FORMULATION OF AQUEOUS DRINKS USING TIGERNUT AND MORINGA SEEDS: EFFECT OF ADDING CHEMICAL HURDLES AND FORTIFICANTS ON PHYSICO-CHEMICAL AND RHEOLOGICAL PROPERTIES

ABSTRACT

The study was undertaken to determine the physico-chemical and rheological properties of tigernut and moringa seeds based aqueous drinks influenced by chemical hurdles and fortificants. The aqueous drink was produced using a 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50(w/w) tigernut (T) moringa seeds (M). These were subjected to sequential affective and descriptive sensory evaluation and yielded (90:10) ratio as the most preferred plain drink (PTMD). The PTMD was then subjected to a 3×3 experimental design comprising 3 levels of sugar (0, 1, 2 %) and 3 levels of citric acid (0, 0.1, 0.2%) treatments followed by sensory evaluation that yielded (PTMD) containing 2% sugar and 0.2% citric acid as most acceptable (TMSC) which was subsequently subjected to triple fortification using 0.15mg KI, 2.0 mg $FeSO_4$ and 1.6mg retinol palmitate/100g each to yield (TMSCFD). The 3 working products (PTMD, TMSC and TMSCFD) were then subjected to physico-chemical analysis and rheological test. Data obtained from rheological tests were fitted into power law model to obtain the flow behaviour indices. The use of hurdles and fortificants significantly ($p<0.05$) increased the proximate composition with the protein ranging from 25.8-26.4, carbohydrates 19.4-27.3, Ash 0.77-4.4, Fibre 20.6-32.3 and moisture 66.1-69.0%. Fortification significantly ($p<0.05$) increased the Fats from 7.01-10.05, Iron, Iodine and Pro-Vitamin A values 2.90-4.80, 0.00-0.15 and 0.94-2.48, the specific gravity of the aqueous drink ranged from 1.64 -1.89, TTA ranged from 0.62-0.66 and pH values ranged from 6.3- 6.8 respectively. The 3 products exhibited pseudo-plastic flow patterns with flow bahaviour indices (n) and consistency indices (m) ranging from 0.66-0.68 (PTMD), 0.65-0.79 (TMSCD) and 0.39-0.48 (n) (TMSCFD), 0.31-0.49 (PTMD), 0.13-0.419 (TMSCD) and 2.19-2.45 Ns/m (TMSCFD) respectively. This results implies that chemical hurdles and fortificants improved the physico-chemical properties of tigernut and moringa seeds aqueous drinks by extending its shelf life and providing adequate nutrients for addressing protein energy malnutrition.

Keywords: Tigernut, Milk, Fortification, Hurdles, Protein, Enrichment, Rheology

1. INTRODUCTION

Among the Nigerian local aqueous non-alcoholic drinks are soy-milk, melon milk and tigernut milk.Tigernut milk which is locally called "*kunu-aya* is a popular and cherished non - alcoholic beverage**.** "*Kunu- aya*" is an aqueous extract from tigernut (*Cyperusesculentus*) which may be plain or sweetened and may contain local spices and herbs for distinct flavours[1].

Earlier reports by Suleima*et al*. [1] indicate that the tigernut milk produced and marketed in Nigeria contains about 8.5% protein, 17.0%, fat, 1.2% ash and 17.8% total carbohydrates. Therefore, the product will require protein enrichment from cheap plant sources such as moringa seeds (*MoringaOleifera*) which is reported to have about 30.1% of protein, in other to meet the 16 % protein as recommended by the Protein Advisory Group (PAG) for children of ages 4 -12 months above.Again *kunu – aya*is highly perishable especially under tropical ambient conditions and will therefore require the use of chemical hurdles such as citric acid and sugar for shelf–life extension. Food fortification especially with fortificants such as vitamin A, iodine and iron require vehicles which are acceptable, safe, cost-effective and commonly consumed [2].The improved *kunu-aya* could therefore act as a potential vehicle for food fortification for addressing vitamin A, iron and iodine deficiencies that are prevalent in most rural Nigerian communities.Food fortification is the addition of nutrients, to consumed foods in order to address identified deficiencies in a given population [3]. Hence combination of tiger nut and moringa seeds aqueous extracts could provide adequate nutrients for addressing PEM while serving as vehicle for triple fortification for addressing MND.

2. MATERIAL AND METHODS

2.1 Sources of Raw Materials

Dried yellow tigernut, sugar and muslin cloth were purchased in Wadata market, Makurdi. Moringa seeds were purchased at a moringa farm opposite international Market, Makurdi, Citric Acid, pro- vitamin A, ion, iodine fortificants were purchase at Emole Nig. Ltd Makurdi Benue State, Nigeria. Analytical reagents, weighing scale, refractometer, spectrophotometer, standardized pH meter, Brookfield viscometer, ovens, a pycnometer and Petri dishes were gotten from Benue State University Chemistry Science Laboratory and Joseph SarwuanTarkaa University, Makurdi Biochemistry laboratory respectively, where the analysis were carried out.

2.2 Sample Treatments

Plain aqueous drinks were formulated using tigernut (T) and moringa seeds (M) at ratios of 100:0, 90:10, 80:20,70:30,60:40 and 50:50 w/w respectively. The products were subjected to sensory evaluation to decide the most preferred formulation. The most preferred formulation was then treated with selected chemical hurdles (Sugar and citric acid) using a 3×3 experimental design comprising 3 levels of sugar (0, 1, 2%) and 3 levels of citric acid (0, 0.1, 0.2%) which yielded 9 experimental groups. The groups were subjected to randomize sensory testing in order to pick the most acceptable.

The most acceptable of the 9 groups was then treated with potassium iodide, ferrous sulphate and 1.6 mg retinol palmitate each /100g sample using standard fortification guide recommended by (Food fortification Regulations, 2021; Guidelines on Food fortification with Micronutrients 2019).

