

**Proximate, Physicochemical and Sensory Properties of Millet (*Pennisetum glaucum*)-Based Kunun-zaki with Groundnut (*Arachis hypogaea*) Inclusion**Towobola Michael^{1, 2*}, Stephen O. Owa², Ayoyinka O. Olojede², James A. Ndako², Emmanuel O. Oludipe², Ewhoritsemogha P. Dottie³, Remilekun M. Thomas²¹Department of Biological Sciences, Federal Polytechnic, Bida, Niger State, Nigeria²Department of Microbiology, Landmark University, Omu-Aran, Kwara State, Nigeria³Department of Agricultural Engineering, Landmark University, Omu-Aran, Kwara State, Nigeria

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ABSTRACT

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Kunun-zaki is a cereal-based fermented refreshing drink popular in the Northern region of Nigeria and has become accepted in other parts of the country. Granted is the fact that protein is deficient in fermented cereal beverages. In an attempt to find solution to this problem, this study investigated the upshot of groundnut inclusion in *kunun-zaki* from millet so as to confirm if the inclusion will increase protein content in the millet-based beverage. Groundnut-fortified *kunun-zaki* (GFK) was prepared at different ratios of millet and groundnut blends. Physicochemical properties were determined. Microbial counts and sensory qualities of the *kunun-zaki* were also evaluated for safety and acceptability. Results showed that addition of groundnut to *kunun-zaki* increased the protein composition significantly ($p < 0.05$) from 1.51 to 5.45%, with 50% millet + 50% groundnut blend (50MT:50GT) having the highest concentration. Groundnut inclusion increased ash and crude fat contents but decreased crude fiber content. The turbidity and pH varied between 60.0-145.1 and 5.2-5.4, respectively. There was no significant difference in the overall consumer's acceptability of the GFK samples compared to the control, however, in appearance, there were significant differences with 50MT:50GT, having the highest score of 7.03 out of 9.0 points. After production, 60MT:40GT sample had the highest lactic acid bacteria and least total aerobic bacteria counts. Coliform bacteria were absent in all the samples. The nutritional quality of *kunun-zaki* was improved with groundnut inclusion and the product had high consumer acceptability, showing that the GFK could be a preferred drink for alleviating protein energy malnutrition.

Keywords: *Kunun-zaki*, Fermented beverage, Groundnut fortification, Millet, Protein malnutrition.

Introduction

Functional foods have attracted a lot of attention in recent years, and researchers continue to investigate how to turn food matrices like cereals, vegetables, and fruits into medicine-like products.¹ Legume-based, cereal-based, coffee, dairy, fruit and vegetable-based and tea are all examples of functional beverages. These beverages' functional characteristics address a variety of situations, including boosting energy, combating the aging process, weariness, and stress.^{2,3}

Kunun-zaki is a sweet and sour taste non-alcoholic fermented beverage made from cereals; it is an indigenous drink in northern Nigeria but is now widely taken in other parts of the country.^{4,5} There are several variations in the product based on the cereal used for its production. Cereals commonly used are millet, sorghum, maize, and guinea corn, which makes up the *kunun-zaki* variant.^{6,7} The processing involves steeping, milling, sieving, cooking and fermentation. The *kunun-zaki* beverage is spiced with mix of ginger, cloves and black pepper, depending on preference as flavor and taste enhancers.

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It holds immense social, economic and medicinal benefits with high carbohydrate content. *Kunun-zaki* is more affordable compared to commercial carbonated beverages, and taken by people of diverse ages, especially as a weaning meal for children in rural and urban areas. Therefore, the need to explore opportunities to enrich the millet based beverage particularly the protein contents.⁸

Groundnut (*Arachis hypogaea*) is a legume and oil seed, rich in protein and also good source of the A, D, E and K vitamins.⁹ It could be snacked on or converted into different product eaten along with several carbohydrate foods such as the Cassava flask known as "*Garri*" in Nigeria. This wide acceptance makes groundnut a good candidate for inclusion into the *kunun-zaki* drink and improves the nutritional value of the cereal beverage.

