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# Effect of Storage Condition on Retention of Vitamins in Selected Commercial Fortified Maize Flour in Kenya

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**Abstract**

Food fortification is one strategy that has been used to overcome micronutrient deficiencies among vulnerable populations. Maize, a common staple food in Kenya, has been used as a suitable fortification vehicle. However, several factors, including storage conditions, impact micronutrient stability in fortified maize flour. This study aimed to assess the influence of storage condition on the retention of retinol and B-vitamins in selected commercial fortified maize flour. Fresh samples of fortified maize flours from two brands (codedXX1 and XY2) were sampled from the manufacturers at the point of production. The storage stability of retinol and B-vitamins in the two brands (XX1 and XY2) was monitored for 6 months at 25°C/ 75% relative humidity and 35°C/ 83% relative humidity. Retinol and thiamine were the least stable vitamins in both flour brands, while riboflavin and folate were relatively stable. Niacin was the most stable vitamin. Retinol was the least stable vitamin for brand XXI at both 25 °C/75% RH and 35 °C/83% RH, followed by thiamine, riboflavin, folate, and niacin. However, brand XY2 showed that under both storage conditions, thiamine was the least stable vitamin, followed by retinol, riboflavin, folate, and niacin.Vitamin retention was higher in samples stored at a lower temperature and relative humidity (25°C/ 75% RH) than in samples stored at higher temperature and relative humidity (35°C/ 83% RH) for both brands.In conclusion, thiamine and retinol were generally more susceptible to storage losses. Although the vitamin content in the flour samples decreased during storage, the changes in both storage conditions (except for riboflavin) and both brands were not significantly different.

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## Introduction

Micronutrient deficiency is one of the main global health concerns. It is projected that more than two billion suffer from micronutrient deficiencies worldwide.[1,2] The majority of people in low-income nations are often lacking in a variety of micronutrients.[2] When people do not have access to micronutrient-rich foods including fruits, vegetables, animal products, and fortified meals, deficiencies arise.

In Kenya, vitamin A deficiency (VAD), vitamin $B_9$ deficiency, and vitamin $B_{12}$ deficiency are the main micronutrient deficiencies of concern.[3] According to the Ministry of Health,[4] folate deficiency affects 32.1 % of expectant women and 30.9 % non-expectant women. Vitamin $B_{12}$ deficiency was found to be more prevalent in women aged 15-19 (47 %) than in older women (31.5 %).[3] Overall, 24% of the Kenyan population are Vitamin A deficient, with preschool children having the highest prevalence at 53 %.[4] A study by Harika, Faber, Mulugeta, Kimiywe and Eilander[5] reported that in Kenya, 15 % of children aged between 0 and 19 years are vitamin A deficient.

Micronutrient deficiencies can be avoided or eliminated if populations consume adequate amounts of the required micronutrients continuously.[6] Food fortification is one of the approaches that has been utilized to alleviate the incidence of micronutrient deficiencies among Kenya's vulnerable groups.[7,8] Food fortification is the deliberate addition of a necessary micronutrient, such as vitamins, minerals, or amino acids, to a food, regardless of whether such nutrients were present in the food prior to processing; with the intention of enhancing its nutritional quality and delivering a public health benefit with a low risk of adverse health effects.[9] A food vehicle for fortification should be widely consumed and readily available to the population.[8] Maize flour, a staple food in most developing countries has been used as a vehicle to increase the intake of iron, zinc, retinol, and vitamins $B_1$, $B_2$, $B_3$, $B_6$, $B_9$, and $B_{12}$.[6]

The effectiveness of a fortification scheme is highly dependent on the retention of micronutrients.[10] The stability of the fortificant is affected by exposure to any chemical orphysical elements, including heat, moisture, oxygen, light,or pH, during manufacturing, packaging, distribution, or storage. Stability and bioavailability of fortificants are among the important factors considered when selecting fortificants used in micronutrient premixes. Folic acid, retinyl palmitate, niacinamide, and riboflavin are the most stable and bioavailable forms of folate, vitamin A, vitamin $B_3$, and vitamin $B_2$.[11]

Despite the fortification programs in many countries, there are still many cases of micronutrient deficiencies.[8] One of the many factors influencing micronutrient stability in maize flour is storage conditions such as temperature and relative humidity. Vitamin losses have been reported to be higher than mineral losses in fortified foods during storage[12] For instance, zinc and iron are relatively stable whereas vitamin A and riboflavin are generally unstable under normal storage conditions.[10,13] Higher temperatures and relative humidity decreases vitamin stability.[10,12] According to Hemery, Laillou, Fontan and Avallone[10] vitamins showed losses of up to 90 % when stored at 40 $^{O}$C temperature and 75 % relative humidity, over a twelve-month storage period.