The plain (PTMD), hurdle treated (TMSCD) and triple fortification (TMSCD) designated as TMSCFD were then subjected to chemical, physical, rheological, sensory evaluation and statistical analysis.

2.3 Preparation of Aqueous Drinks

The tigernut and moringa seeds based aqueous drinks were produced using the method described by Udeozor, [4] as shown in figure 1.

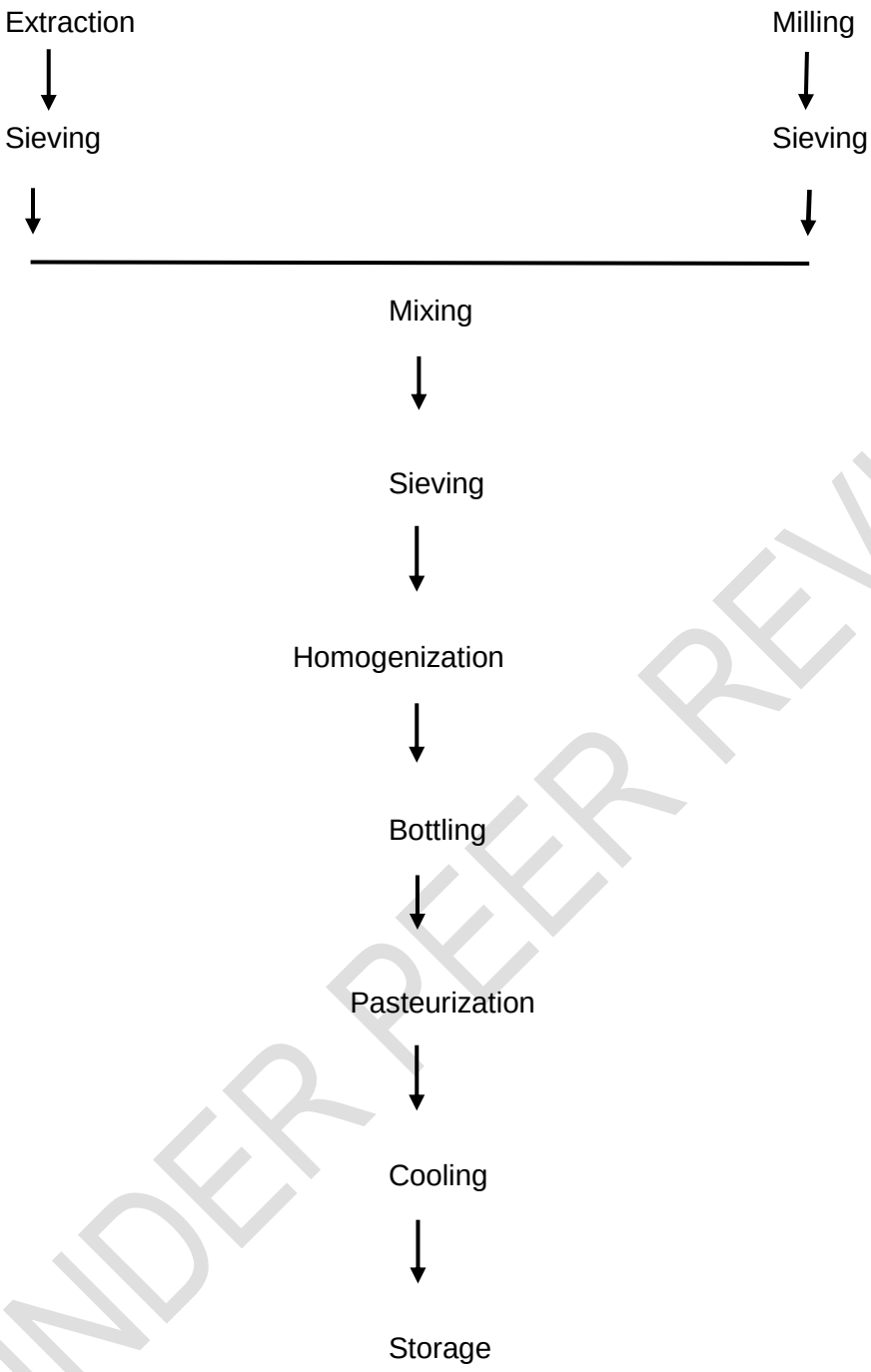

Figure 1: Flow diagram for the production of tigernut milk and moringased-based aqueous extract

Source: [4]

2.4 Chemical Determinations

2.4.1. Proximate Analysis

A.O.A.C [5] standard procedures were used for triplicate determinations of the moisture content (14.06.2), ash (14.06.3), and crude fibre (14.06.4), crude fat (14.06.5), protein (14.06.6) while total carbohydrate was by difference.

2.4.2 Minerals Analysis

Spectrophotometric method was used to determine the mineral content of each sample as described by AOAC, [5]; PyeUnicam, [6]; Pearson, [7]. Samples were analysed for Calcium (Ca), and iron (Fe), magnesium (Mg).

2.4.3 Vitamins Analysis

Vitamin B and K content of tigernut and moringa seeds based aqueous drinks were determined using colourimetric and spectrophotometric methods [5].

2.4.4 Retinol palmitate determination: The retinol palmitate content of the formulated products was analysed using high performance liquid Chromatography (HPLC) as described by Rutkowsi [8].

2.4.5Antinutrient Analysis

Tannins: Tannins were measured using the Burn method [9].

Phytates: The phytate content was determined using the method described by Hassan [10].

Saponins: This was according to the method reported by Aduwamai*et al*.[11].

2.4.6 Thiobarbituric Acid (TBA): The thiobarbituric acid (TBA) value was evaluated using the method reported by Draperand Hadley [12].

