EFFECT OF STORAGE CONDITIONS AND COOKING ON THE STABILITY OF MICRONUTRIENTS AND THE POTENTIAL OF FT-NIR SPECTROSCOPY IN PREDICTING RETINOL IN COMMERCIAL FORTIFIED MAIZE FLOUR IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature ……. Date ……..

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This thesis has been submitted for examination with our approval as the university supervisors.

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DEDICATION

This thesis is dedicated to my parents, Philip and Lilian, for teaching me the construct of life and hard work. To my brother, Brian, for walking this journey with me. You are all my greatest support and motivation. To my little sister, the late Brandie Chebet, our fond memories gave me the courage to keep pushing. I would also like to encourage my extended family to aim high just like I have done and continue to do.

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LIST OF ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BHT	Butylated hydroxytoluene
°C	Degrees centigrade
Fe	Iron
FFAs	Free Fatty Acids
FT-NIR	Fourier Transform Near Infrared
GAIN	Global Alliance for Improved Nutrition
HNO_3	Nitric acid
HPLC	High Performance Liquid Chromatography
hr	Hour
IDA	Iron Deficiency Anaemia
InGaAs	Indium Gallium Arsenide
IR	Infrared
JKUAT	Jomo Kenyatta University of Agriculture and Technology
kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate

KNBS	Kenya National Bureau of Statistics
KOH	Potassium hydroxide
LBW	Low Birth Weight
LDA	Linear Discriminant Analysis
MC	Mean Centering
MeOH	Methanol
mg	Milligram
ml	Millilitres
MLR	Multiple Linear Regression
mM	Millimoles
MMN	Multiple Micronutrient
MNMS	Malawian National Micronutrient Survey
MPLS	Modified Partial Least Squares
MSC	Multiplicative Scatter Correction
NaFeEDTA	Sodium Iron Ethylenediaminetetraacetate
NIR	Near Infrared
NIRS	Near Infrared Spectroscopy
NTDs	Neural Tube Defects
PbS	Lead Sulphide

PCR	Principal Component Regression
PLS-R	Partial Least Squares Regression
R^2	Coefficient of determination
R^2_C	Coefficient of determination for calibration
R^2_V	Coefficient of determination for validation
RH	Relative Humidity
RMSECV	Root Mean Square Error of Cross-Validation
RMSEE	Root Mean Square Error of Estimation
RMSEP	Root Mean Square of Error of Prediction
RPD	Ratio of Performance to Deviation
SD	Standard Deviation
SGA	Small for Gestational Age
SNV	Standard Normal Variate
UK	United Kingdom
µl	Microlitres
UV	Ultra-violet
VAD	Vitamin A Deficiency
Vol	Volume
WHO	World Health Organization

Wt	Weight
Zn	Zinc

DEFINITION OF TERMS

Calibration	a mathematical correlation between the raw NIR data (spectra) of the samples and the chemical constituent or property that is being measured.
Fortificant	a source of a particular micronutrient that is added to food to boost its nutritional value by delivering vital nutrients that the original food product may lack.
Fortification	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.
Fortification standards	micronutrient premix quantity specification for consumption of micronutrient premix while considering its safety and nutritional value.
Mean-centering	An approach that removes additive bias noise from all NIR spectra by subtracting the mean from the spectrum.
Micronutrient deficiency	the lack of essential micronutrients in the human body, making one susceptible to diseases.
Micronutrient premix	a blend of different fortificants developed to deliver specific and determinable levels of micronutrients.
Minerals	are inorganic substances that are essential for various physiological functions in the human body, and are required in relatively small amounts.

Spectrum	the unique wavelengths of electromagnetic radiation (or a fraction thereof) emitted or absorbed by an object or substance, atom, or molecule.
Ugali	a popular food in several East African countries, including Kenya, Tanzania, Uganda, Rwanda, Burundi, and parts of Sudan. It is a simple, dough-like food made from starchy ingredients, primarily maize (corn) flour, though other grains such as millet or sorghum can be used as well.
Validation	a mandatory step after model/method development done to demonstrate the suitability of the method/model for application.
Vitamins	organic compounds that are essential for various biochemical processes in the human body, and are usually required in relatively small quantities.

