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Antioxidant activities and physicochemical properties of spiced Kunu beverage

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Abstract
This work evaluated the antioxidant activities and physicochemical properties of spiced *kunu-zaki* beverages. The ability of the spices (*xylopia aethiopica, Tetrapleura tetraptera, zingiber officinale*) to scavenge free radical actions in *kunu-zaki* beverages was determined by ABTS, DPPH, FRAP and RP assays. The physicochemical properties (chemical and proximate, minerals, vitamins and sensory properties) were determine by standard methods. The result revealed that addition of spices to *kunu-zaki* beverages significantly ($p<0.05$) increased the proximate values and ranged from ash (0.3-0.42%), crude fat (0.20-0.55%), crude protein (1.50-2.45%), carbohydrate (11.60-13.18%) and TSS (16-18). Similarly, the minerals and vitamins were significantly ($p<0.05$) higher in the spiced beverages than the control and ranged from Ca (7.87-14.19 mg/100g), Mg (28.92-52.67 mg/100g), K (49.65-66.29 mg/100g), Fe (0.41-060 mg/100g) and vitamins B_1 (0.03-0.40 mg/100g), B_2 (0.09-0.25 mg/100g), B_3 (0.12-0.27 mg/100g), respectively. With the exception of RP, addition of spices to *kunu-zaki* beverages showed strong antioxidant activities in ABTS (0.30-0.38 mmol/100mL), DPPH (24.02-49.49%), FRAP (4.11-8.62 GAE/mL) and increased polyphenol (1.42-3.66 GAE/mL) than the control. The sensory acceptability scores showed that the spiced *kunu-zaki* beverages were preferred by the panelist to the control in all parameters tested. However, beverage B (*Tetrapleura tetraptera)* had the best overall results in the pH, TSS, proximate, Fe, Mg, vitamins, total polyphenol, strongest antioxidant (ABTS, DPPH and FRAP) activities and best preferred beverage than others.

Keywords: *Kunu-zaki* beverages, spices, antioxidant activities, physicochemical properties, sensory scores

1. Introduction
Kunu-zaki is a traditional non-alcoholic fermented beverage widely consumed in Nigeria, but most especially among the artisans as breakfast meal, low and middle income persons as a refreshing energy food beverage and at low cost. It is characterized by sweet-sour taste, creamy and of a flowing consistency (Obadina *et al*., 2008) [19]. *Kunu-zaki* is a beverage with a high total solid content. Like several other *Kunu* consumed in Nigeria, *Kunu-zaki* is the most popular and it is can be prepared from cereals such as sorghum, maize, millet, guinea corn or rice. Spices such as ginger, clove, alligator pepper, red pepper and black pepper are usually added as flavour and taste improvers. It is a refreshing drink and can be consumed as complimentary food for infants and as a breakfast meal by adults and children of low income groups. It can also be used to entertain guests in social functions in rural and urban places particularly in Northern and Western regions of Nigeria (Onuorah *et al*., 2005) [22]. It is reported to be rich in nutrients, medicinal and of immense social significance (Akoma *et al*., 2006; Ugwuanyi *et al*., 2015) [7, 27]. It is relatively cheap when compared to carbonated and alcoholic beverages (Adejuyitan *et al*., 2008) [3]. Unfortunately, *Kunu-zaki* has a short storage shelf life of less than 24 h at ambient temperature (Adeyemi and Umar, 1994) [5]. Refrigeration storage after pasteurization or refrigeration with sodium benzoate treatment have used to increase the shelf life and prolong the keeping qualities of *kunu-zaki* beverage (Olasupo *et al*., 2000; Osuntogun and Aboada, 2004) [21, 23]. *Kunu-zaki*, being a product of cereal grain contains some essential nutrients such as carbohydrates, fat, protein, minerals and vitamins.

Millet is a cereal crop that ranks the sixth most important grain in the world and is a major crop in Africa and the Indian subcontinent. It is used in soups, and for making whole grain bread, cakes, breakfast porridge and fermented alcoholic and non-alcoholic beverage like *Kunu-zaki* (Ramachandra *et al*., 1977) [25]. Traditional technology through natural fermentation process is often used by the local people in *Kunu-za*ki production. The predominant fermenting natural flora of *Kunu-zaki* belongs mainly to lactic acid bacteria family (*Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Enterococcus)* (Djè *et al*., 2009) [11].