Due to the high demand for foods that provide health advantages with minimal risk to human health, the development of unique functional beverages is a rising sector in the food industry. This research is aimed at investigating the proximate, physicochemical and sensory characteristics of *kunun-zaki* with different ratio of groundnut blend to obtain a protein-enriched fermented cereal beverage.

Materials and Methods*Preparation of materials*

Grains of pear millet (*Pennisetum glaucum*) was purchased in November, 2020 and groundnut (*Arachis hypogaea*) was purchased in August, 2020 and identified as SAMNUT 23 (ICCGV-1596894) developed by ICRISAT Kano and IAR Samaru, Zaria. The grains were transported to Microbiology Laboratory, Landmark University and refrigerated until used. All bench work was carried out in an

aseptic condition, at the Department of Food Science and Microbiology Laboratory, Landmark University (Nigeria).

Kunun-zaki production from millet and groundnut blends

Grains of millet (8.15 kg) and 6.60 kg of groundnut were sorted to remove dirt and pebbles. Blends of the millet and groundnut were prepared by mixing in ratios of 9:1, 8:2, 7:3, 6:4, 5:5 and 10:0 (w: w) accordingly. The blends were washed and steeped in 1 L of water in plastic buckets with lid for 24 hours for primary fermentation and the steeping water were decanted. Then, the grains were washed and wet-milled using a kitchen grinder (Marlex Excella, Daman, India) with added spices (ginger 20 g, clove 5 g, African black pepper 5 g). The slurry was divided into two parts (4:1). The larger part (80% of the bulk) was heated by adding 2.5-3 L of water at 100°C and allowed to cool to a temperature of about 45-50°C; the unheated slurry was then added to the cooked portion and mixed thoroughly. The pooled slurry was left to ferment for 8 hours (secondary fermentation). The slurry was then sieved, bottled and refrigerated for analysis,^{10,6} with slight modifications.

Proximate analysis

The moisture content, crude protein, crude fat, ash contents and total carbohydrate of the “*kunun-zaki*” enriched with different ratio of groundnut blend samples were determined by standard methods as described by AOAC.¹¹ All analysis were carried out in duplicates.

Determination of the physicochemical properties

Tests were carried out for pH, alkalinity, turbidity, total hardness and colour for “*kunun-zaki*”. The test for mineral elements were determined using the Palintest photometer (ELE International UK). Association of Analytical Chemists (AOAC),¹¹ guidelines were used in determining titratable acidity; 20 mL of the “*kunun-zaki*” samples were titrated against 0.1 N NaOH, using 3 drops of phenolphthalein as indicator, and expressed in degrees. One degree (1°) of acidity corresponds to 1 mL of 1 M NaOH required to neutralize the organic acids present in 100 mL of sample. The pH values of *kunun-zaki* were measured using a pH electrode (PP-15; Sartorius, Gottingen, Germany). The rate of acidification - change in pH was observed and recorded (hours), the total titratable acidity (TTA) was calculated using the formula:

$$TTA = \frac{\text{Volume of NaOH}}{\text{Volume of Sample}} \times 0.9$$

The alkalinity, colour, total hardness, concentrations of potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and iron (Fe) in mg/L and turbidity in FTU, of the *kunun-zaki* produced were determined using Palintest Multi-Parameter Photometer (ELE International UK). A 0.5 mL of the sample was thoroughly mixed with 9.5 mL of distilled in Palintest tube and inserted into the Palintest photometer. The photometer displayed the concentrations of the physicochemical parameters and the minerals elements which were then recorded (modified method of Adekola *et al.*)¹²

Sensory evaluation

The organoleptic properties of the different blends of *kunun-zaki* were assessed by thirty-two trained panelists, for appearance, colour, taste, flavour, viscosity and overall acceptability. The assessment was done at random and under privacy, to avoid bias from the panelist. A 9-point hedonic scale was used to grade each preference; from 9 = “extremely liked” to 1 = “extremely disliked”.¹⁵

