Original Research Article

STORAGE CHANGES IN TRIPLE FORTIFIED TIGERNUT AND MORINGA SEEDBASED AQUEOUS DRINKS

ABSTRACT

The study investigated storage changes in triple fortified tigernut and moringa seed based aqueous drinks. During accelerated shelf lifetesting, vitamins C, A, pH, TVB and microbial analysis were determined using standard procedures.From the data obtained, storage life of the drink decreased significantly ($p<0.05$) with temperature and varied from 10-4 weeks, 30-5 weeks and 34-7 weeks within 10-35^{o}C for Plain Tigernut*Moringa* Drink(PTMD), Tigernut*Moringa* seeds plus sugar and Citric AcidDrink(TMSCD)and Tigernut MoringaSeeds plus sugarand citridacid FortifiedDrink (TMSCFD)respectively.TMSCD and TMSCFD significantly ($p<0.05$) lowered microbial growth (<50 cfu/g) as compared toPTMD (1.0×10 cfu/g} after three months of ambient storage. The pH, vitamin C, and retinol palmitate decreased while Total volatile bases (TVB) increased during storage, the reaction rate constant (k) also increased significantly ($p>0.05$) with temperature ranging from 0.2521-0.6343 (wk^{-1}) for PTMD, 0.0818-0.4826, and 0.0924-0.2792 (wk^{-1}) at 40- 70 ^{0}C for TMSCD and TMSCFD for vitamin c and 0.16-0.34 for PTMD, 0.12-0.30 and 0.05-0.23(wk^{-1}) at 40- 70 ^{0}C for pHand 0.0553- 0.179 (wk^{-1}) for retinol palmitatewith critical values of 4.81-1.14 mg/100g, 1.49 mg/100g, 7.3-6.5 and 0.88-2.16 gN/100g respectively. This indicates that PTMD stability is improved by use of the chemical hurdles and also it is an appropriate vehicle for iodine, iron and pro-

vitamin A fortification and protein energy malnutrition (PEM) intervention programmes.

Keywords:Tigernut, Moringa, fortification, storage changes, aqueous drinks, citric acid, sugar

1.0 INTRODUCTION

Tigernut is a staple food for some African tribes. It is regularly collected and eaten by children. Since ancient times, it is cultivated for its small tuberous rhizomes, which are eaten raw or roasted, used as hog fodder, or pressed for juice to make a beverage [1].It can be used to produce delectable cakes and biscuits, as well as to enhance the tastes of fruits. Tigernut and its extract could be used with wheat flour and local flours to produce baked items and gruels [1].

Moringa oleifera (Moringaceae) is a member of the Moringa genus known by many different names around the world, including horseradish tree, drumstick tree, "Guiligandja," "Gagawandalahai," and many others [2]. It is grown in all tropical and subtropical regions, including Pakistan, Arabia, Central America,

the North and South Philippines, Cambodia, the Caribbean Islands, and Africa [2]. Many parts of the plant have pharmacological properties that have been recognized by popular use and confirmed by scientific research. *Moringa oleifera* seeds have antimicrobial properties against fungi and bacteria, antitumor and anti-inflammatory properties [3].

Food fortification, as defined byOlson[4], is the practise of adding micronutrients to regularly consumed foods during processing in order to boost their nutritional value. It's a tried-and-true, risk-free, and low-cost technique for improving diets and avoiding and treating micronutrient deficiencies.

Hurdle Technology is a food processing technique that uses various processing methods to totally eradicate all pathogens in food items, resulting in safer food products with a longer shelf life [5]. This strategy integrates a number of measures (hurdles) to assure microbiological safety and stability, as well as organoleptic and nutritional quality, and economic viability of food items [6].As a result, aqueous extracts of tiger nuts and moringa seeds could supply appropriate nutrients for addressing Protein Energy Malnutrition (PEM) while also functioning as a vehicle for triple fortification for addressing Micronutrient Deficiency (MND).

There is high perishability of the moringa seed based aqueous drinksunder tropical ambient conditions. However,there are limited formulations that act as vehicles for triple food fortification. There is underutilization of non-conventional plant food sources, poor processing and handling of local beverages and low protein quality of local beverages including *kunu-aya*.Use of chemical hurdles such as citric acid and sugar will improve storability and availability of the drinks and will also prevent growth of potential pathogens especially if unknowingly contaminated from utensils and process water. Such product could act as vehicle for multi- fortificatication for vitamin A, iron and iodine. However, there is scanty of information in the suitability of tiger nut and moringa seeds aqueous extracts as acceptable formulations for addressing PEM and MND. Therefore there is the need for investigations of tigernut and moringa seeds based aqueous drinks for addressing the problems highlighted above.

2.0 MATERIALS AND METHODS

2.1 Sources of Raw Materials

Dried yellow tigernut, sugar and muslin cloth were purchased in Wadatamarket, Makurdi. Moringa seeds were purchased at a *moringa* farm opposite International Market, Makurdi.Airtight containers, sample bottles, transparent cups and blender were purchased in Modern Market, Makurdi, Benue State. Citric Acid, pro- vitamin A, ion, iodine fortificants were purchased 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 Preparation

Preliminary affective and descriptive sensory evaluation of *moringa* seed based aqueous drinksconsisting of 90%tigernut milk and 10 % moringa seeds treated with 2% sugar and 0.2% citric acid were the most acceptable and hence were used in this study. The product was divided in to three sub-lots comprising the plain tigernut moringa seeds drink (PTMD) tigernut and moringa seeds plus 2% sugar and 0.2% citric acid (TMSCD) andtigernut and moringa seeds plus 2% sugar and 0.2% citric acid and 0.15 mg KI, 2.0mg $FeSO_4$ and1.6mg retinol palmitate/100g each (TMSCFD)as recommended byFood fortification regulations[7] and were then subjected to ambient (30±2^{o}C) and accelerated storage test.The flow chat of the aqueous drink is as shown in Figure 1.

