Nutritional Composition, Sensory Properties and Microbial Status of Kunun Zaki Fortified with Ground Nut Protein Concentrates

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ABSTRACT

Background: Kunun zaki is a cereal based beverage that is highly consumed in Northern Nigeria, but gaining fast popularity all over the country. However, Kunun zaki is mainly a carbohydrate food that is deficient in protein. Kunun zaki will be nutritionally adequate if its protein content is improved with groundnut protein concentrates which could be locally sourced.

Objective: The study investigated the nutrient composition, sensory and microbial properties of kunun zaki fortified with ground nut protein concentrates.

Methods: Kunun zaki made from millet was fortified with groundnut protein concentrates at levels of 0, 10, 20, and 30%. The proximate composition, essential amino acid, sensory and microbial properties were determined using standard methods of analysis. The data obtained were statistically analyzed using Analysis of variance (ANOVA)

Results: Sample (KN30) had the highest protein (17.8 mg/100g), fat (2.7 mg/100g), ash (2.8 mg/100g), fiber (3.2 mg/100g) and lowest Moisture (65.2 mg/100g) and carbohydrate (8.3 mg/100g) content than other samples. Sample (KN30) had the highest content of lysine (4.3 g/100g), tryptophan (2.6 g/100g) and phenylalanine (3.1 g/100g).Total solids increased from 13% in the control to 25.6% in KN30. All samples had P^H range of 4.0 to 4.2. Samples were microbiologically shelf stable up to 24h of storage at room temperature.

Conclusion: Fortification of kunun zaki with ground nut protein concentrates up to 30% level improved protein, energy, ash and fibre content of the beverage.. Addition of groundnut protein concentrates showed no adverse effects on the sensory and microbial properties of Kunun zaki.

Keywords: Kunun Zaki; Protein; Concentrate; Beverage; Kunun; Fortified.

INTRODUCTION

Beverages are liquid foods that serve as both fluids and nutrients that refresh and nourish the body (1). There are different types of traditional beverages grouped into alcoholic and nonalcoholic drinks. Kunun zaki is a cereal based; nonalcoholic beverage in Nigeria prepared traditionally using one or more of fermented millet, sorghum or maize (2, 3). Although cereals are the major raw materials used in the preparation, other ingredients (ginger, pepper, and clove) that serve as spices are also introduced to give taste and aroma to the final product and also serve as purgative and cure for flatulent conditions. The consumption of Kunun zaki is high particularly in Northern Nigeria and is fast gaining popularity all over Nigeria as it costs less than the conventional carbonated drinks (4,5). Kunun zaki is consumed by both adult and children as a refreshing drink, appetizer and weaning drink for infants.

Kunun zaki is obtained from cereals therefore; it is mainly a carbohydrate food and deficient in protein (6). This makes Kunun zaki nutritionally inadequate. Consumption of Kunun zaki cuts across all age groups and social status with some taking it three to four times daily, this might have a negative effect on the nutritional status on the population that drink kunun zaki especially on the growth rate of infants who are given Kunun zaki as a weaning drink. Deficiencies of protein and calories particularly among the preschool children and lactating women in the developing Nations are still prevalent. The development of nutritionally balanced protein foods to feed the growing population in the developing nations is receiving attention of the food scientists and Nutritionists in order to alleviate the challenges of protein calories malnutrition (6).

Several studies have fortified local starchy foods with different food substances such as soy bean, ground nut flour, bambara ground nut, cowpea among others in other to improve their nutritional qualities (7, 8, 9). One cheap method of enhancing the nutritive value of kunun zaki is by adding ground nut protein concentrate to it. Ground nut seed makes a complete food as it contains adequate quantities of protein, carbohydrate and fat. Protein extracts such as concentrates and isolates rather than flours are preferred in protein supplementation efforts because they provide highly concentrated protein sources than flours, and have reduced objectionable odor, color and anti nutritional factors. They have no flatulence causing sugars (oligosaccharides) and have improved functional properties in food application (10). Therefore, addition of ground nut protein concentrates to kunun zaki could improve the nutritional and sensory properties of the beverage.