2.4.7 pH Determination

Jenway pH metre (Model 3015, serial number 1647, UK) was used to measure the pH of the drink samples. In a beaker, 2 g of each drink sample was put. The pH electrode, which had previously been standardised with buffers of pH 4.01 and 9.20 and cleaned with deionized water, was dipped into the homogenate and allowed to settle before taking readings. Determinations were carried out in duplicate for each sample.

2.4.8 Determination of Total Titratable Acidity (TTA)

Standard method of Antony and Chandra [13] and Ferrati*et al*. [14], were used to measure the titratable acidity.

2.4.9 Rheological Tests: Viscosity Measurements

The rheological characteristics of tigernut from the formulated products were evaluated as described by Dogan and Kayacier, [15]. Data obtained from the viscometry were fitted with the power law rheological model

2.5 Statistical Analysis

The mean and standard deviation of the result data from the experiment was calculated and analyzed using single factor ANOVA in the Statistical Package for Social Science (SPSS) Software (SPSS version 12. 0.1 for windows). The Duncan's New Multiple Range Test and Principal Component Analysis were used to determine the significant difference between mean values.

3.0 Results and Discussion

3.1 Proximate composition

The proximate composition of the plain (PTMD), hurdles treated (TMSCD) and hurdles plus fortificants treated drinks (TMSCFD) are presented in Table 1.

It can be observed Table 1that the hurdles and the fortificants significantly (*P>0.05*)influenced the proximate composition of the aqueous drink, essentially, on a dry weight basis.

Table 1: Proximate composition of tigernut and moringa seeds based aqueous drinks on dry weight basis

Nutrients **(g/100g db)**	**Sample**		
	PTMD	**TMSCD**	**TMSCFD**
Total solids	31.00^{a} ± 1.40	33.00^{a} ± 0.70	34.00^{a} ± 2.00
Protein	25.80^{b} ± 0.20	21.20^{a} ± 1.00	26.40^{b} ± 0.10
Fat	22.60^{a} ± 0.20	24.03^{b} ± 0 .85	26.50^{c} ± 1.00
Ash	0.77^{a} ± 0.01	0.90^{a} ± 0.08	4.40^{b} ± 0.60
Fibre	32.30^{c} ± 1.90	27.30^{b} ±0.64	20.60^{a} ± 0.10
Carbohydrates	19.40^{a} ± 1.23	27.30^{c} ± 1.10	22.10^{b} ± 1.30
Energy (Kcal/100g)	38.420^{a} ± 5.80	412.70^{b} ± 2.50	432.50^{c} ± 2.30

Results are Means ± standard deviation of triplicate determination. Values along each row with common superscripts are not significantly different at p>0.05

Key:

PTMD= Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks

TMSCD = Tigernut and moringa seeds aqueous extract plus 2% Sugar and 0.2% citric acid

TMSCFD = Tigernut (90%) and moringa seeds (10%) based aqueous drink with 2% sugar + 0.2% citric acid and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinol palmitate each/100 g sample.

From the results, it can be observed that the hurdles and the fortificants significantly (*P>0.0*5) influenced the proximate composition the aqeous drink. All the results of the proximate composition of tigernut and moringa seeds based aqueous drinks were within the range of the works reported earlier by Suleiman *et al*. [1] and Abiodun*et al*. [16].

From the results, there was an indication that tigernut and moringa seeds based aqueous drink is high in moisture ranging from 66.03% - 69.03%. Although tigernut and moringa seeds drink is high in moisture content, the high moisture content improves nutrient transportation and other essential metabolic reactions [1]. This also indicates that the formulation is a good beverage that can be used as a synergistic vehicle for fortificants and other micronutrients and can be consumed by children, adults, adolescents, pregnant women, lactating mothers, and as well refreshes the body as earlier reported by Farre, [17].This also implies that the aqueous drink could be easily attacked by micro-organisms, so it should be pasteurized effectively and preserved using various natural barriers to increase its shelf life while preventing microbial attack, this is in accordance with the report byLeistner[18].

Tigernut and moringa seeds aqueous based drink contains an acceptable amount of protein, which may contribute significantly to the body's metabolic processes. The protein content of tigernut and moringa seeds based aqueous drinks in our current study is consistent with the range described by Balewu and Belewu.[19].Tigernut milk protein concentration is approximately 7.01%, on wet basis which is higher than that of most starchy roots and tuber crops such as cassava, sweet potatoes, and yams [20].

Tigernut milk has a higher protein concentration than cereals like rice and sorghum [21]. Tigernut milk may provide more than 17% of an adult's daily protein need and more than 26% of a child's daily protein requirement [22].The greater protein content of fortified tiger nut milk with moringa seeds sample implies that moringa seed may contain adequate protein to alleviate

protein insufficiency common in tiger nut based products as earlier reported by Arijeniwa*et al*. [23].

The protein level of tigernut and moringa seeds could therefore be responsible for the relatively high protein content of the formulated drink. Also the hurdles that were incorporated in to the product increased the protein levels of the product which is in accordance report by Eke *et al*. [24] who stated that application of citric acid greatly increased the protein quality of a food product and also boosted the shelf-stability due to the reduction of microorganism's activities.Eke *et al*. [24] also stated that the use of citric acid, sugar, salt and other spices as hurdles for the preservation of dambu-nama and other food products significantly P>0.05 improved their sensory, microbial stability and nutritional value. The ash percentage of tigernut and moringa seeds aqueous drinks ranged from 0.77% - 4.48%, which is comparable to the ash level of other starchy roots crops such as maize and sorghum [25]. Temple *et al*. [26] reported a similar ash value for tigernut milk. As earlier reported by Willy *et al.*[27]the ash content of a dietary component is an accumulation of minerals, the moderate ash concentration discovered in the fortified tiger nut and moringa seeds drink suggests that fortified tiger nut milk contains vital minerals required by the body.

