Cereal Chemistry

CEREALS & GRAINS ASSOCIATION

RESEARCH ARTICLE OPEN ACCESS

Vitamins Are Retained in Fortified Whole-Grain Maize Meal When Stored Under Tropical Conditions When Optimal Storage Practices Are Used

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Received: 31 March 2025 | **Revised:** 17 July 2025 | **Accepted:** 14 August 2025

Funding: The study was supported by the Rockefeller Foundation (contract number 011957), DSM-Firmenich (grant number 202400026), and the South African National Research Foundation to Tilana Graaff (grant number PMDS22051812333).

Keywords: fortification | storage conditions | vitamin stability | whole-grain maize meal

ABSTRACT

Background and Objective: To ensure provision of essential micronutrients to at-risk maize-consuming populations, their stability in stored fortified whole-grain maize meal (FWGMM) is essential. Vitamin stability in commercial FWGMM was studied over 6 months under two real-world tropical conditions (overall average 25.3°C, 53.5% relative humidity (RH) and 19.4°C, 66.5% RH) in grain stores. The maize was pre-dried before milling and optimal storage practices were employed.

Findings: No loss in Vitamin A (retinol palmitate), B1 and B2 (total and endogenous) occurred, contrary to other fortified maize meal storage studies without pre-drying and/or using suboptimal storage practices. Alternative treatments to maize pre-drying were investigated in an accelerated storage study at 40°C, 65% RH using similar storage practices. There was no loss in Vitamins B1 and B2 (total and endogenous) with any treatment including the not pre-dried maize control.

Conclusions: The vitamin stability in FWGMM during storage is attributable primarily to the use of a polyethylene inner liner in the sacks which largely prevented moisture ingress and to storage in semidarkness which prevented vitamin photo-degradation. Maize pre-drying provides additional assurance against moisture-induced degradation.

Significance and Novelty: These simple practices can help provide the full benefit of micronutrient-fortified WGMM to consumers.

1 | Introduction

Whole-grain cereals contain more and better-quality protein, more lipids and essential fatty acids, more dietary fiber and phytochemicals, and more micronutrients, including most B Vitamins, Vitamin E, and essential minerals than refined cereal grains (Allai et al. 2022). Maize is the major staple cereal in sub-Saharan Africa (Ekpa et al. 2018), the region of the world with the highest incidence of micronutrient deficiencies (Ohanenye et al. 2021). Whole-grain maize contains between 1.7- and 5.8-fold more of iron, magnesium, phosphorus and zinc and between 2.1- and 10.0-fold more of thiamine, riboflavin, niacin and folate compared to refined maize (FAO/Government of Kenya 2018). However, apart from magnesium, 100 g of whole-grain maize meal, prepared as porridge, will typically meet only 10%–20% of the general population's nutrient reference values (NRVs) for these micronutrients (KEBS 2015). Hence, for maize-consuming populations that are at risk of micronutrient malnutrition and its associated diseases, fortification of whole-grain maize meal as well as refined maize is highly beneficial (WHO 2016), and fortification of whole grains in general is being advocated for at-risk populations (Milani et al. 2024).

There are several challenges with replacing refined cereal grain foods with whole-grain foods. These include safety because of their potential to have higher contents of contaminants such as mycotoxins, heavy metals, and pesticide residues (Thielecke and Nugent 2018) and their sensory acceptability as they are darker in color, often have a coarser texture and are in stronger flavor (Heiniö et al. 2016; Bhasin 2020). The stronger flavor of whole-grain foods is primarily due to their flours/meals becoming rancid more rapidly during storage than refined grain flours, which due to their higher content of lipids and endogenous lipase and lipid-oxidizing enzymes (Doblado-Maldonado et al. 2012). This is especially a problem in cereals with a high fat content such as maize (Mestres et al. 2003; Deepa and Umesh Hebbar 2017; Taylor et al. 2024). Lipid rancidity in whole-grain foods is an important factor that can adversely affect their sensory characteristics (Heiniö et al. 2016; Onyeoziri et al. 2021).

The careful control of cereal storage conditions is always essential to prevent insect infestation and mold growth (Butkovsky and Ilana 2023) and this is even more critical with micronutrient-fortified whole-grain flours/meals to also prevent destruction of vitamins and to retard oil rancidity development (Hemery et al. 2018). In an accelerated shelf-life study at 40°C, we have shown that drying the whole kernel maize to below its equilibrium moisture content before milling retarded the development of fat acidity (indicator of hydrolytic rancidity) and greatly retarded peroxide value increase (indicator of oxidative rancidity) in vitamin- and mineral-fortified whole-grain maize meal (Taylor et al. 2024). Descriptive sensory evaluation indicated that the stored dried maize meal and *ugali* stiff porridge prepared from it were more similar to the not-stored control maize meal and its *ugali* than to the equilibrium moisture content-stored maize meal and its *ugali*. Also, except for riboflavin (Vitamin B2), where there was a substantial loss, there was no loss of other fortificant vitamins (Vitamin A, Vitamins B1, B3, B6, and B9) over 47 days storage at 40°C, equivalent to at least 20 weeks storage at 25°C. The loss in riboflavin was likely caused by it being involved in photosensitization-type reactions, to which riboflavin is very susceptible (Cardoso et al. 2012; Sheraz et al. 2014). Subsequent work (publication in preparation) indicated that the maize grain oxidative rancidity-causing peroxidase-type enzymes had been completely inactivated by the hot-air drying treatment, though the lipase enzyme, responsible for hydrolytic rancidity, had not been inactivated.

The objectives of this present study were two-fold: first, to determine the effect of storing the fortified whole-grain maize meal from pre-dried whole kernels for 6 months under actual tropical conditions on its stability, and second, to investigate alternative potentially more effective and economic treatments to grain pre-drying before milling as methods to stabilize the maize meal.

In this paper, we describe the effects of real-time storage and of the alternative treatments on micronutrient stability. The real-time storage study focussed primarily on Vitamins A, B1 and B2, with some data on Vitamins B3, B6, B9, and B12, plus iron and zinc. The alternative treatments study, which was an accelerated storage test at 40°C, focussed primarily on Vitamin B2 stability in view of its known instability, especially to light and our previous research findings (Taylor et al. 2024), but also with data on Vitamins B1.

The alternative treatments to pre-drying the maize investigated were:

Addition of mixed tocopherols to the fortified maize meal with the aim preventing vitamin oxidation (Allen et al. 2006; Sheraz et al. 2014).

Packaging the fortified maize meal in a black polyethylene liner with the aim of reducing exposure to light and hence preventing vitamin photodegradation (Allen et al. 2006; Cardoso et al. 2012; Sheraz et al. 2014).

Fortifying with riboflavin encapsulated in a lipid-based coating with the aim of preventing its degradation by light and oxygen (Cardoso et al. 2012; Sheraz et al. 2014).

2 | Materials and Methods

2.1 | Real-Time Storage Study

2.1.1 | Drying, Milling, and Storage

Commercial white maize of equilibrium moisture content 13.5% was dried using a commercial hot-air dryer at maximum air and grain temperature of 121°C and 62°C, respectively, down to moisture content of 11.3%. Within 24 h it was then milled into whole-grain maize meal using a commercial hammer mill fitted with an 0.8 mm opening screen. The maize meal was fortified using a manually dosed, batch-type ribbon-blending system with a commercial pre-mix containing Vitamin A (as retinol palmitate), Vitamins B1, B2, B3, B5, B6, B9, B12, iron (as NaFeEDTA), and zinc (as zinc oxide) obtained from DSM-Firmenich (Johannesburg, South Africa). The premix conformed to the East African Standard for fortified milled maize products (East African Community 2019). The maize drying, milling and fortification operations were kindly performed by Minimex Ltd., Kigali, Rwanda. The fortified whole-grain maize meal (FWGMM) had a moisture content of 9.76% (as is basis), similar to the low moisture content treatment FWGMM in our previous study (9.5% moisture) (Taylor et al. 2024). The FWGMM was packaged in 50 kg woven polypropylene (PP) sacks with polyethylene (PE) liners containing the maize meal. The PE liner was sealed by sewing into the top seam of the woven PP sack. At the same time, whole-grain maize meal from the dried maize that had not been fortified was produced and packaged in the same way.