This non-alcoholic beverage may be regarded as a functional food due to its bioactive compounds contributed mainly by spices added during production. Spices like *xylopia aethiopica, Tetrapleura tetraptera, zingiber officinale*, and *allium sativum* have antioxidant compounds (Ojimelukwe and Ukom, 2017) [20], and when used in *Kunu-zaki* production may not only enhance the nutrient quality and flavour, but also increase the antioxidant potential that act as free radical scavengers to improve physiological health (Ojimelukwe and Ukom, 2017) [20]. The knowledge of dietary antioxidant content in *Kunu-zaki* beverage is quite scarce. Dietary antioxidants are polyphenolic compounds which compliments endogenous antioxidants that play important roles in physiological health through quenching oxidative reactions in the human body. The objective of this work was to produce and evaluate the antioxidant potential, physicochemical properties and sensorial acceptability of a *xylopia aethiopica, Tetrapleura tetraptera* and *zingiber officinale* spiced-millet *kunu-zaki* beverage.

2. Materials and methods

2.1 Ingredients

Pearl millet, potato, sugar, ginger (*Zingiber officinale), Tetrapleura tetraptera), Xylopia aethiopica* and clove were purchased from Ubani market in Umuahia, Abia state. All chemicals were of analytical grades.

2.2 Preparation of *Kunu-zaki* slurry

Kunu-zaki slurry was prepared according to the modified method of Akoma *et al*. (2002) [6]. Five hundred (500) grams of pearl millet was cleansed and soaked in 1000 mL of tap water (1:2, w/v) for 24 h at 30-32 °C. The steeped water was decanted off the grain and thoroughly washed with running tap water. It was then wet milled using local attrition mill with 1000 mL distilled water, 20 grams dried sweet potato chips and 2 grams cloves into a slurry. To the slurry contained in a bowl was added 7.5 grams of ground ginger (*Zingiber officinale*). The same process was repeated with the addition of 7.5 grams of ground *T. tratraptera* and 7.5 grams of ground *Xylopia aethiopica* separately. Each of the *Zingiber officinale, Tetrapleura tratraptera* and *Xylopia aethiopica* spiced slurry was thoroughly mixed with a wooden spatula.

2.3 Production of *Kunu-zaki* beverages

Each spiced (*Z. officinale, T. tratraptera, X. aethiopica*) slurry was divided into two unequal portions (1/3 and 2/3 v/v). To the larger portion (2/3) was added 1.4 L of boiled water at 98 ^{0}C and covered until gelatinized. It was then allowed to cool to 40 °C and added to the un-gelatinized (1/3) portion. The slurry was stirred vigorously for about 2 min and then allowed to ferment for 8-10 h. The fermented *kunu-zaki* was sieved through 350 µm diameter mesh and sweetened with 50 grams of sugar to taste.

Table 1: Formulations for *kunu zaki* beverage production (g)

Sample	Millet	Clove	Potato	Sugar	Ginger	*T.tetraptera*	*X. aethiopica*
A	500	2	20	50	7.5		
B	500	2	20	50		7.5	
C	500	2	20	50			7.5
D	500	2	20	50			

Modified from Akoma *et al*. (2002)

2.4 Determination of proximate composition

The proximate composition of moisture, crude protein (Kjeldhal method, %N x 6.25), crude fat (Soxhlet extraction method), ash (Muffle furnace 550 ^{0}C) and fibre contents were determined by the method of AOAC, (2005) [8]. The carbohydrate content was calculated by difference as the nitrogen free extract: % NFE = 100 - % (crude protein + crude fat + ash + crude fibre + moisture)

2.5 Determination of the mineral content

Mineral content was obtained by the dry ash extraction method described by James (1995) [17]. Five grams of each *kunu-zaki* sample was burnt to ash and was dissolved in 5 mL of dilute 0.1 M HCL acid solution, and made up to 100 mL in volumetric flask. The extracts were used for the specific mineral analysis. Phosphorus content was analyzed by the molybdo-vandate colorimetric method described by James (1995). Calcium and magnesium content was determined by the Versanate EDTA titrimetric method described by Carpenter and Hendricks (2003) [10]. Iron was determined by atomic absorption spectrophotometric method as described by James (1995).

