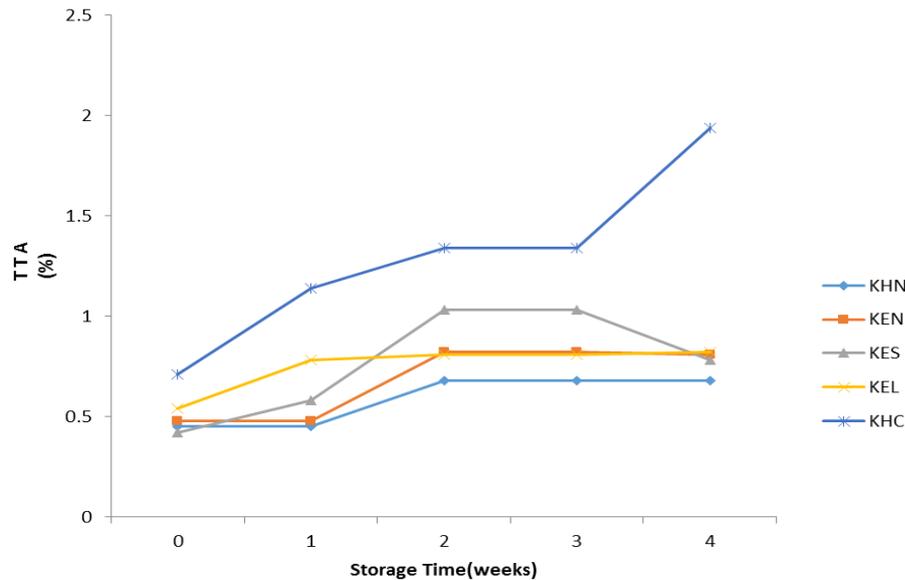


Fig 3b. TTA of the Enriched *Kununzaki* Drink Samples during Storage at Refrigerated Temperature

KHN- 100% *Kununzaki* with no preservative
 KEN- 80/20% *Kununzaki*/cocoa with no preservative
 KES- 80/20% *Kununzaki*/cocoa with sodium benzoate
 KEL- 80/20% *Kununzaki*/cocoa with lime and lemon
 KHC - 100% Commercial *Kununzaki*
 Number represents the numbers of weeks of storage.

Total solid content of enriched *kununzaki* drinks

The total solid contents of the enriched *Kununzaki* samples in storage at both ambient and refrigerated temperatures are shown in Figures 4a and b respectively. The total solids ranged from 14 to 30% when freshly prepared. Commercial *Kununzaki* sample (KHC) had the least total solid contents (14%) while 100% *kununzaki* (KHN) had the highest value (30%). The result obtained in this study can be compared with the value 13.42% of *Kununzaki* prepared from wet milled sorghum by Adejuyitan *et al.* (2008). Abel *et al.* (2011) reported the total solid contents for *Kununzaki* treated with benzoate chemicals to be 9.20 to 12.5 %. Also Adeyemi and Umar (1994) reported total solids of 6.85 g/100 ml of *Kununzaki*. Addition of cocoa powder to the *Kununzaki* increased the total solids of the drinks.

After a storage period of four weeks, total solids contents in the samples stored at ambient and refrigerated temperatures ranged from 9.35 to 19.78% and 9.25 to 18.99% respectively. A reduction was observed in the total solids

content of the enriched *Kununzaki* samples throughout storage period at both refrigerated and ambient temperatures respectively. It was most obvious at ambient temperature. This might be due to breakdown of the solids by microorganisms at room temperature. Metabolism of carbohydrate and protein content of the samples with release of water could be responsible for the reduction in total solids content during the storage period. Reduction in total solids of *Kununzaki* samples were also observed during storage by Abel *et al.* (2011) on effect of chemical treatment of *Kununzaki*. Commercial *Kununzaki* sample (KHC) had the least total solids contents (9.35%, 9.25%) at both ambient and refrigerated temperatures after storage for four weeks; this may be due to the nature of starch in the cereal and method of preparation. At the end of the storage period, there was a significant difference ($P < 0.05$) that there was decrease in the total solid contents of all samples.

However, higher total solids content had effect on consumer acceptability as it imparts texture to the beverage (Adejuyitan *et al.*, 2008).

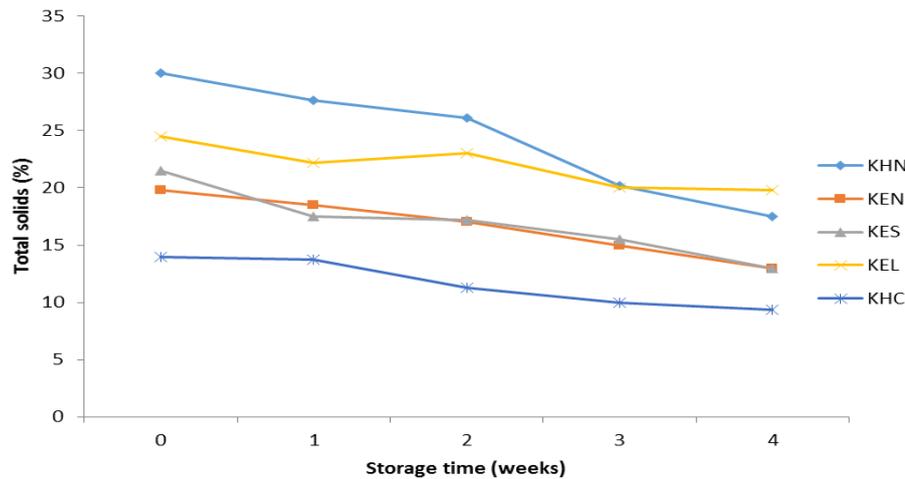


Fig. 4a Total Solids of the Enriched *Kununzaki* Drink Samples during Storage at Ambient Temperature

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *Kununzaki*

Number represents the numbers of weeks of storage.