Within 24 h, the sacks of maize meal were transported to grain stores at two locations in Rwanda: Kicukiro (altitude 1428 m) and Burera (altitude 2059 m), designated lower altitude (LA) and higher altitude (HA), respectively. They were stored for up to 6 months over the May–November 2023 period at the two locations. The sacks were stored on slatted wooden pallets under well-ventilated conditions and not exposed to direct sunlight or artificial light. The maximum light intensity in the LA store was 11–12 Lux and in the HA store it was 40 Lux. The light intensities in the grain stores were measured at approximately 12 noon using a Lux meter model MT-30 (R & D Instruments, Chenai, India).

The average daily temperatures at 08:00 and 14:00 over the 6 months in the two stores were: LA—23.0°C and 27.6°C, respectively and HA- 17.4°C and 21.3°C, respectively (Table 1). The minimum and maximum temperatures recorded were: LA—20.3°C and 30.7°C, respectively and HA—14.2°C and 24.5°C, respectively. The average relative humidities (RHs) were: LA—62% and 45%, respectively and HA—74% and 59%, respectively. The minimum and maximum RHs recorded were: LA—28% and 89%, respectively and HA—35% and 99%, respectively.

2.1.2 | Sampling and Analyses

At 1-month intervals, a sack of FWGMM and a sack of unfortified whole-grain maize meal were opened, and the entire contents of each sack were spread out on a plastic sheet and thoroughly mixed manually. The contents were then divided into four portions and a 250 g sample drawn from each. These were then thoroughly mixed together. The 1 kg sub-samples were vacuum-packed and covered with aluminum foil, then

TABLE 1 | Temperature and relative humidity in the two grain stores over 6-months storage, and moisture content and water activity (A_w) of the stored fortified whole-grain maize meal.

Grain store		**LA storage**				**HA storage**			
Parameter	**Month**	**08:00**	**14:00**	**Min.**	**Max.**	**08:00**	**14:00**	**Min.**	**Max.**
Grain store temperature (°C) monthly average at 08:00 and 14:00, minimum and maximum	1	24.0	28.1	21.7	30.0	17.4	20.5	16.0	23.2
	2	22.8	28.6	21.0	29.5	17.2	21.4	16.0	23.2
	3	23.7	28.4	21.6	31.0	17.6	22.5	15.8	24.5
	4	23.2	28.1	20.6	30.7	17.3	21.6	15.8	23.8
	5	22.6	27.1	20.7	30.7	18.2	22.1	15.5	24.4
	6	21.8	25.1	20.3	28.2	16.8	19.4	14.2	22.9
	Mean	**23.0**	**27.6**	**NA**	**NA**	**17.4**	**21.3**	**NA**	**NA**
Grain store relative humidity (%) monthly average at 08:00 and 14:00, minimum and maximum	1	59	42	36	65	75	63	52	80
	2	53	36	34	62	64	53	38	74
	3	48	35	28	56	72	47	35	87
	4	63	42	32	74	72	57	40	87
	5	70	50	39	81	72	59	49	86
	6	79	62	46	89	86	74	40	99
	Mean	**62**	**45**	**NA**	**NA**	**74**	**59**	**NA**	**NA**
Maize meal moisture content (%) (East Africa Standard: Max. 14.0%)	0	9.76				9.76			
	1	9.49				9.55			
	2	8.55				8.70			
	3	10.12				9.91			
	4	9.99				10.10			
	5	9.89				10.49			
	6	9.76				10.50			
	Mean	**9.65**				**9.86**			
Maize meal water activity (A_w) (decimal fraction)	0	0.378				0.378			
	1	0.395				0.395			
	2	0.373				0.393			
	3	0.428				0.413			
	4	0.409				0.420			
	5	0.473				0.472			
	6	0.450				0.466			
	Mean	**0.415**				**0.421**			

Abbreviations: HA, higher altitude; LA, lower altitude; NA, not applicable.

airfreighted to an analytical laboratory accredited for micronutrient fortificant assays in Italy.

Vitamin A was determined by HPLC according to European Standard method CEN EN 12823-1:2014 (BSI Knowledge 2024). The expanded uncertainty of measurement for the assay was ±15.9%.

Total (endogenous plus fortificant) contents of Vitamins B1, B2, B3, and B6 were determined by enzymatic digestion to release the vitamins and assayed by LC-MS/MS according to AOAC method 2015.14 (McClure 2020). The expanded uncertainty of measurement for the assay was ±20.3%. Endogenous Vitamin B2 was determined by the same method on stored maize meal that had not been fortified.

Total content of Vitamin B9 in terms of folate was determined by enzymatic digestion to release the vitamin isomers and assayed by LC-MS/MS according to AOAC method 2011.06 (Bhandari et al. 2018). The expanded uncertainty of measurement for the assay was ±30.8%.

Total content of Vitamin B12 was determined by extraction with a buffer containing cyanide and assayed by liquid chromatography with UV detection according to AOAC method 2014.02 (Giménez et al. 2018). The expanded uncertainty of measurement for the assay was ±20.8%.

Total contents of iron and zinc were determined by an ICP-MS procedure based on AOAC method 2015.06 (Pacquette et al. 2018). The expanded uncertainty of measurement for the assay was ±19.5%.

All vitamins and minerals were assayed in duplicate. Vitamin A and Vitamin B1, and total and endogenous Vitamin B2 were determined every month (months 0–6) during the study because of the potential particular instability of Vitamin A and Vitamin B2 (Parrish et al. 1980; Allen et al. 2006; Dunn et al. 2014; Sheraz et al. 2014). Vitamins B3, B6, B9, and B12, iron, and zinc were determined only at months 0 and 6.

Water activity (A_w) at 25°C was determined by ISO International Standard 18787:2017 (International Organization for Standardization 2017).

2.2 | Maize Treatments, Accelerated Storage Study

2.2.1 | Treatments, Milling, and Storage

Two batches (designated A and B) of commercial white maize (grade WM1) were obtained from Premier FMCG, Kroonstad, South Africa. The maize was stored at 8°C in the dark in woven PP sacks, with intermittent brief exposure to fluorescent light. Six treatments were applied to the maize as set out below. All fortified treatments were performed in duplicate.

2.2.1.1 | Maize Hot-Air Pre-Dried, Fortified (Dried; Fortified) Treatment (as in the Real-Time Study). Maize (2.5 kg batch size) of equilibrium moisture content 13.8% was hot-air dried in a pilot-scale forced convection, continuous tumble grain roaster (Roastech, Bloemfontein, South Africa) set at an air temperature of 100°C. The first kernel took approximately 13 min to exit the roaster and the last kernel ± 20 min to exit. The maize was dried to a moisture content of 10.9%. After drying, the kernels were cooled on stainless steel trays to a maximum of 30°C. The dried maize was milled using a Drotsky S1 hammer mill (Drotsky, Alberton, South Africa) fitted with a 500 µm opening screen. The resulting meal was cooled to ambient temperature (~25°C) before fortification. It was then fortified with a vitamin and mineral fortificant pre-mix of the composition and source described in Section 2.1.1, by very thorough mixing using a spiral dough-type mixer. The FWGMM was then packaged in 0.5 kg batches into woven PP bags with clear PE liners (zip-lock type bags) containing the maize meal.

