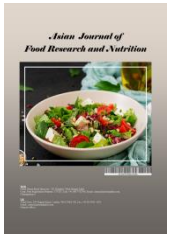

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Storage Changes in Triple Fortified Tigernut and Moringa Seed Based Aqueous Drinks

Ashaver V. A. [a*], Ariahu C. C. [b], Yusufu M. I. [c], Ariahu E. C. [d] and Gbuusu B. [e]

[a] Department of Chemistry, Benue State University, Makurdi, Benue State, North Central, Nigeria.
[b] Centre for Food and Research, Benue State University, Makurdi, Benue State, North Central, Nigeria.
[c] Department of Food Science and Technology, Federal Polytechnic Idah, Kogi State, North Central, Nigeria.
[d] Department of Pharmaceutical Sciences, Bingham University, Nasarawa State, Nigeria.
[e] Department of Food Science and Technology, University of Mkar, Mkar, Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study investigated storage changes in triple fortified tiger nut and moringa seed based aqueous drinks. During accelerated shelf life testing, vitamins C, Retinol Palmitate, pH, Total volatile bases (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 10-35oC for Plain Tigernut *Moringa* Drink (PTMD), Tigernut *Moringa* seeds plus sugar and Citric Acid Drink (TMSCD) and Tigernut Moringa Seeds plus sugar and citric acid Fortified Drink (TMSCFD) respectively. TMSCD and TMSCFD significantly

**Corresponding author: E-mail: veraashaver@gmail.com;*

(p<0.05) lowered microbial growth (<50 cfu/g) as compared to PTMD (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 pH and 0.0553- 0.179 (wk^{-1}) for retinol palmitate with 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 Retinol Palmitate fortification and protein energy malnutrition (PEM) intervention programmes.

Keywords: Tigernut; moringa; fortification; storage changes; aqueous drinks; citric acid; sugar.

1. 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 rawor 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].

Moringaoleifera (Moringaceae) is a member of the Moringa genus known by many different names around the world, including horseradish tree, drumstick tree, "Guiligandja," "Gagawandalahai," and manyothers [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. *Moringaoleifera* 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 inorder 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 asorganoleptic 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 drinks under 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 multifortificatication for (Retinol Palmitate), 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. MATERIALS 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* farmopposite International Market, Makurdi. Airtight containers, sample bottles, transparent cups and blender were purchased in Modern

Market, Makurdi, Benue State. Citric Acid, Retinol Palmitate, 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 Sarwuan Tarkaa University, Makurdi Biochemistry Laboratory respectively, where the analysis were carriedout.\

2.2 Sample Preparation

Preliminary affective and descriptive sensory evaluation of *moringa* seed based aqueous drinks consisting 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) and tigernut and moringa seeds plus 2% sugar and 0.2% citric acid and 0.15 mg KI, 2.0 mg $FeSO_4$ and 1.6 mg retinol palmitate/100g each (TMSCFD) as recommended by Food fortification regulations [7] and were then subjected to ambient (30±2oC) and accelerated storage test. The flow chat of the aqueous drink is as shown in Fig. 1.

2.3 Normal Storage Tests

Microbiology: Total plate count, coliforms, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, Yeast and molds were evaluated within four weeks of storage at (30±2^{o}C) as described by Garbutt, [9].