Microbiological analysis

A ten-fold serial dilution was made from each bottled *kunun-zaki* stock; 1 mL of the *kunun-zaki* sample up to 10 mL with sterile distilled water in a test tube to obtain a dilution of 10.¹ The procedure was repeated till the 10⁵ dilution. The microbiological analysis was performed on Nutrient agar (Oxoid UK, CM 0003) for total viable bacteria, Man Rogosa Sharpe agar (HIMEDIA) for the isolation of lactic acid bacteria, MacConkey agar (Oxoid, UK) for total coliform count and Potatoes dextrose agar (Oxoid, UK) with addition of chloramphenicol (500mg/500mL) for total yeast count. All media were prepared according to the manufacturers’ instructions and sterilized in an autoclave at 121°C for 15 minutes. The pour plate technique was employed in inoculation using 1 mL of 10⁵ dilutions of the *kunun-zaki* samples with sterile molten media at 45°C. The Petri dishes were allowed to set, placed in an inverted position and incubated aerobically at 37°C for 24hour for aerobic bacteria, while, MRS plates were incubated anaerobically at 35°C for 48hour, PDA plates were incubated aerobically at 25°C for 120hours. The Characteristics observed colonies were counted, calculated and expressed as logarithm colony forming unit per millimeter (Log cfu/mL).¹⁴

Statistical analysis

Analysis of Variance (ANOVA) was used to analyze the sensory data, mineral analysis, proximate analysis, total titratable acid (TTA) and pH of the groundnut inclusion millet based *kunun-zaki*. Data was expressed as mean and standard deviation (SD). Significant differences were determined using the Duncan multiple range test (p<0.05) for comparison of means using Statistical Package for Social Sciences (SPSS), version 15.0.¹⁵

Results and Discussion

The proximate composition of *kunun-zaki* produced from the blend of millet with groundnut inclusion is presented in Table 1. The crude protein was in the range of 3.05 and 5.50% which was significantly different (p<0.05) and higher than that obtained in the control sample (100MT). Crude protein and crude fat generally increased along with increasing groundnut inclusion, while crude fiber and carbohydrate generally increased along increasing inclusion of millet. Thus, energy is highest with higher millet inclusion. The protein contents obtained in this work were comparable to the findings of Aderinola T and Oluwamukomi¹⁶ and Verni *et al.*,¹⁷ who reported that the presence of protein and dietary fiber in a food makes it functional food, hence the millet groundnut *kunun-zaki* can be a functional food beverage.

Table 1: Proximate composition of *Kunun-zaki* from different blend of millet and groundnut at different ratios

Parameter (%)	100%MT	90MT:10GT	80MT:20GT	70MT:30GT	60MT:40GT	50MT:50GT
Moisture	85.14±0.36 ^b	84.44±0.08 ^a	84.59±0.43 ^{ab}	84.38±0.21 ^a	84.64±0.1 ^{ab}	84.73±0.08 ^{ab}
Ash	0.28±0.007 ^a	0.25±0.03 ^a	0.30±0.02 ^a	0.32±0.01 ^a	0.34±0.09 ^a	0.24±0.12 ^a
Crude fat	0.39±0.00 ^a	0.53±0.2 ^b	0.63±0.04 ^c	0.72±0.1 ^d	0.74±0.02 ^d	0.87±0.06 ^e
Crude protein	1.50±0.07 ^a	3.05±0.07 ^b	3.80±0.01 ^c	4.81±0.13 ^d	5.05±0.07 ^e	5.50±0.15 ^f
Crude fiber	0.06±0.00 ^d	0.05±0.01 ^c	0.03±0.00 ^b	0.03±0.00 ^b	0.03±0.00 ^b	0.02±0.00 ^a
Carbohydrate	14.65±0.46 ^d	12.22±0.17 ^c	11.12±0.47 ^b	9.30±0.01 ^a	9.58±0.13 ^a	9.05±0.08 ^a
Energy Value (kcal/g)	68.71±0.71 ^d	60.66±1.63 ^a	63.10±0.35 ^b	64.22±0.15 ^{bc}	65.26±0.40 ^c	64.13±0.34 ^{ab}