2.6 Determination of vitamin content

Vitamin B_1 (thiamine) content was determined by the method of AOAC (2005), while vitamin (B_2) (riboflavin) and vitamin B_3 (niacin) were determined according to the method of James (1995).

2.7 Detemination of antioxidant activities

2.7.1 Modified methanolic extraction

A modified method of Ukom *et al*. (2014) [28] was used for *kunu-zaki* methanolic extract. Five (0.5) gram of *kunu-zaki* drink was mixed with 5 mL absolute methanol. It was rested for 10 sec., capped and re-mixed in a Vortex mixer for 1min. and then placed on a multi-purpose rotator for 30 min. at 600 rpm. It was centrifuged for 5 mins at 6000 rpm. Two (2) mL of the sample extract was collected and stored in the dark at 4 ^{0}C for use in the determination of total polyphenol, flavonoids and antioxidant activities assayed by ABTS, DPPH, FRAP and RP.

2.7.2 Determination of total polyphenol content (TP)

Total phenolic content of the *kunu-zaki* methanolic extract was determined using Folin-Ciocalteu reagent according to the method of Jagadish *et al*. (2009) [16] with slight modification. Half (0.5) mL of the methanolic extract was added to 10 mL of distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu phenol reagent in a 25 mL volumetric flask. After 5 mins, 2 mL of 2% Na_2CO_3 solution was added to the mixture. The solution was diluted to 25 mL volume with distilled water and allowed to stand for 90 min. Reagent blank using distilled water was prepared instead of the sample. The sample absorbance and blank were measured at780 nm. Gallic acid was used as standard for the calibration curve. Total phenolic content was calculated as mg Gallic acid equivalent per gram fresh weight of sample.

2.7.3 Determination of total flavonoid content (TFC)

The $AlCl_3$ method of Jagadish *et al.* (2009) was used for the determination of the total flavonoid content of the spiced *kunu-zaki* methanolic extract. One and half (1.5) mL of the extract, 5 mL of distilled water and 0.3 mL of 5% $NaNO_2$ were mixed in a 10 mL volumetric flask. After 5 mins, 1.5 mL of 2% methanolic $AlCl_3$ solution was added, followed by 2 mL of 1 mol dm^{-3} NaOH. Then the volume was made up to 10 mL with distilled water and shaken. Reagent blank using distilled water instead of sample was prepared. The absorbance was read at 367nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin. The flavonoid content was expressed as mg quercetin per gram of fresh weight of sample.

2.7.4 Determination of antioxidant activity by ABTS assay

Antioxidant activity of spiced *kunu-zaki* methanolic extract was measured in ABTS assay according to the method described by Seeram *et al*. (2006) [26]. $ABTS^{\cdot+}$ was prepared by adding 80 mg of solid manganese dioxide to a 5 mM aqueous stock solution of ABTS (20 mL of 75 mM Na/K buffer at pH 7). *Kunu-zaki* methanolic extract was diluted in Na/K buffer pH 7 and was mixed with 200 *µL* of ABTS•+ radical cation solution. The absorbance was read at 750 nm after 5 min (Spectrophotometer, Jenway digital). TEAC

values was calculated from Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) standard curve and expressed as Trolox Equivalents in µM.

2.7.5 Determination of antioxidant activity by DPPH assay

The DPPH radical scavenging activity of spiced *kunu-zaki* methanolic extract was determined by the method described by Manzocco *et al.* (1998) [18]. The sample extract (0.2 mL) was diluted with 10 mL methanol and 2 mL DPPH solution (0.5 mM). After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging activity was calculated using the equation given below

% Inhibition of DPPH radical = $(A_0 - A_S) \times 100 / A_0$

Where: A_0 = absorbance of the control reaction containing all reagent except test compound, A_S= absorbance of the test compound

2.7. 6 Determination of antioxidant activity by FRAP assay

Antioxidant activity was determined by FRAP assay according to the method of Benzie and Strain (1999) [9]. Three (3) mL of FRAP reagent was mixed with 100 µL of diluted spiced *kunu-zaki* methanolic extract. The absorbance at 593 nm was recorded after 30 min incubation at 37 ^{0}C. FRAP values was obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+}. The result was expressed as µM of Fe^{2+} equivalents per kg.

