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PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF NOVEL PLANT MILK BEVERAGE DEVELOPED FROM EXTRACTS OF TIGER NUT, CASHEW NUT AND COCONUT

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ABSTRACT

Plant milk was obtained from three different materials namely tiger nut, cashew nut and coconut. The milk extract was blended together in varying proportions and four plant milk formulations was developed. The formulated milk beverages were evaluated for their physicochemical properties, proximate composition, antioxidative properties and consumer acceptability. Lactose-free beverages developed from blends of plant seed is a potential feeding alternative with improved nutritional and functional properties to individuals with milk-related food restrictions. Plant-based milk alternatives also serve as inexpensive alternative to poor economic group in developing countries having insufficient cow milk supply. Plant milk beverages showed significant amount of protein, fat and ash contents. Formulations with greater proportion of tigernut milk exhibited highest phenolic content as well as significant ability to reduce Fe^{3+} *to* Fe^{2+}*. Similarly, the milk beverage with the highest content of coconut milk exhibited high DPPH radical scavenging ability and was the most preferred by the panellists. The formulated novel beverage has potential as a functional beverage to contribute to nutritional well-being as well protects cardiovascular health.*

Keywords: vegetable milk, lactose-free, beverage, phenolic, radical scavenging

INTRODUCTION

There is an increase in global demand and consumption of beverages from alternative animal milk sources due to health problems related to milk nutrients (Granato et al., 2010). These products are often derived from plant/vegetable sources which are regarded as healthy foods with positive cardiovascular function (Jenkins et al., 2006). Plant milk refers to food product obtained from plant source, have similar appearance to milk but neither contains milk fat, sugar (lactose) or other components. The plant derived beverage could be useful for individuals with lactose intolerance, allergy to cow milk proteins or individuals following a vegetarian diet. Raw materials that can be used as substitutes for milk should possess excellent nutritional characteristics. The best known and most popular vegetable milk is derived from soy, although the demand for almond, rice, and coconut milks is on the increase (Bernat et al., 2014).In Nigerian markets, imported soymilk beverages are the most common plant milk which are currently sold at high prices because of the devalued currency (Abdulfatai et al., 2013).Plant nuts (especially coconut, cashew nut and tiger nut) fall into the category of suitable substitute as they possess good nutritional and antioxidant properties. These nuts can be combined in various proportions to improve taste, consistency and nutritional profile. Consumers do not regard beverages as mere thirst quenchers, as they look out for specific functionalities such as energy booster, fatigue, stress and ageing fighter (Sethi et al., 2016). This has led to development of newer products which can meet consumers need, prevent nutrition-related chronic diseases and improve physical and mental well-being.Beverages from vegetable sources have been reported as healthy foods because they act as protectors against cardiovascular diseases (Jenkins et al., 2006). They contain beneficial bioactive compounds, flavonoids and other phytosterols. Cashew nut (*Anarcadiumoccidentale* L.) belongs to the Anacardiaceae family, the third most produced nut worldwide. It is a tree nut with excellent nutritional characteristics and variety of bioactive substances, antioxidant activity and mineral elements (Alasalvar et al., 2009).Tiger nut is an edible perennial plant with health promoting properties associated to its consumption. The major components are complex carbohydrates mainly starch and dietary fibre which provides milk with low glycemic index and positive effect on cholesterol level (Bernat et al., 2014). Other properties include enhanced blood circulation, reduced risk of colon cancer, prevention of heart disease (Chukwuma et al., 2010; Adejuyitan, 2011). Coconut milk refers to a sweet, milky-white, natural oil-in-water emulsion extract obtained from mature coconut meat. Coconut milk is increasingly becoming an essential raw material in industrial food processing as it is recently used as milk substitute in confectionery products. Several researchers have evaluated the physicochemical and nutritional composition of extract of the individual plant nut. However, novel beverage from blends of the nut have not be developed, likewise no existing reports on antioxidative potential of beverage formulated from a blend of different nut extracts. The study therefore seeks to formulate a novel beverage from blends of the milk extracted from three plants (tiger nut, cashew nut and coconut), evaluate the physicochemical properties, antioxidant activity and consumer acceptability of the plant milk blend beverage.

MATERIALS AND METHODS

Mature tiger nuts, cashew nuts and coconuts were purchased from Oba market in Akure, Ondo State, Nigeria. All reagents used were of analytical grade.