Data are means of two independent experiments ± standard deviation (n=2); 90MT:10GT: 90% millet/10% groundnut; 80MT:20GT: 80% millet/20% groundnut, 70MT:30GT: 70% millet/ 30% groundnut, 60MT:40GT: 60% millet/40% groundnut, 50MT:50GT: 50% millet/50% groundnut and 100%MT: (control).

There was increase in the alkalinity, turbidity, colour and total hardness readings in sample 100% MT (control) and significantly different ($p < 0.05$) from other samples with groundnut inclusion (Table 2). The pH showed an acidic range of 4.8 to 5.3 for all samples, the control and sample 70MT: 30GT significantly different ($p < 0.05$) with pH reading of 5.2. Total titratable acidity increased with increase in groundnut ratio from 0.26 in the control sample (100MT) to 0.32 in sample 50MT:50GT (highest groundnut inclusion). The observed decrease in pH readings of the samples is parallel to increases in the titratable acidity readings in the *kunun-zaki* samples produced in this work which agrees with the findings of Ratau.¹⁸ However, the total titratable acidity was highest in the sample with 50MT:50GT millet/groundnut ratio while, it was least in the control, this owing to the inclusion of groundnut in the *kunun-zaki*. High pH was reported by Oyedo *et al.*,¹⁹ in fermentation of peanut and cowpea by *Lactobacillus spp.* This is as a result of inclusion of groundnut in

the *kunun-zaki* because groundnut is fermented at pH range of 4.5-6.5, groundnut fermentation dragged the *kunun-zaki* pH to the values obtained in this work which is also comparable with the findings of Ekwere.²⁰

Table 3 shows the mineral composition of *kunun-zaki* from different blends of millet and groundnut. The control (100% MT) has K, Ca, Mg, P and Fe contents of 3.60, 70.40, 38.10, 70.60 and 4.0 mg/L, respectively, which represent the highest values for each of the elements. While sample 50MT:50GT (highest groundnut inclusion) has the lowest values of 2.65, 63.50, 18.0, 33.65 and 2.08 mg/L, respectively. From the result of the mineral compositions, it can be deduced that the level of each cation in the beverage correlates with the percentage of millet in the blend. This suggests that millet, the dominant component of these blends, has a higher content of the cations. As reported.^{21,22}

Table 2: Physicochemical parameters of *kunun-zaki* from different blend of millet and groundnut at different ratios

Millet to Groundnut ratio	Alkalinity (mg/l)	Turbidity (FTU)	Colour (mg/l)	Total hardness (mg/l)	pH	TTA (mg/mL)
100%MT	225.0±21.2 ^a	137.50±3.5 ^c	787.50±9.2 ^d	122.00±4.2 ^c	5.2±0.7 ^{bc}	0.26±0.00 ^a
90MT:10GT	175.0±14.1 ^c	117.50±10.6 ^{bc}	740.0±42.4 ^{cd}	112.5±3.5 ^c	4.8±0.0 ^a	0.68±0.00 ^c
80MT: 20GT	150.0±7.1 ^{bc}	112.5±23.4 ^{bc}	675.0±7.1 ^c	77.5±3.5 ^b	5.1±0.1 ^b	0.72±0.06 ^c
70MT : 30GT	102.5±3.5 ^a	102.5±3.5 ^b	415.0±21.2 ^b	70.0±7.0 ^b	5.2±0.1 ^{bc}	0.76±0.01 ^c
60MT : 40GT	135.0±7.1 ^b	98.5±0.7 ^b	415.0±21.2 ^b	32.5±3.5 ^a	5.1±0.0 ^b	0.55±0.1 ^b
50MT : 50GT	103.0±1.4 ^a	63.5±2.1 ^a	230.0±56.6 ^a	37.5±3.5 ^a	5.3±0.0 ^c	0.50±0.03 ^b