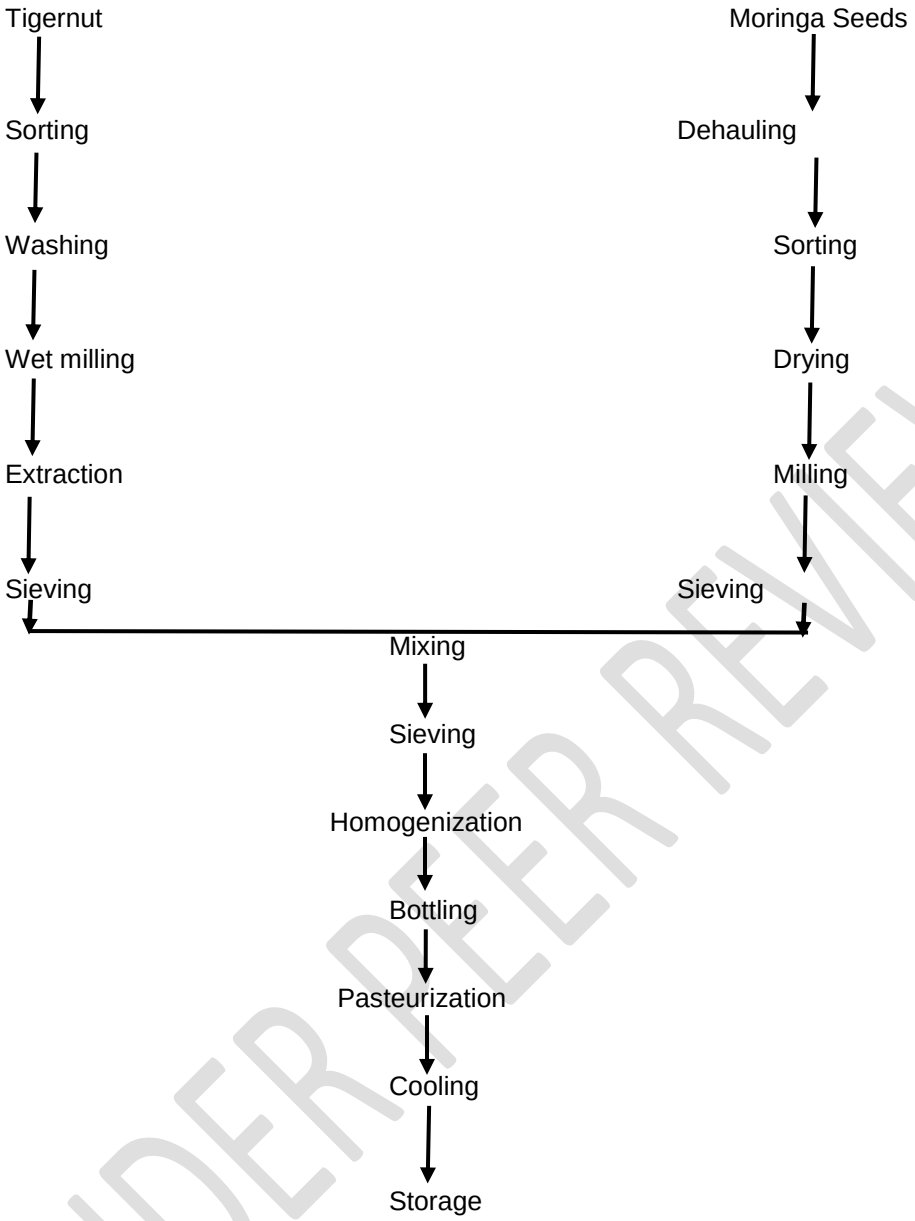

Figure 1: Flow diagram for the production of tigernut milk and moringa sed-based aqueous extract. Source: [8].

2.3 Normal Storage Tests

Microbiology: Total plate count, coliforms, *E. coli*, *Bacillus sibtilis* ,*Staphylococusaerus*, Yeast and moulds were evaluated within four weeks of storage at (30±2^{o}C) as described byGarbutt, [9].

2.4Accelerated shelf storage

The most direct way to estimate the shelf life of a product is to conduct simulation tests which are time consuming and expensive. Conversely, accelerated shelf-life tests can be successfully used for stable

products having long expected shelf life. The following temperatures 40^{o}C, 50^{o}C, 60 ^{o}C and 70 ^{o}C were used for the accelerated shelf storage for the fortified samples, non- fortified, non- fortified and non-hurdle samples respectively. Data obtained from accelerated storage tests were evaluated using zero and first order reaction kinetics.

2.4.1Vitamins Analysis

Vitamin C was determined using 2, 4 dinitrophenyl hydrazine method as described byBall [10].

Pro-Vitamin A determination: The pro- vitamin A content of the formulated products was analysed using high performance liquid Chromatography (HPLC) as described byRutkowsi, [11].