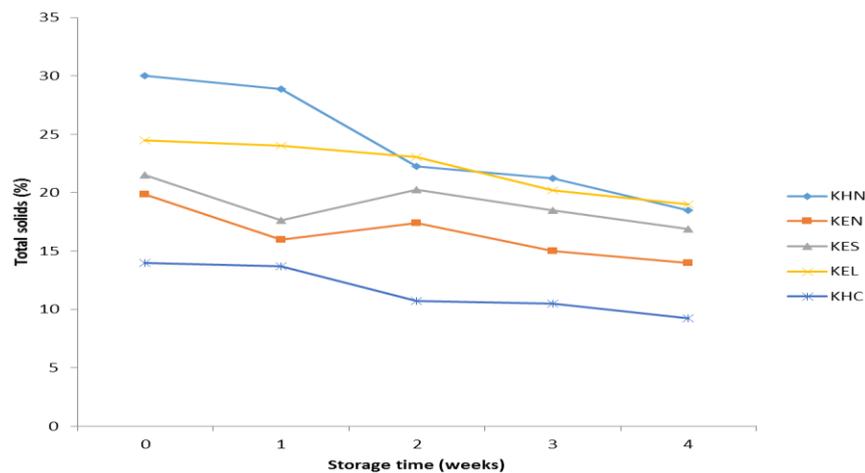


Fig. 4b. Total Solids of the Enriched *Kununzaki* Drinks during Storage at Refrigerated Temperature

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *Kununzaki*

Number represents the numbers of weeks of storage

Minerals compositions of the Enriched *Kununzaki* Drinks

The mineral compositions of the enriched *Kununzaki* samples when freshly prepared and after storage for four weeks at ambient and refrigerated temperatures are shown in Table 3. The calcium content ranged from 4.1 to 5.9 mg/100 g; 4.2 to 6.0 mg/100 g, 4.3 to 6.1 mg/100 g for freshly prepared samples and for

samples after storage for four weeks at ambient and refrigerated temperatures. The calcium values obtained in this study are similar to the value 5.18 mg/100 g reported by Oluwalana and Adedeji, (2012) for *Kununzaki*. An increase of 31.71% to 43.90% was observed in the calcium content with the addition of cocoa powder. Use of the preservative did not increase calcium content significantly.

Commercial sample (KHC) made from millet is rich in calcium, as the value (5.2 mg/100 g) is higher than Sample KHN (4.1 mg/100 g) made from sorghum. Effect of storage time on calcium varied among samples. Calcium content of sample containing sodium benzoate (KES) increased, those containing lime and lemon (KEL) decreased while there was no difference in laboratory 100% sample (KHN) with storage time at both temperatures.

Zinc content of the samples ranged from 3.4 to 7.4 mg/100 g in freshly prepared samples and 2.3 to 7.2 mg/100 g, 3.3 to 7.4 mg/100 g for samples after storage for four weeks at ambient and refrigerated temperatures respectively. Addition of cocoa powder increased the zinc contents by 3.3% to 23.33% in the samples. There was a significant difference ($P < 0.05$) in the zinc content of the samples. Sample KEN had the highest (7.4 mg/100 g) value and sample KHC, the least (3.4 mg/100 g) value of zinc content. This may be due to difference in the cereal (millet) used. Values obtained in this study (3.4 to 7.4 mg/100 g) were higher than the value 3.30 mg/100 g reported by Oluwalana and Adedeji, (2012) for zinc in *Kununzaki*. Zinc is a trace element which is needed in minute amount that enhances body functions. There was no significant difference ($P > 0.05$) in the zinc content of all the samples after storage that is there was negligible change in the calcium content after storage.

Magnesium content ranged from 85 to 182 mg/100 g for freshly prepared samples and 85 to 204 mg/100 g, 85 to 194 mg/100 g for samples stored for four weeks at ambient and refrigerated temperatures. Addition of cocoa powder increased the magnesium content by 4.9% to 13.3% and the presence of the preservatives increased it further, there was some increase in magnesium content of the sample with the addition of lime and lemon. Sample KEL had the highest magnesium values (182, 198 and 194 mg/100 g) when freshly prepared and after storage. This might be due to the presence of lime and lemon. Storage time did not affect the magnesium content. There was no significant difference ($P > 0.05$) after storage of four weeks in the

magnesium content. This result agrees with the findings (145.04 mg/100 g) of Oluwalana and Adedeji (2012) but the values of this work were much higher than the results 31.23 mg/100 g obtained by Makinde and Oyeleke, (2012). Magnesium helps in keeping the muscle relaxed and the formation of strong bones and teeth. It plays fundamental roles in most reactions involving phosphate transfer. It is believed to be essential in the structural stability of nucleic acid and intestinal absorption while deficiency of magnesium in man is responsible for severe diarrhea, hypertension and stroke (Romani and Andrea, 2013).