2.7.7 Determination of antioxidant activity by Reducing Power assay

This was determined by the method of Oyaizu (1986) [24]. Two and half (2.5) mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 mL of spiced *kunk-zaki* methanolic extract and mixed. The mixture was incubated at 5 ^{0}C for 20 min, after which 2.5 mL of Trichloro acetic acid (10% w/v) was added. The mixture was centrifuged at 3000 rpm for 10 min, and 2.5 mL of the upper solution was collected and mixed with 2.5 mL distilled water plus 0.5 mL $FeCl_3$ (0.1%, w/v). The absorbance of the test sample and blank was measured at 700 nm.

2.8 Sensory acceptability scores of spiced kunu-zaki beverages

Fresh *kunu-zaki* beverages were subjected to sensory acceptability for taste, aroma, texture, appearance and overall acceptability using a 9-point hedonic scale, with 9 as like extremely and 1 as dislike extremely (Iwe, 2002) [15]. Twenty panelists familiar with *kunu-zaki* beverage were involved in the assessment. The panelists rinsed their mouth with water after tasting each *kunu-zaki* sample.

2.9 Statistical analysis

Data obtained from duplicate samples were subjected to analysis of variance (ANOVA) and mean differences separated using Duncan's Multiple Range Test (DMRT).

3. Results and discussion

3.1 Chemical properties of spiced kunu-zaki beverages

Table 2 shows the result of the chemical properties of the spiced *kunu-zaki* beverages. There were significant ($p<0.05$) variations in the results obtained. For the chemical properties, the pH value ranged from 4.00 – 4.25. The result shows that the *kunu-zaki* beverage spiced with ginger (A) had highest pH, followed by the un-spiced (control). *Kunu-zaki* beverages spiced with *T. tetraptera* and *X. aethiopica* showed lower pH values (4.0 and 4.05). At these pH values, low temperature preservation at 24 h can be achieved. The result compared with the pH range (2.0-4.15) reported by Abiodun *et al.* (2017) [1] for *kunu zaki* sweetened with Black *velvet tamarind* pulp, but were lower than 4.70 – 5.75 reported by Akoma *et al.* (2006) [7]. The total titratable acidity of the spiced kunu-zaki ranged from 0.65 – 0.73 % and this result compares with the report of Abiodun *et al*. (2017). These results demonstrate an inverse relationship with pH values. Lactic acid production by microorganisms in the natural fermentation of *kunu-zaki* slurry was responsible for this levels of acidity. The total soluble solid ranged from 16.00 – 18.00 ^{0}Brix. The result shows that the spiced *kunu-zaki* beverages were not significant ($p>0.05$), but were higher than the un-spiced (16 ^{0}Brix). This implies that each spice increased the total soluble solid content of their various *kunu-zaki* beverage. Overall, the TSS was higher than 4.40 – 7.85 ^{0}Brix reported by Abiodum *et al*. (2017) [1].

Table 2: Chemical properties of spiced *kunu-zaki* beverages

***Kunu-zaki* Beverages**	**pH**	**TTA**	**TSS**
A	$4.20^{a}\pm0.00$	$0.65^{b}\pm0.34$	$18.00^{a}\pm0.00$
B	$4.05^{bc}\pm0.71$	$0.71^{a}\pm0.06$	$17.50^{a}\pm0.71$
C	$4.00^{c}\pm0.71$	$0.73^{a}\pm0.06$	$17.00^{a}\pm0.00$
D	$4.15^{ab}\pm0.00$	$0.70^{ab}\pm0.06$	$16.00^{b}\pm0.00$

Values are mean ± SD. Values on the same column with different superscripts are significantly different *($p < 0.05$)*. Keys: A (*Kunu zaki* spiced with Ginger), B (*Kunu zaki* spiced with *T. tetraptera)*, C (*Kunu zaki* spiced with *X. aethiopica*) and D (*Kunu zaki* without spice: Control).