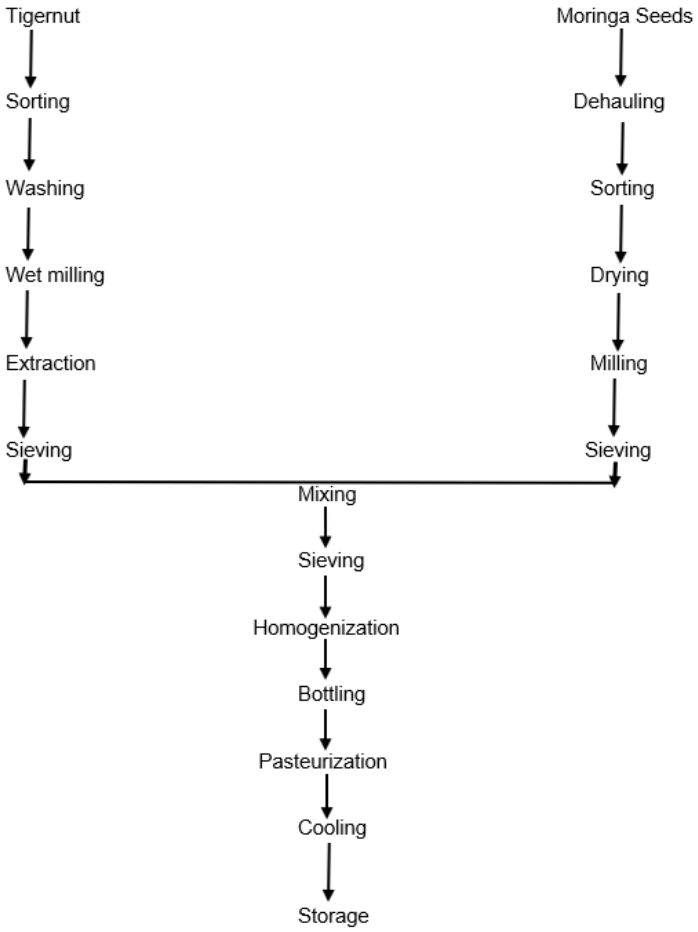

Fig. 1. Flow diagram for the production of tigernut milk and moringa sed-based aqueous extract. Source: [8]

2.4 Accelerated 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 40ºC, 50ºC, 60ºC and 70ºC 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.

2.4.1 Vitamins analysis

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

Retinol Palmitate determination: The Retinol Palmitate content of the formulated products was analysed using high performance liquid Chromatography (HPLC) as described by Rutkowsi, [11].

2.4.2 Total volatile bases

Total Volatile Base (TVB) is related to protein for breakdown and includes NH_3, indole, skatole, mercaptan. This was done according to the method described by Idakwo et al. [12]. 100 g of flesh of fresh fish sample was weighed and blended with 300 ml of 5% Tricholoroacetic acid. The blend was then centrifuged at 3000 ×g for 1 h toobtain clear extract. 5 ml of the extract was pipetted into the Markhan apparatus and 5 ml of 2 M Sodium hydroxide (NaOH) was added. This was steam distilled into 15 ml of standard 0.01 M hydrochloric acid (HCl) containing 0.1 ml rosolic indicator. After distillation, the excess acid was then titrated in the receiving flask using standard 0.01 M NaOH to a pale pink end point. A procedural blank was done using 5 ml Trichloroacetic acid with no sample and titrated as before. The concentration of TVBN (in mg N/100 g sample) was computed as follows:

$$\text{TVBN (mg N/100 g sample)} = \frac{(M)(VB-VS)(14)(300+W)}{5} \quad (1)$$

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 of Anthony, U. & Chandra [13] was used to measure the titratable acidity.

2.4.5 Sensory 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 by Ihekoronye and Ngoddy [14] using dilute solution s 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, S oftware (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. 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±2ºC) 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 *Staphylococcus 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, Staphylococcus 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 *Staphylococcus aureus* were absent while yeast and molds ranged from 0-20 cfu/ml within four weeks of storage.

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 theosmotic 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 theosmotic 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 withover 2.00×10 are bad for consumption, and samples with higher bacteria load are also considered unsafe for consumption, therefore, the larger the

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

Parameters	Microbial Count (c.fu/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 Spos	0	0	0.1×10^{2}	0.1×10^{2}	0.2×10^{2}
	Staphylococcus aureus	0	0.2×10^{2}	0.2×10^{2}	0.2×10^{2}	0.2×10^{2}
	Yeast and molds	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 subtilis	0	0	0	0.1×10^{2}	0.2×10^{2}
	Staphylococcus aureus	0	0	0	0	0
	Yeast and molds	0	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 subtilis	0	0	0	0	0
	Staphylococcus aureus	0	0	0	0	0
	Yeast and molds	0	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 palmitate

microbial load, the more unsafe and susceptible the sample is to spoilage [17]. These findings are also consistent with the findings ofonovo andogaraku [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 effecton 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 qualityon 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 40oC, 50oC, 60oC and 70oC respectively for the test products. The dataobtained were best described by zeroorder ($r^2\geq0.998$) reaction kinetics. The predicted shelf life at ambient (10-35oC) using the zeroorder kinetics are presented in Table 2. From Table 2 it can beobserved 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 - 35oC respectively.

3.2.1 General acceptability

General acceptability decreased with storage time and were lower at higher temperatures andon a nine-point Hedonic scale within four weeks of storage for all the products. It can beobserved from the results that, theoverall 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 byocheme et al. [21], who reported a decrease in sensory attributes of tigernut c offee 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 effectson theoverall quality of the drink.

The zeroorder 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 theorder of reactions equation using 6 as the acceptability outgoing quality level for general acceptability to determine the shelf life of the drink at lower

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

Temperature (ºC)	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)

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 acceptability	r^2	≥0.985	≥0.993	≥0.965
	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

storage temperatures of 10-35oC. 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 or ganoleptically during storage.