ABSTRACT

Food fortification is one of the strategies that has been used to overcome micronutrient deficiencies among vulnerable populations. Maize, a common staple food in Kenya, is used as a suitable fortification vehicle. However, several factors including storage conditions and consumer preparation methods involving heat processing impact micronutrient stability in fortified maize flour. Additionally, non-compliance with fortification standards hinders the success of fortification programs due to lack of techniques to rapidly check the amounts of the added fortificants. Fourier Transform Near-Infrared (FT-NIR) spectroscopy has been proposed as a fast and reliable analytical technique for vitamin determination. The objectives of this study, therefore, were to assess the influence of storage condition on the retention of retinol and B-vitamins in selected commercial fortified maize flour, to assess the impact of heat processing on retinol, B-vitamins, iron, and zinc in fortified maize flour and to develop an FT-NIRS-based model for determination of retinol in a fortified maize flour sample. Fortified maize flours from two brands (coded XX1 and XY2) were sampled from two manufacturers at the point of production. The stability of retinol and B-vitamins in the two brands (XX1 and XY2) was determined periodically over a storage period of 6 months at 25°C/ 75% relative humidity and 35°C/ 83% relative humidity. Cooking stability was assessed by comparing the amounts of micronutrients in sample XX1 before (uncooked maize flour samples) and after (*ugali*) cooking. For FT-NIR studies, 150 fortified maize flour samples were randomly collected from 10 counties in Kenya. Retinol reference values obtained by high performance liquid chromatography (HPLC) and NIR spectra of the fortified maize flour samples were used to develop calibration models using partial least squares regression (PLS-R). In storage stability studies, 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 cooking stability studies, retinol was the least stable vitamin (43.2% retention) while niacin was the most stable vitamin (61.1% retention). As expected, iron (91.4% retention) and zinc (95.6% retention) were the most heat-stable among the micronutrients analysed. In FT-NIR model development studies, two calibration models were developed to predict retinol above and below 1.0 mg/kg. The performance metrics of model one developed to predict retinol < 1.0 mg/kg were: $R^2c = 0.81$, RMSEE = 0.08, RPD = 2.29 and $R^2v = 0.82$, RMSEP = 0.09, RPD = 2.07 for the calibration and validation, respectively. The second model developed to predict retinol ≥ 1.0 mg/kg had the following performance metrics: $R^2c = 0.93$, RMSEE = 0.16, RPD = 3.58 and $R^2v = 0.81$, RMSEP = 0.22, RPD = 2.43 for the calibration and validation, respectively. In conclusion, the stability of vitamins for both brands XX1 and XY2 progressively declined over the six-month storage period. There was a significant difference ($p < 0.05$) in the amounts of retinol, thiamine, riboflavin, folate, and niacin in the flour samples after 6 months of storage at 25 °C/ 75% RH, and 35 °C/ 83% RH for both brands XX1 and XY2. The amounts of all the vitamins assessed differed significantly ($p < 0.05$) between cooked and uncooked fortified maize flour, while the amounts of iron and zinc in cooked and uncooked fortified maize flour were not significantly different ($p > 0.05$). FT-NIRS model development studies results demonstrated that NIR spectroscopy can be used to adequately predict retinol in fortified maize flour. NIR spectroscopy, by replacing time-consuming and laborious wet chemistry laboratory procedures, has the potential to be used for rapid regulatory monitoring of fortification compliance for a large number of samples.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Micronutrient deficiency is one of the main global health concerns. It is projected that more than two billion people suffer from micronutrient deficiencies worldwide (Bailey et al., 2015; Singh et al., 2016). A variety of micronutrients are commonly inadequate in the diets of the majority of people in low-income countries (Bailey et al., 2015). Deficiencies arise when people do not have access to micronutrient-rich foods including fruits, vegetables, animal products, and fortified meals.