Tigernut extract was processed as described by Udeozor (2012). Coconut milk was extracted as described by Edem and Aniekpeno (2016) while cashew nut was processed into milk according to the method previously described by Muhammad et al.(2017). The milk extract from the individual nuts was blended together in different proportions to obtain a homogeneous nutty milk beverage as described in Table 1.

Table 1.Proportion for formulation of nutty milk beverage

Sample code	Tiger nut milk (ml)	Cashew nut milk (ml)	Coconut milk (ml)
TCCo1	60	60	60
TCCo2	30	60	90
TCCo3	60	90	30
TCCo4	90	30	60

TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2:tigernut, cashewnut, coconut (1:2:3); TCCo3:tigernut, cashewnut, coconut (2:3:1); TCCo4:tigernut, cashewnut, coconut (3:1:2)

Determination of physicochemical parameters

pH was measured using a standardized pH meter (Hanna instruments, HI 8314 membrane pH meter). Total soluble solids (TSS) was determined using a bench type Abbé refractometer (0-32^o) ATAGO N1 (model 2313 MASTER-M) and expressed as ^{o}Brix. The titratable acidity of the beverages was determined by titration with 0.1 M NaOH and phenolphthalein indicator.

Determination of proximate composition

Proximate composition of the formulated milk beverage was carried out. Protein, ash, crude fibre, fat and moisture contents were determined as described in AOAC (2012), carbohydrate was calculated by difference.

Evaluation of antioxidant properties

Free radical scavenging ability of the milk beverage against DPPH (1, 1- diphenyl-2-picryhydrazyl) was evaluated using the method described by Gyamfi et al. (1999). Briefly, appropriate dilution of sample (1 ml) was mixed with 1 ml of 0.4mM methanolic DPPH solution. The mixture was left in the dark for 30 min and absorbance measured at 516 nm.

The total flavonoid content was determined using a colorimeter assay developed by Bao et al. (2005) Aliquot (0. 2 ml) of the sample was added to 0.3 ml of 5% $NaNO_3$ at zero time. After 5min, 0.6 ml of 10% $AlCl_3$ was added and after 6min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank.The total phenolic content (TPC) was determined according to modified Folin-Ciocalteau method (Singleton et al., 1999). Briefly 0.2 ml of diluted drink was added to 1 ml of Folin-Ciocalteau reagent (prediluted 10-fold with distilled water) and shaken well. Mixture was allowed to stand at room temperature for 8 min. Then 0.8 ml of sodium carbonate (7.5 %) was added to mixture, shaken and left at room temperature for 30 min. Absorbance was measured at 765 nm in a spectrophotometer (Ultrospec 4300 Pro UV/Vis). The

TPC was assessed by plotting the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per ml of sample. The reducing property was determined as described by Pulido et al. (2000), 0.25 ml of the drink sample was mixed with 0.25 ml of 200 mM Sodium phosphate buffer (pH 6.6) and 0.25 ml of 1% KFC. The mixture was incubated at 50°C for 20 min, thereafter 0.25 ml of 10% TCA was also added and centrifuged at 2000rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.1% of $FeCl_3$ and the absorbance was measured at 700nm.

Sensory evaluation

Sensory evaluation of the formulated beverage was carried out as follows. Twenty (20) panellists experienced in the evaluation of plant milk were chosen for the assessment of the sensory attributes of nutty beverage. A 30 mins training session was conducted to evaluate the use of the attributes by the panellists during sensory analysis. The sensory attributes allowed the differentiation of samples in terms of appearance (colour), texture (viscosity, consistency/flow), flavour (flavour and aroma) and taste (palatability). Samples were coded and served to the panellists for independent evaluation, all sensory attributes assessed by the panellists were rated using 9-point hedonic scale (1= dislike extremely, 9= like extremely).

Statistical analysis

Data was generated in triplicate and subjected to Analysis of Variance using SPSS V.19. Results were expressed as mean values ± standard deviation.

RESULTS AND DISCUSSION

Table 2 presents the physicochemical properties of the plant milk beverage. The pH ranged from 4.33 to 5.60 while the titratable acidity ranged from 0.28 to 0.44 % lactic acid. The pH of the milk blend was significantly ($P<0.05$) lower than that of soymilk and this may be attributed to the formulation comprising of different quantities of plant milk with different degrees of acidity. Contrary to the present findings, Bristone et al. (2015) reported higher pH and lower titratable acidity for soy-tigernut yogurt. The variation in result may be owing to the latter being a product of microbial fermentation. Moreso, the pH values fell within the values reported by Belewu et al. (2010) for yoghurt prepared from blend of tiger nut and coconut milk. Soluble solids content (°Brix) has been reported to correspond greatly to the concentrations of sugar, organic acids and minerals (King et al., 2006). The blend with the highest tigernut milk content had the highest °Brix (21.60) which suggests that tiger nut milk contributed to increased soluble solids content of the formulated beverage (TCCo2).