Tigernut and moringa seeds based aqueous drinks fibre values of 20.6% - 32.3% from these findings are in line with the report of Umerie*et al.*[28]. If tigernut milk is consumed, the content plays an important function in reducing pressure and transit time of food through the body, hence improving digestion and weight loss [29]. *Moringaoleifera* on the other hand contain a reasonable quantity of fibre. Since fibre aids in the relief of flatulence, the fibre content of tigernut milk could be explored in the formulation of diets for treating indigestion, constipation, and non-communicable illnesses such as obesity, colon cancer, diverticulosis, and coronary heart disease as also reported earlier by Wardlaw*et al.* [30].

Tigernut and moringa seeds drink includes a moderate quantity of carbohydrates, 100 g of tigernut milk can offer more than 40% of a child's (4-9) daily carbohydrate requirement and more than 32% of an adult's daily carbohydrate requirement as recommended by FAO/ WHO/UNU [22].

Tigernut and moringa seeds drink has a higher fat content than other starchy roots and tubers and may contribute to a child's daily fat requirement as recommended by FAO/WHO/UNU [22]. Fortification with retinol palmitate significantly (P>0.05) increased the formulated tigernut and moringa seeds drink (22.6-26.5 g/100g .db) for PTMD-TMSCFD respectively.Tigernut milk has a fat level that is comparable to nuts and seeds but greater than cereals and similar to soya beans [31]. Retinol palmitate is a pro vitamin A substance, the higher crude fat content of the fortified product could be attributed to retinol palmitate which is pro vitamin A substance that is fat soluble and hence extractable with petroleum ether. Although in the current study cholesterol was not determined in this study since the raw materials used for the product formulation were of plant origin and hence it can be assumed that the tigernuts and moringa seeds based aqeous drinks are cholesterol free unlike formulations containing animal fats [32].Tigernuts and moringa seeds drinks are appropriate for, adolescents, the elderly, and athletes, and they are also highly nutritious[33]. For many years, tigernuts were thought to have sufficient characteristics to combat respiratory infections and some gastrointestinal ailments this therefore justifies the use of tigernut in the aqueous drinks formulation [34]. Zimmermann [35] concluded that tigernuts minimize the incidence of colon cancer and are acceptable for diabetics and obese people. According to common belief in Valencia, Spain [34], Horchata is still a good cure for diarrhoea. “Horchata" (tigernut milk) is natural sweet tasting vegetable milk that could be produced directly from tigernuts and used as a pleasant drink as well as a substitute for cow milk. According to the authors, the tigernut milk based dink is an excellent substitute for other vegetable milk. It is an excellent milk for lactose intolerant people.

3.2 Effects of hurdles and fortificants on selected mineral contents of the drink

Table 2 shows the Ca, Fe, Mg, I_2 contents of the plain (PTMD), hurdles treated (TMSCD), hurdles and fortificants treated (TMSCFD) aqueous drinks. As expected, fortification resulted in higher iron and Iodine contents with values of (4.80 and 0.15 mg/100 g) as compare to (2.90 and 0.00 mg/100 g) respectively in the plain drink.

Table 2: Mineral composition of formulated tigernut and moringa seeds based aqueous drink.

Mineral	**Sample**		
(mg/100g)			
	PTMD	**TMSCD**	**TMSCFD**
Ca	48.20^{a}±0.07	50.2^{b} ±0.07	55.5^{c}±3.53
Fe	2.90^{a}±0.06	2.94^{a}±0.10	4.80^{c}±0.08
Mg	119.89^{a}±0.07	120.48^{b} ±0.07	121.66^{c}±0.47
I_2	0.00^{a} ±0.00	0.00^{a}±0.00	0.15^{c}±0.02

Results are Means ± standard deviation of triplicate determination. Values along each row with common superscripts are not significantly different at p>0.05

Key:

PTMD= Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks

TMSCD = Tigernut and moringa seeds aqueous extract plus 2% Sugar and 0.2% citric acid

TMSCFD = Tigernut (90%) and moringa seeds (10%) based aqueous drink with 2% sugar + 0.2% citric acid and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinylpalmitate each/100g sample.

The high values of calcium found in the Chufa, (tigernut milk) are adequate for bone and teeth development in infants. Calcium is required for muscular contraction, nerve impulse transmission, heartbeat regulation, and fluid equilibrium within cells [36]. The requirements are higher during periods of growth, such as childhood, pregnancy, and breast feeding [37].

Tigernut and moringa seeds drink contains high levels of calcium which aids in the maintenance of healthy bones and teeth. It plays a part in the formulation of ATP (Adenosine Triphosphate) an energy compound indispensable for activating glucose fatty acids and improvement of intellectual performance[30].The range of values obtained in this study are in line with the findings of Moore, [38]. Calcium also plays an important role in blood clotting; helping muscles to contract and regulates normal heart rhymes and nerve functions [30]. The higher calcium in this study is in line with the report by Shaker*et al*. [39] which revealed that Chufa tubers have high calcium, magnesium, sodium and phosphorus and low manganese, iron, iodine, zinc and copper as mineral , although the values are higher than the values of the work that was carried out and reported by Suleima*et al*. [40] and Ndubuisi*et al*. [21] which attributable to the addition of moringa seeds to the tigernut milk as a blend although the result falls within the range that was reported by Aanuoluwa*et al*. [40].