Data are means of two independent experiments ± standard deviation (n=2); 90MT:10GT: 90% millet/10% groundnut; 80MT:20GT: 80% millet/20% groundnut, 70MT:30GT: 70% millet/ 30% groundnut, 60MT:40GT: 60% millet/40% groundnut, 50MT:50GT: 50% millet/50% groundnut and 100%MT: (control). TTA: Total titratable acid

Table 3: Mineral elements of *kunun-zaki* from different blend of millet and groundnut at different ratios

Millet to Groundnut ratio	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Phosphorous (mg/L)	Iron (mg/L)
100%MT	3.70±0.1 ^c	70.20±4.0 ^b	37.65±2.2 ^d	70.40±1.8 ^d	3.85±0.1 ^d
90MT:10GT	3.10±0.0 ^b	68.00±1.0 ^b	22.50±0.5 ^b	66.30±1.6 ^d	2.50±0.0 ^b
80MT: 20GT	3.70±0.0 ^c	67.50±1.5 ^b	40.50±1.5 ^d	58.25±1.7 ^c	3.80±1.0 ^d
70MT: 30GT	2.65±0.1 ^a	64.50±0.5 ^a	31.00±1.0 ^c	39.45±2.1 ^b	3.75±0.1 ^d
60MT: 40GT	2.70±0.0 ^a	68.50±0.5 ^b	23.00±1.0 ^b	33.90±1.5 ^a	3.00±0.1 ^c
50MT: 50GT	2.95±0.1 ^b	63.50±0.5 ^a	18.00±0.0 ^a	33.65±1.3 ^a	2.08±0.0 ^a

Data are means of two independent experiments ± standard deviation (n=2); 90MT:10GT: 90% millet/10% groundnut; 80MT:20GT: 80% millet/20% groundnut, 70MT:30GT: 70% millet/ 30% groundnut, 60MT:40GT: 60% millet/40% groundnut, 50MT:50GT: 50% millet/50% groundnut and 100%MT: (control).

Sensory properties of *kunun-zaki* from millet and groundnut blend showed significant differences ($p < 0.05$) in the appearance, taste, flavour and viscosity of the samples with groundnut inclusion (Table 4). However, there were no significant differences ($p < 0.05$) in the overall acceptability among all the samples in comparison with the control. *Kunun-zaki* produced from 50% millet and 50% groundnut was preferred by the sensory panelist in taste, flavour, appearance and colour, the significance may be due to the percentage groundnut addition which did not alter the known traditional *kunun-zaki* taste but improved on the flavour, texture and overall quality of the product. Pan²³ reported that ammonia nitrogen is produced by microbial decomposition of protein during fermentation. Thus, the product of protein fermentation (ammonia) may have impacted taste and flavour on sample 50MT: 50GT which could be the reason the sensory properties was most significant and rated high by the panelist. The total bacteria count during *kunun-zaki* production is shown in Figures 1, 2, 3 and 4. Sample 90MT:10GT, 80MT:20GT and 70MT:30GT had a lower total aerobic bacteria count of 5.1 (logcfu/mL) during fermentation compared to steeping. The total aerobic bacteria count generally increased during fermentation stage. Sample 60MT:40GT