Table 2. Physicochemical properties of plant milk beverage

Nutty milk formulations	pH	Brix (°)	TTA (%)
SOY	5.60 ± 0.01^{a}	18.70 ± 0.10^{b}	0.28 ± 0.10^{a}
TCCo1	4.53 ± 0.00^{bc}	17.00 ± 0.00^{c}	0.42 ± 0.07^{b}
TCCo2	4.57 ± 0.00^{b}	21.60 ± 0.50^{a}	0.44 ± 0.03^{b}
TCCo3	4.63 ± 0.01^{b}	12.00 ± 1.00^{e}	0.38 ± 0.11^{c}
TCCo4	4.53 ± 0.00^{bc}	14.70 ± 0.50^{d}	0.40 ± 0.10^{b}

Values are mean ±SD, values with different superscripts (a-c) on the same column are significantly different ($P<0.05$) TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2: tigernut, cashewnut, coconut (3:2:1); TCCo3: tigernut, cashewnut, coconut (1:3:2); TCCo4: tigernut, cashewnut, coconut (2:1:3)

The formulated beverage with the highest proportion of cashew nut milk (TCCo3) had the highest fat content (Table 3) attributable to the high fat content of cashew nut kernel. On the other hand, beverage with the highest proportion of tiger nut (TCCo2) had the least fat content probably because tiger nut is a low-fat seed compared to the other nut used for beverage formulation. TCCo2 showed the highest ash content which suggests that this plant-milk blend may be a rich source of mineral elements. Variations in ash content may be due to variations in inorganic compounds present in the milk extract especially tiger nut and cashew nut. The ash content observed in the present study was higher than those reported by Awonorin and Udeozor (2014) for tiger nut-soymilk blends. Beverage with the highest tiger nut milk content (TCCo2) and the lowest tiger nut milk content (TCCo3) showed significant difference (P< 0.05) in carbohydrate content with the later having the least (3.39 %) and the former having the highest (9.93 %). This agrees with the result from other studies, the researchers reported that tiger nut contributes to increased carbohydrate content of resulting product (Awonorin and Udeozor, 2014; Bristone et al., 2015).

Table 3. Proximate composition of plant milk beverage

	Composition (%)					
Nutty milk formulations	Moisture	Fat	Ash	Crude Fibre	Protein	Carbohydrate
SOY	73.62±0.11^{a}	8.12±0.10^{b}	0.85±0.10^{d}	0.19±0.05^{b}	10.52±0.33^{b}	6.70±0.14^{c}
TCCo1	72.28±0.22^{b}	8.14±0.20^{b}	1.88±0.07^{b}	0.22±0.02^{b}	9.86±0.11^{c}	7.62±0.23^{c}
TCCo2	72.97±0.33^{ab}	5.15±0.50^{c}	2.98±0.13^{a}	0.33±0.05^{a}	8.64±0.14^{d}	9.93±0.13^{a}
TCCo3	70.91±0.25^{c}	10.96±0.83^{a}	1.90±0.19^{b}	0.24±0.02^{b}	12.60±0.53^{a}	3.39±0.54^{d}
TCCo4	70.13±0.21^{c}	8.44±0.35^{b}	1.63±0.22^{c}	0.13±0.08^{c}	10.89±0.21^{b}	8.78±0.35^{b}

Values are mean±SD, values with different superscripts (a-d) on the same column are significantly different (P<0.05)
TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2: tigernut, cashewnut, coconut (3:2:1); TCCo3: tigernut, cashewnut, coconut (1:3:2); TCCo4: tigernut, cashewnut, coconut (2:1:3)

The total flavonoid and total phenolic content (TFC/TPC) of the beverages follow similar trend (Fig. 1). The control (soy milk drink) exhibited the highest TFC and TPC. However, the formulation with the highest content of tiger nut milk (TCCo2) also exhibited significant phenolic content (33.25 mg GAE/mL). The plant milk formulations showed significant ability to scavenge the DPPH radical (Fig. 2). However, the blend with highest coconut milk exhibited the highest scavenging activity (52.18 %). There was no significant difference (P<0.05) in DPPH radical scavenging activity of soymilk drink and TCCo2.The result of the present study corroborates earlier reports by Badejo et al. (2014) who reported high radical scavenging activity in beverage produced from tiger nut extract. The formulated nutty milk beverage showed significant ferric reducing antioxident power (Fig. 3). TCCo2 also exhibited the highest capacity to reduce Fe^{3+} to Fe^{2+} (0.11 mmol Fe^{2+}/mg), the blend with the highest coconut milk content (TCCo4) also possessed significant reducing ability.