The aim of this study, therefore, was to assess the influence of storage condition on the retention of retinol and B-vitamins in selected commercial fortified maize flour.

## Materials and Methods

### Sample Collection

Fresh samples of fortified maize flours from two brands, coded XX1 and XY2, were sampled from the manufacturers at the point of production, on the same day that the fortificants were added. The fortificant premixes used to fortify the two different brands were from different suppliers. The samples were from large-scale commercial millers. The samples were thereafter brought to the laboratory for analysis.

### Sample Preparation

Samples from brands XX1 and XY2 were first conditioned for 72 hours at 25 °C and 75 % RH. After conditioning, a sample (0 month storage) was drawn and analysed for retinol, folate, niacin, riboflavin, and thiamine. The flour samples were then each divided into two equal batches, re-packaged in brown khaki bags to eliminate biasness due

to differences in packaging material and then labeled with unique sample codes. The samples were then stored in two separate incubation chambers; one set at 25°C and a relative humidity of 75% and the other set at 35°C and a relative humidity of 83%. Relative humidity of 75% and 83% were achieved using saturated salt solutions of sodium chloride and potassium chloride respectively. These conditions were selected to reflect normal storage conditions commonly encountered in Kenya, namely storage conditions around Nairobi (25 °C/ 75 % RH) and storage conditions at the Kenyan Coast (35 °C/ 83 % RH). Under both storage conditions, sampling was done at monthly intervals for six months. At each sampling interval, the content of retinol, folate, niacin, riboflavin, and thiaminein the flour samples were analysed.

**Determination of Retinol**

Extraction and quantification of retinol were carried out according to the method described by Zahar and Smith[14] with little modifications. Two grams of flour was weighed into a centrifuge tube. This was followed by adding 5 ml of ethanol containing 0.1 % (wt/vol) ascorbic acid and then 2 ml of 50% (wt/vol) potassium hydroxide. The centrifuge tubes were capped, shaken well, and put in a water bath (Memmert WNB AC 230 V-50/60 HZ, Germany) at 80 ºC for 20 minutes. The tubes were shaken intermittently throughout this period. Using running water, the tubes were cooled before 20 ml of hexane containing 0.01% BHT (wt/vol) was added. The contents of the tubes were thoroughly mixed on a vortex mixer for one minute, and after standing for two minutes, the contents of the tubes were again mixed for another minute. Fifteen ml of cold water (1ºC) was added to each centrifuge tube and then the tubes were inverted 10 times. The samples were centrifuged at 10000 rpm for 10 min. Afterward, the upper-organic layer was pipetted into a tube and the solvent was evaporated under vacuum at 40 ºC using a rotary vacuum evaporator (Hahnshin HS-2005S, water bath HS-3001, Korea). The residue was dissolved in 1 ml of methanol, ready for HPLC analysis. Twenty(20) µl of the sample was injected into reverse-phase HPLC (Shimadzu RF-20A, Japan) fitted with column C-18 ODS size 250 mm × 4.6 mm × 0.5 µm. The mobile phase was methanol and water in a ratio of 95:5 and the flow rate was 0.8 ml/min. A UV-visible diode-array detector (SPD - M20A) was used for the identification of retinol at 325 nm. Concentrations of retinol were calculated using peak areas of the samples and the standard curves of the retinol standards.

**Determination of B-vitamins**

Determination of vitamin $B_1$ (thiamine), vitamin $B_2$ (riboflavin), vitamin $B_3$ (niacin), vitamin $B_6$ (pyridoxine), and vitamin $B_9$ (folate) was carried out according to Ekinci and Kadakal[15] with little modifications. To 5 g of sample, 20ml of acidified deionized water was added followed by vortexingat medium speed for 1 minute. The mixture was then centrifuged for 10 minutes at 10000 rpm. The supernatant was drawn and filtered through 0.45 µm pore size membrane filters. Twenty µl of the sample was injected into reverse-phase HPLC (Shimadzu RF-20A, Japan); column C-18 ODS size 250 mm × 4.6 mm × 0.5 µm. The mobile phase constituted 100 mM $KH_2PO_4$ and Me OH in the ratio of 90:10 (v:v) and the flow rate was 1 ml/min. A UV-visible diode-array detector (SPD- M20A) was used. Thiamine, riboflavin pyridoxine, folic acid, and niacin were identified at 254 nm. The integrated peak areas of the samples obtained and the calibration curves of the corresponding standards were used to calculate the concentrations of the specific B-vitamins.