and 50MT:50GT had a count of 6.3 (logcfu/mL) and 7.2 (logcfu/mL) respectively during fermentation. The total lactic acid bacteria count significantly ($p < 0.05$) increased from 5.2 (logcfu/mL) in sample 60MT:40GT during steeping to 7.4 (logcfu/mL) in the final product, with other sample following similar pattern of increase in total lactic acid bacteria count. Conversely, there was no presence of coliform bacteria throughout the production process. The total yeast/mould count in Figure 3 ranged from 5.2 (logcfu/mL) to 5.7 (logcfu/mL) during steeping, 5.2 (logcfu/mL) to 5.7 (logcfu/mL) during fermentation and 5.1 (logcfu/mL) to 5.6 (logcfu/mL) in the final product. The microbial counts of millet groundnut *kunun-zaki* indicated that the microbial succession showed geometric progression and the peak of activities occurred during fermentation, this agrees with Adesulu-Dahunsi's report.²⁴ There was no coliform count in all the plates after 48hour incubation at 37°C, owing to the fact that all aseptic techniques were observed in the production process to eliminate possible source of contamination in the beverage. Hazard analysis critical control point (HACCP) as described by Bello *et al.*²⁵ and Akoma *et al.*,²⁶ was carefully observed which eliminated pathogens and food spoilage microorganisms in the *kunun-zaki*.

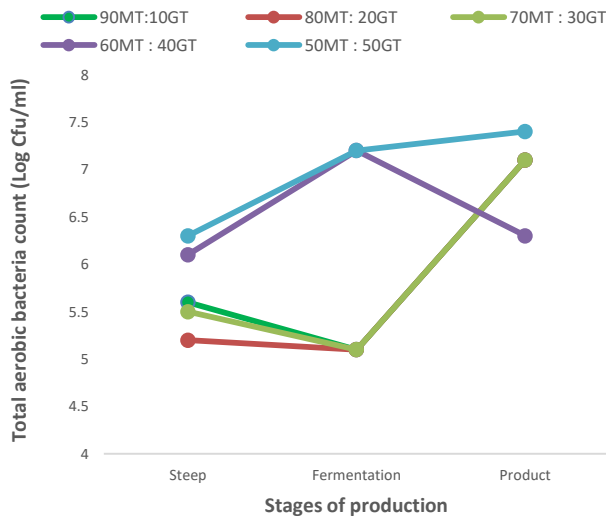


Figure 1: Total aerobic bacteria count of *kunun-zaki* from different blends of millet and groundnut blend during production MT, millet; GT, groundnut.

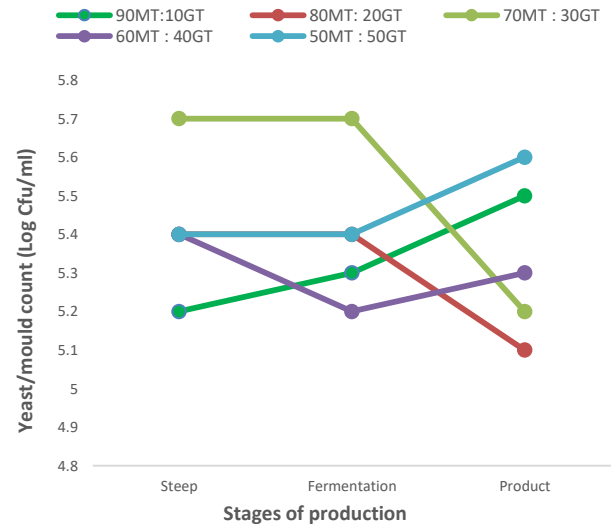


Figure 3: Total Yeast/mould count of *kunun-zaki* from different blends of millet and groundnut blend during production.

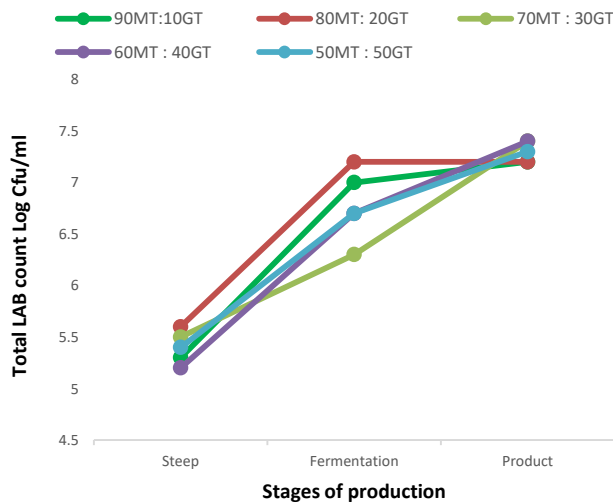


Figure 2: Total LAB count of *kunun-zaki* from different blends of millet and groundnut blend during production MT, millet; GT, groundnut.