Iron content of the enriched *Kununzaki* drinks ranged from 4.6 to 7.0 mg/100 g for freshly prepared and 4.2 to 6.9 mg/100 g, 4.2 to 7.0 mg/100 g for samples after storage for four weeks at ambient and refrigerated temperatures. The iron content increased (9.1% to 27.3%) with the addition of cocoa powder and the preservatives. Sample KHC (commercial *Kununzaki*) had the lowest iron value (4.6 mg/100 g) followed by KHN (5.5 mg/100 g), the low value of the iron is likely because sorghum and millet seeds have been reported to have small amount of iron (Shobha *et al.*, 2008). The iron content of this work compares with results reported by Kayode, (2006), 3 to 11 mg/100 g and Makinde and Oyeleke, (2012), 2.7 to 4.2 mg/100 g to be the iron concentration of sorghum grains. The addition of the preservatives such as lime and lemon, sodium benzoate enhanced the iron content when in storage because there was no significant reduction in the iron content of the stored drinks. Iron is an important element in the diet of pregnant women, nursing mothers, infants convulsing patients and elderly to prevent anaemia and other related diseases (Oluyemi *et al.*, 2006). Potassium content of the enriched *Kununzaki* drinks ranged from 129 to 199 mg/100 g for freshly prepared samples and 125 to 176 mg/100 g, 126 to 176 mg/100 g for samples after storage for four weeks at ambient and refrigerated temperatures respectively.

Table 3: Mineral Compositions (mg/100 mg) of Enriched *Kununzaki* Drink Samples at different Storage Temperatures (Ambient and Refrigerated) and Time

| | KHN 100% | KEN 80/20 | KES 80/20 | KEL 80/20 | KHC 100% |
|---------------------|-----------------------|------------------------|------------------------|-----------------------|-----------------------|
| Calcium (mg/100g) | | | | | |
| Wk 0 | 4.1±0.02 ^c | 5.8±0.00 ^a | 5.4±0.04 ^b | 5.9±0.40 ^a | 5.2±2.2 ^b |
| Wk 4(A) | 4.2±0.0 ^b | 5.8±0.06 ^a | 5.8±0.01 ^a | 5.1±0.2 ^{ab} | 5.7±0.05 ^a |
| Wk 4(R) | 4.3±0.02 ^c | 5.9±0.03 ^b | 6.2±0.00 ^a | 5.1±0.13 ^b | 6.0±0.06 ^a |
| Zinc (mg/100 g) | | | | | |
| Wk0 | 6.0±0.04 ^b | 7.4±0.02 ^a | 7.0±0.01 ^a | 6.2±0.24 ^b | 3.4±0.0 ^c |
| Wk4(A) | 6.6±0.02 ^b | 7.2±0.02 ^a | 7.1±0.04 ^a | 6.4±0.21 ^b | 2.3±0.06 ^c |
| Wk4(R) | 5.2±0.10 ^c | 7.4±0.04 ^a | 7.0±0.05 ^a | 6.5±0.60 ^b | 3.3±0.04 ^d |
| Magnesium (mg/100g) | | | | | |
| Wk0 | 162±0.02 ^c | 170±0.03 ^b | 177±0.21 ^{ab} | 182±0.04 ^a | 85±0.04 ^d |
| Wk4(A) | 161±0.02 ^c | 170±0.04 ^{bc} | 176±0.02 ^b | 198±0.02 ^a | 85±0.02 ^d |
| Wk4(R) | 160±0.00 ^c | 170±0.04 ^{bc} | 177±0.14 ^b | 194±0.06 ^a | 85±0.20 ^d |
| Iron (mg/100g) | | | | | |
| Wk 0 | 5.5±0.06 ^b | 6.0±0.04 ^b | 6.9±0.30 ^a | 7.0±0.02 ^a | 4.6±0.04 ^c |
| Wk4(A) | 5.4±0.04 ^b | 6.4±0.02 ^{ab} | 6.7±0.06 ^a | 6.9±0.02 ^a | 4.2±0.04 ^c |
| Wk4(R) | 5.5±0.02 ^b | 6.0±0.02 ^b | 6.8±0.06 ^a | 7.0±0.20 ^a | 4.2±0.00 ^c |
| Potassium (mg/100g) | | | | | |
| Wk 0 | 129±0.03 ^d | 155±0.02 ^c | 191±0.68 ^a | 178±0.00 ^b | 199±0.04 ^a |
| Wk4(A) | 125±0.21 ^e | 152±0.00 ^c | 142±0.08 ^d | 176±0.10 ^a | 163±0.01 ^b |
| Wk4(R) | 126±0.18 ^d | 152±0.07 ^{bc} | 156±0.00 ^b | 176±0.04 ^a | 149±0.04 ^c |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same row are not significantly different at 5 % probability level

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *kununzaki*

WK 0-Week 0, WK 4A- Week 4 at Ambient Temperature, Week 4 at Refrigerated Temperature

There was an increase of (20.2 to 48.1%) in potassium content with the addition of cocoa powder and the preservatives. Sample KHC had highest (199 mg/100 g) potassium value and sample KHN the least value (129 mg/100 g). This may be because of the cereal (millet) used in the production of sample KHC. The result in this work agrees with the findings (134.27 mg/100 g) of Oluwalana and Adedeji (2012) for *Kununzaki*. There was no significant difference ($P > 0.05$) in the drinks after storage in the potassium level. Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins (Malik and Scrivastava, 1982). Generally, the addition of cocoa powder increased the mineral levels of *Kununzaki* drinks. The use of preservatives especially the lime and lemon furthermore enhanced the mineral content and also helped in stabilising it during storage especially at refrigerated temperature.