**Statistical Analysis**

The standard deviations and mean values of all samples were computed. The means for the storage experiment were then analysed by two-way analysis of variance (ANOVA) using Gen Stat statistical tool (19th Edition, 2018) to assess the interaction effect between storage time, storage conditions, and brands on micronutrient stability. Tests were conducted at a 95% confidence level and the interactions between storage conditions and storage time; storage time and brand were regarded as significant atp ≤0.05.

**Results and Discussion**

**Vitamin A (Retinol)**

At the beginning of storage, retinol concentration in brand XX1 and XY2 was 0.63 mg/kg and 0.51 mg/kg respectively. At 25ºC/ 75% RH, retention of retinol after six months of storage was 66.4% and 73.3% for brands XX1 and XY2 respectively (Figure 1). The amount of retinol retained after a 6-month storage period at 35 ºC/ 83 % RH was 54.3% and 61.9% for brands XX1 and XY2 respectively (Figure 2). For both brands at each

monthly analysis, retention of retinol was higher for samples stored at 25 ºC/ 75 % RH as compared to those stored at 35 ºC/ 83 % RH. Overall, the results show significantly higher losses of retinol at high temperatures and high relative humidity. Retinol is more susceptible to losses in hot and humid surroundings than in cold and dry conditions.[16] Temperature may influence the oxidation rate of fat-soluble vitamins such as retinol, resulting in decreased vitamin stability.[17] It is also known that retinol is susceptible to isomerization because its structure contains double bonds.[18] One of the most unstable vitamins is vitamin A, with retinol being more unstable as compared to other retinyl esters.[18]