2.2.1.2 | Maize not Pre-Dried, Fortified, With Added Tocopherols (Designated Fortified; Tocopherols). The maize (not hot-air pre-dried) was milled and fortified as described above. Then dry mixed tocopherols (30%), (DSM-Firmenich, Product code: 50 1572 1) in the form of a free-flowing powder, were very thoroughly mixed in as described at a dosage of 105 mg tocopherols/kg. The fortified meal was then packaged as described for the Dried; Fortified treatment.

2.2.1.3 | Maize not Pre-Dried, Fortified, Black Pigmented Additional HDPE Liner (Fortified; Black Additional Liner). The maize (not hot-air pre-dried) was milled and fortified as described for Dried; Fortified. It was then packaged as described above but including an additional black pigmented high-density PE liner between the clear PE liner and the woven PP bag.

2.2.1.4 | Maize not Pre-Dried, Fortified, Riboflavin Encapsulated (Fortified; Encapsulated Riboflavin). The maize (not hot-air pre-dried) was milled and fortified as described above, except that the riboflavin was in a lipid-encapsulated form (DSM-Firmenich). The fortified meal was then packaged as described for the Dried; Fortified treatment.

2.2.1.5 | Maize not Pre-Dried, Fortified (Fortified; Control). The maize (not hot-air pre-dried) was milled, fortified and packaged as described for Dried; Fortified treatment.

2.2.1.6 | Maize not Pre-Dried, Unfortified (Endogenous Micronutrient Content) (Unfortified; Control. Maize (not hot-air pre-dried) was milled and packaged as described for Dried; Fortified but without fortification.

The packaged maize meal was stored under accelerated storage conditions at 40°C, 65% RH in a LHS-150HC-I incubator (Bluepard Instruments, Shanghai, China) without internal illumination and with the incubator window masked to reduce external light penetration, that is, storage was in semidarkness (maximum light intensity 13–14 Lux). Light intensity was measured at approximately 12 noon using a Wanbang Model HTC1 Lux meter (Henan Wanbang Ep Tech, Shangqiu, China).

2.2.2 | Sampling and Analysis

Sampling was at 0, 1, 3, 5, and 9 weeks. When a Q_{10} temperature factor of 2 is applied, 9 weeks storage at 40°C corresponds to approximately 25 weeks at the LA storage temperature (average 25.6°C) and at 36 weeks at the HA storage temperature (temperature 19.7°C). The samples were treated as described in Section 2.1.1, except that the complete PP bags were sent for analysis and the bags were vacuum packed then stored at −20°C before sending a single consignment of samples for analysis. Vitamins B1 and B2 were analyzed at every sampling point over the 9-week storage period, and Vitamins B6 and B9, iron and zinc were determined only at week 0.

The vitamins and minerals were analyzed as described in 2.1.2.

2.3 | Statistical Analyses

The effects of storage time in the real-time and accelerated storage studies on the contents of Vitamins A, B1, and B2 were assessed by one-way analysis of variance (Tables 2 and 4). The effects of storage condition (LA vs. HA storage), treatment (Fortified, no treatment, not dried; Fortified, dried; Fortified, tocopherols; Fortified, black additional liner), storage time and their interactions (Tables 3 and 5) were assessed by two-way analysis of variance, using XLSTAT (Addinsoft).

The data for these vitamins were also plotted on scatter-plot graphs (Figures 1 and 2) and linear regression lines fitted and their correlations determined using Excel (Microsoft).

3 | Results and Discussion

3.1 | Real-Time Storage Study

On average, the temperature in the LA store was 6°C warmer than the HA store (25.3°C vs. 19.4°C) and the RH was, on average, 13% lower (53.5% vs. 66.5%), although there were large differences in the recorded maximum and minimum temperatures and RHs during each day and over the 6-month storage time (Table 1). The moisture content of maize meal in the LA store remained constant over 6-months storage (M0 and M6 both 9.76%), whereas it increased slightly to 10.5% at 5- and 6-months storage in the HA store. This can be attributed to the very high RH later during storage (daily average 80% at 6 months in the HA store). This was a result of this period being during the country's annual short rainy season. Notwithstanding this, the moisture content of the maize meal remained considerably below its equilibrium moisture content, which for whole kernel maize is approximately 16.8% at that RH (Hellevang, undated). This can be attributed to the sewn-in PE liner in the woven PP sack, which is specified by the World Food Programme (WFP 2020), which was clearly a very effective moisture barrier.

There was a slight increase in the water activity of the maize meal in both stores over the 6 months, from 0.378 to 0.450 and to 0.466 in the LA and HA stores, respectively (Table 2). That the increase in water activity occurred in the meal stored in the LA store where there was no increase in meal moisture content indicates that a small proportion of the water that was bound to the maize components, especially the carbohydrates, was freed. Nevertheless, the water activity of the maize meal in both storage conditions remained well below that required for mold growth on flour, > 0.7 (Carter et al. 2015), again attributable to the effectiveness of the PE liner.

Figure 1 shows the effect of storage of the FWGMM under the two conditions on the stability of Vitamins A, B1 and B2. There was no significant trend ($p > 0.05$) in the content of Vitamin A (almost entirely from fortificant, as there is very little provitamin A in white maize) under either condition over the 6 months of storage (Figure 1A). However, there was a relatively large variation in the analytical values between months (Table 2), with a significant effect of storage time ($p = 0.001$) (Table 3). There was also a significant effect of storage conditions ($p = 0.003$) (Table 3), with the measured quantity of Vitamin A being generally some 10% lower in the LA store (Figure 1A) but nevertheless it still conformed to the East African minimum standard for fortified maize (East African Community 2019).

Concerning Vitamin B1, there was no reduction in its content (fortificant plus endogenous) during storage, but similarly some variation in analytical values (Figure 1B and Table 2). However, there was an apparent significant trend in increasing content from months 0 to 6 ($p < 0.01$) (Figure 1B) and a significant increase in content from months 0 to 6 ($p \leq 0.05$) (Table 2) in the LA store, which was also reflected by two-way analysis of variance ($p < 0.001$) (Table 3). There was no significant effect of storage condition ($p > 0.05$).

With Vitamin B2, there was also no reduction in its content (fortificant plus endogenous), with some variation in analytical values. Like Vitamin B1, there was also an apparent significant increasing trend in Vitamin B2 content ($p < 0.001$) (Figure 1C) and a significant increase in content from months 0 to 6 ($p \leq 0.05$) (Table 2) in the LA store, which was also reflected by two-way analysis of variance ($p < 0.001$) (Table 3). There was no significant effect of storage condition ($p > 0.05$). That Vitamins B1 and B2 followed the same trend is logical as they are assayed simultaneously together with Vitamins B3 and B6 by AOAC method 2015.14 (McClure 2020).

With endogenous Vitamin B2, there was no significant trend in content over storage time under either storage condition ($p > 0.05$) (Figure 1C). It is noteworthy that endogenous Vitamin B2 contributed a small but meaningful proportion (approximately 17%) of the total Vitamin B2 in the FWGMM.

The contents of Vitamins B1 and B2 greatly exceeded the East African minimum standard for fortified maize (East African Community 2019) throughout the 6-month storage period under both the HA and LA storage conditions (Figure 1B,C).