Antinutrient Contents in the Enriched *Kununzaki* Drink

The results of the anti-nutrient contents (oxalate, tannin and saponin) of the enriched *Kununzaki* samples when freshly prepared and after storage for four weeks at ambient and refrigerated temperatures are shown in Table 4. The oxalate content of the samples ranged from 0.44 to 1.32 mg/100 g. After storage for four weeks at ambient and refrigerated temperatures, it ranged from 0.66 to 1.32 mg/100 g, and 0.44 to 2.62 mg/100 g respectively. The oxalate content of the samples increased (33.3 to 66.7%) with the addition of cocoa powder. This might be because cocoa powder has high oxalate content of 650 to 783 mg/ 100 g (USDA, 2011). The oxalate content of the samples are presented in the following order KEL>KHC>KES>KEN>KHN when freshly prepared. Sample KEL had d highest oxalate content (1.32 mg/100 g) and sample KHN had

the least value (0.44 mg/100 g) oxalate content when freshly prepared. For samples after storage at both ambient and refrigerated temperatures, the oxalate contents were in the following orders: KEN>KES>KHC>KEL>KHN and KES>KHN>KEN>KHC>KEL respectively. The oxalate content of samples KHN, KEN, KES increased with storage time while samples KEL and KHC decreased with storage time. These values were significantly ($P < 0.05$) different from each other. However, there was no difference in the oxalate content of sample KEN between the week zero and week four of samples stored at refrigerated temperature. The result also revealed that samples containing cocoa powder (KEN, KES and KEL) had higher value of oxalate than the samples without cocoa powder (KHN and KHC). This might be due to the fact that cocoa powder contains oxalate content of 650 to 783 mg/ 100 g (USDA, 2011). The preservatives did not affect the oxalate content. Oxalate forms complexes with calcium thereby making it unavailable when consumed and more so high oxalate diets can increase the risk of renal calcium absorption (Osagie and Eka, 1998).

The total tannin contents of the samples ranged from 3.66 to 3.95 mg/100 g, 0.58 to 2.82 mg/100 g and 1.84 to 2.26 mg/100 g for freshly prepared samples and samples after storage for four weeks at ambient and refrigerated temperatures respectively. The addition of cocoa powder increased the tannin content of the drink slightly but the increase in the samples with preservatives was not as high as the one without cocoa powder. The tannin content of the samples reduced with storage time both at ambient and refrigerated temperatures. The results also showed that samples stored at ambient temperature had reduced tannin content when compared with the freshly prepared samples. However, samples containing preservatives (KES and KEL) had lower values of tannin when compared with samples without preservative both for freshly prepared and the stored samples. Tannins have been reported to affect nutritive value of food products by binding the metals such as iron and zinc and reduced the absorption of the nutrient and also form complex with protein thereby inhibiting their digestion and absorption (Obloh *et al.*, 2003).

Table 4: Antinutrient Content of Enriched *Kununzaki* Drink Samples at different Storage Temperatures (Ambient and Refrigerated) and Time

| Sample | KHN 100% | KEN 80/20% | KES 80/20% | KEL 80/20% | KHC 100% |
|------------------|--|---|---|---|---|
| Oxalate(mg/100g) | | | | | |
| WK0 | 0.44±0.01 ^e | 0.66±0.00 ^d | 0.88±0.01 ^c | 1.32±0.12 ^a | 1.1±0.01 ^b |
| WK4(A) | 0.66±0.03 ^d | 1.32±0.01 ^a | 1.1±0.01 ^b | 0.66±0.08 ^d | 0.88±0.02 ^c |
| WK4(R) | 0.68±0.02 ^b | 0.66±0.12 ^b | 2.62±0.00 ^a | 0.44±0.00 ^c | 0.66±0.01 ^b |
| Tanin(mg/100g) | | | | | |
| WK 0 | 3.66±0.01 ^c | 3.87±0.01 ^b | 3.67±0.04 ^c | 3.69±0.13 ^c | 3.95±0.01 ^a |
| WK (A) | 2.82±0.02 ^a | 2.68±0.01 ^b | 2.26±0.06 ^c | 1.98±0.06 ^d | 0.58±0.01 ^e |
| WK(R) | 1.84±0.00 ^c | 2.12±0.05 ^b | 1.84±0.01 ^c | 1.84±0.01 ^c | 2.26±0.01 ^a |
| Saponin(mg/100g) | | | | | |
| WK(0) | 5.54x10 ⁻⁵ ±0.01 ^a | 3.29 x10 ⁻⁵ ±0.06 ^e | 4.94 x10 ⁻⁵ ±0.01 ^c | 3.44 x10 ⁻⁵ ±0.06 ^d | 5.44 x10 ⁻⁵ ±0.11 ^b |
| WK(A) | 1.137 x10 ⁻⁴ ±0.01 ^c | 9.42x10 ⁻⁵ ±0.01 ^d | 1.182x10 ⁻⁴ ±0.01 ^b | 7.18x10 ⁻⁵ ±0.00 ^e | 1.197x10 ⁻⁴ ±0.12 ^a |
| WK 4(R) | 1.017x10 ⁻⁴ ±0.05 ^b | 5.24x10 ⁻⁵ ±0.03 ^d | 5.83x10 ⁻⁵ ±0.00 ^c | 4.34x10 ⁻⁵ ±0.01 ^e | 1.406x10 ⁻⁴ ±0.00 ^a |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same row are not significantly different at 5 % probability level