In Kenya, the major micronutrient deficiencies of concern are vitamin A deficiency (VAD), iron deficiency, and zinc deficiency (Harika et al., 2017; KNMS, 2011). Overall, 24% of the Kenyan population are Vitamin A deficient, with preschool children having the highest prevalence at 53% (Ministry of Health, 2018). A study by Harika et al. (2017) also reported that in Kenya, 15% of children aged between 0 and 19 years were vitamin A deficient. Iron deficiency affects 28.7% of women aged 15 to 49 years in Kenya while 51% of children under the age of five are zinc deficient (Global Nutrition Report, 2018). Additionally, folate deficiency affects 32.1% of expectant women and 30.9% non-expectant women (KNMS, 2011).

Micronutrient deficiencies can be avoided or eliminated if populations regularly consume adequate amounts of the required micronutrients (WHO, 2009). Food fortification is one of the approaches that has been utilized to alleviate the incidence of micronutrient deficiencies among Kenya's vulnerable groups (Harrison, 2010; Ministry of Health, 2018). 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 (WHO & FAO, 2006). A fortification food vehicle should be widely consumed and readily available to the population (Ministry of Health, 2018). 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} (WHO, 2009).

The retention of added micronutrients (fortificants) is crucial for the effectiveness of a fortification scheme (Hemery et al., 2018). The stability of the fortificant is affected by exposure to any chemical or physical elements, including heat, moisture, oxygen, light, or pH, during manufacture, 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, respectively. Similarly, the most soluble and bioavailable forms of iron and zinc are sodium iron ethylenediaminetetraacetic acetate (NaFeEDTA) and zinc oxide, respectively (East African Community, 2011).

Despite the fortification programs in many countries, there are still many cases of micronutrient deficiencies, especially of vitamin A, iron and zinc (Ministry of Health, 2018). One of the 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 (Dunn et al., 2014). For instance, zinc and iron are relatively stable whereas vitamin A and riboflavin are generally unstable under normal storage conditions (Hemery et al., 2018; Khamila et al., 2020). Higher temperatures and relative humidity decreases vitamin stability (Dunn et al., 2014; Hemery et al., 2018). According to Hemery et al. (2018), vitamins showed losses of up to 90 % in fortified wheat flour when stored at 40 ℃ temperature and 75 % relative humidity, over a twelve-month storage period.

Additionally, consumer preparation methods involving heat processing impacts micronutrient stability in fortified maize flour (Suri & Tanumihardjo, 2016). It is well known that cooking alters the nutrients in food (Fabbri & Crosby, 2016; Suri & Tanumihardjo, 2016; Yong et al., 2019). Prior to consumption, maize flour is cooked by boiling and heat treatment can have a significant negative impact on the stability of vitamins (Lee et al., 2018).

Moreover, non-compliance with fortification standards due to lack of techniques to rapidly check the amounts of the added fortificants is a challenge. To ensure that manufacturers comply with food fortification standards, routine laboratory analysis is done. Conventional methods such as High-Performance Liquid Chromatography (HPLC) have been used but it is time-consuming and tedious as it takes long to generate results especially if a large number of samples is involved. As a result, there is need to develop a non-destructive and rapid analytical technique for vitamin determination such as Fourier Transform Near-Infrared (FT-NIR) spectroscopy.

The objectives of this study, therefore, were to assess the influence of storage condition on the retention of retinol and B-vitamins in selected commercial fortified maize flour, to assess the impact of heat processing on retinol, B-vitamins, iron, and zinc in fortified maize flour and to develop an FT-NIR-based model for retinol determination in a fortified maize flour sample.

1.2 Problem statement

In Kenya, the major micronutrient deficiencies of concern are vitamin A deficiency (VAD), iron deficiency, and zinc deficiency (Harika et al., 2017; KNMS, 2011). The most vulnerable groups include infants, pre-school children, school-age children, young women, and pregnant women (WHO, 2009). The Kenyan government introduced fortification of commonly consumed foods such as salt, maize flour, edible fats and oils, wheat flour, and sugar to provide a consistent supply of micronutrients to Kenyans (Fiedler et al., 2014). In Kenya, mandatory maize flour fortification has been used as an intervention to lower the prevalence of micronutrient deficiencies among the most vulnerable populations (Ministry of Health, 2018).