Magnesium levels in tiger-nut and moringa seeds based aqueous drinks were observed to be (119.89-121.66 mg/100g).It aids in the production of bone for strength, as well as the functions of enzymes, nerves, and the heart. It helps improve bone formation for strength, as well as enzyme, nerve, and heart function. Tigernut milk contains magnesium, which aids in immune system function and blood level maintenance. The formulation is a good source of magnesium, which when consumed will helps the body's muscle, immune system, as well as nerve function perform properly as earlier also reported by Wardlaw and Kessel, [40] for tigernut. Additional advantage of the tigernut and moringa seeds based aqeous drinks can also be deduced from earlier investigations on human blood pressure which indicated that a diet rich in potassium and

magnesium but low in sodium can lead to a decrease in blood pressure within days of starting a specific diet [40].

Magnesium is also involved in numerous enzyme systems, including those involving the currency of energy in the body, ATP, as well as protein synthesis, energy production, and muscular contraction [36]. Low magnesium consumption may raise the risk of coronary heart disease [41] and type 2 diabetes [42].The high content of key minerals such as magnesium, potassium, calcium, sodium, and phosphorus in the fortified aqueous drinks shows that fortification of tiger nut milk will aid in the reduction of micronutrient shortage in humans. In agreement with earlier studies by Osagie*et al*. [43], tiger nut milk fortified with moringa seed could be consumed by both children and adults (including pregnant and breastfeeding mothers) due to its high energy content and preventive or protective nutrients. These nutrients may contribute considerably to the body's metabolic activities while also rejuvenating the body.

The iron (Fe) content of tigernut and moringa seeds aqueous drinks significantly (*P>0.05*) increased fortification. Iron helps in preventing anaemia. Iron is a functional component of haemoglobin as well as other important molecules involved in respiration, immunological function, and cognitive development [22]. The higher iron content found in this present study for tigernut and moringa seeds based aqueous drinks were higher than the values earlier reported by Ndubuisi*et al.*[21]. Iron absorption is aided by vitamin C. Vitamin C is a reducing agent that converts iron into a more absorbable form. An acidic environment also aids in Fe absorption.

Iron aids in the prevention of anaemia. It participates in several enzyme processes, including those that generate energy from carbohydrate, fat, and protein. It also plays a function in cell division, carbon dioxide and oxygen transport in the blood, and immunity [36].

Hence the formulated tigernut and moringa seeds based aqueous drinks iron content are adequate to meet the daily needs recommended by FAO/WHO/UNU [22] for children, adolescents and expectant mothers.

As shown in the results, the iodine value in tigernut and moringa seeds based aqueous drinks ranged from 0.00 – 0.15 mg/100 g, there was a significant (*P>0.0*5) increase in the iodine content of the drink, the range is in agreement with the report by Mendez *et al.* [44]. Iodine is a mineral contained in some foods that the body need in order to produce thyroid hormones. This hormone regulates the body's metabolism as well as many other vital activities.Thyroid hormones are also required by the body for appropriate bone and brain development during pregnancy and infancy. For healthy people (over the age of 18), the dietary reference value (DRV) is 150µg of iodine per day. During pregnancy and lactation, requirements might reach 200µg per day [45]. The tigernut and moringa seeds based aqueous drinks (0.15mg/100g) are adequate to provide the dietary reference value for pregnant women and infants as recommended by [45].

3.3 Vitamins composition

Table 3 shows the selected vitamins contents of the aqueous drinks. From the results, it can be observed that fortification significantly (*P>0.05*) increased the pro vitamin A and vitamin C contents.

Table 3: Vitamins composition of formulated tigernut and moringa seeds based aqueous drink

Vitamin (mg/100g)	Sample		
	PTMD	**TMSCD**	**TMSCFD**
K	$15.10^{b} \pm 0.59$	$14.10^{a} \pm 0.55$	$14.20^{a} \pm 0.77$

B1	$19.30^{b} \pm 0.07$	$20.70^{c} \pm 0.05$	$20.90^{c} \pm 0.00$
C	$28.80^{b} \pm 0.50$	$26.90^{b} \pm 0.10$	$26.50^{a} \pm 0.32$
Retinol Palmitate	$0.94^{a} \pm 0.60$	$0.99^{a} \pm 0.01$	$2.48^{b} \pm 0.06$

Results are Means ± standard deviation of triplicate determination. Values along each row with common superscripts are not significantly different at p>0.05

Key:

PTMD= Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks

TMSCD = Tigernut and moringa seeds aqueous extract plus 2% Sugar and 0.2% citric acid

TMSCFD = Tigernut (90%) and moringa seeds (10%) based aqueous drink with 2% sugar + 0.2% citric acid and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinol palmitate each/100g sample

Some selected vitamin contents of the aqueous drinks were determined. From the results, it can be seen as expected that fortification increased the pro vitamin A content of the formulation while the vitamin C decreased with fortification. The vitamin C value of the formulated tigernut and moringa seeds based aqueous drink ranged from 28.83- 26.5 mg/100 g. The lower vitamin c content for the fortified drink could be attributed to the dilution effects of the chemical hurdles and fortificants. However, if children aged 4 to 9 years old consume 100 g of tigernut and moringa seeds per day, the vitamin C level will be adequate, delivering 88 - 100% of their recommended dietary requirement as recommended by FAO/WHO/UNU, [22]. Moringa seeds are rich in vitamin C which when blended with tigernut milk will play integral roles in a multitude of physiological processes such as, vision, bone health, immune function and coagulation as reported earlier by Willy *et al*. [27]. Vitamin C is required for the formation of collagen, hormones, and neurotransmitters. 100 g of tigernut milk could also cover 77% of teenagers' daily vitamin C needs, 69% of adults' needs, and 52% of expecting mothers' needs. In addition,

the high vitamin C level in tigernut and moringa seeds aqueous drink will aid to make the iron content soluble and more available as earlier reported by MAFF, [46] for moringa seeds drink. The formulated product from this present study therefore will be adequate in providing the vitamin c needed for the prevention of cardiovascular disease and cancer [47].