The data obtained for overall acceptability, vitamin C, Total volatile bases (TVB), pH and Retinol palmitate degradation were best described by Zero and firstorder reaction kinetics (r^2 ≥ 0.998). The regression parameters are presented in Table 3. From the results it can be observed thatoverall acceptability, vitamin C, TVB, pH and Retinol palmitate degradation rate constant increased with temperature and were highest (1.31-1.83 wk^{-1}) for TMSCD and lowest (1.19 – 1.59 wk^{-1}) for TMSCFD. 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- 70oC respectively. For pH, data shows that the highest (0.16 – 0.34) was for PTMD and lowest (0.05-0.23) was for TMSCFD.

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 accept ability outgoing quality level for general acceptability to determine the shelf life of the drink at lower storage temperatures of 10- 35oC. 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 firstorder regression parameters for the degradation of vitamin C in tigernut and *moringa* seeds based aqueous drink at 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- 35oC respectively. The best retention of vitamin c content was at 10oC, this was in agreement with earlier report by Abbasi et al. [23].

The data obtained for changes in Total volatile bases (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 Total volatile bases (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 firstorder 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 Total 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 Total volatile bases (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 40oC, 48.7 mN/100 g at 50oC, 50.2 mN/100 g at 60oC and 52.5 mN/100 g at 70oC of PTMD within zero to four weeks of accelerated storage. For TMSCD the TVB ranged from 25.5-44.7 mN/100 g at 40oC, 46.8 mN/100g at 50oC, 48.5 mN/100 g at 60oC and 48.9 mN/100 g at 70oC respectively from zero to four weeks of accelerated storage. For TMSCFD the TVB varied from 20.2 mN/100 g at 40oC, 36.4 mN/100 g at 50oC, 40.0 mN/100 g at 60oC and 42.0 mN/100 g at 70oC 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 0.1196-0.2202 wk^{-1} for PTMD, 0.1374-0.1717 wk^{-1} for TMSCD and 0.1424-0.1852 wk^{-1} for TMSCFD within 40 – 70oC respectively.

Total volatile bases (TVB) 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 Total volatile bases (TVB) levels of the Food Chemistry and Fish in Nutrition Guidelines [26]. This is an indication that the hurdles and fortificants that were applied in the tigernut and moringa seeds based aqueous drinks had stabilizing effectson the freshness and quality of the drink.

3.2.3 Changes in pH

The changes in pH with storage time at 40-70oC are presented in Table 5 while the regression parameters from the zeroorder plots are given in Table 3. The pH increased with storage time and

temperature and varied from 6.8- 7.4 at 40oC, 6.8- 7.7 at 50oC, 6.8-7.9 at 60oC and 6.8 – 8.2 at 70oC within four weeks of storage for PTMD. For TMSCD, the pH values ranged from 6.5-7.0 at 40oC, 6.5-7.2 at 50oC, and 6.5-7.4 at 60oC and 6.5-7.7 at 70oC within four weeks of storage. For TMSCFD the pH varied from 6.3-6.5 at 40oC, 6.3-6.7 at 50oC, 6.3- 7.0 at 60oC, and 6.3-7.2 at 70oC 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 -70oC respectively within four weeks of storage.

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, skatole, mecaptole asother non- protein nitrogenous 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 beobserved that the pro vitamin A reduced with increase in storage time and temperature with the values ranging from 100- 80.7% at 50oC, 100-76.4% at 50oC, 100-68.0% at 60oC, 100-60.1% at 80oC and 100- 50.5% at 90oC respectively for the TMSCFD. The regression parameters for the firstorder plots of the retinol palmitate 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.

The percentage retention during the accelerated test indicated that the retinol palmitate 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.

Table 4. Storage changes in total volatile bases (TVB) of formulated tigernut milk and moringa seeds-based aqueous drinks

Sample	Time (wks^{-1})	Temperatures (^oC)			
		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

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

Sample	Time (wks^{-1})	Temperatures (ºC)			
		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 retinol palmitate each/100g sample

Table 6. Absolute retinol palmitate degradation (mg/kg) of formulated tigernut milk and moringa seeds-based aqueous drinks

Time (wk)	Temperatures (ºC)				
	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 retinol palmitate each/100g sample

3.2.5 Predicted critical values ofobjective 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 theorder 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.

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 dependenton the products

Table 7. Critical Values ofobjective 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 (kj/mol)	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 (kj/mol)	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(kj/mol)	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

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 Total volatile bases (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 Total volatile bases (TVB) are the concentrations corresponding to the subjective general acceptability predicted shelf lives of the products. Therefore the critical values of theobjective 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 palmitate value wasonly 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 Acceptableoutgoing 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.

4. CONCLUSION

1. 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- 35oC ambient storage.
2. At the end of storage the retinol was still high and stable at ambient conditions indicating that the product is a good vehicle for pro-vitamin A (retinol palmitate) fortification.

5. 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 effecton the drink.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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