Despite various interventions including fortification, there are still many cases of micronutrient deficiencies. This could be attributed to lack of access to the required amounts of micronutrients as a result of non-compliance to fortification standards, the impact of different storage conditions and micronutrient degradation during heat processing. The stability of micronutrients added to flour determines the success of a fortification program (Dunn et al., 2014). Temperature, relative

humidity, oxygen levels, light, heat and alkaline/acidic conditions are among the factors affecting micronutrient stability (Osendarp et al., 2018). Exposure to these factors often occur during processing, distribution, storage and preparation.

Minerals are generally very stable, and storage factors like temperature and relative humidity are unlikely to cause losses. However, vitamins such as retinol and B-vitamins in fortified maize flour have been reported to be more susceptible to storage losses (Dunn et al., 2014; Hemery et al., 2018). Little has been reported on the stability of retinol and B-vitamins stored under different ecological zones in Kenya. Vitamins such as retinol, thiamine, riboflavin, pyridoxine and folate are also heat-sensitive and therefore vitamin losses could occur in fortified maize flour during cooking (Lee et al., 2018).

The effectiveness of fortification as an intervention to curb micronutrient deficiencies is further hindered by non-compliance with fortification standards, which is not easily determined due to a lack of techniques for quickly determining the quantity of fortificants added. Laboratory analysis is essential in monitoring successful fortification by checking compliance with fortification standards (GAIN, 2015). High-Performance Liquid Chromatography (HPLC) is a conventional method that is used to determine the amounts of retinol in fortified maize flour as a way of monitoring compliance to fortification standards. However, due to the lengthy extraction operations, HPLC as a monitoring approach is time-consuming and tedious especially when a large number of samples are involved (Yang and Irudayaraj, 2001). Additionally, use of HPLC for quantification of retinol in samples is highly prone to oxidation and many interfering compounds which is a source of likely errors in the method (Zhang et al., 2018). There is need therefore, to develop a non-destructive, more accurate, and rapid technique for vitamin A determination such as the Fourier Transform Near-Infrared (FT-NIR) spectroscopy.

1.3 Justification

Maize is the main staple food in Kenya. Maize is widely grown in Kenya and is affordable to the poor, making it one of the preferred fortification vehicles (Fiedler et al., 2014). According to a survey conducted in Kenya to assess fortification

coverage, 59% of Kenyans consumed hammer-milled (posho-milled) maize flour, 33% consumed sifted (roller-milled) maize flour, and 86% consumed both types of maize flour (Fiedler et al., 2014). Maize flour fortification will help greatly in securing the health status of the nation, particularly the vulnerable groups in the Kenyan population. Micronutrient deficiencies such as iron deficiency anaemia (IDA) and vitamin A deficiency (VAD), as well as zinc and iron deficiencies, can be reduced if fortification is effective. Reduced micronutrient deficiencies result in many advantages including reduced miscarriages, stillbirths, blindness, and stunted growth in infants (Kamenwa, 2017).

Additionally, assessment of micronutrient losses during storage and cooking of fortified maize flour will largely inform the optimization of storage conditions (storage temperature and relative humidity) and cooking conditions to ensure adequate micronutrient intake. This will also help the Kenyan government and maize millers in making sound reforms and adjustments in the legislated food fortification program. Such adjustments include taking into account projected micronutrient losses, which necessitates the incorporation of additional specific micronutrients in fortification premixes to compensate for expected losses during storage and cooking.

In order to determine compliance with fortification standards, it is necessary to use innovative techniques like FT-NIR spectroscopy for vitamin A (retinol) determination in developing nations like Kenya. Fourier Transform Near-Infrared (FT-NIR) spectroscopy technique is convenient, rapid, accurate, and non-destructive (Roggo et al., 2007). FT-NIR spectroscopy has substantial potential as a quantitative quality control tool for the food industry. Furthermore, the development of an FT-NIR model for determining retinol in a flour sample will considerably shorten the time required for scholars and scientists seeking more understanding in the field to obtain results during laboratory analysis.

1.4 Objectives

1.4.1 General objective

To evaluate the effect of storage conditions and cooking on the stability of micronutrients and the potential of FT-NIR spectroscopy in predicting retinol in commercial fortified maize flour in Kenya.