2.4.2 Total Volatile Bases

Total Volatile Base is related to protein for breakdown and includes NH_3, indole, skatole, mercaptan and a distillation apparatus was set-up. 10 g of sample + 2 g MgO + 300 ml tap water was added to the sample. 25 ml of 2% boric acid solution + 3 drops of screened methyl red indicator was added to receiving flask. The apparatus was connected with receiver tube dipping below the boric acid solution. The distilling flask was heated and the liquid boiled in exactly 10 mins and using the same heating, it was distilled for exactly 25 min. The condenser was washed down with distilled water. The distillate was titrated with 0.1 M H_2SO_4 and multiplied (less blank) by 14 to obtain TVN as mg N/100 g samplePearson[12].

2.4.3 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.

2.4.4 Determination of Total Titratable Acidity (TTA)

Standard method ofAnthony, U. & Chandra[13] was used to measure the titratable acidity.

2.4.5Sensory Evaluation

A consistent panel of 25 judges were used for sensory evaluation. The panellists were chosen based on a preliminary testing of their perceptions of sweetness, sourness and bitterness as described byIhekoronye andNgoddy[14] using dilute solutions of fructose, vinegar and quinine ranging from extremely sweet to

slightly sweet, extremely sour to slightly sour and extremely bitter to slightly bitter respectively. Individuals with the right perceptions were then selected for sensory evaluation of the test products.

2.4.6 Statistical Analysis

The mean and standard deviation of the result data from the experiment was calculated and analysed 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 was used to determine the significant difference between mean values. Least significant difference (LSD) test was used for mean separation at 5% probability level of significance.

3.0 Results and Discussion

3.1 Storage changes in the aqueous drinks

Microbiological changes at ambient storage: the changes in the microbiological qualities during ambient storage (30±2 ^{o}C) over a period of four weeks of the aqueous drinks are shown in Table 1 for the plain drink (PTMD), for the hurdles treated drink (TMSCD) and for the hurdles and fortificants treated drink (TMSCFD). For the PTMD, total plate count ranged from 0 – 40 cfu/ml, the coliform, *E. coli,* yeast and molds were absent, *Bacillus subtilis* ranged from 0-20 cfu/ml while *Staphylococus aureus* ranged from 0-20 cfu/ml within zero to four weeks of storage. As can be seen in table 1 for TMSCD, the total plate count ranged from 0-20 cfu/ml, *Bacillus substilis* ranged from 0-20 cfu/ml while coliforms, *E. coli, Staphylocucus aureus* and yeast and molds were all absent within zero to four weeks of storage.

For the hurdles and fortificants treated drink the total plate count ranged from 0-20 cfu/ml, coliform, *E.Coli, Bacillus subtilis* and *Staphylococus aureus* were absent while yeast and molds ranged from 0-20 cfu/ml within four weeks of storage.

Table 1: Microbial Quality of formulated tiger nut and moringa seeds aqueous drink

Parameters	Microbial Count (cfu/ml)	Ambient storage time (wks^{-1})				
		0	**1**	**2**	**3**	**4**
	Total plate count	0	0.2×10^2	0.3×10^2	0.3×10^2	0.4×10^2
	Coliform	0	0	0	0	0
PTMD	*E. Coli* 0	0	0	0 0		
	Bacillus Spps 0	0	0.1×10^2	0.1×10^2	0.2×10^2	
	Staphylococus aureus 0	0.2×10^2	0.2×10^2	0.2×10^2	0.2×10^2	
	Yeast and moulds	0	0	0	0	0
	Total plate count	0	0	0	0.1×10^2	0.2×10^2
	Coliform	0	0	0	0	0
TMSD	*E. coli*0	0	0	0	0	
	*Bacillus sibtilis*0	0	0	0.1×10^2	0.2×10^2	
	*Staphylococcus aureus*0	0	0	0	0	
	Yeast and moulds0	0	0	0	0	
TMSCFD	Total plate count	0	0	0	0.1×10^2	0.2×10^2
	Coliform	0	0	0	0	0
	*E. coli*0	0	0	0	0	
	Bacillus sibtilis 0	0	0	0	0	
	*Staphylococus aureus*0	0	0	0	0	
	Yeast and moulds0	0	0	0.1×10^2	0.2×10^2	

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

The changes in the microbiological qualities during ambient storage (30±2 ^{o}C) indicated that total plate count was lower for the TMSCFD compared to the plain drink (PTMD). The lower values can be attributed to the inhibitory effects of the sugar and citric acid. The sugar increases the osmotic gradient