There is dearth of information on Kunun zaki fortified with ground nut protein concentrates. The main objective of this study was to evaluate the nutritional composition, sensory and microbial properties of kunun zaki prepared from millet and ground nut protein concentrates.

MATERIALS AND METHODS

Pearl millet grains (*Pennisetum glaucum*), groundnut (*Arachis hypogaea* L.), sweet potatoes (*Ipomea batatas* L. (Lam)), ginger (*Zingiber officiale*), red pepper (*Capsicum frutescens*) and cloves (Syzygium *aramaticum*) were obtained from central market, Anyigba in Dekina Local Government Area, Kogi state, Nigeria.

Production of Kunun zaki

The method of Ayo *et al.* (11) was adopted in the production of Kunun zaki. One kilogram (1Kg) of cleaned millet grains was washed and steeped in twice of clean water (1:2 w/v) for 48h in a covered plastic bucket at room temperature to soften the seed. The grains were washed to remove stones and wet milled using a well cleaned attrition mill along with added spices (50g ginger, 10g red pepper, 5g cloves) and 10g sweet potatoes into slurry. Two third of the slurry was mixed with 2500ml. of boiling water and stirred to form a gel; this was allowed to cool for 3h. The remaining one third of the slurry was added to the gel, mixed with boiled cold water (1000mL.) and left to ferment for 12h. The mixture was thoroughly mixed and sweetened with 10 % granulated Sugar, filtered and bottled before pasteurizing at 65 °C for thirty minutes.

Production of groundnut protein concentrates

The method described by Andres *et al.* (10) with slight modification was used to produce protein concentrates from groundnut flour. Groundnut flour was mixed with deionized water using flour to solvent ratio 1:10 and the P^H adjusted to 11 with 1M NaOH. The solution was stirred mechanically for 2h and centrifuged at 3000 × g for 15min. The residue was discarded and the supernatant was adjusted to P^H 4.5 by adding 0.1 N HCl and centrifuged at 3000 × g for 15 min. The supernatant was discarded and the precipitate was washed three times with deionized water and allowed to dry in open air at room temperature for 24h. The dry precipitate was milled in an electric blender and sieved with 150 μm screen size, packaged and stored at 4 °C ± 1.

Formulation of fortified Kunun zaki

Kunun zaki and reconstituted groundnut protein concentrates (20g sample and 50ml of water) were mixed in three different proportions of 90: 10, 80: 20, 70: 30 (v / v) of kunun zaki: protein concentrates and coded as KN10, KN20, and KN30 respectively. Samples of commercially sold and Laboratory prepared kunun zaki with no addition of protein concentrates (KN0 and KN01) served as control samples. The formulated samples were pasteurized at 65 ° C for 30 min.

Proximate and amino acid determination.

Proximate composition and amino acid content of freshly prepared samples were determined using the method of Association of Official Analytical

chemists (17).

Physicochemical analysis.
The pH, specific gravity and total solid of the samples were determined using the standard methods outlined by AOAC (12).

Microbial Assay.
Microbial analysis was carried out according to the method outlined by Adegoke (13). Ten milliliter of samples was thoroughly mixed in 90ml Sterile peptone water to obtain 10^{-1} The dilution was made to 10^{-2}, 10^{-3}, 10^{-3}, 10^{-4}, and 10^{-5}. One milliliter of appropriate dilutions was mixed with molten medium (45 ° C) using acidified potato dextrose agar (PDA) for molds and yeast; MacConkey agar for coliforms and nutrient agar (NA) for total viable bacteria. Incubation period was 48h at 37 °C except for yeast and mold (25 °C, 72h). Determination was carried out in triplicates and counts were expressed as colony-forming unit per ml of sample (CFU/ml).