1.4.2 Specific objectives

1. To assess the influence of storage conditions on the retention of retinol and B-vitamins in selected commercial fortified maize flour.
2. To assess the impact of heat processing on retinol, B-vitamins, iron, and zinc in selected commercial fortified maize flour.
3. To develop an FT-NIRS-based model for determination of retinol in fortified maize flour.

1.5 Null hypotheses

1. Storage conditions have no effect on the stability of retinol and B-vitamins in fortified maize flour.
2. There is no significant difference in the amounts of retinol, B-vitamins, iron, and zinc content between raw and processed (*'ugali'*) fortified maize flour.
3. There is no significant relationship between the FT-NIRS spectra of fortified maize flour samples and their retinol content.

CHAPTER TWO

LITERATURE REVIEW

2.1 Micronutrient deficiencies

Micronutrient deficiency is universally defined as the lack of essential micronutrients in the human body, making one susceptible to diseases (Ramakrishnan, 2003). Micronutrients are essential in sustaining life and for optimal physiological body functioning (Samuel et al., 2018). Micronutrient deficiencies (MNDs) are widespread globally, posing the greatest risk to expectant mothers and children under the age of five. Micronutrient deficiencies is estimated to affect more than two billion people worldwide (Bailey et al., 2015). The most prevalent MNDs globally are deficiencies in iron, iodine, folate, vitamin A, and zinc. According to a World Health Organisation (WHO) report, anaemia affected more than 40% of children and pregnant women in 2016 (Ritchie, 2017). Additionally, more than 250 million children become blind as a result of vitamin A deficiency, and half of them die within a year of being blind (World Health Organization, 2009).

Pre-school children and pregnant women in Africa have the highest prevalence rates of anaemia, at 67.6% and 57.1%, respectively (Bailey et al., 2015). In 2019, prevalence of anaemia in women of reproductive age (15 - 49 years) and children under 5 in Sub-Saharan Africa was 40.5% and 60.5%, respectively. The WHO database reported Vita min A deficiency among pregnant women in Sub-Saharan Africa to be between 20-25%, and between 60-70% in children under the age of five (World Health Organization, 2009).

Micronutrient deficiencies are also of major public health concern in Kenya. Vitamin A, folate, iron, and zinc deficiencies are top-ranked as major public health concerns in Kenya (KNMS, 2011). Children from food-insecure households are especially vulnerable to micronutrient deficiencies (WHO & FAO, 2006). According to Evang et al. (2020), iron deficiency was more common among children living in rural areas (14.3%) than those living in urban areas (2.9%). The prevalence of Vitamin A deficiency (VAD) was however, higher in children living in the urban areas (14.7%)

than in rural areas (8.6%) while 34.3% of children were zinc deficient (Evang et al., 2020).

In developing countries, micronutrient deficiencies are prevalent due to increasing population, lack of resources, and lack of legislation and regulatory supervision (Akhtar et al., 2011). Furthermore, as a result of industrialization and changing lifestyles, there is a tendency to rely on one or a few food components, which ultimately leads to a lesser intake of micronutrients. Unfortunately, communities in developing countries rely heavily on plant-based diets and consume less of the often costly meat and meat products, resulting in an increase in micronutrient deficiencies (Akhtar et al., 2011). The most vulnerable groups include children and women of child-bearing age.

Consumption of adequate amounts of the required micronutrients on a continuous basis can help control micronutrient deficiencies (WHO, 2009). When addressing micronutrient deficiencies, there should be a combination of effective and efficient interventions. A variety of strategies have been employed to supply specific micronutrients that are thought to be limited for a population and vulnerable groups (Harrison, 2010). Among the strategies used to address micronutrient deficiencies in Kenya are dietary diversification, supplementation, biofortification, and food fortification (Harrison, 2010).

2.2 Interventions to combat micronutrient deficiency in Kenya

2.2.1 Dietary diversification

Dietary diversification is a strategy that changes food consumption at the household level, such as increasing the consumption of animal-food sources (Gibson & Hotz, 2001). The objective of dietary diversification is to increase the variety and quantity of micronutrient-rich foods in order to decrease micronutrient deficiencies (Bhutta et al., 2013).