and pressure of the product while the citric acid lowers the pH and increases titratable acidity of liquid foods and therefore could explain the lower plate count for both the TMSCD and the TMSCFD. The survival of *Bacillus sp* which are spore formers especially in the plain tiger nut drink (PTMD) could be attributed to heat resistance of the micro-organisms while their suppression in the hurdle treated and fortified treated product could be due to the osmotic and acidity effect. Towards the end of the storage, yeast and molds were isolated in the fortified product this could be attributed to recovery of injured cells in the enriched medium, however the values were lower than 52 – 100 cfu/ml specified by the NIS for liquid beverages. Also the values for TMSCFD were within the acceptable range of the FAO/WHO standards for microbiological quality of milk and dairy products [15]. The presence of yeast and molds in the fortified sample also indicated that, the fortificants aided the injured micro-organisms to recover. It can therefore be postulated that the fortificants were also needed by micro-organisms. Montville and Mathew [16] stated that all pasteurised milk samples within this range are good. This also suggests that the pasteurisation temperatures and barriers used in this investigation were adequate for microbial elimination and the aqueous drinks shelf life extension. According to [14], samples with 0.4×10^{3}cfu/ml of bacteria are good for consumption, samples with 0.5×10^{5} are fairly good and manageable, samples with over 2.00×10 are bad for consumption, and samples with higher bacteria load are also considered unsafe for consumption, therefore, the larger the microbial load, the more unsafe and susceptible the sample is to spoilage [17]. These findings are also consistent with the findings of Onovo and Ogaraku [18], who stated that heat treatment, proper storage, and heat processing have the potential to suppress microbial growth and are sufficient to kill any type of microbe. The levels of microbial growth at ambient temperature were within the Codex Alimentation Commission's standard of acceptance for dairy milk, which is 2.0×10^{5}cfu/ml [19]. It was also consistent with the findings of Abubakar *et al.*[20], who found that adding preservatives during processing had a substantial effect on the chemical properties of tigernut milk samples. During storage, the samples which had no preservative and were stored at room temperature dropped significantly in quality after 2 days, whereas the preserved samples without pasteurization deteriorated significantly ($P<0.05$) in quality on the first week, while the preserved samples that received pasteurization were found to stay more than a week with fair quality. At the third week, all samples went below the permissible range, according to Abubakar *et al*. [20].

3.2 Accelerated storage test

The accelerated storage test was at 40 ^{o}C, 50 ^{o}C, 60 ^{o}C and 70 ^{o}C respectively for the test products. The data obtained were best described by zero order ($r^2 \geq 0.998$) reaction kinetics. The predicted shelf life at ambient (10-35 ^{o}C) using the zero order kinetics are presented in Table 2. From Table 2 it can be observed as expected that the predicted shelf life increased with decrease in storage temperature and ranged from 10-4wk^{-1} for PTMD, 30-5wk^{-1} for TMSCD and 34-7wk^{-1} for TMSCFD within 10- 35 ^{o}C respectively.

Table 2: Predicted parameters based on general acceptability of formulated tigernut milk and moringa seeds-based aqueous drinks

Temperature (^{o}C)	Parameter	Sample		
		PTMD	TMSCD	TMSCFD
10	k (wk^{-1})	0.2986	0.0993	0.0871
	t_s (wk)	10	30	34
20	k (wk^{-1})	0.4329	0.1988	0.1699
	t_s (wk)	7	15	18
25	k (wk^{-1})	0.5213	0.2810	0.2372
	t_s (wk)	6	11	13
30	k (wk^{-1})	0.6278	0.3975	0.3313
	t_s (wk)	5	8	9
35	k (wk^{-1})	0.7560	0.5621	0.4627
	t_s (wk)	4	5	7

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; k: Reaction rate constant (wk-1)

3.2.1 General Acceptability

General acceptability decreased with storage time and were lower at higher temperatures and on a nine-point Hedonic scale within four weeks of storage for all the products. It can be observed from the results that, the overall acceptability of all the products decreased with an increase in storage time and temperature, the decrease was highest for the plain product and was lowest for the hurdle treated and

fortified product which is in line with earlier report by Ochemee*t al*. [21], who reported a decrease in sensory attributes of tigernut coffee with an increase in temperature and storage period. The lowest decrease in general acceptability of the product with hurdles and fortificants indicated that the hurdles and fortificants used had significant effects on the overall quality of the drink.

The zero order regression parameters for changes in general acceptability showed high reaction rates at high temperatures with storage time, for all the products.The predicted parameters were generated for all the quality indices by fitting k values in the order of reactions equation using 6 as the acceptability outgoing quality level for general acceptability to determine the shelf life of the drink at lower storage temperatures of 10- 35^{o}C. It can be observed as expected that the predicted shelf life increased with decrease in storage temperature which recorded the longest shelf life of 34 weeks for the fortified products and 10 weeks as the lowest shelf life for the plain drink, indicating that the preservatives and the fortificants that were used were effective in extending the product shelf life. The results also indicated that the plain drink was the least stable while the fortified drink was the most stable organoleptically during storage.

The data obtained for overall acceptability, vitamin C, TVB, pH and Retinol palmitate degradation were best described by Zero and first order reaction kinetics (r^2≥0.998).The regression parameters are presented in Table 3. From the results it can be observed that overall acceptability, vitaminC, TVB, pH and Retinol palmitate degradation rate constant increased with temperature and were highest (1.31-1.83 wk^{-1}) forTMSCD and lowest (1.19 – 1.59 wk^{-1}) forTMSCFD. For vitamin C degradation the data obtained was highest (0.2521- 0.6343 wk^{-1}) for PTMD, and lowest (0.0924-0.2792 wk^{-1}) for TMSCFD. For TVB, the data obtained was highest (0.1196 – 0.2202) for PTMD and lowest (0.1374 – 0.17.17) within four weeks of accelerated storage at 40- 70 ^{o}C respectively. For pH, data shows that the highest (0.16 – 0.34) was for PTMD and lowest (0.05-0.23) was for TMSCFD.