Sensory evaluation.
Samples were subjected to sensory evaluation for the attributes of taste, aroma, appearance and acceptability. A semi trained twenty member panel who were familiar with the product was used and score were allocated to the attributes (taste appearance aroma and overall acceptability) based on a 9 – point hedonic scale ranging from 1 (dislike extremely) to 9 (Like extremely). The coded samples were served in clean, transparent cups at room temperature (30° C ± 2). Water was given to each panelist for oral rinsing in between tasting of the samples. The results obtained were analyzed using statistical methods of analysis.

Statistical analysis
Data were analyzed using SPSS (Statistical Package for Social Sciences) version 20.0.) The mean and standard error of means (SEM) of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine the level of significant differences between the means using Duncan Multiple Range Test.

RESULTS

Proximate composition
The proximate composition of Kunun zaki fortified with groundnut protein concentrates is presented in Table 1. The Moisture content of formulated samples raged from 74.1mg/100g to 65.2mg/100g while the moisture content of the control samples were 76.8mg/100g (KN01) and 77.3mg/100g fo (KN00) respectively. Fibre and ash contents ranged from 3.2 to 0.8 mg/100g, 2.8 to 1.5 mg/100g respectively. The fat and protein composition ranged between 2.7 and 1.o mg/100g, 17.8 and 3.3 mg/100g respectively. Carbohydrate content ranged from 15.8 in KN00 to 8.3 mg/100g in KN30 while energy value ranged between 128.7 and 85.4 Kcal respectively.

Table 1: Proximate composition (mg/100g) and energy (Kcal) of kunun zaki fortified with ground nut protein concentrate.

Sample	Moisture	Fiber	Ash	Fat	Protein	CHO	Energy Kcal
KN00	$77.3^{a} \pm 0.03$	$0.8^{c} \pm 0.09$	$1.8^{d} \pm 0,11$	$1.0^{b} \pm 0.00$	$3.3^{d} \pm 0.12$	$15.8^{a} \pm 0.08$	$85.4^{c} \pm 0.31$
KN01	$76.8^{a} \pm 0.01$	$0.9^{c} \pm 0.06$	$1.5^{d} \pm 0.13$	$1.1^{b} \pm 0.03$	$3.8^{d} \pm 0.15$	$15.9^{a} \pm 0.14$	$79.7^{c} \pm 0.33$
KN10	$74.1^{b} \pm 0.04$	$2.0^{b} \pm 0.12$	$2.1^{c} \pm 0.15$	$2.0^{a} \pm 0.05$	$8,6^{c} \pm 0.16$	$11.2^{b} \pm 0.16$	$97.2^{c} \pm 0.21$
KN20	$69.0^{c} \pm 0.02$	$2.6^{b} \pm 0.08$	$2.4^{b} \pm 0.07$	$2.1^{a} \pm 0.12$	$13.4^{b} \pm 0.11$	$10.5^{b} \pm 0.17$	$114.5^{b} \pm 0.22$
KN30	$65.2^{d} \pm 0.00$	$3.2^{a} \pm 0.05$	$2.8^{a} \pm 0.01$	$2.7^{a} \pm 0.04$	$17.8^{a} \pm 0.09$	$8.3^{c} \pm 0.13$	$128.7^{a} \pm 0.11$

Values are means ± standard deviation of triplicate determinations. Values followed by the same superscript letter in the same column are not significantly ($p > 0.05$) different from each other.

Kn00: Commercially procured Kunun zaki
KN01: Laboratory prepared Kunun zaki
KN10: Kunun zaki fortified with 10% groundnut protein concentrate.
KN20: Kunu zaki fortified with 20% ground nut protein concentrate.
KN30: Kunun zaki fortified with 30% ground nut protein concentrate.

Essential amino acid profile

Table 2 shows the essential amino acid profile of kunun zaki fortified with ground nut protein concentrates. Essential amino acid of kunun zaki samples showed that isoleucine and leucine ranged from 0.8 to 2.8 and 3.5 to 4.3 g/100g respectively. Lysine and tryptophan ranged from 0.9 to 4.4 to 0.7 to 2.6 g/100g respectively. Treonine and methionine content ranged between 1.4 and 2.3g/100g, 2.2 and 2.4 g/100g while phenilealanine, valine and histidine ranged from 1.3 to 3.1 g/100g, 1.0 to 2.5g/100g and 1.3 to 2.8g/100g respectively.