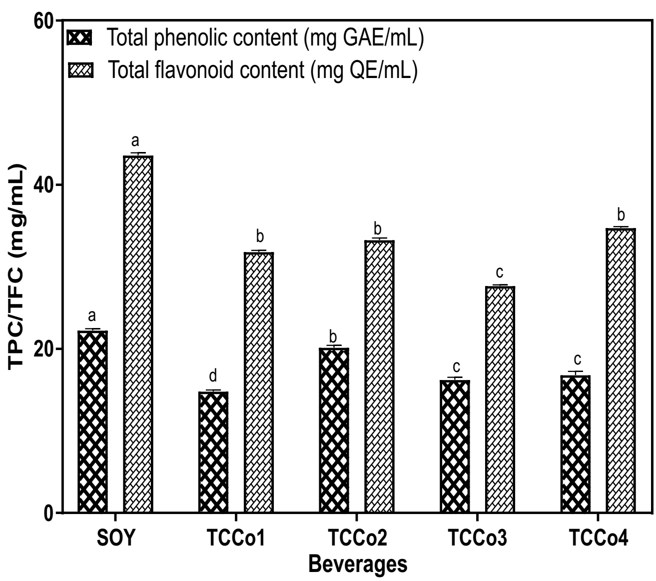

Figure 1: Total phenolic and total flavonoid content of plant milk beverages
Values are mean ± SD; Bars with different letters (a-d) are significantly different (P<0.05)
TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2: tigernut, cashewnut, coconut (1:2:3); TCCo3: tigernut, cashewnut, coconut (2:3:1); TCCo4: tigernut, cashewnut, coconut (3:1:2)

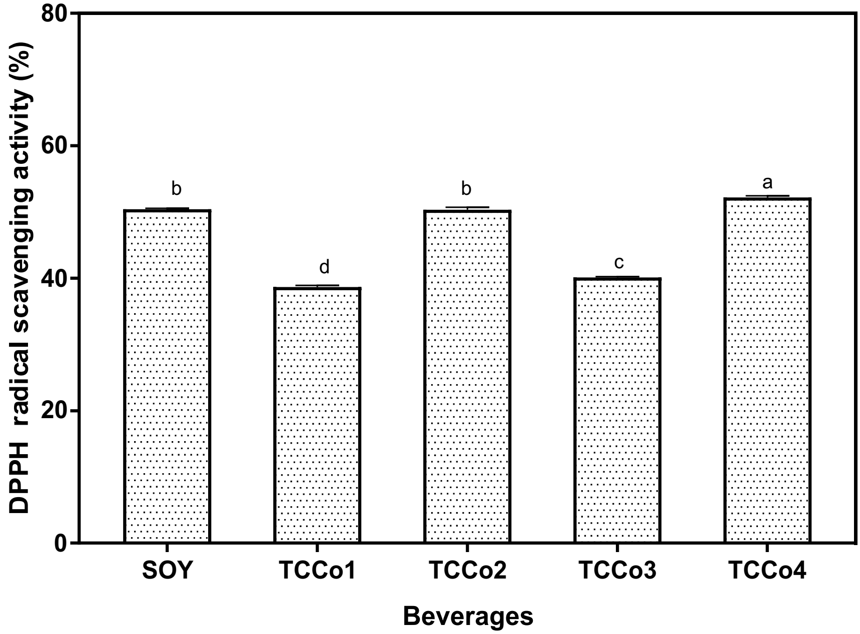

Figure 2: DPPH radical scavenging activity (%) of plant milk beverages
Values are mean ± SD; Bars with different letters (a-d) are significantly different (P<0.05)
TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2: tigernut, cashewnut, coconut (1:2:3); TCCo3: tigernut, cashewnut, coconut (2:3:1); TCCo4: tigernut, cashewnut, coconut (3:1:2)