3.2 Proximate composition of spiced kunu-zaki beverage

The proximate content of spiced *kunu-zaki* beverage is presented in Table 3. The result shows significant ($p<.05$) variations in all the parameters analyzed. The moisture content ranged from 83.53 – 86.81 % with the un-spiced (D) showing the highest value, followed by beverage C, while beverage B was the least. Similar moisture range of 82 – 85 % was reported for *kunu-zaki* made from rice and acha blend (Egwin *et al*., 2009) [12].

The ash content of the *kunu-zaki* beverage ranged from 0.30 – 0.42 %. The results show that the ash content was enhanced by the added spices, especially with *X. aethiopica*. Values obtained in this study agreed with the result reported by Egwin *et al*. (2009).

The fat content of the *kunu-zaki* beverages ranged from 0.20 - 0.55 %. *Kunu-zaki* spiced with *T. tetraptera* had the highest value (0.55%), followed by *kunu-zaki* spiced by *X. aethiopica* (0.46%). The fat content of ginger spiced *kunu-zaki* and the un-spiced were not statistically ($p>0.05$) different. Uyoh *et al*. (2013) [29] reported that *T. tetraptera* has a high fat content and this was confirmed in our study. The protein content of the *kunu-zaki* beverage was enhanced by spice addition. Crude protein values ranged from 1.50 – 2.45 % with *T. tetraptera* spiced *kunu-zaki* (B) having the highest crude protein, while the un-spiced (D) had the least value. These values are comparable to the crude protein content of 0.4 – 1.98 % in *kunu-zaki* reported by Essien *et*

al. (2009) [14], but lower than that of 2.47 – 3.98 % reported by Adelekan *et al.* (2013) [4]. This difference may be due to the cereal type used in the *kunu-zaki* production.

Table 3: Proximate composition of the spiced *Kunu zaki* beverage (%)

Kunu-zaki beverages	Moisture	Ash	Fat	Protein	Carbohydrate
A	84.30^{c}±0.14	0.33^{c}±0.01	0.24^{c}±0.00	1.95^{b}±0.14	13.18^{a}±0.14
B	83.53^{d}±0.25	0.37^{b}±0.01	0.55^{a}±0.42	2.45^{a}±0.00	13.11^{a}±0.20
C	85.01^{b}±0.00	0.42^{a}±0.00	0.46^{b}±0.00	1.68^{c}±0.11	12.44^{b}±0.11
D	86.81^{a}±1.14	0.30^{d}±0.00	0.20^{c}±0.00	1.50^{c}±0.00	11.60^{c}±0.28

Values are mean ± SD. Values on the same column with different superscripts are significantly different *(p < 0.05)*. Keys: A (*Kunu zaki* spiced with Ginger), B (*Kunu zaki* spiced with *T. tetraptera)*, C (*Kunu zaki* spiced with *X. aethiopica*) and D (*Kunu zaki* without spice: Control).

The carbohydrate content of the *kunu-zaki* beverage ranged from 11.60 – 13.78 %. The result shows that the un-spiced (D) had the least carbohydrate content (11.60%) when compared to the spiced samples. The ginger spiced beverage (A) had the highest value, followed by *T. tetraptera* spiced beverage (B). The carbohydrate values in this study falls within the range obtained by Essien *et al* (2009) [14] and Adelekan *et al*. (2013) [4]. Our result show that spice addition to *kunu-zaki* production increased the overall nutrient content.

3.3 Mineral content of spiced kunu-zaki beverages

Table 4 shows the result of the mineral contents of the *kunu-zaki* beverages. The results indicates significant ($p<0.05$) differences in the mineral contents. Addition of spices to the millet *kunu-zaki* increased the mineral contents significantly ($p<0.05$) especially with *X. aethiopica* (C) and *T. tetraptera* (B). The results show that calcium content was highest in *X. aethiopica* spiced *kunu-zaki* (14.19 mg/100g), followed by *T. tetraptera* sample (11.46 mg/100g) and the least was the un-spiced beverage (7.87 mg/100g). *X. aethiopica* has high calcium content (328 mg/100g) as reported by Abolaji *et al.* (2007) [2]. Lower value of 0.88 – 11.55 mg/100g was reported for *kunu zaki* by Adelekan *et al*. (2013) [4].