Table 3: Zero and first Order Regression Parameters for change in quality indices of formulated tigernut milk and moringa seeds-based aqueous drinks

Quality Indices	Parameter	PTMD	TMSCD	TMSCFD
Overall	r^2	≥0.985	≥0.993	≥0.965
acceptability	k	1.34-1.65	1.31-1.83	1.19 – 1.59

Vitamin C	r^2	≥0.995	≥0.997	≥0.991
	k	0.2521-0.6343	0.0818 -0.4826	0.0924-0.2792
TVB	r^2	≥0.974	≥0.962	≥0.991
	k	0.1196-0.2202	0.1374-0.1717	0.1424-0.1852
pH	r^2	≥0.997	≥0.999	≥0.998
	k	0.16 -0.34	0.12 -0.30	0.05 -0.23
Retinol palmitate	r^2	≥	≥	≥0.969
	k	-	-	0.179

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; k = Reaction rate constant (wk-1); r2 = Regression coefficient

The zero order regression parameters for changes in general acceptability showed high reaction rates at high temperatures with storage time, for all the products. The predicted parameters were generated for all the quality indices by fitting k values in the order of reactions equation using 6 as the acceptability outgoing quality level for general acceptability to determine the shelf life of the drink at lower storage temperatures of 10- 35^{o}C. It can be observed as expected that the predicted shelf life increased with decrease in storage temperature which recorded the longest shelf life of 34 weeks for the fortified products and 10 weeks as the lowest shelf life for the plain drink,indicating that the preservatives and the fortificants that were used were effective in extending the product shelf life. The results also indicated that the plain drink was the least stable while the fortified drink was the most stable organoleptic ally during storage.

The first order regression parameters for the degradation of vitamin C in tigernut and *moringa* seeds based aqueous drinkat the various storage temperatures and time conditions were studied with high r^2 values of (0.968-0.991) conversely. The coefficients were much higher than zero order and fitted better; the reaction rates (k) of tigernut and *moringa* seeds based aqueous drinks were higher at high temperatures and lower at low temperatures which was in line with the report by [22].

The predicted vitamin c reaction rate constant at ambient storage of the formulated tigernut and moringa seeds based aqueous drinks, showed that vitamin c degradation rate constant increased with temperature and were highest for plain drink and lowest for fortified drink within four weeks of accelerated storage at 10- 35 ^{o}C respectively. The best retention of vitamin c content was at 10 ^{o}C, this was in agreement with earlier report by Abbasi *et al*. [23].

The data obtained for changes in TVB during storage were best fitted with first order reaction kinetics ($r^2 \geq 0.97$) with the reaction rate constants. The results indicated that there was a high reaction rate at high temperatures due to protein breakdown into non protein nitrogens (NPN) such as NH_3, H_2S, Mecaptan, skatole and indole. This is also caused as a result of spoilage microorganisms which during storage convert many nitrogenous compounds into volatile bases (Kirk *et al*., 1991). Sara *et al*. (2021) reported that, the volatile nitrogen compounds produced during longer storage period is due to the result of destructive activities of microorganisms and chemical interactions especially at elevated temperatures and are considered as one of the most important freshness indicators to monitoring the quality and safety of food products.

The predicted TVB reaction rate constant at ambient storage of the aqueous drinks, indicated that the reaction rates in TVB were low at low temperatures and increased as the temperatures increased although the reaction rates were relatively low compare to the rates at elevated temperatures.

The zero order regression parameters for changes in pH in the formulated tigernut and moringa seeds based aqueous drinks indicated that the reaction rate was high at higher temperatures and low at lower temperatures, although the fortified drink had the lowest reaction rate.

The regression parameters for retinol palmitate degradation of formulated tigernut and moringa seeds based aqueous drinks indicated that the reaction rate constant values best fitted the first order reaction (0.901-0.969) while the reaction rates were high with increase in temperature and storage time (0.0553-0.179) which was in agreement with earlier report by Bhawana *et al*. [24].

3.2.2 Volatile Bases (TVB)

Variations in total volatile bases of the aqueous drink during accelerated storage are shown in Table 4. From the results it can be seen that TVB increased with storage time and temperature and were lowest

for TMSCFD and highest for PTMD with the values ranging from an initial value 29.8 - 44.2 mN/100g at 40 ^{o}C, 48.7 mN/100 g at 50 ^{o}C, 50.2 mN/100 g at 60 ^{o}C and 52.5 mN/100 g at 70 ^{o}C of PTMD within zero to four weeks of accelerated storage. For TMSCD the TVB ranged from 25.5-44.7 mN/100 g at 40 ^{o}C, 46.8 mN/100g at 50 ^{o}C, 48.5 mN/100 g at 60 ^{o}C and 48.9 mN/100 g at 70 ^{o}C respectively from zero to four weeks of accelerated storage. For TMSCFD the TVB varied from 20.2 mN/100 g at 40 ^{o}C, 36.4 mN/100 g at 50 ^{o}C, 40.0 mN/100 g at 60 ^{o}C and 42.0 mN/100 g at 70 ^{o}C respectively within zero to four weeks of accelerated storage. The data obtained for changes in TVB with storage were best fitted with first order reaction kinetics ($r^2 \geq 0.97$) with the reaction rate constants as shown in Table 3, ranging from 0.1196-0.2202wk^{-1} for PTMD, 0.1374-0.1717 wk^{-1} for TMSCD and 0.1424-0.1852wk^{-1} for TMSCFD within 40 – 70 ^{o}C respectively.