Several studies have been conducted in Kenya to study the impact of dietary diversification on population health. A study conducted to assess the contribution of

fish farming to household wellbeing of fish farmers in Kitui Central Sub-county, reported an increase in diet diversity by 33.3 % and a positive correlation between fish farming and household wellbeing (Nzevu et al., 2018). Another study on the impact of dietary diversity on child malnutrition in rural Kenya reported that higher dietary diversity scores were associated with lower levels of child malnutrition (Bukania et al., 2014). Jones, (2017) also investigated the link between agricultural diversity (producing a variety of crops) and child nutritional status in Kenya. The findings demonstrated a link between increased agricultural diversification and improved child nutrition. Similarly, according to Walingo and Ekesa, (2013) there was a positive association between agricultural biodiversity, dietary diversity, and education level. Underweight and stunting were significantly influenced by morbidity and dietary diversity. Consideration of agrobiodiversity in terms of dietary diversity can improve the nutrition and health status of pre-school children.

There have also been reports on reduced micronutrient deficiencies due to dietary diversification in other developing countries. Between 2001 and 2009, the Malawian population experienced a relative reduction in vitamin A deficiency (VAD) of more than 60% (Williams et al., 2021). Evidence that nearly half of the women used a supplementary feeding program that included a corn-soya blend fortified with vitamin A led to speculation about what caused this decline (Williams et al., 2021). In northern Mozambique, a recent study found that the introduction of orange-fleshed sweet potatoes in the diet increased vitamin A intake among women of reproductive age and children (Olson et al., 2021).

In terms of the overall success of dietary diversification in Kenya, the studies reviewed imply that there is a positive relationship between dietary diversity and improved nutrition outcomes. The success of dietary diversity as an intervention can, however, be influenced by various factors, such as socioeconomic conditions, access to different and nutritious foods, cultural preferences, and educational initiatives.

Dietary diversification is a food-based strategy which makes it a long-term sustainable intervention. It is also one of the cheaper approaches once the consumers understand the benefits of diversifying their diets using the locally available foods.

Dietary diversification has an additional advantage of targeting multiple micronutrients (Nair et al., 2016). However, the technique has significant drawbacks, including lack of evidence basis, slow results, lack of quantifiable end points, and concerns of affordability, which must be addressed during implementation (Nair et al., 2016). Rising incomes have accelerated the nutritional shift by increasing demand for and consumption of nutrient-rich foods such as fruits, vegetables, whole grains, and seafood (Korir et al., 2023). People prefer to diversify their diet as their income rises, owing to affordability. As a result, a lack of affordability (being poor or unable to get preferred foods) frequently leads to insufficient intake of diverse diets that are nutritious (Korir et al., 2023).

Dietary diversification is essential for acquiring a diverse range of nutrients from a variety of food sources. Even when a diverse diet is maintained, there are occasions when fortification of foods or supplementation may be more impactful, such as when curbing specific micronutrient deficiencies, focusing on specific target population groups, and when there are issues associated with dietary restrictions, and bioavailability.

2.2.2 Supplementation

Supplementation is another intervention that is highly recommended in developing countries to combat chronic and acute deficiencies (Pritwani & Mathur, 2016). Supplementation is the provision of nutrients in the pharmaceutical form to prevent or reduce one or more micronutrient deficiencies (Harrison, 2010). Micronutrient supplements can be taken orally as pills, capsules, tablets, liquids, powders, or other forms (Melina et al., 2016).

Iron and folic acid supplementation (IFAS) is one of the most cost-effective global intervention strategies for controlling anaemia in pregnancy, with the added benefit of lower maternal-child morbidity and death (Kamau et al., 2018; Müngen, 2003). This is necessary because the body demands a higher level of nutrients during pregnancy that is usually not sufficiently provided by the regular diet. Following WHO guidelines (World Health Organization, 2012), Kenya implemented the IFAS programme, with the goal of reaching 80% coverage by 2017 (Kamau et al., 2018).

Indeed, IFAS tablets are now routinely distributed free of charge during antenatal treatment at all public health facilities for daily usage during pregnancy. Despite the government's efforts to deliver IFAS for free, compliance has remained low throughout the years. According to various studies, low compliance hinders IFAS success, resulting in poor maternal-child outcomes (Kamau et al., 2018).