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *Kununzaki*

WK 0-Week 0, WK 4A- Week 4 at Ambient Temperature, Week 4 at Refrigerated Temperature

The results also showed that samples stored at ambient temperature had reduced tannin content when compared with the freshly prepared samples. However, samples containing preservatives (KES and KEL) had lower values of tannin when compared with samples without preservative both for freshly prepared and the stored samples. Tannins have been reported to affect nutritive value of food products by binding the metals such as iron and zinc and reduced the absorption of the nutrient and also form complex with protein thereby inhibiting their digestion and absorption (Oboh *et al.*, 2003). The total saponin contents of the samples ranged from 3.29×10^{-5} to 5.54×10^{-5} mg/100 g, 7.18×10^{-5} to 1.197×10^{-4} mg/100 g and 4.34×10^{-5} to 1.406×10^{-4} mg/100 g for four weeks at ambient and refrigerated temperatures respectively. Addition of cocoa powder decreased the saponin content of the enriched *Kununzaki* drinks. There was decrease in the saponin level with storage time. The results revealed that saponin contents of the freshly prepared samples were higher than the values in either ambient or refrigerated stored samples. With respect to the preservative used, samples containing preservatives had lower saponin contents than the samples without preservatives. Also, samples without cocoa powder, (KHN and KHC) had higher saponin content than samples containing cocoa powder. The trend observed in this study agreed with the findings of Makinde and Oyeleke, (2012) on the effect of sesame seeds on the anti-nutritional properties of *Kununzaki* enriched with sesame seed flour. Antinutrients have potential in helping to reduce the risk of several deadly diseases in man if they are below the recommended or permitted level in the body (Fagbemi *et al.*, 2005). Saponins have been found to cause haemolytic activity by reacting with sterols of erythrocyte membrane.

Antioxidant Properties of the Enriched *Kununzaki* Drink

DPPH radical scavenging activities

Table 5 shows the result of the DPPH free radical scavenging capabilities of the enriched

Kununzaki samples when freshly prepared and after storage for four weeks at ambient and refrigerated temperatures. For freshly prepared samples at different concentrations, the values ranged from 10 to 69.41(KHN), 14 to 74.41 (KEN), 15 to 78.50 (KES), 15 to 80.02 (KEL), 10 to 69.01 (KHC) %. After storage for four weeks at ambient temperature, the values ranged from 6.20 to 62.01 (KHN), 8.6 to 65 (KEN), 9.25 to 70 (KES), 12.01 to 75.50 (KEL) and 6 to 47.02% (KHC). After storage for four weeks at refrigerated temperature, the values ranged from 10 to 64.04% (KHN), 12.50 to 69.02% (KEN), 12 to 76 % (KES), 13 to 78.01% (KEL), 8 to 67.35% (KHC).

The DPPH free radical scavenging activities of all the extracts were concentration dependent as shown in Table 4.

The free radical scavenging activities as measured by DPPH assay increased with increasing sample concentrations for all the samples from 0.5 to 2.5 mg/ ml. There was increase in the DPPH activity with the addition of cocoa powder, this may be due to the antioxidant activities of cocoa powder. Counnet *et al.* (2006) showed that cocoa exhibits a good antioxidant capacity and that cocoa powder is a potentially rich dietary source of flavonoids. Among the different samples tested, sample KEL exhibited the highest radical scavenging activity value (80% at 2.5 mg/ ml) and sample KHC had the least radical scavenging activity value (69% at 2.5 mg/ ml) when freshly prepared and it follows the same trend after storage. DPPH radical scavenging activity of these extracts showed antioxidant potency when compared with ascorbic acid as shown in the Table 5.

Just like the effect of cocoa powder, samples with preservatives had higher DPPH scavenging abilities than the samples without preservatives. With respect to the storage method, samples stored at refrigerated temperature also exhibited higher free radical scavenging abilities than samples stored at room temperatures.

Table 5: Effect of Storage Temperature and Time on DPPH inhibition (%) of Enriched *Kununzaki* Drink Samples