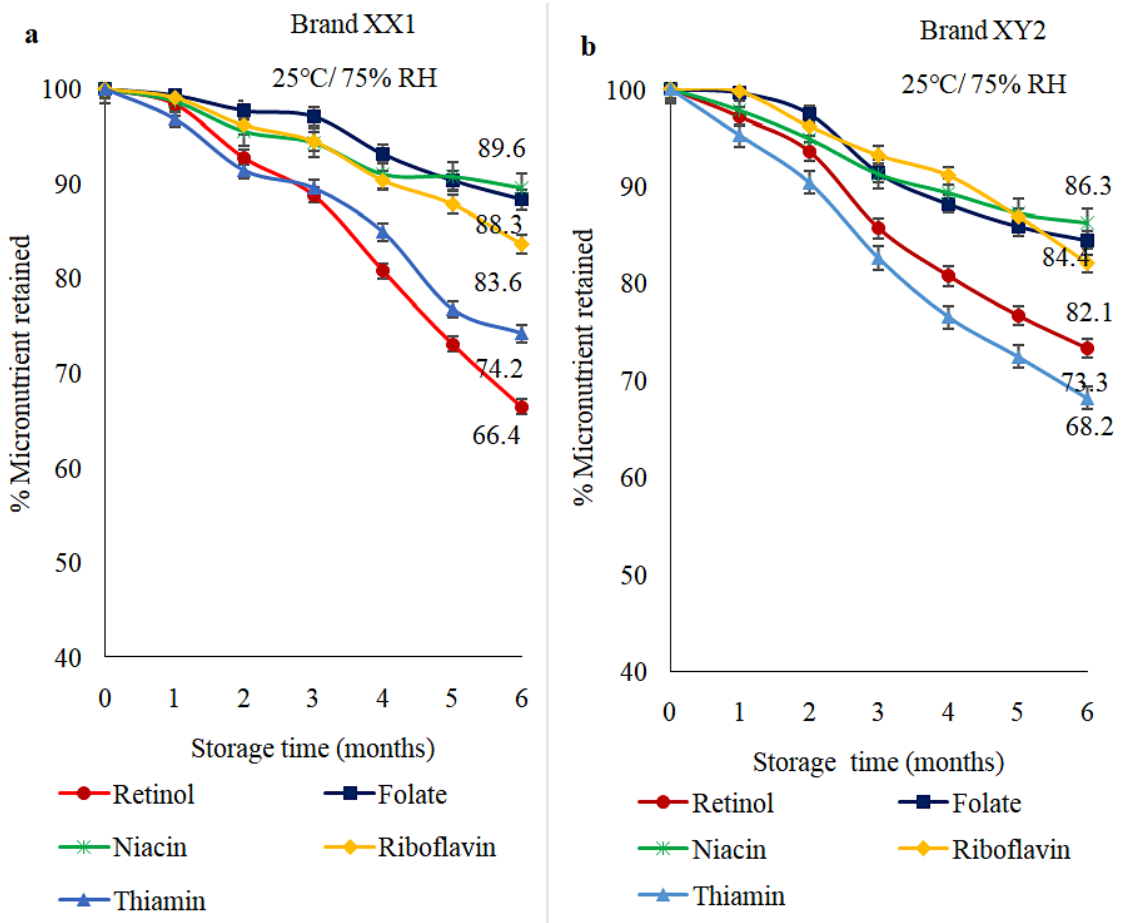


**Fig. 1: Retention of micronutrients in fortified maize flour from (a) brand XX1 and (b) brand XY2 stored at 25 ºC/ 75 % RH**

Retention of retinol was also brand-dependent (Figures 1 and 2), however, both brands depicted similar trends. The differences in retention capacity across the brands could probably be attributed to the different forms of vitamin A fortificant and the quality of the premixes used by different millers. Usually, fortificants are added to the selected food vehicle in the form of a micronutrient premix.[6] Fortificants are defined as the source of micronutrients, while micronutrient premixes are a blend of different fortificants formulated to provide specified and determinable amounts of micronutrients.[11] Fortification premixes vary in the type of encapsulates and antioxidants used in their formulation, which may affect the the stability of retinol across brands.[19] Retinyl acetate, retinyl palmitate, and provitamin A (β-carotene) are some of the vitamin A forms (retinyl esters) that may be

added to food during the fortification process.[19] The protected form of retinyl palmitate, which is specially coated is the most stable and is usually preferred for use in flour fortification.[12]