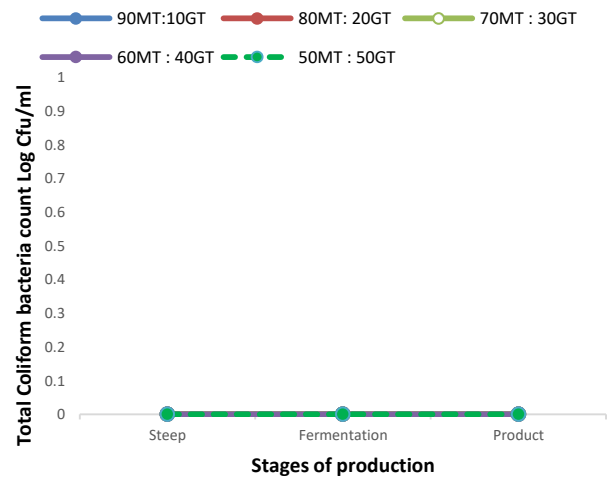


Figure 4: Total coliform bacteria count of *kunun-zaki* from different blends of millet and groundnut blend during production MT, millet; GT, groundnut.

Table 4: Sensory evaluation of *kunun-zaki* from different blend of millet and groundnut at different ratios

Millet to Groundnut ratio	Appearance/Colour	Taste	Flavour	Viscosity	Overall acceptability
100%MT	6.81±1.1 ^{a,b}	6.64±2.0 ^b	6.62±1.2 ^b	6.77±1.5 ^b	6.9±1.1 ^a
90MT:10GT	6.48±2.0 ^{a,b}	5.64±1.8 ^{a,b}	6.13±1.9 ^{a,b}	5.32±2.3 ^a	5.90±1.9 ^a
80MT:20GT	6.55±1.8 ^{a,b}	5.48±2.6 ^{a,b}	5.48±2.1 ^a	6.22±2.0 ^{a,b}	6.29±1.9 ^a
70MT:30GT	5.87±2.1 ^a	5.64±2.2 ^{a,b}	5.74±2.0 ^{a,b}	6.10±1.8 ^{a,b}	6.45±2.0 ^a
60MT:40GT	6.12±2.4 ^{a,b}	5.00±2.5 ^a	5.68±2.0 ^{a,b}	6.10±2.5 ^{a,b}	6.23±2.2 ^a
50MT:50GT	7.03±2.0 ^b	5.81±2.3 ^{a,b}	6.39±1.8 ^{a,b}	6.74±2.1 ^b	6.45±2.2 ^a

Data are means of two independent experiments ± standard deviation (n=2); 90MT:10GT: 90% millet/10% groundnut; 80MT:20GT: 80% millet/20% groundnut, 70MT:30GT: 70% millet/ 30% groundnut, 60MT:40GT: 60% millet/40% groundnut, 50MT:50GT: 50% millet/50% groundnut and 100%MT: (control).

Conclusion

The inclusion of groundnut in the production of millet based *kunun-zaki* increased the nutritional value of the beverage without altering its sensory characteristics thereby making it a potential functional food beverage. Groundnut though contains anti nutritive substances such as tannin, phytate and oxalate, fermentation by LAB hydrolyses the protein thereby aiding the bio accessibility of available nutrients in groundnut. Hence, the inclusion of groundnut significantly increased the protein content of millet blend of *kunun-zaki* beverage

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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