Tigernut and moringa seeds based aqeous drinks pro- vitamin A and vitamin C concentration could serve as a useful source of antioxidant and it is recommended for newborns and the elderly due to its high vitamin content and anti-oxidant advantages in the cell membrane [48]. Vitamin C has anti-infective qualities, improves wound healing, may stimulate the immune system, and aids in the prevention of infections, whilst vitamin A aids in the maintenance of excellent vision and eye health, supports skin cell health, it also supports cell growth, immune function, fetal development and the prevention of certain eye illnesses. From the results obtained from this present study there was a significant (*P>0.05*) increase in pro- vitamin A content due to the fortification of the drink with retinol palmitate, despite the fact that tigernut milk is not a good source of vitamin A, [1],however, due to vitamin A fortification and the inclusion of moringa seeds to the tigernut milk, the vitamin A content increased from 0.94 – 2.48mg/100g. Since 0.3microgram of retinol is equivalent to 1 iu (International Unit) then the 2.48mg/100g retinol/100 g translate to about 8,266.6 iu which is adequate for human nutrition.

The vitamin K (Phytonadione and Menaquinones): Moringa seeds and tigernut are high in vitamin K. Vitamin K value in the drink ranged from 14.05 - 15.08 mg/100 g, which is within the FDA's recommended daily intake levels. Vitamin K is needed by the body for blood clothing, bone development and it can also reduce risk of fractures, blood loss in the event of an injury; however, a lack of it can cause bleeding, increases calcium deposition which can lead to coronary artery calcification and the development of heart disease [28]. Although vitamin K insufficiency is uncommon, it can have long-term effects on the body. A daily dose of 120mcg of vitamin K is usually sufficient for adult males and less for females and children [49]. Hence

indicating that the vitamin k content in the formulated product from this study are adequate for human nutrition purposes.

Vitamin B1 (thiamine) levels in tigernut and moringa seeds based aqueous drinks were determined to be 19.27 - 20.86 mg/100 g, which is required for body cells to operate normally. Vitamin B aids the body in the conversion of food into energy (metabolism), the formation of new blood cells, and the maintenance of healthy skin cells, brain cells, and other physiological components. B vitamins are frequently found together in the same foods. Many people can obtain adequate amounts of B vitamins by consuming a range of nutrient-dense meals. People who do not obtain enough B vitamins in their diets may develop B deficiencies. The daily value for vitamin B 1 (thiamin) is 1.2 mg for adults and children aged 4 and up, and 1.4mg for pregnant and lactating mothers as recommended by FAO/WHO/UN, [22]. Hence indicating that the values from the products satisfy the requirements. The body requires vitamin B (thiamin) to break down sugar (carbohydrate) molecules from diet, produce neurotransmitters, fatty acids, and synthesise some hormones [36].

3.4 Antinutrients and Thiobabuturic acid

Table 4 shows the antinutrient and Thiobabuturic Acid (TBA) composition of formulated tigernut and moringa seeds based aqueous drinks.

The chemical indices of quality evaluated were antinutrients (Tannins, Phytate and Saponins), then pH, titratable acidity and TBA in addition to specific gravity of the drinks as influenced by the chemical hurdles and fortificants from Table 4.

Physical qualities: the specific gravity of the aqueous drinks was as presented in Table 4 also.

Table 4: Chemical indices of formulated tigernut and moringa seeds based aqueous drink.

Parameter	Sample		
	PTMD	TMSCD	TMSCFD
TANNINS (mg/100g)	5.30^{c} ±0.00	5.28^{b}±0.08	4.98^{a}±0.03
PHYTATES (mg/100g)	0.44^{b}±0.05	0.43^{a}±0.00	0.41^{a}±0.06
SAPONNINS (mg/100g)	3.90^{c}±0.00	3.89^{b} ±0.00	3.88^{a}±0.00
pH	6.80^{b} ±0.01	6.50^{b} ± 0.03	6.30^{a} ± 0.04
TTA (% lactic acid)	0.66^{a} ±0.07	0.63^{a} ± 0.28	0.62^{b} ± 0.02
TBA (mg MDA)/kg)	0.43^{a}±0.00	0.43^{a}±0.01	0.55^{b}±0.03
Sp. Gr	1.64^{a} ±0.09	1.84^{a} ± 0.07	1.89^{b} ± 0.01

Results are Mean ± standard deviation of triplicate determination. Values along each row with common superscripts are not significantly different at p>0.05

Key:

PTMD= Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks

TMSCD = Tigernut and moringa seeds aqueous extract plus 2% Sugar and 0.2% citric acid

TMSCFD = Tigernut (90%) and moringa seeds (10%) based aqueous drink with 2% sugar + 0.2% citric acid and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinol palmitate each/100 g sample

TTA: Titratable Acidity

The chemical indices of quality evaluated were antinutrients (Tannins, Phytate and Saponnins), then pH, titratable acidity and TBA in addition to specific gravity of the drinks as influenced by the chemical hurdles and fortificants. Tannins are a type of antioxidant polyphenol that may

hinder food digestion. Tannins bind to proteins, making them harder to digest in the body [50]. Although soaking, boiling, and other heat treatments can decrease the antinutrient content of tigernuts[50]. Tigernut and moringa seeds based aqueous drinks contained 4.98 mg/100 g - 5.30 mg/100 g of tannins, the fortified product had the lowest content of about 4.98 mg/100 g, the results of tannins in this investigation were higher than those published by Chuckwuma*et al.* [51] but lower than those reported by Wayah*et al*. [52] for tigernut residue, while still within the acceptable range. The tannin content in this study could be as a result of the moringa seeds that were incorporated in the drink, the lower content of tannin in this study when compared with other reports could be as a result of the treatments given to the moringa seeds such as drying and pasteurization of the drinks during processing as reported by earlier researchers such as [51] and [52] for other related products.