| Samples/ Concentrations (ml) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
|------------------------------------|--------------------------|----------------------------|--------------------------|---------------------------|--------------------------|
| KHN Wk 0 | 10.00±0.01 ^{cd} | 23.01±0.15 ^{de} | 42.00±0.02 ^{gh} | 62.01±0.03 ^{cd} | 69.41±0.03 ^d |
| Wk 4(A) | 6.20±0.07 ^{ef} | 19.50±0.14 ^{fg} | 33.00±0.01 ^j | 56.01±0.04 ^b | 62.01±0.15 ^f |
| Wk 4(R) | 10.00±0.02 ^{cd} | 21.00±0.03 ^{efg} | 39.01±0.03 ⁱ | 60.02±0.04 ^{de} | 64.04±0.03 ^{ef} |
| KEN Wk 0 | 14.00±0.24 ^{ab} | 28.00±0.02 ^{bc} | 50.01±0.16 ^{cd} | 67.00±0.09 ^{bc} | 74.41±0.19 ^c |
| Wk 4(A) | 8.60±0.04 ^d | 22.50±0.1 ^{def} | 42.50±0.01 ^{gh} | 60.01±0.01 ^{de} | 65.00±0.00 ^{ef} |
| Wk 4(R) | 12.50±0.04 ^b | 25.00±0.01 ^{cd} | 47.00±0.13 ^{ef} | 65.00±0.12 ^{bcd} | 69.02±0.11 ^d |
| KES Wk 0 | 15.00±0.02 ^a | 31.50±0.13 ^a | 53.50±0.13 ^{ab} | 72.01±0.02 ^a | 78.50±0.01 ^{ab} |
| Wk 4(A) | 9.25±0.01 ^d | 26.76±0.01 ^{bc} | 43.00±0.03 ^f | 65.02±0.04 ^{bcd} | 70.00±0.01 ^d |
| Wk 4(R) | 12.00±0.05 ^{bc} | 28.02±0.16 ^{bc} | 50.50±0.06 ^{cd} | 69.31±0.05 ^{ab} | 76.00±0.00 ^{bc} |
| KEL Wk 0 | 15.00±0.01 ^a | 32.01±0.02 ^a | 56.01±0.01 ^a | 73.00±0.01 ^a | 80.02±0.01 ^a |
| Wk 4(A) | 12.01±0.1 ^{bc} | 25.00±0.23 ^{cd} | 49.00±0.02 ^{de} | 67.50±0.00 ^b | 75.50±0.01 ^{bc} |
| Wk 4(R) | 13.00±0.4 ^{ab} | 29.00±0.13 ^{ab} | 52.02±0.17 ^{bc} | 70.10±0.19 ^{ab} | 78.01±0.01 ^{ab} |
| KHC Wk 0 | 10.00±0.04 ^{cd} | 22.02±0.07 ^{defg} | 46.00±0.10 ^f | 60.12±0.07 ^{de} | 69.01±0.07 ^d |
| Wk 4(A) | 6.00±1.0 ^{ef} | 12.50±0.05 ^h | 30.00±0.10 ^j | 40.00±0.01 ^f | 47.02±0.01 ^g |
| Wk 4(R) | 8.00±0.01 ^{de} | 19.00±0.03 ^g | 40.00±0.20 ^{hi} | 57.00±0.01 ^e | 67.35±0.01 ^{de} |
| Ascorbic Acid | 20.29±0.01 ^e | 45.83±0.01 ^d | 62.09±0.01 ^c | 82.62±0.01 ^b | 95.34±0.01 ^a |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same row are not significantly different at 5 % probability level

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *Kununzaki*

WK 0-Week 0, WK 4A- Week 4 at Ambient Temperature, Week 4 at Refrigerated Temperature

Metal Chelating activity

Table 6 shows the result of the metal chelating ability of the enriched *Kununzaki* samples when freshly prepared and after storage for four weeks at ambient and refrigerated temperatures respectively. At the concentration of the samples (6.25, 12.5, 25, 50, 100 mg/ ml), the metal-chelating values for freshly prepared samples ranged from 8.8 to 76.01 (KHN), 8 to

73.01 (KEN), 7.75 to 72.40 (KEN), 7.35 to 69.02 (KEL) and 9.2 to 82.01 (KHC) %. After storage for four weeks at ambient temperature, the values ranged from 7.08 to 50.01 (KHN), 7.2 to 70 (KEN), 6.0 to 63 (KES), 5.8 to 61.50 (KEL) and 8.1 to 70.02% (KHC). For samples after storage for four weeks at refrigerated temperature, the values ranged from 8.08 to 53.28% (KHN), 8.0

to 74.02% (KEN), 7.20 to 68.02 % (KES), 6.8 to 64.9% (KEL), and 9.0 to 79.05% (KHC).

The result showed the ability of the *Kununzaki* drink enriched with cocoa powder to chelate and deactivate transition metals. The ferrous ion-chelating ability of all the samples increased as the concentrations of the samples increased from 6.25 to 100 µg/ ml. The metal chelating ability is such that the samples without cocoa powder had higher values than

samples with cocoa powder at all concentrations between 6.25 and 100 µg/ ml when freshly prepared and when stored at both ambient and refrigerated temperatures. At a concentration of 100 µg/ ml, sample KHC exhibited the highest ferrous ion-chelating ability value (82%) and sample KEL had the least value (69.02%).