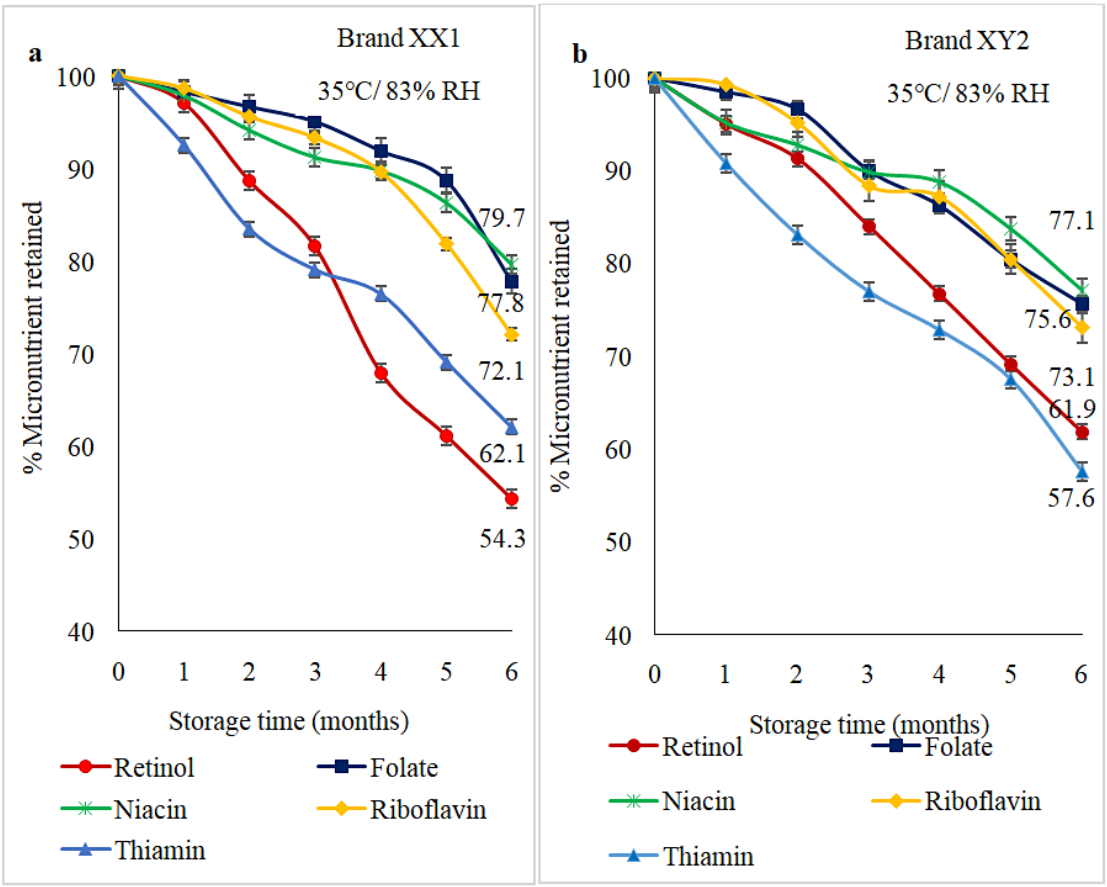


**Fig. 2: Retention of micronutrients in fortified maize flour from (a) brand XX1 and (b) brand XY2 stored at 35 ºC/ 83 % RH**

These results were different from those reported by Dunn, Jain and Klein12 of 75 % vitamin A retention in cornmeal flour stored at 27 ºC for six months. Higher retention values of 83.8 % and 75.7 % were reported by Khamila, Sila and Makhoha[13] after storage of fortified maize flour samples at 25 ºC / 60 % relative humidity and 35 ºC / 75 % relative humidity respectively for six months.

**Vitamin $B_1$ (Thiamine)**

The amount of thiamine in brand XX1 and XY2 at the start of storage, was 2.91 mg/kg and 2.77 mg/ kg.As shown in Figure 1, thiamine retention was higher in flour samples stored at 25 °C/75 % RH after six months, with 74.2 % and 68.2 % retention for brands XX1 and XY2, respectively. Like for retinol, samples stored at 35ºC/ 83% RH for six months showed lower retention values of 62.1% and 57.6 % for brand XX1 and brand XY2 respectively (Figure 2). For all the brands investigated, the overall rate of thiamine degradation was higher in samples stored at 35 °C/ 83% RH than in samples stored at 25 °C/75% RH.For both storage conditions, thiamine was the least stable vitamin for brand XY2, while it was the second least stable vitamin for brand XX1. This variation may probably be due to the make-up differences in premixes sourced from different suppliers.Thiamine hydrochloride

and thiamine mononitrate are the two commonly used salts for fortification and are both heat-labile and sensitive to both humidity and oxygen.[20] If the makeup ingredients are not stable, the premix may not be stable.[21] Thiamine is a vital micronutrient, however delivering it in food products is challenging due to its instability under heat, alkaline pH, and various processing/storage conditions.[22] However, thiamine hydrochloride and thiamine mononitrate are both quite stable to oxygen in the absence of light and moisture and extremely stable when used in dry goods with light and moisture-resistant packaging.[18] Thiamine hydrochloride, despite being sold in a crystalline state, also exists in an amorphous form, particularly infortificants, which is more labile.[23] Thiamine degradation rate increases with increased relative humidity.[24]

**Vitamin $B_2$ (Riboflavin)**

Riboflavin was relatively stable amongst the vitamins assessed. Initial riboflavin concentration for brand XX1 was 2.41 mg/kg and 2.34 mg/kg for brand XY2. Figure 1 illustrates that at 25ºC/ 75% RH, retention of riboflavin after six months of storage was 83.6% and 82.1% for brands XX1 and XY2 respectively. At 35ºC/ 75% RH, the amount of riboflavin retained after a 6-month storage period was 72.1% and 73.1% for brands XX1and XY2 respectively (Figure 2). For both brands, retention of riboflavin was greater at 25 °C/75% RH than at 35 °C/75% RH under the specified storage conditions after each month. These values were different from those reported by Khamila, Sila and Makhoha[13] where fortified maize flour samples stored at 25ºC/ 60% RH and 35ºC/ 75% RH, 66% and 54.4% of riboflavin were retained respectively. Higher retention values of 94% after storage for 24 months at 30ºC have been reported by Coad and Bui.[25] According to Ottaway,[18] riboflavin is relatively stable during heat processing, storage, and cooking. It degrades, though, when exposed to light. A combination of light and high temperatures make riboflavin generally unstable in food products.[26] Riboflavin is degraded through cleavage of the isoalloxazine ring, yielding a number of unstable compounds.[26]