These findings confirm the results of our previous accelerated (40°C) storage study (Taylor et al. 2024) with respect to fortification of whole-grain maize meal with Vitamin A and B1. However, in apparent contrast to the previous study, there was no loss of Vitamin B2 during the 6-month storage period. The most likely reason for the different finding is that in this present

TABLE 2 | Effect of storage over 6 months in the two grain stores on the contents of Vitamins A, B1, B2, B3, B6, B9, B12, iron, and zinc in fortified whole-grain maize meal.[a,b]

Grain store			
Micronutrient	**Month**	**LA storage**	**HA storage**
Vitamin A (fortificant) (mg Retinol Activity Equivalents/kg) (East African Standard Min. 0.5, Max. 1.4)	0	$0.51^{b} \pm 0.0$ (±0.08)[c]	$0.51^{ab} \pm 0.0$ (±0.08)
	1	$0.55^{b} \pm 0.0$ (±0.09)	$0.60^{abc} \pm 0.0$ (±0.10)
	2	$0.52^{b} \pm 0.0$ (±0.08)	$0.75^{c} \pm 0.1$ (±0.12)
	3	$0.40^{a} \pm 0.0$ (±0.06)	$0.49^{a} \pm 0.0$ (±0.08)
	4	$0.50^{ab} \pm 0.0$ (±0.08)	$0.60^{abc} \pm 0.0$ (±0.10)
	5	$0.49^{ab} \pm 0.1$ (±0.08)	$0.62^{abc} \pm 0.0$ (±0.10)
	6	$0.73^{c} \pm 0.0$ (±0.12)	$0.65^{bc} \pm 0.0$ (±0.10)
	Mean	**0.53**	**0.63**
Vitamin B1(thiamine) (mg/kg) (East African Standard Min. 3.0)	0	$6.0^{ab} \pm 0.1$ (±1.2)	$6.0^{ab} \pm 0.1$ (±1.2)
	1	$5.6^{a} \pm 0.3$ (±1.1)	$5.7^{a} \pm 0.1$ (±1.2)
	2	$6.7^{bc} \pm 0.1$ (±1.4)	$8.7^{de} \pm 0.1$ (±1.8)
	3	$7.9^{d} \pm 0.2$ (±1.6)	$9.6^{e} \pm 0.5$ (±1.9)
	4	$6.7^{bc} \pm 0.2$ (±1.4)	$7.1^{bc} \pm 0.8$ (±1.4)
	5	$7.3^{cd} \pm 0.5$ (±1.5)	$7.9^{cd} \pm 0.5$ (±1.6)
	6	$7.9^{d} \pm 0.1$ (±1.6)	$5.4^{a} \pm 0.1$ (±1.1)
	Mean	**6.9**	**7.2**
Vitamin B2 (riboflavin) (mg/kg) (East African Standard Min. 2.0)	0	$2.5^{a} \pm 0.0$ (±0.5)	$2.5^{a} \pm 0.0$ (±0.5)
	1	$2.7^{ab} \pm 0.1$ (±0.5)	$2.9^{a} \pm 0.2$ (±0.6)
	2	$3.4^{c} \pm 0.1$ (±0.7)	$4.4^{c} \pm 0.1$ (±0.9)
	3	$4.2^{d} \pm 0.1$ (±0.9)	$5.0^{c} \pm 0.0$ (±1.0)
	4	$3.1^{bc} \pm 0.2$ (±0.6)	$3.7^{b} \pm 0.2$ (±0.8)
	5	$4.0^{d} \pm 0.1$ (±0.8)	$4.8^{c} \pm 0.4$ (±1.0)
	6	$5.1^{e} \pm 0.0$ (±1.0)	$2.7^{a} \pm 0.0$ (±0.5)
	Mean	**3.6**	**3.7**
Vitamin B3 (niacin) (mg/kg) (East African Standard Min. 14.9)	0	16.4 ± 0.3 (±3.3)	16.4 ± 0.3 (±3.3)
	6	35.2 ± 0.5 (±7.1)	20.3 ± 1.3 (±4.1)
Vitamin B6 (pyridoxine) (mg/kg) (East African Standard Min. 2.0)	0	4.3 ± 0.1 (±0.9)	4.3 ± 0.1 (±0.9)
	6	6.4 ± 0.2 (±1.2)	4.0 ± 0.0 (±0.8)
Vitamin B9 (folate) (mg/kg) (East African Standard Min 0.6, Max. 1.7)	0	0.67 ± 0.0 (±0.21)	0.67 ± 0.0 (±0.21)
	6	0.59 ± 0.0 (±0.18)	0.39 ± 0.0 (±0.12)
Vitamin B12 (cobalamin) (μg/kg) (East African Standard Min. 7)	0	20.0 ± 0.0 (±4.2)	10.0 ± 0.0 (±2.1)
	6	20.0 ± 0.0 (±4.2)	10.0 ± 0.0 (±2.1)
Iron (mg/kg) (East African Standard Min. 21)	0	41.4 ± 0.9 (±8.1)	41.4 ± 0.9 (±8.1)
	6	62.0 ± 2.0 (±12.1)	40.7 ± 0.2 (±7.9)
Zinc (mg/kg) East African Standard Min. 33, Max. 65)	0	44.6 ± 0.7 (±8.7)	44.6 ± 0.7 (±8.7)
	6	70.5 ± 1.5 (±13.7)	39.7 ± 0.9 (±7.7)

Abbreviations: HA, higher altitude; LA, lower altitude.

[a]Values are for total (fortificant+endogenous) micronutrients, unless stated otherwise.

[b]Means and standard deviations of duplicate determinations (replicate analysis), values with different superscript letters differ significantly over storage time ($p \leq 0.05$).

[c]Values in brackets are the expanded uncertainty of measurement.

study the FWGMM was exposed to much less light, which prevented photodegradation of the Vitamin B2, to which it is highly susceptible (Cardoso et al. 2012; Sheraz et al. 2014). Although the packaging was the same in both studies, here the maize meal was stored in 50 kg sacks as opposed to 500 g bags in the previous study. The former has a smaller surface area to volume ratio. Further, in this present study, the grain stores were of a design (perforated brick walls with an overhanging

TABLE 3 | Two-way analysis of variance of the effects of storage condition (higher and lower altitude stores) and storage time (months) on Vitamin A, B1, and B2 contents.

Vitamin A **Effects**	**DF (number)**	**DF (denominator)**	***F*-value**	**Probability**
Storage condition	1	14	12.505	0.003
Months of storage	6	14	8.409	0.001
Storage condition × Month	6	14	3.298	0.031
Vitamin B1				
Storage condition	1	14	3.896	0.068
Months of storage	6	14	22.436	< 0.001
Storage condition × Month	6	14	10.472	< 0.001
Vitamin B2				
Storage condition	1	14	2.224	0.158
Months of storage	6	14	48.854	< 0.001
Storage condition × Month	6	14	27.452	< 0.001

Abbreviations: DF, degress of freedom; HA, higher altitude; LA, lower altitude.

roof) that prevented exposure to direct sunlight and there was little or no artificial illumination. As stated in Section 2.1.1, the light intensity at noon in the LA store was 11–12 Lux and 40 Lux in the HA store. The previous accelerated study (Taylor et al. 2024) was performed in an incubator with internal illumination (fluorescent light) with a light intensity of 560–562 Lux, that is, some 48-fold and 14-fold higher than in the LA and HA stores, respectively. The light intensity in the grain stores compare with a measured full sunlight intensity of 70,000 Lux and an outside shade light intensity of 7000 Lux, which are in good agreement with published values of 100,000 Lux for a bright summer day and 10,000 Lux for full daylight (Sorsa-Leslie 2022).