The magnesium content ranged from 28.92 – 52.67 mg/100g. The result shows that *T. tetraptera* spiced beverage (B) had the highest value indicating that *T. tetraptra* was a rich source of magnesium.

The potassium content of the beverages ranged from 49.65 – 66.29 mg/100g. *Kunu-zaki* (C) spiced with *X. aethiopica* had the highest value followed by beverage B (*T. tetraptera*), while the un-spiced had the least. Uyoh *et al*. (2013) [29] reported that *T. tetraptera* contain higher potassium content (240 – 270 mg/100g).

Table 4: Mineral content of the spiced *Kunu zaki* beverages (mg/100g)

Kunu-zaki beverages	Calcium	Magnesium	Potassium	Iron
A	8.36^{c}±0.13	42.29^{b}±1.70	56.69^{c}±0.28	0.49^{b}±0.00
B	11.46^{b}±0.37	52.67^{a}±0.44	60.00^{b}±0.74	0.60^{a}±0.04
C	14.19^{a}±0.80	31.92^{c}±0.66	66.29^{a}±0.00	0.52^{b}±0.00
D	7.87^{d}±0.25	28.92^{d}±0.36	49.65^{d}±0.00	0.41^{c}±0.28

Values are mean ± SD. Values on the same column with different superscripts are significantly different ($p < 0.05$). Keys: A (*Kunu zaki* spiced with Ginger), B (*Kunu zaki* spiced with *T. tetraptera*), C (*Kunu zaki* spiced with *X. aethiopica*) and D (*Kunu zaki* without spice, control).

The iron content of the *kunu-zaki* beverage ranged from 0.41 – 0.60 mg/100g. *Kunu-zaki* (B) spiced with *T. tetraptera* had the highest value, followed by beverage C (*X. aethiopica*) and A (ginger), while D (un-spiced) had the least. Uyoh *et al*. (2013) and Adelekan *et al.* (2013) reported that *T. tetraptera* contain high amount of iron which directly contributed to the high iron content of beverage B when compared to others. The *kunu zaki* produced in this study can supply appreciable amount of minerals for various metabolic functions (Emmanuel-Ikpeme *et al*., 2012) [13]. Weaver and Heaney (2006) [30] reported that calcium is a micronutrient essential to health and well-being, which performs diverse biological functions in the human body. The Food and Nutrition Board (FNB, 1980) recommends a dietary allowance of 360 mg and 1200 mg calcium for infants and young adults. Deficiency of calcium can lead to ricket in children. Magnesium is essential to health because it helps to maintain normal muscle and nerve function, keeps heart rhythm steady and supports healthy immune system. Potassium reduces high blood pressure (Emmanuel-Ikpeme *et al*., 2012). Iron deficiency anemia is very common around the world, especially in women and children in developing Nations. The high mineral concentration in spiced kunu zaki shows the importance of spices addition to *kunu-zaki* beverage. Depending on the type of cereal employed in *kunu-zaki* production, the mineral concentration may vary.

3.4 Vitamin content of spiced kunu-zaki beverages

The result of the vitamin content of *kunu-zaki* beverages is presented in Table 5. The results show significant variations ($p<0.05$) with vitamin B_1 ranging from 0.03 – 0.40 mg/100g. *Kunu-zaki* A (ginger) had the highest value, followed by B (*T. tetraptera*) and C (*X. aethiopica*), while the un-spiced had the least. The higher value obtained in sample A suggests that ginger contains B_1 vitamin more than other spices. Vitamin B_2 content of the *kunu-zaki* beverages ranged from 0.09 – 0.25 mg/100g with the un-spiced (D) beverage showing the lowest value. *Kunu-zaki* beverage B (*T. tetraptera* spiced) had the highest value, followed by C (*X. aethiopica*) and A (ginger). Vitamin B_3 content of the *kunu-zaki* beverages ranged from 0.12-0.27 mg/100g. The result showed that beverage B (*T. tetraptera* spiced) had the highest value of B_3, followed by beverage C and A, while the un-spiced had the least. The addition of spices to *kunu-zaki* beverage increased both the vitamin and

mineral contents. This implies nutrient value addition to *kunu-zaki* beverage.