Table 4: Storage Changes in TVB of formulated tigernut milk and moringa seeds-based aqueous drinks

Sample	**Time (wks^{-1})**	**Temperatures (^{o}C)**			
		40	**50**	**60**	**70**
	0	29.8	29.8	29.8	29.8
	1	30.2	30.9	31.5	32.5
PTMD	2	35.7	38.2	39.8	40.5
	3	45.4	40.4	47.5	48.7
	4	44.2	48.7	50.2	52.5
		40	**50**	**60**	**70**
	0	25.5	25.5	25.5	25.5
	1	27.6	28.7	29.8	30.0
TMSCD	2	30.0	30.2	31.6	32.9
	3	35.5	38.9	42.5	45.4
	4	44.7	46.8	48.5	48.9
		40	**50**	**60**	**70**
	0	20.2	20.2	20.2	20.2
	1	22.3	23.5	25.8	27.0

TMSCFD	2	25.7	29.6	28.6	30.5
	3	30.0	30.5	38.9	39.8
	4	35.5	36.4	40.0	42.0

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

Total volatile bases in food stuffs can be a strong indicator of the foodstuffs freshness, and therefore gives an idea of whether the foodstuff is safe for consumption or low quality. The unpleasant ammonia-like odour is typical of compounds that contribute to TVB [25]. The volatile compounds such as dimethylamine, ammonia, trimethylamine, hydrogen sulphide, indole, mecaptan and sckatole are all considered as the total volatile bases (TVB) and are used as indices of protein breakdown. The storage changes in Total volatile bases (TVB) of the formulated tigernut and moringa seeds based aqueous drinks, increased with storage time and temperature and were lowest for the plain drink and highest for fortified drink within zero to four weeks of accelerated storage but were within the acceptable TVB levels of the Food Chemistry and Fish in Nutrition Guidelines (2012) [26]. This is an indication that the hurdles and fortificants that were applied in the tigernut and moringa seeds based aqueous drinks had stabilizing effects on the freshness and quality of the drink.

3.2.3 Changes in pH

The changes in pH with storage time at 40-70 ^{o}C are presented in Table 5 while the regression parameters from the zero order plots are given in Table 3. The pH increased with storage time and temperature and varied from 6.8- 7.4 at 40 ^{o}C, 6.8- 7.7 at 50 ^{o}C, 6.8-7.9 at 60 ^{o}C and 6.8 – 8.2 at 70 ^{o}C within four weeks of storage for PTMD. For TMSCD, the pH values ranged from 6.5-7.0 at 40 ^{o}C, 6.5-7.2 at 50 ^{o}C, and 6.5-7.4 at 60 ^{o}C and 6.5-7.7 at 70 ^{o}C within four weeks of storage. For TMSCFD the pH varied from 6.3-6.5 at 40 ^{o}C, 6.3-6.7 at 50 ^{o}C, 6.3- 7.0 at 60 ^{o}C, and 6.3-7.2 at 70 ^{o}C within zero to four weeks of storage. The rates constants for changes in pH are shown in Table 3 ranging from 0.16-0.34 wk^{-1} for PTMD, 0.12-0.30 wk^{-1} for TMSCD and 0.05-0.23 wk^{-1} for TMSCFD within 40 -70 ^{o}C respectively within four weeks of storage.

Table 5: Storage Changes in pH of formulated tigernut milk and moringa seeds-based aqueous drinks

Sample	Time (wks^{-1})	Temperatures (^{o}C)			
		40	**50**	**60**	**70**
	0	6.8	6.8	6.8	6.8
	1	6.9	7.0	7.2	7.2
PTMD	2	7.1	7.3	7.4	7.5
	3	7.3	7.6	7.6	7.8
	4	7.4	7.7	7.9	8.2
		40	**50**	**60**	**70**
	0	6.5	6.5	6.5	6.5
	1	6.6	6.7	6.8	6.8
TMSCD	2	6.7	6.8	6.9	7.1
	3	6.8	7.0	7.2	7.4
	4	7.0	7.2	7.4	7.7
		40	**50**	**60**	**70**
	0	6.3	6.3	6.3	6.3
	1	6.35	6.4	6.5	6.5
TMSCFD	2	6.4	6.5	6.6	6.7
	3	6.45	6.6	6.7	7.0
	4	6.5	6.7	7.0	7.2

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 retinyl palmitate each/100g sample

The pH increased with storage time and temperature. Tigernut and moringa seeds based aqueous drinks pH was increasing with an increase in temperature and storage time, which was due to protein breakdown into volatile bases such as NH_3, H_2S, indole, skaloe, mecaptole as other non- protein

nitrogeneous compounds due to enzymic reactions and action of putrifatic microbes especially the pseudomonas species [26].

3.2.4 Changes in Retinol palmitate during accelerated test

The percentage retention during the accelerated test is shown in Table 6. From the results it can be observed that the pro vitamin A reduced with increase in storage time and temperature with the values ranging from 100- 80.7% at 50 ^{o}C, 100-76.4% at 50 ^{o}C, 100-68.0% at 60 ^{o}C, 100-60.1% at 80 ^{o}C and 100- 50.5% at 90 ^{o}C respectively for the TMSCFD. The regression parameters for the first order plots of the pro vitamin A degradation are presented in Table 3 with the degradation rate constants ranging from 0.0553-0.179 wk^{-1} within zero to four weeks of storage.