Two recent studies of vitamin stability of stored fortified maize flour from Kenya have found significant losses of Vitamins A, B1, and B2 and other vitamins. Khamila et al. (2020) reported 16% loss in Vitamin A and a 40% loss in Vitamin B2 over 6-months storage at 25°C, 60% RH (similar temperature to the LA storage condition) in commercially fortified maize flour kept in "brown khaki" bags (unbleached kraft paper bags). Chepkoech et al. (2022) reported an average 16% loss in Vitamin A, 29% in Vitamin B1, and 17% in Vitamin B2 over 6 months storage at 25°C, 75% RH in two samples of commercial fortified maize flour, also kept in "brown khaki" paper bags. The type of maize flour used in these studies was not reported but was presumably semi-refined as currently there is little fortified whole-grain maize flour in that country. It is likely that the retention of these vitamins in this present study was due primarily to the packaging material and pre-drying the maize to below equilibrium moisture content before milling. As stated, the sewn-in PE liner acted as an effective moisture barrier. In contrast, paper packaging is moisture permeable (Awol et al. 2024). Unfortunately, the authors of the two papers did not state the moisture content of the flours. However, the equilibrium moisture content of maize stored under these conditions is approximately12.5% at 25°C, 60% RH and 15.1% at 25°C, 75% RH (Hellevang 2020), to which the maize flour would have equilibrated to over the 6 months storage. According to the FAO (1999), the ideal moisture content for long-term storage of maize grain is < 13.0%. Thus, the latter would be considerably above this moisture content and substantially above the East African maximum 14% moisture standard for fortified maize flour (East African Community 2019).

Several reasons were probably responsible for the relatively large variation in vitamin analytical values during storage observed in this present study and apparent trends. These include: the fortificant premix not being totally uniformly mixed into the maize meal, the fortificants settling during transport and storage of the sacks, not totally uniform sampling and sub-sampling, and intrinsic and extrinsic issues with the analytical methods and actual analyses. The analysis methods used to determine vitamin content, although CEN and AOAC standard methods, are subject to quite large variations. According to the accredited laboratory that performed the analyses, the expanded uncertainty of measurement for Vitamin A was ±15.9% and ±20.3% for Vitamins B1 and B2. The expanded uncertainty of measurement indicates that there is a 95% probability that the true value is within this range (US National Institute of Standards and Technology 2025). Hence, the differences in measured vitamin content between months are largely apparent rather than real, as is shown in Table 2 where the expanded uncertainty of measurement is provided for the vitamin values.

As to why the content of Vitamin A was generally lower throughout storage in the FWGMM stored under the LA condition (Figure 1A), a likely explanation is that most of the bags delivered to this store contained a little less retinol palmitate fortificant than those delivered to the HA store. As explained in Section 2.1.1, the fortificant pre-mix was incorporated into the maize meal using a batch-type ribbon-blending system. Purpose-built automated, continuous fortificant dosifiers are preferred for consistent blending (Johnson et al. 2004).

Concerning the increasing trend in Vitamins B1 and B2 during storage under the LA condition (Figure 1B,C), it is possible that

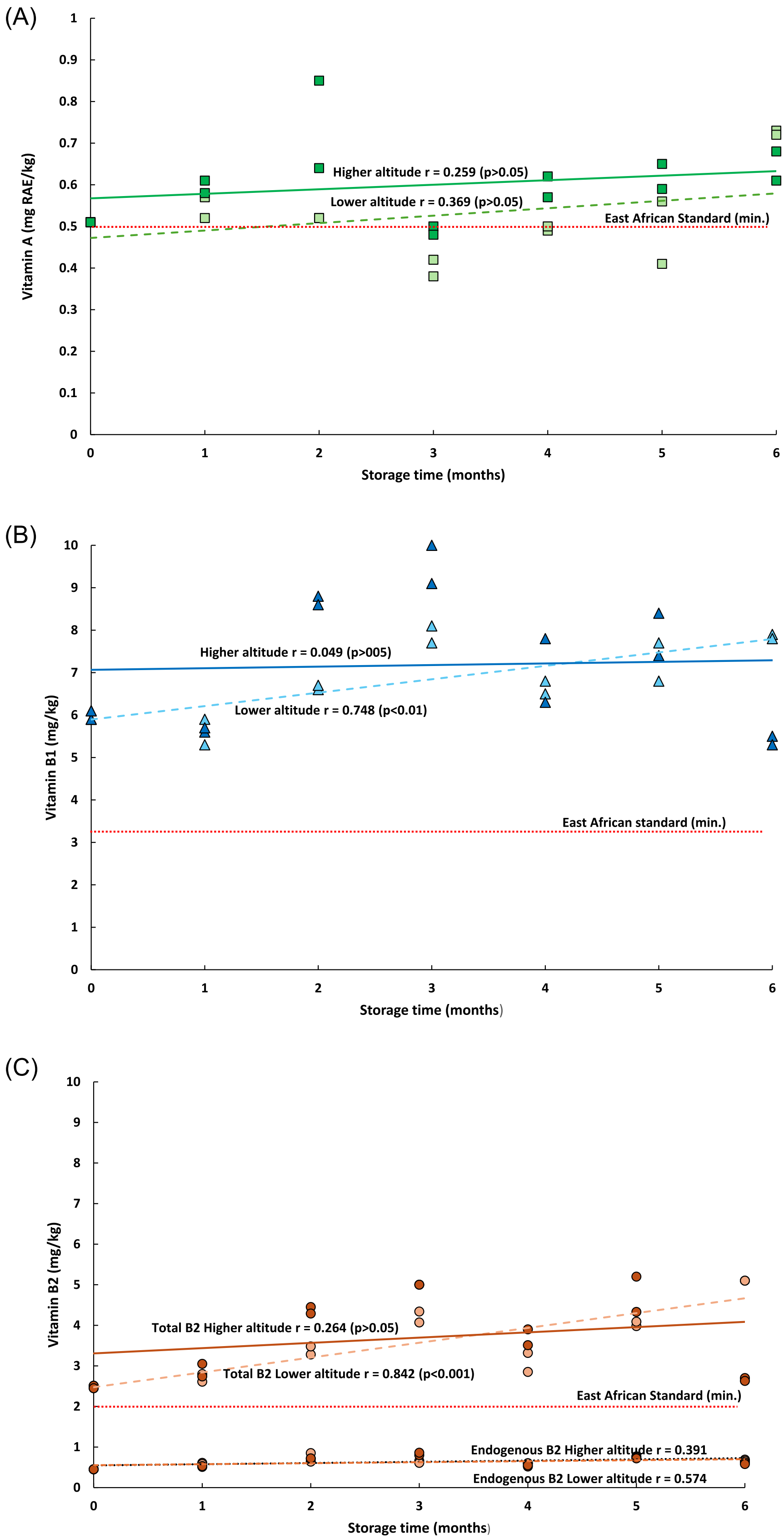

FIGURE 1 | Effects of storage of fortified whole-grain maize meal for 6 months under two different tropical conditions (overall average 25.3°C, 53.5% ambient relative humidity—lower altitude and 19.4°C, 66.5% ambient relative humidity—higher altitude) on its contents of vitamins. (A) Vitamin A. (B) Vitamin B1. (C) Vitamin B2.