Table 5: Vitamin Composition of the spiced Kunu-zaki beverages (mg/100g)

Kunu-zaki beverages	Vitamin B_1	Vitamin B_2	Vitamin B_3
A	0.40^{c}±0.00	0.12^{c}±0.00	0.15^{c}±0.01
B	0.16^{a}±0.01	0.25^{a}±0.00	0.27^{a}±0.04
C	0.08^{b}±0.00	0.18^{b}±0.01	0.21^{b}±0.28
D	0.03^{c}±0.01	0.09^{d}±0.01	0.12^{c}±0.00

Values are mean ± SD. Values on the same column with different superscripts are significantly different ($p < 0.05$). Keys: A (*Kunu zaki* spiced with Ginger), B (*Kunu zaki* spiced with *T. tetraptera*), C (*Kunu zaki* spiced with *X. aethiopica*) and *Kunu zaki* without spice.

3.5 Antioxidant activities of spiced kunu-zaki beverages

Table 6 shows the result of the antioxidant potential of the *kunu-zaki* beverages. There were significant ($p<0.05$) variations in antioxidant potentials of the *kunu-zaki* beverages. The result showed that the total polyphenol content ranged from 1.42 – 3.66 mgGAE/mL. *T. tetraptera* spiced *kunu-zaki* beverage (B) had the highest polyphenol content (3.66 mgGAE/mL), followed by ginger spiced *kunu-zaki* (2.87 GAE/mL) and *X. aethiopica* spiced *kunu-zaki* beverage (2.24 GAE/mL), while the un-spiced (D) was the least (1.42 GAE/mL). The spiced *kunu-zaki* beverages had higher polyphenol content than the un-spiced, an indication that the spices contributed to high polyphenolic content of the beverages. The flavonoids values ranged from 0.42 – 0.86 mgQE/mL with the un-spiced (control) having higher flavonoids content than the spiced beverages.

For antioxidant activity assays, ferric reducing power (FRAP) ranged from 4.11 – 8.22 mgGAE/mL. The strongest FRAP activity was obtained in beverage B (*T. tetraptera*), followed by A (ginger) and C (*X. aethiopica*), while the least was the un-spiced *kunu-zaki* beverage (4.11 mgGAE/mL). The *kunu-zaki* beverage assayed by ABTS ranged from 0.30 – 0.38 mmol/100 mL. *Kunu-zaki* spiced with *T. tetraptera* (B) had the highest value (0.38 mmol/100mL), while beverages A (ginger), C (*X. aethiopica*) and un-spiced (control) were not significantly ($p>0.05$) different. The result confirms the report of Ojimelukwe and Ukom (2017) [20] that spices are good sources of antioxidant. The percentage DPPH inhibition ranged from 24.02- 49.64% and was highest in *kunu-zaki* B (*T. tetraptera*) (49.46%), followed by *kunu-zaki* beverage A (ginger) (43.19%) and C (*X. aethiopica*) (24.02%), respectively. It was obvious that % DPPH inhibition significantly ($p<0.05$) increased with the addition of the spices, indicating a strong antioxidant activity. The reducing power of *kunu zaki* beverages ranged from 0.51 – 0.68 mgQE/g. A high reducing ability was obtained in the un-spiced *kunu-zaki* beverage, followed by ginger spiced (A) and lastly *T. tetraptera* spiced (B) and *X. aethiopica* spiced (C) beverages, respectively. Millets contain phytic acid, tannins, and phenols which can contribute to antioxidant activity. Ojimelukwe and Ukom (2017) reported that spice extracts of *Terapluera tetraptera* and *Xylopia aethiopica* have high content of polyphenolic compounds and strong antioxidant activities. These are indicative of the high polyphenolic compounds and strong antioxidant activities of the spices ability to scavenge free radical action in the body as result of *kunu-zaki* beverage consumption.