Physicochemical properties

Physicochemical properties of fresh Kunun zaki samples are presented in Table 3. Samples KN00 and KN01 had P^H of 4.1 and 4.0 respectively while fortified samples had 4.2. Total solids ranged between 13.6 and 25.6% while specific gravity ranged from 1.81 to 0.72 respectively.

Table 2: Essential amino acid profile of Kunun zaki fortified with ground nut protein concentrate in g/100g protein

Amino acid	KN00	KN01	KN10	KN20	KN30	FAO/WHO
Isoleucine	$0.8^a \pm 0.03$	$0.7^a \pm 0.05$	$0.9^a \pm 0.11$	$1.3^b \pm 0.09$	$2.8^c \pm 0.15$	4.2
Leucine	$3.5^a \pm 0.07$	$3.6^a \pm 0.13$	$3.5^a \pm 0.15$	$3.8^a \pm 0.16$	$4.3^b \pm 0.17$	4.8
Lysine	$0.9^a \pm 0.01$	$1.0^a \pm 0.08$	$1.2^a \pm 0.04$	$3.2^b \pm 0.05$	$4.4^c \pm 0.11$	4.2
Tryptopahan	$0.7^a \pm 0.04$	$0.7^a \pm 0.06$	$0.9^a \pm 0.02$	$2.0^b \pm 0.03$	$2.6^b \pm 0.13$	1.2
Threonine	$1.4^a \pm 0.12$	$1.5^a \pm 0.14$	$1.6^a \pm 0.11$	$1.9^a \pm 0.13$	$2.3^b \pm 0.14$	2.8
Methionine	$2.4^a \pm 0.17$	$2.7^a \pm 0.16$	$2.5^a \pm 0.17$	$2.3^a \pm 0.12$	$2.2^a \pm 0.00$	2.2
Phenylalanine	$1.5^a \pm 0.06$	$1.3^a \pm 0.11$	$1.6^a \pm 0.07$	$2.2^b \pm 0.17$	$3.1^c \pm 0.12$	2.8
Valine	$1.1^a \pm 0.03$	$1.0^a \pm 0.01$	$1.2^a \pm 0.05$	$1.9^b \pm 0.06$	$2.5^c \pm 0.07$	4.2
Histidine	$1.3^a \pm 0.00$	$1.3^a \pm 0.00$	$1.3^a \pm 0.01$	$2.0^b \pm 0.16$	$2.8^b \pm 0.12$	NS

Means along the same row with different superscript are significantly different ($p < 0.05$)

Kn00: Commercially procured Kunun zaki
KN01: Laboratory prepared Kunun zaki
KN10: Kunun zaki fortified with 10% groundnut protein concentrate.
KN20: Kunu zaki fortified with 20% ground nut protein concentrate.
KN30: Kunun zaki fortified with 30% ground nut protein concentrate
FAO/WHO: Food and Agricultural Organization/ World Health Organization Recommended allowance for infants.
NS: Not specified.

Table 3: Physicochemical properties of Kunun zaki fortified with ground nut protein concentrate.

Sample	P^H	Total soluble solids (%)	Specific gravity (g/ cm^3)
KN00	$4.1^a \pm 0.0$	$15.0^d \pm 0.09$	$0.83^b \pm 0.01$
KN01	$4.0^a \pm 0.0$	$13.6^{de} \pm 0.14$	$0.74^b \pm 0.03$
KN10	$4.2^a \pm 0.0$	$18.5^c \pm 0.08$	$0.72^b \pm 0.02$
KN20	$4.2^a \pm 0.0$	$20.5^b \pm 0.12$	$0.82^b \pm 0.00$
KN30	$4.2^a \pm 0.0$	$25.6^a \pm 0.11$	$1.81^a \pm 0.12$

Values followed by the same superscript letter in the same column are not significantly ($p > 0.05$) different from each other.