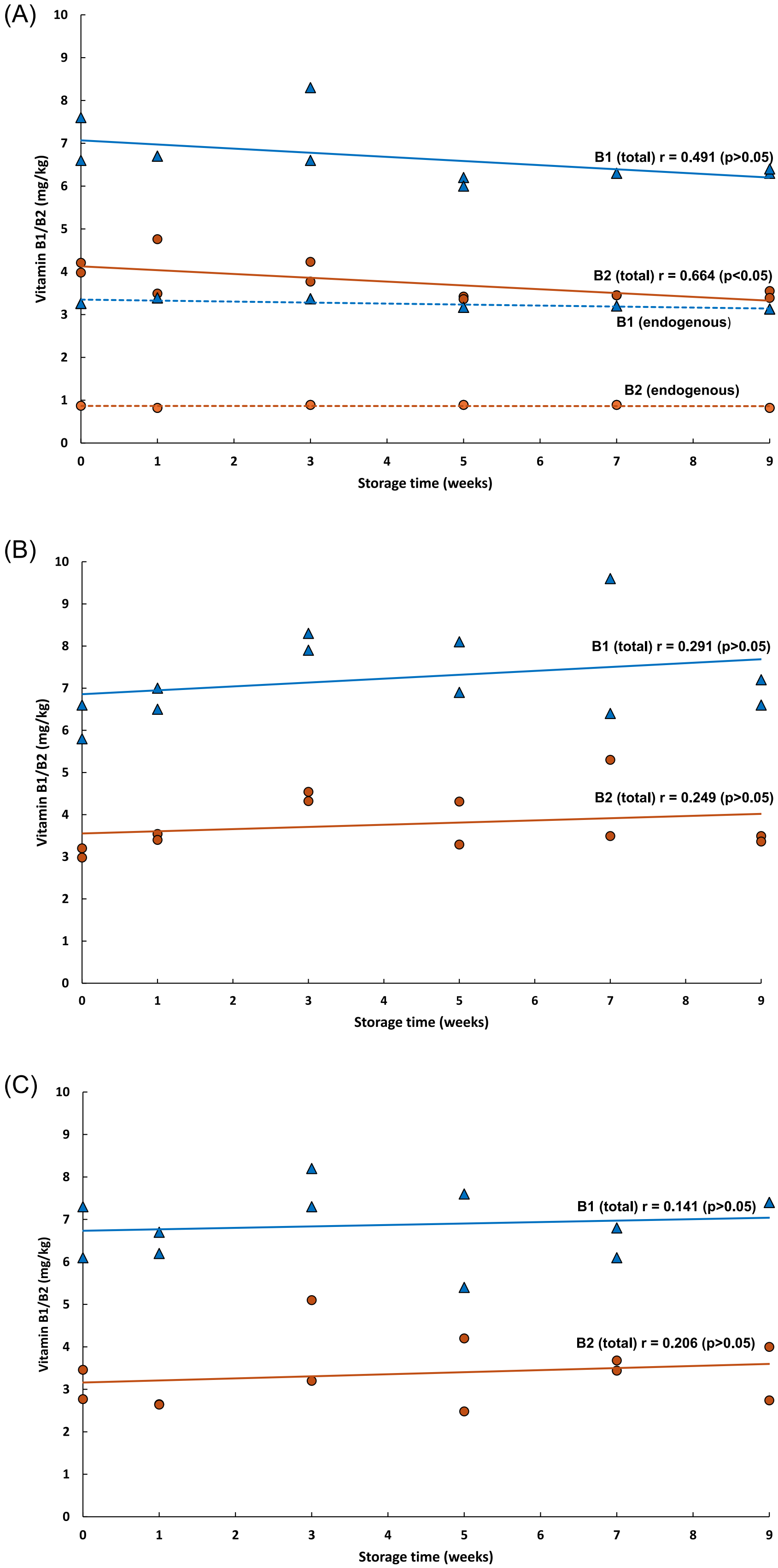

FIGURE 2 | Effects of different treatments on the contents of Vitamins B1 and B2 in fortified whole-grain maize meal stored at 40°C, 65% ambient relative humidity for 9 weeks. (A) No treatment; maize not pre-dried. (B) Fortified; maize pre-dried. (C) Fortified; Tocopherols, maize not pre-dried. (D) Fortified; Black additional liner, maize not pre-dried. (E) Fortified; encapsulated riboflavin (Vitamin B2), maize not pre-dried.

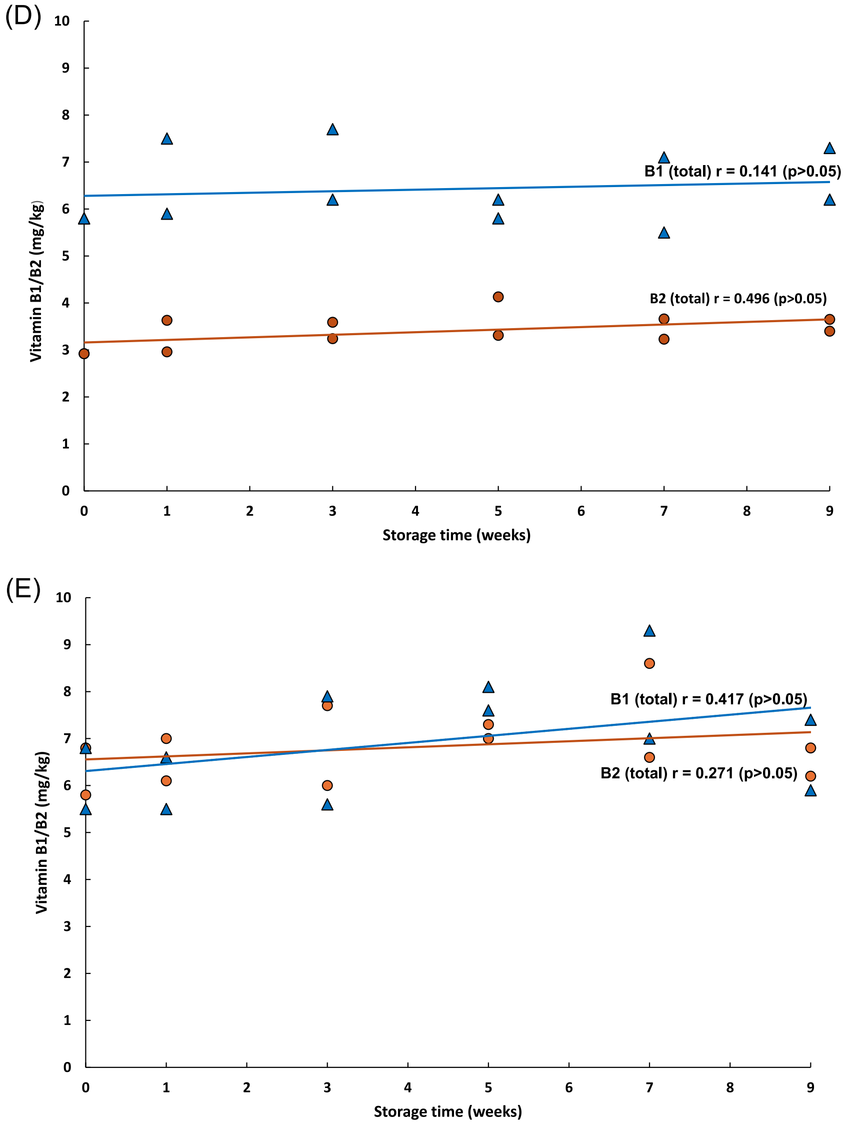

FIGURE 2 | (Continued)

this was a result of the increase in water activity, which rendered these water-soluble vitamins easier to extract. However, as water activity similarly increased in the meals under both conditions and maize meal moisture content only increased under the HA condition (Table 1), this seems highly improbable. It is more likely that the increasing trend under the LA condition, although statistically significant ($p < 0.01$), was due to a combination of the above-mentioned factors. Of these, differences in the quantity of fortificants in individual sacks seems to be the most important because the month 6 LA condition sample had high contents of all the fortificant micronutrients, most notably iron and zinc (Table 2). As these are inorganic minerals, their content cannot change over time.

Regarding the effects of storage under the two conditions on the contents of the other micronutrients, as with Vitamins A, B1, and B2, there were relatively large variations in the measured contents. Unfortunately, due to cost constraints these other micronutrients could not be measured at time intervals during the storage period. Notwithstanding this, Vitamins B3, B6, B12, iron, and zinc all met the East African minimum standard for fortified maize (East African Community 2019) after the 6 months storage under both conditions (Table 2). There are reports of small losses in Vitamin B3 during storage of commercial fortified maize flour (probably semi-refined flour). Khamila et al. (2020) found a 12% loss over 6 months at 25°C, 60% RH and Chepkoech et al. (2022) found an average loss of 14% over 6 months at 25°C, 75% RH. Their findings are, however, not directly comparable with the present study as they used moisture-permeable packaging.