Table 6: Absolute Retinol Degradation (mg/kg) of formulated tigernut milk and moringa seeds-based aqueous drinks

Time (wk)	**Temperatures (^{o}C)**				
	50	**60**	**70**	**80**	**90**
0	2.50	2.50	2.50	2.50	2.50
1	2.49	2.49	2.48	2.44	2.46
2	2.42	2.41	2.31	2.35	2.26
3	2.32	2.37	2.22	2.16	1.99
4	2.28	2.22	2.13	2.01	1.80
% Retention					
0	100	100	100	100	100
1	99.8	99.4	98.0	96.6	91.8
2	95.5	94.3	88.5	83.8	78.9
3	88.4	85.7	76.6	73.3	60.1
4	80.7	76.4	68.0	60.1	50.5

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 retinyl palmitate each/100g sample

The percentage retention during the accelerated test indicated that the pro vitamin A reduced with increase in storage time and temperature for all the products, this could be as a result of heat, time and antioxidant activity, this result is in agreement with earlier report by Bhawana *et al*. [24] who reported great vitamin A loss in fortified milk during high pasteurization and storage of the milk.

The 100% retinol retention showed a significant retinol retention at ambient conditions throughout the storage period which was an indication that retinol palmitate was stable during storage at ambient conditions which indicates that the product is a good vehicle for vitamin A fortification.

3.2.5 Predicted Critical Values of objective indices

The predicted critical values for vitamin C, TVB, pH, Retinol palmitate and general acceptability are the predicted best before dates of the products are provided in Table 7 together with their $Q_{10,}$ k_o and E_a values. The Q10 were of the order of 1.5 - 2.4 for vitamin C, 1.4 - 1.7 for TVB, 2.1 - 2.3 for pH, 2.5 (TMSFD) for Retinol palmitate and 1.9-2.0 for general acceptability.

Table 7: Critical Values of Objective Indices at Ambient (10 – 35^{o}C)

Quality Index	Quotient	Sample		
		PTMD	TMSCD	TMSCFD
Vitamin C (mg/100g)	C_r	4.81	1.14	1.81
	Q_{10}	2.4	5.5	2.4
	Ko	16.064	203.185	34.691
	Ea	28.97	1.15	33.5
TVB (gN/100g)	C_r	2.16	0.88	0.90
	Q_{10}	1.4	1.8	1.7
	Ko	351.5	1.8144	4.170
	Ea	20.925	6.69	8.89
pH	C_r	7.3	6.7	6.5
	Q_{10}	2.2	2.3	2.1
	Ko	1.84	3.65	0.12
	Ea	33.46	26.84	45.21

Retinol(mg/100g)	C_r	-	-	1.49
	Q_{10}	-	-	2.5
	Ko	-	-	2.03
	Ea(kj/mol)	-	-	7.32
General Acceptability	C_r	6.0	6.0	6.0
	Q_{10}	2.0	1.9	2.0
	Ko	15.496	56.225	49.689
	Ea(kj/mol)	5.94	9.92	9.74

Key: PTMD: Plain tigernut (90%) + moringa seeds (10%) based aqueous drinks; TMSCD: Tigernut (90%) and moringa seeds (10%) based aqueous drinks plus 2% sugar and 0.2% citric acid; TMSCFD: Tigernut (90%) and moringa seeds (10%) based aqueous drink with 0.2% citric acid, 2% sugar + and 0.15 mg, potassium iodide, 2.0 mg of ferrous sulphate and 1.6 mg of retinol palmitate each/100g sample; **Cr** =Critical value; **Q10** = Temperature quotient for changes in reaction rates with respect to changes in temperature.

The predicted critical values for vitamin C, are the vitamin c concentration at the expiration dates of the products while the Q_{10} is the temperature quotient for changes in reaction rates with respect to changes in temperature. The critical values are dependent on the products and were 4.81, 1.14 and 1.81 mg/100 g, the differences in the critical values can be attributed to differences in chemical interactions in the food systems. This implies that the three products do not have the same shelf life and also that treatment with hurdles and the fortificants extended the critical vitamin C content to significantly lower values. Q_{10} values for vitamin C were 2.4, 1.5 and 2.4 indicating that tigernut and moringa seeds aqueous drinks should be removed from the shelf when it's vitamin c content falls within 1.14- 4.81(C_r), it could also be termed the vitamin C rejection value of the product. The critical value for vitamin C was high at the end of storage indicating that, the quality of the drink was maintained till it's expiration, this is also an indication that tigernut and moringa seeds drink is highly nutritious and healthy for consumption. The Q_{10} values showed an increase in reaction rate with increase in temperature, this was in agreement with earlier report by Monica et *al*. [27].

The TVB critical values were 2.16, 0.88 and 0.90 mN/100g respectively while the Q_{10} values were 1.4, 1.8 and 1.7 respectively for PTMD, TMSCD and TMSCFD. The predicted critical values for TVB are the concentrations corresponding to the subjective general acceptability predicted shelf lives of the products.

Therefore the critical values of the objective measurements are correlated with the subjective determinations of shelf lives.

The pH critical values were 7.3, 6.7 and 6.5 respectively, the Q_{10} values were 2.2, 2.3 and 2.1 respectively indicating that at the pH of 7.3, the should be removed from the shelf.Retinol value was only analysed for (TMSCFD) which was fortified with 0.15 mg/100g potassium iodide, 2.0 mg/100g of ferrous sulphate and 1.6 mg/100g and the critical value was 1.49 mg/100g and Q_{10} value was 2.5, at the end of the shelf life, the retinol palmitate value was 1.49mg/100g which was still high indicating that tigernut and moringa seeds based aqueous drink is a good vehicle for fortification.

General Acceptability critical value also regarded as the Acceptable outgoing Quality Level indicated that during storage the drink was subjected to sensory evaluation and at the end of the storage the product which scored 6.0 (like moderately) will be discarded while the Q_{10} values were 2.0, 1.9 and 2.0 respectively.