**Vitamin $B_3$ (Niacin)**

When compared to other vitamins analyzed, niacin was the most stable in both brands and both storage conditions. At the beginning of storage, the amount of niacin in brand XX1 and XY2 was 21.53 mg/kg and 17.20 mg/kg respectively. Niacin retention for the samples stored at higher temperatures and relative humidity was lower than those stored in lower temperatures and relative humidity (Figures 1 and 2). At 35 ºC/ 83 % RH, 79.7 % and 77.1 % niacin content were retained for brands XX1 and XY2 respectively at the end of storage (Figure 2). Higher retention values of 89.6 % and 86.3 % for brandsXX1 and XY2 respectively were observed for samples stored at 25 ºC/ 75 % RH for six months (Figure 1). At every month of analysis for both brands XX1 and XY2, niacin degradation rate was lower for samples stored at 25 ºC/ 75 % RH than those stored at 35 ºC/ 83 % RH. Niacin retention values observed in this study were consistent with those reported by Khamila, Sila and Makhoha13 of 87.7% and 75.6% for flour stored for six months at 25ºC/ 60% RH and 35ºC/ 75% RH respectively. Beizadea[27] reported higher niacin retention values of 94% when fortified spaghetti was kept in the darkfor three months at a temperature of 25 °C and 60% relative humidity. Niacin exists as nicotinamide in fortified foods, and is usually stable during thermal processing, and exposure to oxygenand light.[18] Although considered the most stable vitamin, niacin losses are mainly attributed to leaching into cooking water.[28] Generally, maize has low niacin content. Fortification of milled flour reduces the risk of pellagra among the vulnerable population.[29]

**Vitamin $B_9$ (Folate)**

For brand XX1 and XY2,there was a folate concentration of 1.56 mg/kg and 1.40 mg/kg respectively, at the start of storage. As shown in Figure 1, there was 88.3% and 84.4% retention in samples stored at 25 ºC/ 75 % RH for brandsXX1 and XY2 respectively after a 6-month storage period. On the other hand, brand XX1 and brand XY2 flour samples stored at 35 ºC/ 83 % RH retained 77.8 % and 75.6 % amounts of folate at six months (Figure 2). Folate degradation rate was lower for samples stored at 25 °C/ 75% RH than it was for samples stored at 35 °C/ 83% RH during every monthly analysis for both brands XX1 and XY2. These values compare favorably with the findings of Khamila, Sila and Makhoha[13] who reported 87.3% and 75.9% retention of vitamin B9 for flour stored at 25ºC/ 60% RH and 35ºC/ 75% RH respectively. Another study by Hemery, Fontan, Laillou, Jallier,

Avallone and Berger 30reported a retention range of 81-83%, regardless of storage temperature, after 3 months when wheat samples were kept at 65 % RH, which was fairly consistent with the findings of this study. Folate is relatively stable to humidity and heat.[27] Light, temperature, oxygen, and pH are all environmental factors that can cause interconversion or degradation of folates, resulting in irreversible loss of activity.[31] Folic acid is an essential micronutrient in the diet.[32] The mechanism of folate degradation is determined by the vitamin's structure and the chemical environment.[33] Folate degradation typically involves alterations to the bond structures, the pteridine ring system, or both.[31] In the presence of oxidants or reductants, folic acid can be cleaved and inactivated.[31] A study conducted by Scientific Advisory Committee on Nutrition[34] reported that mandatory fortification at levels of 300 µg of folic acid per 100 g of flour increased the average folic acid intake of the UK population by about 80 µg/day and would be effective in reducing neural tube defects (NTD) risk by about 11-18%.

**The Interaction between Storage Time, Storage Conditions and Maize Flour Brands on Vitamin Stability**

Based on previous research conducted by Dunn, Jain and Klein,[12] and Khamila, Sila and Makhoha[13] on vitamin stability in fortified foods during storage, it is expected that vitamin losses will occur over an extended storage period. In agreement, the current study indicated that vitamins in fortified maize flour are less stable after an extended storage time (Figures 1 and 2). However, the interaction between storage time and storage conditions was not significant ($p > 0.05$) for the amounts of retinol, thiamine, niacin, and folate in fortified maize flour stored for six months (Table 1). Additionally, as shown in Table 1, the F-observed values were less than the F-critical (2.24). These statistics indicate that there was no significant interaction between time and storage condition on the levels of of retinol, thiamine, niacin, and folate retained in the flour samples. There was, however, a significant ($p < 0.05$) interaction between storage time and storage condition on the amounts of riboflavin retained in the flour samples (Table 1). Further, as indicated in Table 1, the F-observed (6.04) is greater than the F-critical (2.24) hence the interaction of time and storage condition on riboflavin retention in fortified maize flour was significant. Overall, this observation means that despite the decrease in the vitamin content in the samples during storage, there was no significant difference in the changes at 25 ºC/ 75 % RH and 35 ºC/ 83 % RH except for riboflavin. Meaning even though the vitamin retention was higher in samples stored at a lower temperature and relative humidity (25°C/ 75% RH) than in samples stored at higher temperature and relative humidity (35°C/ 83% RH), the difference was not significant.