In the present study, the one exception was Vitamin B9 (folate), where after 6 months storage the measured content was marginally below the specification of 0.6 mg/kg in the LA condition (0.59 mg/kg), whereas it was considerably below it in the HA condition (0.391 mg/kg) (Table 2). However, when looking at the values the expanded uncertainty of measurement for the AOAC Vitamin B9 assay method used has to be taken into consideration, which was particularly high, ±30.8%. There are also reports of Vitamin B9 loss during storage of commercial fortified maize flour (probably semi-refined flour). Issa-Zaccharia et al. (2024) found a 33% loss over 6 months under ambient conditions, whereas Khamila et al. (2020) found only a 13% loss and Chepkoech et al. (2022) found an average loss of 14%. As with Vitamin B3, their findings are, however, not directly comparable. Vitamin B9 fortificant is relatively stable (Allen et al. 2006). Hence, taking this into account and the findings with the other vitamins, it is possible that the measured low content after 6 months storage in the HA condition was due to that bag having a low content of Vitamin B9 and the particularly high expanded uncertainty of measurement of its assay method.

3.2 | Maize Treatments, Accelerated Storage Study

The purposes of the four test treatments were as follows:

- Dried; Fortified–Hot-air pre-drying the maize kernels to below their equilibrium moisture content, as described previously (Taylor et al. 2024) and as performed in the real-time storage study, was with the dual aim of retarding the enzymatic reactions which are primarily responsible for fat rancidity in stored whole-grain cereal flours (Doblado-Maldonado et al. 2012) and retarding moisture-accelerated degradation of vitamins. Hemery et al. (2018) found that RH and packaging (moisture-proof vs. moisture permeable) were two factors that highly significantly affected vitamin stability in fortified flour ($p < 0.001$).
- Fortified; Tocopherols–Tocopherols were added as an antioxidant with the dual aim of retarding oxidative rancidity (Doblado-Maldonado et al. 2012) and protecting the susceptible vitamins against oxidation. Pure Vitamin A is very unstable when exposed to oxygen and air (Allen et al. 2006) and the degradation of Vitamin B2 can involve oxidation in addition to photodegradation (Sheraz et al. 2014).
- Fortified; Black additional liner–Packaging using a black pigmented additional PE liner was aimed at preventing light-catalyzed destruction of the vitamins. Both Vitamin B2 (Cardoso et al. 2012; Sheraz et al. 2014) and not encapsulated Vitamin A (Allen et al. 2006) are very susceptible to photodegradation.
- Fortified; Riboflavin encapsulated–Vitamin B2 was encapsulated in a lipid-based coating with the aim of preventing its degradation by light and oxygen.

With all of the test treatments and with the maize not pre-dried fortified control there were no significant effects ($p > 0.05$) on Vitamin B1 or Vitamin B2 (both not encapsulated and encapsulated) contents during storage (Table 4). There were also no significant trends ($p > 0.05$) with respect to the content of these vitamins over storage time (Figure 2), except for the Vitamin B2 fortified; not pre-dried treatment, where there was a slight but significant declining trend, $p < 0.05$ (Figure 2A). However, two-way analysis of variance showed that there was no significant effect ($p > 0.05$) of either storage time or treatment on the contents of Vitamin B1 or B2 with any treatments (Table 5). Thus, both Vitamin B1 and Vitamin B2 were stable over the 9-weeks storage period at 40°C (equivalent to approximately 25 weeks at 25°C and 36 weeks at 20°C) at an RH of 65% irrespective of the treatment applied, including pre-drying the maize kernels to below their equilibrium moisture content before the milling treatment.

The finding for Vitamin B1 is the same as we reported previously for accelerated storage of FWGMM stored in the same packaging (woven PP bag with PE liner), made from ambient moisture maize and from maize that had been pre-dried to below its equilibrium moisture content (Taylor et al. 2024), where there was no loss. It is different, however, from our previous Vitamin B2 data where a substantial loss occurred over storage, which was higher in the FWGMM made from ambient moisture maize (Taylor et al. 2024). The findings of the present study are also different from those of studies of fortified maize flour (probably semi-refined), where substantial losses of Vitamin B1 and a rather smaller loss of Vitamin B2 during storage were reported (Khamila et al. 2020; Chepkoech et al. 2022), as described in Section 3.1.

Concerning Vitamin B1, its fortificant forms are sensitive to heat, alkali, oxygen, and radiation (Voelker et al. 2018). Although PE is a relatively poor oxygen barrier (Krochta 2002), the PE liner may have been sufficient to prevent its loss by oxygen exposure in this study and our previous study (Taylor et al. 2024). As stated, the fortified maize flour storage studies of Khamila et al. (2020) and Chepkoech et al. (2022) utilized "brown khaki" paper packaging, which is very gas permeable as well as moisture permeable (Hamdani et al. 2022).

That there was no loss in Vitamin B2 during storage with any of the treatments including the fortified maize not pre-dried treatment where the flour moisture content was 12.0% (Table 4) can be attributed to the FWGMM being stored in semidarkness in this study, maximum light intensity 13–14 Lux. This is in contrast to our previous study (Taylor et al. 2024) where the bags of FWGMM were exposed to a continuous light intensity of 560–562 Lux. As explained, Vitamin B2 is highly susceptible to photodegradation (Cardoso et al. 2012; Sheraz et al. 2014). Unfortunately, the illumination conditions in the fortified maize flour storage studies of Khamila et al. (2020) and Chepkoech et al. (2022) were not stated. In this regard, the comment of Hemery et al. (2018) that it could be useful to model the impact of exposure time to light, various temperatures and RH conditions on Vitamin A stability in stored fortified flour is clearly also applicable to Vitamin B2 and probably other vitamins.

4 | Conclusions

If optimal storage practices are applied, micronutrient-fortified whole-grain maize meal can be stored for several months without loss of fortificant- or endogenous vitamins under tropical conditions, average daily temperature up to approximately 25°C and average ambient relative humidity up to approximately 65%. Logically, this should also apply to fortified refined maize meal. The environmental parameters, both in storage and during distribution, that all need to be controlled are maize meal moisture content, ambient light intensity, ventilation, and possibly oxygen (to some extent). Additionally, application of standard grain and flour storage practices including storage of sacks on pallets and correct stacking,

TABLE 4 | Effects of various treatments on the moisture content and contents of Vitamins B1, B2, B6, B9, iron, and zinc in fortified whole-grain maize meal when stored at 40°C (accelerated storage), 65% relative humidity.[a,b]