Table 6: Effect of Storage Temperature and Time on Metal Chelating Ability (%) of Enriched *Kununzaki* Drink Samples

| Samples/ Concentrations (ml) | 6.25 | 12.5 | 25 | 50 | 100 |
|------------------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
| KHN | | | | | |
| Wk 0 | 8.8±0.01 ^{abc} | 15.50±0.11 ^{ab} | 24.00±0.02 ^{ab} | 50.01±0.03 ^{abc} | 76.01±0.03 ^{abc} |
| Wk 4(A) | 7.08±0.03 ^{de} | 11.00±0.14 ^{cd} | 11.06±0.01 ^f | 35.01±0.04 ^h | 50.01±0.15 ^h |
| Wk 4(R) | 8.08±0.02 ^{bcd} | 11.10±0.03 ^{cd} | 11.62±0.03 ^f | 38.67±0.04 ^{fg} | 53.28±0.03 ^{gh} |
| KEN | | | | | |
| Wk 0 | 8.00±0.24 ^{bcd} | 14.00±0.02 ^{bc} | 22.01±0.16 ^{abc} | 48.01±0.09 ^{cd} | 73.01±0.19 ^{bcd} |
| Wk 4(A) | 7.20±0.04 ^{def} | 13.50±0.11 ^{abc} | 21.05±0.01 ^{bcd} | 43.01±0.01 ^{ef} | 70.00±0.00 ^{cde} |
| Wk4(R) | 8.00±0.04 ^{bcd} | 14.9±0.01 ^{ab} | 23.00±0.13 ^{abc} | 45.00±0.12 ^{de} | 74.02±0.11 ^{abc} |
| KES | | | | | |
| Wk 0 | 7.75±0.02 ^{abcde} | 13.80±0.13 ^{abc} | 22.30±0.13 ^{abc} | 47.80±0.02 ^{bcd} | 72.40±0.01 ^{bcd} |
| Wk 4(A) | 6.00±0.01 ^{fg} | 12.01±0.01 ^{bcd} | 18.01±0.03 ^{de} | 43.02±0.04 ^{ef} | 63.00±0.01 ^{ef} |
| Wk 4(R) | 7.20±0.05 ^{bcdef} | 12.90±0.16 ^{abc} | 20.05±0.06 ^{cd} | 44.00±0.05 ^{de} | 68.02±0.00 ^{cdef} |
| KEL | | | | | |
| Wk 0 | 7.35±0.01 ^{abcdef} | 13.01±0.02 ^{abc} | 20.50±0.01 ^{cd} | 44.00±0.01 ^{de} | 69.02±0.01 ^{cdef} |
| Wk 4(A) | 5.80±0.1 ^g | 09.00±0.23 ^d | 16.02±0.02 ^e | 39.30±0.00 ^{fg} | 61.50±0.03 ^{fg} |
| Wk 4(R) | 6.80±0.04 ^{efg} | 11.00±0.13 ^{cd} | 16.01±0.17 ^e | 42.01±0.19 ^{ef} | 64.9±0.01 ^{def} |
| KHC | | | | | |
| Wk 0 | 9.2±0.04 ^a | 16.50±0.07 ^a | 25.03±0.10 ^a | 54.02±0.07 ^a | 82.01±0.07 ^a |
| Wk 4(A) | 8.10±1.0 ^{bcd} | 12.03±0.05 ^{bcd} | 23.01±0.10 ^{abc} | 46.00±0.01 ^{cde} | 70.02±0.01 ^{cde} |
| Wk 4(R) | 9.00±0.01 ^{ab} | 14.01±0.03 ^{abc} | 24.00±0.20 ^{ab} | 52.00±0.01 ^{ab} | 79.05±0.01 ^{ab} |
| EDTA | 16.00±0.01 ^e | 20.72±0.02 ^d | 30.34±0.02 ^c | 50.67±0.01 ^b | 96.3±0.01 ^a |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same row are not significantly different at 5 % probability level

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *kununzaki*

WK 0-Week 0, WK 4A- Week 4 at Ambient Temperature, Week 4 at Refrigerated Temperature

EDTA: Ethylene diamine tetra-acetate;

Table 7: Effect of Storage Temperature and Time on Ferric Reducing Antioxidant Power (FRAP) (AAE µg/ g) of Enriched Kununzaki Drink Samples

| Sample | KHN | KEN | KES | KEL | KHC |
|---------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Wk 0 | 60.2±0.01 ^b | 60.9±0.11 ^c | 68.1±0.03 ^a | 68.8±0.02 ^a | 58.2±0.00 ^c |
| Wk 4(A) | 42.8±0.01 ^b | 49.1±0.03 ^b | 50.1±0.04 ^a | 51.9±0.02 ^a | 31.9±0.01 ^c |
| Wk 4(R) | 51.0±0.04 ^c | 52.7±0.04 ^c | 61.7±0.11 ^b | 68.0±0.01 ^a | 50.1±0.03 ^c |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same row are not significantly different at 5 % probability level

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC - 100% Commercial *Kununzaki*

WK 0-Week 0, WK 4A- Week 4 at Ambient Temperature, Week 4 at Refrigerated Temperature

The chelating ability of the extract measures how effective the compounds in the sample can compete with ferrozine for ferrous ion. There was no significant difference ($p < 0.05$) in storage temperature of the samples on the metal chelating activity. Sample containing preservative had lower chelating effect than samples without preservatives. The results also showed that refrigerated samples had higher chelating abilities than the samples stored at ambient temperatures. This might be because of low activities in low temperature.