Saponin reduces the absorption of some nutrients such as glucose and cholesterol in the gut via an intraluminal physicochemical interaction [53]. Tigernut and moringa seeds aqueous based drinks contained 3.70 mg/100 g - 3.90 mg/100 g, the quantity of saponin in the tigernut and moringa seeds drink was within the FAO/WHO approved limit. The interaction of soaking, grinding, and boiling (pasteurisation) with other chemical processes in the grain may explain the decrease in anti-nutrients in the drink. The anti-nutritional components of the drinks were significantly lowered due to the pretreatments and hence may not interfere with protein and minerals metabolisms in accordance with earlier report by Obizoba*et al*.[54]. Processing procedures increases the nutritional quality of legumes and grains by altering their chemical composition and removing anti-nutritional components.As a result, processing could diminish the antinutrient content of raw tigernuts and moringa seeds. Antinutrient levels were minimal when compared to those found in nuts such as peanuts [55]. The phytochemical composition in this study for phytate were lower than the results reported by Waya*et al*. [52], which could be

due to the heat treatment given to the drink during processing, but it was within the same range as Obioma*et al*. [56].

Phytates are mostly present in seeds, grains, and legumes and they limit mineral absorption from a meal[51].Phytic acid may have a number of beneficial health effects, including protection against oxidative damage and insulin resistance. Phytates content tigernut and moringa seeds based aqueous drinks was 0.41 mg/100 g - 0.44 mg/100 g. The presence of phytates in biological systems may bind divalent metals such as calcium and magnesium, or it may inhibit the absorption of important minerals in the gastrointestinal tract [57] thus decreasing their bioavailability [58]. Phytate chelates with mineral elements, having a major impact on mineral utilization, although this is dependent on the amount of phytate in the dietary product. They also react with basic protein residues [57]. Wise and Gilbert [59] reported that phytate as a calcium salt can protect against dietary Pb^{2+} in experimental animals and human volunteers, hence phytate has the ability to counteract acute aral Pb^{2+} toxicity. Phytic acid at levels of 0.44 mg/100g as obtained from the formulated product may protect against a fatty liver resulting from elevated lipogenesis and that the anti- nutrient effect of phytic acid on mineral absorption will only occur at 10 fold higher levels which is in accordance with earlier reported by Gilbert [59]. Phytates should be lowered as much as as possible for best health benefits, ideally to 25mg per 100g or to about 0.035% - 0.5% of phytate containing food eaten [60]. The phytate content in this study was within the acceptable levels by other researchers and [61].Tigernut and moringa's health advantages are attributed to the presence of many phytochemicals with antioxidant potential as reported by Willis *et al*. [62]. Based on their chemical composition, tigernut drinks can be employed as functional foods.[63]Tigernut consumption increases antioxidant processes and may reduce the incidence of obesity and diabetes due to its ability to inhibit -amylase and lipase [64].

The phytochemicals in tigernut milk have been shown to protect rats from drug-induced liver damage by either increasing glutathione formation or acting as antioxidants [65]. The anti-cancer benefits of tigernut'squercetin and beta-sitosterol are well recognised[66].

The result for pH and titratable acidity of formulated tigernut milk and moringa seeds based aqueous drinks showed an inverse relationship as expected. Hence the higher the pH the lower the titratable acidity which is in agreement with earlier reports by Ogbonna*et al.* [67]. The hurdles treated and fortified product (TMSCFD) had the lowest pH and and hence higher titratable acidity which indicated that, the hurdles treatment contributed to the low pH which was good for the drink since earlier report by several researchers [67] have indicated that low acidity foods inhibit toxigenic organisms and promote minimal heat treatment for commercial sterilization. Tigernut and moringa seeds aqueous drinks pH and acidity is capable of extending its shelf life because most bacteria do not thrive in acidic conditions. It also makes growth unfavourable for most food poisoning mesophilic microbes [68]. In most circumstances, acidity inhibits the growth of bacteria [67].

Thiobarbituric acid: Thiobarbituric acid test measures malonaldehyde (MDA) produced due to the oxidation of fatty acids with three or more double bonds and it measures other TBA reactive substances such as 2- alkenals and 2,4 alkadienals. Tigernut and moringa seeds based aqueous drinks contained 0.43 mg/kg - 0.55 mg/kg, from the result obtained in this study, the values were lower than the values reported by Akama*et al.* [69] fortigernut milk. The lower the TBA value, the lower the oxidative state of the product. The lower TBA values obtained from the formulated Tigernut and moringa seeds drink could be attributed to the stabilizing effects of the chemical hurdles as reported by earlier by Djikeng*et al.* [70] for chemical hurdles.

3.4 Physical properties

3.4.1 Viscometry

Generally, viscosity decreased with shear rate and temperature in all the products respectively. The power law regression parameters are shown in Table 5 for PTMD, TMSCD and TMSCFD respectively. From the results it can be observed that, the flow behaviour indices were all >1, indicating pseudoplastic flow behaviour for the drinks.