Kn00: Commercially procured Kunun zaki
KN01: Laboratory prepared Kunun zaki
KN10: Kunun zaki fortified with 10% groundnut protein concentrate.
KN20: Kunu zaki fortified with 20% ground nut protein concentrate.
KN30: Kunun zaki fortified with 30% ground nut protein concentrate.

Microbial analysis

Microbial analysis of fortified kunun zaki and the control samples are presented in Table 4. The total viable population ranged from 2.9×10^2 cfu / ml to 3.9×10^2 cfu / ml during 48h of storage at room temperature. Fungi count ranged between 1.5×10^2 cfu / ml and 4.8×10^3 cfu / ml. No coliform group of organism was detected in all the samples analyzed.

The results of the mean sensory scores are shown in Tables 5. The mean scores of sensory evaluation showed that taste and appearance ranged between 7.5 and 7.8, 7.3 and 8.6 respectively while aroma ranged from 8.2 to 8.5. The results of the overall acceptability ranged between 7.1 and 7.3.

DISCUSSION

Fortification of Kunun zaki with protein concentrates significantly ($p < 0.05$) reduced its moisture content as shown in Table 1. The results were similar with the reports of Olusegun *et al.*, and Abidoye (3, 1) who fortified kunun zaki with tiger nut extract and cocoa powder respectively. The lower moisture content of Kunun zaki with groundnut protein concentrates addition, indicates that the solid content of the fortified kunun zaki is high, which means that the product can last longer in the stomach before digestion as solid foods digest slowly compared with liquid foods.

Fortification of Kunun zaki with varying levels of groundnut protein concentrates (10 %, 20 %, and 30 %) resulted in dose dependent increase in protein, fiber, ash, fat and energy content of Kunun zaki. The increase in protein content and energy value of the formulated Kunun zaki could be attributed to the high content of protein and fat in ground nut protein concentrates. Studies have shown that a single plant based food such as Kunun zaki, is low in protein content and is energy dense, when combined with protein rich food materials like legumes or its extracts, the protein content of the mixes usually improved (14). Intake of cereal based gruels have been implicated in the etiology of protein— energy malnutrition among children where such beverages serve as sole complementary food (15). Protein- energy malnutrition (PEM) has continued to pose challenges in Nigeria and other developing countries due to low quality protein commonly associated with plant- based single diets. The recommended dietary allowance (RDA) for crude protein in children food is ≥ 16.0 mg/100g (16). In the present study, sample KN30 (17.8mg/100g) met this requirement. The ash content increased correspondingly with increased incorporation of protein concentrates (Table 1). The present result is in tandem with previous results (17).who reported increase in the ash contents of kunun zaki enriched with extract of moringa seeds flour over the control sample without the extract. The higher the ash content, the more the mineral content of the food (2). Dietary fiber consists primarily of the indigestible complex carbohydrate of cell wall in plants. High dietary fiber can have some beneficial biological effects such as laxative effect on gastro intestinal tract, increased fecal bulk and help reduce plasma cholesterol level (18). In this study, crude fiber values were found to be higher in the fortified kunun zaki.

The carbohydrate content of the enriched kunun zaki showed a reverse trend. It decreased with increasing substitution of protein concentrates. The decrease in the carbohydrate content of the fortified kunun zaki observed in this study agreed with the observation of previous researchers that addition of legume or legume derivative decreases the carbohydrate content of cereal based traditional foods (2, 7, 8,).

Food protein quality is traditionally dependent on its amino acid content (9). Essential amino acids are necessary for new protein structures as they cannot be produced by the human body in physiologically significant amounts and therefore must be supplied as crucial components of a balanced diet. In the present study, kunun zaki fortified with 30% (KN30) ground nut protein concentrate significantly ($p < 0.05$) inproved in lysine, tryptophan and phenylalanine. According to FAO/WHO [16], the recommend dietary allowance for lysine, tryptophan and phenylalanine are 4.2 g/100g, 1.2 g/100g and 2.8 d/100g respectively (Table 2). It can be inferred from the present study, that kunun zaki fortified with 30% ground nut protein concentrate (KN30) satisfied the dietary requirement of lysine, tryptophan and phenylalanine for infants.