Maize batch Treatment			A Unfortified; (endogenous micronutrient content) No treatment (not dried)	B Fortified; no treatment (not dried)	Fortified; Dried	Fortified; Tocopherols	Fortified; Black additional liner	Fortified; Encapsulated riboflavin
Moisture content % (average over storage) (East African Standard Max 14.0%)			11.4	12.0	9.0	12.6	12.0	11.7
Micronutrient	**Weeks at 40°C**	**Weeks approx. equiv. at 25°C**						
Vitamin B1(thiamine) (mg/kg) (East African Standard Min. 3.0)	0	0	3.3 (±0.7)[c]	$7.1^a \pm 0.5$ (±1.6)	$6.2^a \pm 0.4$ (±1.3)	$6.7^a \pm 0.6$ (±1.4)	5.8^a (±1.2)	$6.3^a \pm 0.5$ (±1.3)
	1	3	3.4 (±0.7)	$6.7^a \pm 0.0$ (±1.4)	$6.8^a \pm 0.3$ (±1.4)	$6.5^a \pm 0.3$ (±1.3)	$6.7^a \pm 0.8$ (±1.4)	$6.6^a \pm 0.5$ (±1.3)
	3	9	3.4 (±0.7)	$7.5^a \pm 0.9$ (±1.5)	$8.1^a \pm 0.2$ (±1.6)	$7.8^a \pm 0.5$ (±1.6)	$7.0^a \pm 0.8$ (±1.4)	$6.9^a \pm 0.9$ (±1.4)
	5	14	3.2 (±0.6)	$6.1^a \pm 0.1$ (±1.2)	$7.5^a \pm 0.6$ (±1.5)	$6.5^a \pm 1.1$ (±1.3)	$6.0^a \pm 0.2$ (±1.2)	$7.2^a \pm 0.2$ (±1.5)
	7	20	3.2 (±0.6)	$6.3^a \pm 0.0$ (±1.3)	$8.0^a \pm 1.6$ (±1.6)	$6.5^a \pm 0.4$ (±1.3)	$6.3^a \pm 0.8$ (±1.3)	$7.6^a \pm 1.0$ (±1.5)
	9	26	3.1 (±0.6)	$6.4^a \pm 0.1$ (±1.3)	$6.9^a \pm 0.3$ (±1.4)	$6.9^a \pm 0.5$ (±1.4)	$6.8^a \pm 0.6$ (±1.4)	$6.5^a \pm 0.3$ (±1.3)
	Mean		**3.3**	**6.7**	**7.3**	**6.3**	**6.4**	**6.9**
Vitamin B2 (riboflavin) (mg/kg) (East African Standard Min. 2.0)	0	0	0.9 (±0.2)	$4.1^a \pm 0.1$ (±0.8)	$3.1^a \pm 0.1$ (±0.6)	$3.1^a \pm 0.3$ (±0.6)	2.9^a (±0.6)	$6.2^a \pm 0.7$ (±1.3)
	1	3	0.8 (±0.2)	$4.1^a \pm 0.6$ (±0.8)	$3.5^a \pm 0.1$ (±0.7)	$2.6^a \pm 0.0$ (±0.5)	$3.3^a \pm 0.3$ (±0.7)	$6.1^a \pm 0.6$ (±1.2)
	3	9	0.9 (±0.2)	$4.0^a \pm 0.2$ (±0.8)	$4.4^a \pm 0.1$ (±0.9)	$4.2^a \pm 1.0$ (±0.9)	$3.4^a \pm 0.2$ (±0.7)	$6.8^a \pm 1.2$ (±1.4)
	5	14	0.9 (±0.2)	$3.4^a \pm 0.0$ (±0.7)	$3.8^a \pm 0.5$ (±0.8)	$3.3^a \pm 0.9$ (±0.7)	$3.7^a \pm 0.4$ (±0.8)	$7.9^a \pm 0.3$ (±1.6)
	7	20	0.9 (±0.2)	$3.5^a \pm 0.0$ (±0.7)	$4.4^a \pm 0.9$ (±0.9)	$3.6^a \pm 0.1$ (±0.7)	$3.4^a \pm 0.2$ (±0.7)	$8.2^a \pm 1.2$ (±1.7)
	9	26	0.8 (±0.2)	$3.5^a \pm 0.1$ (±0.7)	$3.4^a \pm 0.1$ (±0.7)	$3.4^a \pm 0.6$ (±0.7)	$3.5^a \pm 0.1$ (±0.7)	$6.7^a \pm 0.8$ (±1.4)
	Mean		**0.9**	**3.8**	**3.8**	**3.4**	**3.4**	**7.0**
Vitamin B6 (pyridoxine) (mg/kg) (East African Standard Min. 2.0)	0	0	1.4 (±0.3)	4.2 ± 0.0 (±0.9)	3.9 ± 0.2 (±0.8)	4.0 ± 0.1 (±0.8)	3.4 (±0.7)	4.3 ± 0.4 (±0.9)

(Continues)

TABLE 4 | (Continued)

Maize batch Treatment			A Unfortified; (endogenous micronutrient content) No treatment (not dried)	B Fortified; no treatment (not dried)	Fortified; Dried	Fortified; Tocopherols	Fortified; Black additional liner	Fortified; Encapsulated riboflavin
Vitamin B9 (folate) (mg/kg) (East African Standard Min 0.6, Max. 1.7)	0	0	0.17 (±0.05)	0.62 ± 0.00 (±0.19)	0.86 ± 0.40 (±0.26)	0.51 ± 0.10 (±0.16)	1.13 (±0.39)	0.65 ± 0.20 (±0.20)
Iron (mg/kg) (East African Standard Min. 21)	0	0	19.4[d]	45.0 ± 1.9 (±8.8)	41.2 ± 0.9 (±8.0)	39.3 ± 7.2 (±7.7)	43.5 (±8.5)	40.7 ± 3.4 (±7.9)
Zinc (mg/kg) East African Standard Min. 33, Max. 65)	0	0	16.8 (±3.3)	59.0 ± 5.0 (±11.5)	53.3 ± 2.8 (±10.4)	46.4 ± 5.7 (±9.0)	51.4 (±10.0)	56.0 ± 2.0 (±10.9)

[a]Values are for total (fortificant+endogenous) micronutrients, unless stated otherwise.
[b]Means and standard deviations of two independent replicate experiments, values with different superscript letters in a column for a particular vitamin differ significantly over storage time ($p \leq 0.05$).
[c]Values in brackets are the expanded uncertainty of measurement.
[d]Own data not available, endogenous iron content value from Dunn et al. (2014), which is the mean of 1215 samples.

TABLE 5 | Two-way analysis of variance of the effects of treatment (Fortified, no treatment, not dried; Fortified, dried; Fortified, tocopherols; Fortified, black additional liner) and storage time (weeks) on Vitamin B1 and B2 contents.

Effects	DF (number)	DF (denominator)	*F*-value	Probability
Vitamin B1				
Treatment	3	24	2.088	0.128
Weeks of storage	5	24	1.842	0.143
Treatment × Week	15	24	0.681	0.778
Vitamin B2				
Treatment	3	24	1.740	0.186
Weeks of storage	5	24	1.505	0.225
Treatment × Week	15	24	0.936	0.541

fumigation against insects, prevention of rodents and birds, and maintenance of cleanliness are essential.

The moisture content of the maize meal must be kept below 13.0% (preferably rather lower) to prevent general moisture-induced degradation of vitamins (Allen et al. 2006; Hemery et al. 2018). Moisture ingress into the meal under high relative humidity conditions can be largely prevented by using sacks with a polyethylene inner liner, which may also act as a partial oxygen barrier. Drying the grain to $\leq 11.5\%$ moisture directly before milling provides a further assurance against moisture-induced vitamin loss (Taylor et al. 2024). Good ventilation, including correct stacking of sacks to prevent temperature gradients within the store, is necessary to avoid moisture migration within the grain/flour mass (Joffe 1958). Storage in semidarkness is essential to prevent previously observed losses in Vitamin A (Hemery et al. 2018) and Vitamin B2 (Khamila et al. 2020; Chepkoech et al. 2022; Taylor et al. 2024), which are caused primarily by light-catalyzed reactions.

If all these storage practices can be rigorously applied, other measures such as light-proof packaging, inclusion of chemical antioxidants and encapsulation of Vitamin B2 (in addition to the required practice of encapsulating Vitamin A) appear to be unnecessary. They could, however, provide additional assurance under extreme tropical storage conditions. Additionally, systematic studies into the effects of light exposure in combination with other storage conditions on the stability of Vitamin A, B2 and other B vitamins, especially Vitamin B9, in fortified whole-grain maize meal are desirable.

Acknowledgments

The management and staff of Minimex Ltd, Rwanda. for drying and milling the maize, the World Food Programme and its staff at Kicukiro, and Mr. J.C. Ngwijabanzi and Mr. F. Rukundo Francois in Burera for storing the maize meal in their school's facilities, Ms Clarissa van Heerden and Dr John Lubaale for technical support at the University of Pretoria. Financial support was provided by the Rockefeller Foundation (contract number 011957), DSM-Firmenich (grant number 202400026), and the South African National Research Foundation to Tilana Graaff (grant number PMDS22051812333).

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