**Table 1: The interaction between storage time, storage conditions, and maize flour brands on micronutrient stability**

| Micronutrients | Time vs Condition | | Time vs Brand | |
|---|---|---|---|---|
| | P-values | F-values (observed) | P-values | F-values (observed) |
| Vitamin A (Retinol) | 0.768 | 0.55 | 0.140 | 1.68 |
| VitaminB1 (Thiamine) | 0.075 | 2.02 | 0.650 | 0.70 |
| Vitamin B2 (Riboflavin) | 0.001 | 6.04 | 0.935 | 0.30 |
| Vitamin B3 (Niacin) | 0.978 | 0.19 | 0.975 | 0.20 |
| Vitamin B9 (Folate) | 0.759 | 0.56 | 0.147 | 1.65 |

Level of significance = 0.05; F-critical value = 2.24

This may be explained by the small differences in temperature (10 ºC) and relative humidity (8 %) between the two storage conditions. Larger variations in storage temperature and relative humidity would probably indicate significant differences in the combined effect of storage time

and the storage conditions on vitamin stability. The difference observed in riboflavin stability could be attributed to sample exposure to light both during sampling and sample preparation for analysis. Ribeiro, Pinto, Lima and Sousa [35] observed that after storage of vitamin formulations at 25ºC for 72 hours, riboflavin retention was 99% and 94.7% with and without photo protection, respectively. This indicated that light influenced riboflavin stability during storage. Among all the vitamins assessed, light had the biggest impact on riboflavin's stability.[18,27]

Furthermore, the current study found that the combined effects of time and brand on the stability of retinol, thiamine, riboflavin, niacin, and folate concentrations in fortified maize flour stored for six months did not significantly differ ($p > 0.05$) (Table 1). Furthermore, as described in Table 1, the F-observed values were less than the F-critical (2.24). These statistics indicate that there was no significant interaction between time and brand on the levels of all vitamins retained in the flour samples. These results can be interpreted that although the vitamin content in the samples decreased during storage, there was no significant difference in the changes between brandXX1 and brand XY2. It therefore does not matter what brand the fortified maize flour is from since the vitamin changes are not significantly different. This observation may be attributed to the fact that vitamin premixes from different manufacturers are homogeneous in their technological development, therefore, reducing the differences in vitamin stability. These findings are in agreement with Yang, Wang, Li, Zhang and Ma[36] who reported that the vitamin supplier had no significant influence on vitamin A stability in vitamin premixes stored for 12 months.

## Conclusions

With regards to storage stability tests,retinol was the least stable vitamin for brand XXI at both 25 °C/75% RH and 35 °C/83% RH, followed by thiamine, riboflavin, folate, and niacin. However, brand XY2 showed that under both storage conditions, thiamine was the least stable vitamin, followed by retinol, riboflavin, folate, and niacin. In comparison to samples stored at higher temperatures and relative humidity (35 °C/ 83% RH), samples stored at lower temperatures and relative humidity (25 °C/ 75% RH) retained more vitamins for both brands XX1 and XY2. Despite the decrease in the vitamin content in the stored samples, there was no significant difference in the changes at 25 ºC/ 75 % RH and 35 ºC/ 83 % RH except for riboflavin. The stability of vitamins for both brands XX1 and XY2 progressively decreased over the six-month storage period however, there was no significant difference in the changes between the two brands. The results of this study conclusively show that most vitamin losses in fortified maize flour occur during storage. There is need for more studies to assess the bioaccessibility of the vitamins added to maize flour during the fortification process. This is because the overall goal of fortification of flour is to make micronutrients available to vulnerable groups through the consumption of fortified flour products.

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## Conflict of Interest

The authors declare no conflict of interest.

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