Ferric Reducing Activity Power (FRAP)

The result of the ferric reducing ability power of all the samples is shown in Table 7. The values ranged from 58.2 to 68.8 AAEµg/ g when freshly prepared and after storage for four weeks at ambient and refrigerated temperatures, it ranged from 31.9 to 51.9 AAEµg/ g and 50.1 to 68.0 AAEµg/ g respectively. The ferric reducing ability of all the drinks increased by (1.5 to 14.3%) with the addition of cocoa powder. The result of this work is comparable with the results (72.32 AAEµg/ g) of Elena *et al.* (2007) on the ferric reducing abilities of fibre-rich product from cocoa. The sample containing cocoa powder had higher reducing abilities than the samples without cocoa powder. The results from this study showed that freshly prepared samples had higher reducing abilities than either of the samples stored at ambient or refrigerated temperatures. The ferric reducing ability reduced with storage time at both ambient and refrigerated storage. The refrigerated storage

samples had better ferric reducing abilities than the samples stored at ambient temperature. With respect to the preservatives used, samples with preservatives had better reducing effects than samples without preservatives. The reducing abilities of the samples were all significantly ($p < 0.05$) different for all samples.

Sensory Scores of the Freshly Prepared Preserved Enriched *Kununzaki* Drinks

Table 8 shows the sensory evaluation for the samples as judged by the panelists. The scores for the colour of the samples ranged from 1.4 to 5.0. There was a significant difference ($p > 0.05$) in the colour of the samples. 100% *Kununzaki* samples (KHN) had a score of 1.4 and Commercial *Kununzaki* sample (KHC) had 5.0, this might be because of the difference in the cereal used. Millet was used for sample KHC while the dark red sorghum was used for sample KHN. Enriched samples containing sodium benzoate (KES) and those containing lime and lemon (KEL) had colour scores of 3.4 and 3.0 respectively which suggest that the presence of the preservatives did not improve the product's colour. The samples with cocoa powder blended with the sorghum base *Kununzaki*. Sample KHC (5.0) was mostly preferred in term of colour. This might be because the panelists were used to the whitish colour of the commercial *Kununzaki*. The scores for the taste of the samples ranged from 1.6 to 3.3. The addition of cocoa powder to the drinks reduced the likeness for the taste of the samples.

Table 8: Sensory Scores of the Enriched *Kununzaki* Drink Samples

| Samples | KHN | KEN | KES | KEL | KHC |
|-----------------------|-----------------------|-----------------------|------------------------|------------------------|-----------------------|
| Colour | 1.4±0.32 ^d | 2.4±0.04 ^c | 3.4±0.23 ^b | 3.0±0.02 ^b | 5.0±0.01 ^a |
| Taste | 1.8±0.01 ^b | 3.3±0.01 ^a | 2.6±0.01 ^{ab} | 3.0±0.06 ^{ab} | 1.6±0.01 ^b |
| Flavour | 1.4±0.04 ^b | 3.1±0.03 ^a | 2.6±0.00 ^{ab} | 3.0±0.05 ^a | 2.8±0.01 ^a |
| Texture | 1.8±0.04 ^b | 3.0±0.10 ^a | 3.2±0.14 ^a | 3.0±0.01 ^a | 2.0±0.02 ^b |
| Overall Acceptability | 1.8±0.02 ^b | 3.4±0.05 ^a | 2.8±0.01 ^{ab} | 2.7±0.11 ^{ab} | 1.8±0.01 ^b |

Mean ± standard deviation of triplicate determinations

Mean with the same superscripts in the same row are not significantly different at 5 % probability level

KHN- 100% *Kununzaki* with no preservative

KEN- 80/20% *Kununzaki*/cocoa with no preservative

KES- 80/20% *Kununzaki*/cocoa with sodium benzoate

KEL- 80/20% *Kununzaki*/cocoa with lime and lemon

KHC- 100% Commercial *Kununzaki*

This might be because cocoa powder had a bitter taste. Samples with cocoa powder had poorer scores than samples without cocoa powder. Laboratory 100% samples (KHN) and commercial sample (KHC) are similar in taste because they do not contain cocoa powder. Sample KEL had better score for taste than sample KES. This may be because lemon enhances the taste of food. The presence of preservatives (lime and lemon) improved the taste of the *Kununzaki* drinks.

The scores for the flavour of the samples ranged from 1.4 to 3.1. The addition of cocoa powder to the *Kununzaki* drink improved the flavour of the drinks. The scores were within the acceptable range. Sample KEN was rated higher in taste than other samples.

The presence of lime and lemon improved the flavour of the samples (3.1).

The scores for the mouthfeel of the samples ranged from 1.8 to 3.2. The addition of cocoa powder to the drink affected the mouthfeel of the samples with cocoa powder. There might be poor solubility of the cocoa powder in dilution. Samples KHN and KHC had similar scores. Samples with cocoa had poorer scores than 100% samples. Addition of preservatives did not affect the mouthfeel of the drink (3.0-3.2).

The scores for the overall acceptability of the samples ranged from 1.8 to 3.4. Sample KEN was generally more accepted followed by samples KES and KEL. Addition of preservatives improved the acceptability of the cocoa enriched samples.

4. CONCLUSIONS

The study showed that enrichment of *Kununzaki* with 20% cocoa powder resulted in *Kununzaki* drink with improved nutritional values such that there was increase in the antioxidant values, protein, carbohydrate, fats, pH, TTA and good overall acceptability in terms of the colour, taste, flavor and mouthfeel. The provided information on the suitability of using organic (lime and lemon) preservatives in the *Kununzaki*. Samples containing sodium benzoate were stable up to the fourth week but samples with lime and lemon were stable up to the third week but had better taste than the samples with sodium benzoate.

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