Table 6: Antioxidant potentials of spiced *Kunu zaki* beverage

Kunu-zaki beverages	Polyphenol (mg/GAE/mL)	Flavonoid mgQE/mL)	FRAP (GAE/mL)	ABTS (mmol/100 mL)	DPPH (%)	R. power (mgQE/g)
A	2.87^{b}±0.02	0.49^{c}±0.00	8.01^{b}±0.01	0.31^{b}±0.01	43.16^{b}±0.28	0.63^{b}±0.00
B	3.66^{a}±0.02	0.42^{c}±0.01	8.62^{a}±0.02	0.38^{a}±0.00	49.64^{a}±0.03	0.57^{c}±0.01
C	2.24^{c}±0.02	0.64^{b}±0.05	5.31^{c}±0.00	0.31^{b}±0.00	37.27^{c}±0.04	0.51^{d}±0.00
D	1.42^{d}±0.01	0.86^{a}±0.00	4.11^{d}±0.02	0.30^{b}±0.00	24.02^{d}±0.01	0.68^{a}±0.02

Values are mean ± SD. Values on the same column with different superscripts are significantly different ($p < 0.05$). Keys: A (*Kunu zaki* spiced with Ginger), B (*Kunu zaki* spiced with *T. tetraptera*), C (*Kunu zaki* spiced with *Xylopia aethiopica*), D (*Kunu zaki* without spice: control)

3.6 Sensory evaluation of spiced kunu-zaki beverages

The result of the sensory evaluation is presented in Table 7. The overall acceptability score ranged from 60 – 70%. The spiced *kunu-zaki* beverage maintained the best acceptability than the un-spiced (control). The *kunu-zaki* beverage B (*T.tetraptera*) scored 70%, followed by beverage A (ginger) (68%) and *kunu-zaki* C (*X. aethiopica*) (64%) were more preferred by the panelists than the un-spiced (60%). The different *kunu-zaki* beverages scored above 50% in a 9-point hedonic scale which make them a good nourishing drink judging from their taste, appearance, flavor and texture. The scores for appearance and taste show that beverage B had the highest acceptability, followed by beverage A. The *kunu-zaki* spiced with *T. tetraptera* was most preferred by the panelists followed by the *kunu-zaki* spiced with ginger. On the other hand, texture score show that *kunu-zaki* spiced with ginger was more preferred than the other spiced beverages

Table 7: Sensory Acceptability scores of spiced *kunu zaki* beverage

Kunu-zaki beverages	Taste	Appearance	Flavour	Texture	Acceptability
A	6.80^{a}±0.76	6.60^{ab}±0.65	6.40^{b}±0.99	7.00^{a}±1.22	6.80^{ab}±0.93
B	6.60^{b}±0.98	6.80^{a}±0.78	7.00^{a}±0.75	6.40^{b}±1.33	7.00^{a}±1.33
C	6.20^{c}±0.67	5.60^{d}±0.98	6.20^{c}±0.67	6.80^{a}±0.85	6.40^{c}±1.34
D	6.00^{d}±0.88	6.00^{c}±0.79	5.60^{d}±1.54	6.20^{c}±0.85	6.00^{d}±1.44

Values are mean ± SD. Values on the same column with different superscript are significantly different ($p < 0.05$). Keys: A (*Kunu zaki* spiced with Ginger), B *(Kunu zaki* spiced with *T. tetraptera*), C (*Kunu zaki* spiced with *X.aethiopica*), D (*Kunu zaki* without spice: control)

4. Conclusion

The result obtained from this study show that the proximate composition, minerals, vitamins and antioxidant activities of the *kunu-zaki* beverage increased with the addition of spices (ginger, *T. tetraptera* and *X. aethiopica*). *X. aethiopica* increased the ash, calcium and potassium contents of the kunu-zaki beverages. *T. tetraptera* increased the fat, protein, magnesium, iron, vitamin B_2 and B_3 contents of the *kunu-zaki* beverages, while ginger increased the carbohydrate and vitamin B_1 contents of the *kunu-zaki* beverages. These results indicate that the spiced millet *kunu-zaki* beverages have high antioxidant compounds and possessed strong antioxidant activities when compared to the un-spiced (control) sample. The *kunu-zaki* beverages show that the sensory scores were accepted by the panelists, mostly the spiced once and *T. tetraptera* beverage was adjudged the best in overall acceptability.

5. References

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