Physicochemical results in Table 4 showed no significant differences ($p > 0.05$) in the P^H values among samples. These values are within the range of 3.8 to 4.5 reported by Otaru *et al.* (19), but lower than 5.0 and 6.0 reported by Olusegun *et al.* (3). The acidity of the Kunun zaki drinks may be due to the presence of some bacteria which help in acid fermentation of the Kunun drink as well as added spices like ginger, cloves and pepper which are acidic in nature. The values

obtained for specific gravity did not differ significantly ($p > 0.05$) among the samples. The values are comparable to those reported by Taiwo *et al.* (20).Total solids content of the samples ranged between 13.6 % and 25.6 %. The results of total solids content obtained in this work is higher than 9.20 to 12.5 % reported by Abel et al. [21] for kunun zaki treated with chemicals but compare with the results reported by Abidoye *et al.* (1) who fortified kunu zaki with cocoa powder.

The results of microbial population in Table 4 show that fresh formulated samples had no detectable growth for total viable count (TVC), but fresh commercially produced sample presented a total viable count of 2.9×10^2 cfu / ml. The non detection of organisms in the formulated samples could be as a result of good manufacturing and hygiene practices observed during production. The presence of organisms in the fresh commercially produced Kunun zaki (KNOO), suggests that it must have been contaminated after cooking and cooling process of the drink. Sources of contamination could be as a result of fermentation vessels, storage containers, sieves used for filtration, hands of the handlers and even the plastic bottles used for packaging before sales. The microbial counts recorded in this study were similar to those reported by Ayo *et al.* (11).and Adeleke *et al.* [6]. The authors reported average microbial loads of 3.0×10^2 and 2.5×10^2 cfu / ml in kunun zaki.. The latter also reported an average fungal count of 2.2×10^2 in the beverage and this corroborates those recorded in the present study. These organisms are responsible for the fermentation of the beverage during storage at room temperature (11).The microbial load of the drink became too high to be counted after 24h of storage at room temperature. Efiuwere and Akoma (22) also reported high bacteria populations in Kunun zaki prepared and sold in Jos metropolis. The high microbial load after 24h of storage is an indication of spoilage (13). No coliform bacteria were detected in Kunun zaki samples in the present study. Coliforms are majorly of fecal origin and their presence in foods indicate contamination from fecal sources and suggests inadequate processing which is highly undesirable; this is because some coliforms such as *Escherichia coli*, *Clostridium pafringens* can cause diseases such as gastroenteritis, diarrhoea and urinary tract infections.

The analysis of variance of sensory evaluation presented in Table showed no significant differences ($p > 0.05$) among the samples in terms of taste, aroma and overall acceptability. This could mean that, enrichment of kunun zaki with ground not protein concentrates had no adverse effects on taste, aroma and the general acceptability of the beverage. There were significant differences ($p < 0.05$) in appearance between the control samples and the enriched Kunun zaki. This difference may be due to the addition of groundnut protein concentrates to kunun zaki. All the samples of Kunun zaki evaluated were generally accepted by the consumers.

CONCLUSION

The present study has demonstrated that fortification of kunun zaki with ground nut protein concentrates significantly ($p < .0.05$) improves its nutrient content. Fortification of millet with ground nut protein concentrates up to 30% in the production of kunun zaki has no adverse effects on its sensory properties and microbial stability. It can therefore be concluded that kunun zaki fortified ground nut protein concentrate is nutritionally superior to kunun zaki obtained from 100% millet. However, the microbial content of the hawked Kunu zaki drink was high and suggests poor processing and handling method. Therefore good manufacturing and hygiene practices should be given utmost importance during production of Kunun zaki to avoid microbial contamination that may cause food borne diseases.

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