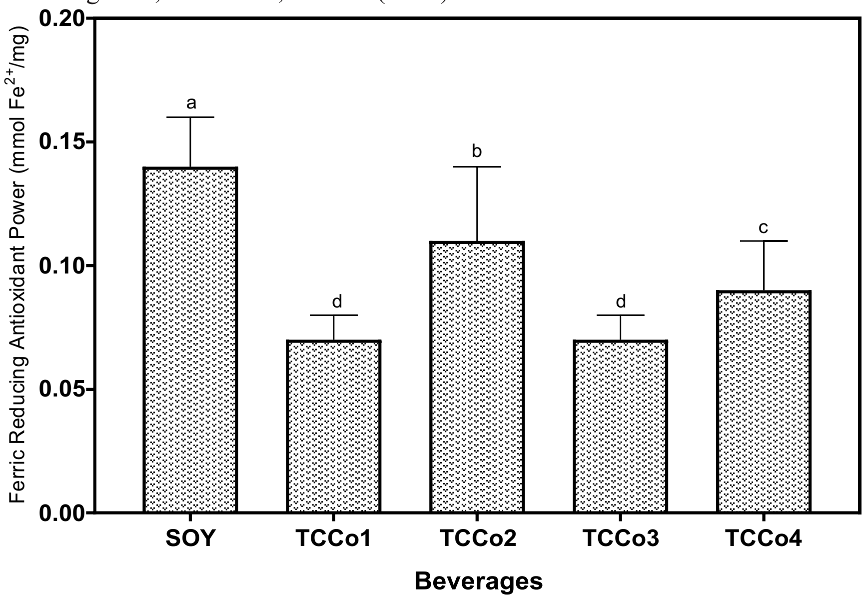

Figure 3: Ferric reducing antioxidant power of plant milk beverages
Values are mean ± SD; Bars withdifferentletters (a-d) are significantlydifferent (P<0.05)
TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2: tigernut, cashewnut, coconut (1:2:3); TCCo3: tigernut, cashewnut, coconut (2:3:1); TCCo4: tigernut, cashewnut, coconut (3:1:2).

The sensory characteristics of the formulated plant milk beverage evaluated by twenty panellists are presented in Table 4. There was no significant difference in some sensory parameters (appearance, consistency and overall acceptability) between control (soymilk) and beverage with equal proportion of the nut milk (TCCo1). TCCo1 also had superior taste to the other beverage formulations. On the other hand, TCCo2 exhibited superior mouth feel and consistency while the beverage with high coconut and tiger nut milk content (TCCo4) had the highest overall acceptability. Samples TCCo2 and TCCo3 with high cashew nut milk content were the least preferred.

Table 4. Sensory attributes of formulated plant milk beverages

	Sensory parameters					
Nutty milk formulations	Taste	Appearance	Flavour	Mouthfeel	Consistency	Overall acceptability
SOY	6.47±0.39[b]	6.60±0.45[a]	6.40±1.09[b]	6.20±1.05[b]	6.27±0.96[b]	6.33±0.11[b]
TCCo1	6.73±1.10[a]	6.53±1.36[a]	6.33±0.50[d]	5.87±1.20[d]	6.27±0.19[b]	6.27±1.03[b]
TCCo2	6.33±1.13[c]	5.93±1.03[c]	6.40±1.13[d]	6.80±0.50[a]	6.40±0.98[a]	6.02±1.08[c]
TCCo3	5.47±0.68[d]	5.93±0.80[c]	5.93±1.16[e]	5.67±0.98[e]	6.47±1.53[a]	6.02±0.54[c]
TCCo4	6.47±0.64[b]	6.04±0.49[b]	6.67±1.18[a]	6.00±0.25[c]	5.67±0.21[c]	6.47±0.30[a]

Values are mean±SD, values with different superscripts (a-d) on the same column are significantly different (P<0.05)
TCCo1: tigernut, cashewnut, coconut (1:1:1); TCCo2: tigernut, cashewnut, coconut (3:2:1); TCCo3: tigernut, cashewnut, coconut (1:3:2); TCCo4: tigernut, cashewnut, coconut (2:1:3)

CONCLUSION

The formulated beverages were was fairly rich in protein, fat and ash contents. Formulation of novel beverage from locally abundant plant materials may enhance the industrial utilization of the crops and serve as a suitable alternative to imported brands. Also, the drink may provide a functional animal milk alternative capable of supplying nutrients as well protecting human health from the possible side effects of oxidative stress. More importantly, tiger nut milk can be suggested to constitute a higher proportion in formulating a nutritionally functional beverage.

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