Table 5: Power law regression parameters for influence of shear rate on viscosity of tigernut milk + moringa seed aqueous drinks

Sample	Regression parameter	Temperature (^{0}C)					
		15	**20**	**25**	**30**	**35**	**40**
	N	5	5	5	5	5	5
	r^2	0.970	0.996	0.994	0.991	0.995	0.904
PTMD	Slope	-0.329	-0.344	-0.301	-0.333	-0.331	-0.326
	∴ n	0.67	0.66	0.68	0.67	0.67	0.67
	Intercept	-0.747	-0.718	-0.825	-0.843	-0.955	-1.157
	∴ m	0.47	0.49	0.42	0.41	0.39	0.31
	N	5	5	5	5	5	5
	r^2	0.985	0.978	0.979	0.975	0.952	0.896
TMSCD	Slope	-0.347	-0.285	-0.325	-0.280	-0.262	-0.209
	∴ n	0.65	0.71	0.68	0.72	0.74	0.79
	Intercept	-0.885	-1.212	-1.172	-1.575	-1.718	-2.056
	∴ m	0.41	0.30	0.31	0.21	0.18	0.13
	N	5	5	5	5	5	5
	r^2	0.995	0.996	0.991	0.992	0.997	0.999
TMSCFD	Slope	-0.521	-0.546	-0.526	-0.589	-0.611	-0.618
	∴ n	0.48	0.45	0.47	0.41	0.39	0.39

Intercept	0.817	0.890	0.798	0.801	0.815	0.786
∴ m	2.26	2.45	2.22	2.23	2.26	2.19

Key:

PTMD= Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks

TMSCD = Tigernut and moringa seeds aqueous extract plus 2% Sugar and 0.2% citric acid

TMSCFD = Tigernut (90%) and moringa seeds (10%) based aqueous drink with 2% sugar + 0.2% citric acid and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinol palmitate each/100g sample; n = Number of points; r2 = Coefficient regression; n = Flow behaviour index; m = Consistency index (Ns/mn)

The changes in viscosity with shear rate for the aqueous drinks generally indicated that, viscosity decreased with shear rate and temperature in all the products. It can be observed from this study that, the flow behaviour indices were all >1, indicating pseudoplastic flow behaviour for the drinks. The activation energy (E_a) values which showed a definite trend suggesting that the citric acid and sugar that were used as hurdles have a stabilizing effect on the molecular orientations and alignments, this observation is in agreement with Koksoy*et al*. [71].

The decrease in viscosity with increasing shear rate of aqueous beverages was in line with earlier report by Lokumco*et al*. [72]. The consistency index (m) indicated little variation with temperature with no definite trend. Viscosity is described as the resistance to flow and deformation, such a freer interaction is expected to minimize the resistance. The Arrhenius-type of equation has been used to assess the temperature sensitivity and dependency of foods systems [73]; [74]. The Arrhenius equation is suitable for describing the relationship between temperature and flow behaviour index (n).

Table 6: Temperature dependency of flow of formulated tigernut milk and moringa seeds aqueous drinks at different shear rates

Regression Parameter	PTMD	TMSCD	TMSCFD
r^2	≥0.989	≥0.997	≥0.972
E_a(kJ/mol)	8.9-15.7	16.5-27.4	6.8-19.7

Key:

r2 = Coefficient regression; *Ea* = Activation energy;

PTMD = Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks;

TMSCD = Tigernut and moringa seeds aqueous extract plus 2% Sugar and 0.2% citric acid;

TMSCFD = Tigernut (90%) and moringa seeds (10%) based aqueous drink with 2% sugar + 0.2% citric acid and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinol palmitate each/100g sample

3.4.2 Specific Gravity

Specific gravity is a dimensionless quantity which relates the ratio of the density of a material to that of pure water at the same temperature.Materials with a specific gravity less than one are less dense than water and will float on it; substances with a specific gravity greater than one are denser than water and will sink.The specific gravity of the tigernutand moringa seeds drink increased with the incorporation of sugar, citric acid and fortificants. The higher values for the formulated could then be attributed to the composition of the formulated products which contained dissolved solids in addition to water. The values obtained in this study especially the fortified sample falls within the range reported by FAO, [75].

The temperature dependency of flow of tigernut and moringa seeds based aqueous drinks activation energy (E_a) ranged from 8.9-15.7-10.2-12.7 and 9.9 (kj/mol) within values rpms of 5-100 for (PTMD), showed the instability of the product molecules due to alignment, realignment and disruption of molecular orientation of the constituents with changes in shear rates, values ranging from 27.4 - 16.5 (kj/mol) for (TMSCD) of the activation energy (E_a) showed a definite

trend suggesting that the citric acid and sugar that were used as hurdles have a stabilizing effect on the molecular orientations and alignments. It also showed that less energy input is required to initiate flow than in the plain tigernut and moringa seeds based aqueous drinks (PTMD) as shear rate increases.

For TMSCFD, the activation energy values ranged from 6.8-19.7 (kj/mol) this indicated that, the energy required to initiate flow increases with increase in shear rate. The molecular spices have in definite and stable orientation. This stable orientation can be attributed to the effect of the additives especially the fortificants. In terms of pumping requirements, the hurdles and fortificants treated products will require less energy for transporting the product from one point to another in scale up operations.

Conclusion

Triple fortification of the formulated tigernut and moringa seeds aqueous drinks with potassium iodide, ferrous sulphate and retinol palmitate improved the physico-chemical qualities of the drink, making it a good vehicle for micronutrient fortification which can be used to address Protein Energy Malnutrition and Micronutrient Deficiency (PEM and MND)

Applications of citric acid and sugar as chemical hurdles to formulated tigernut and moringa seeds aqueous drinks extended the shelf life of the drink and improve the keeping quality of the drink by eliminating all the pathogenic micro-organisms that are capable of causing deterioration of the product.

The pH and acidity values obtained in this study are capable of extending shelf life of the product.

Organoleptically acceptable tigernut and moringa seeds based aqeous drink can be produced using 90 tigernut milk and 10% moringa seeds.

Recommendation

Tigernut and moringa seeds based aqueous drinks should be considered in the National Food Security Programs for combating PEM and as vehicle for micronutrient fortification.

Data obtained from the studies are recommended as base- line indices for scale up production and regulatory purposes of tigernut and moringa seeds based aqueous drinks.

Further work is recommended to ascertain the bio–availability of the fortificants especially through haematological and bio- utilization aspects.

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