Conclusion

Accelerated storage temperature increased pH and total volatile bases and decreased the vitamin C, pro vitamin A and microbial load of tigernut and moringa seeds based aqueous drinks.Hurdles treatment and fortification extended the shelf life of the formulated aqueous drink by a factor of 3.4 -1.8 at 10- 35 ^{o}C ambient storage.At the end of storage the retinol was still high and stable at ambient conditions indicating that the product is a good vehicle for vitamin A (retinol) fortification.

Recommendations

Hurdles treatment and fortification in this research has proven to improve the nutritional and also extends the shelf life of the drink hence the treatment is recommended to be adopted for commercial and local production.

Antinutritional properties should be examined in further works to ascertain toxicity effect on the drink.

References

1. Abdullahi, N, Badau, H. M., Umar, N. B., Yunusa, A. K., Rilwan, A., Jibril, H. &Illyasu, R. Tigernut: A Nutrient-Rich underutilized crop with many potentials. FUDMA Journal of Sciences. 2022;6(2), 103-115.

2. Morton, J. F. The horseradish tree, Moringa pterygosperma (Moringaceae): A boon to arid lands.Economic Botany. 1991; *45*(3), 318–333

3. Donli, P. O. and Dauda, H. Evaluation of aqueous moringa seed extract as a seed treatment biofungicide for groundnuts. Pest Management Sci. 2003;59(9), 1060–1062.

4. Olson, R., Gravin – Smith, B., Ferraboschi, C. & Kraemer, K. Food Fortification: The advantages, Disadvantages and lessons from sight and life programs nutrient. 2021; 13-118.

5. Bareera, S. and Owais, Y. Hurdle Technology in Food Processing. 2021; 2(4), 88-90.

6. Alasalvar, C. *Seafood quality, safety, and health application*. John Wiley and Sons.2010.

7. Food fortification regulations. 2021.

8. Udeozor, L. O. Tigernut – soy milk drink: Preparation, Proximate Composition and Sensory qualities. International Journal of Food Sciences and Nutrition, 2012; 1, 18-26.

9. Garbutt, J. Essentials of food microbiology. Arnold Publishers.1997.

10. Ball, G.F.M. Water Soluble Vitamins: Assays in Human Nutrition. Chapman and Hall. 1994; Pp 17 – 157.

11. Rutkowsi, M. &Grzegorcyk, K. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Sci., Pol. Technol. Aliment.2007; 6(3), 17-28.

12. Pearson, D. The Chemical Analysis of Foods; Manual of Analysis CBA – 301 Series (7th Ed) Churchill, Livingstone London; 1970 Pp 7 – 11

13. Anthony, U. & Chandra, T.S. Microbial population and biochemical changes in fermenting finger millet. *World Journal of Microbiology Biotechnology.* 1997; 13(5), 533-537.

14. Ihekoronye, A.I. &Ngoddy, P.O. *Integrated food science and technology for the tropics*. Macmillan Publishers, New York; 1994.

15. FAO/WHO/UNU. Human vitamin and mineral requirements (Recommended Dietary Intakes): Report of a joint food and agricultural organization, World Health Organization and United Nations University; 2002.

16. Montville, T.J. & Matthews, K. Food microbiology: An introduction. ASM Press, Washington DC 2005.

17. Brooks, A. and Asamudo, N.U. Microbiological and Physico-ChemicalAnalysis of soymilk and soy flour sold in Uyo Metropolis (Nigeria). Journal of pure and applied Science 2003; 9:12-17.

18. Onovo, J.C. andOgaraku, A. O. Studies on some microorganisms associated with exposed tigernut (*Cyperus esculentus L.*) milk. Journal of Biological Sciences,2007;7, 1548-1550.

19. FAO/WHO.Milk and Milk Products. Joint FAO/WHO Food Standards Programme. CODEX Alimentarius Commission; 2002. pp 42-50.

20. Abubakar, H., Atiku, M.K., Alhassan, A. J. & Muhammad, A, S. Effects of processing tigernuts on the chemical composition of tigernut (*Cyperus esculentus*) milk products; 2018.

21. Ocheme, O. B., Ariahu, C. C., Ingbian, E. K. &Igyor, M.A. A survey of the traditional methods of processing dakuwa (a cereal/legume based snack food) in Niger State. Nigerian Food Journal, 2011; 29(1), 103-112.

22. Al-Zubaidy MMI and Khalil RA. Kinetic and prediction studies of ascorbic acid degradation in normal and concentrate local lemon juice during storage. Food Chem 2007; 101(1):245-259.

23. Abbasi A and Niakousari M. Kinetics of ascorbic acid degradation in un-pasteurised Iranian lemon juice during regular storage conditions. Pak j Bio Sci 2008; 11(10): 1365-1369.

24. Bhawana, S., Ravinder., K., Sumit, A. & Azhar, K. Effect of processing conditions on stability of native Vitamin A and fortified retinol acetate in milk. International Journal for Vitamin and Nutrition Research. 2019; 91(1-2), 1-10.

25. Conway and Byrne. Determination of the Total Volatile Basic Nitrogen(TVBN) in fish; 2014.

26. Kirk R.S, Sawyer R. Pearson's Composition and Analysis of foods, 9th ed.(student edition), England Addision Wesley Longman Ltd. 1991; Pp.33-36.

27. Monica P., Tao F.,Nuria A., Tong W. Efects of food additives on egg yolk gelation induced by freezing. Food chem. 2018:263:142-150.