A study conducted by Ngethe et al. (2020) reported a significant relationship between compliance to IFAS and anaemia prevalence in pregnant women in Nyeri county, Kenya, and concluded that IFAS supplementation is only effective when adhered to. Another study demonstrated that weekly iron supplementation for five months resulted in a significant rise in haemoglobin levels among adolescent schoolgirls in Western Kenya (Leenstra et al., 2009). Additionally, the effect of routine vitamin A supplementation on the nutritional status of young children aged 6 to 59 months in Wajir County was studied. The study found that the prevalence of stunting was higher in children who did not get vitamin A supplementation (63.4%) than in those who did (36.6%) (Abdi et al., 2021).

Tam et al. (2020) also reported that through administration of multiple micronutrient supplementation (MMN), anaemia among children under five in low- and middle-income countries was reduced. Vitamin A supplementation was also reported to have reduced all-cause mortality, whereas zinc supplementation reduced the incidence of diarrhoea (Tam et al., 2020). According to another study, MMN supplementation during pregnancy reduced the number of low birth weight (LBW) infants by 14% and the number of small for gestational age (SGA) infants by 13% (Bhutta et al, 2013).

One of the advantages of supplementation is that it is a short-term solution to micronutrient deficiencies (Bailey et al., 2015). Micronutrient supplementation is also the most widely used intervention for the prevention and management of single or multiple micronutrient deficiencies. However, supplementation as a strategy necessitates the availability of adequate educational programs to ensure compliance and supplements which are quite expensive. Additionally, supplementation

is limiting because it does not address the underlying cause of the deficiency (Bailey et al., 2015).

Food fortification and supplementation are both important strategies for addressing nutrient deficiencies, but they serve slightly different purposes. Supplementation unlike fortification does not have a wide-reaching impact, requires behavioural change as it cannot be integrated into the normal diet, and can be associated with the risk of overconsumption (Harrison, 2010; Tam et al., 2020).

2.2.3 Biofortification

Biofortification is another sustainable intervention that involves breeding crops to increase nutritional value either by selective breeding, or genetic modification (Siwela et al., 2020). There is a significant increase in the nutrient density of bio-fortified crops as compared to non-bio-fortified crops (Osendarp et al., 2018). Consumption of bio-fortified foods therefore can improve specific micronutrient intake hence resulting in reduced malnutrition among vulnerable groups of the population (Siwela et al., 2020).

A study was carried out by Talsma et al. (2016) to assess biofortified yellow cassava link with vitamin A status among Kenyan children, and demonstrated that consumption of biofortified yellow cassava resulted in modest increase in serum retinol concentration and a significant rise in b-carotene concentration. Elsewhere, Girard et al. (2017) studied the promotion of orange-fleshed sweet potato (OFSP) to increase Vitamin A intake among postpartum Kenyan women. According to Girard et al. (2017) consumption of orange-fleshed sweet potato (OFSP) by pregnant and lactating women is an effective approach for improving vitamin A intake. Haas et al. (2005) also assessed the efficacy of high-iron rice consumption in Filipino women over a 9-month feeding trial with a double-blind dietary intervention. They concluded that the consumption of bio-fortified rice alone, without any other dietary variations, is beneficial in improving the iron stores of women in developing countries with iron-deficient diets.

One advantage of biofortification is that it focuses on poor people that live in rural areas with little to no access to industrially fortified foods. Additionally, biofortification can help food systems supply more nutrient-dense foods at a lower cost when properly targeted (Olson et al., 2021). Some of the disadvantages of genetic biofortification are: heavy funding requirements and time needed for initial genetic research, and issues with regulatory approval and public skepticism of genetically modified crops (Singh et al., 2016).

Food fortification and biofortification are both strategies to increase the nutrient content of foods, but they differ in their implementation and target populations. Some situations where food fortification might be more suitable than biofortification include: when there is need for targeted nutrient addition, rapid response to deficiencies, and when targeting specific public health issues, such as neural tube defects (addressed by fortifying grains with folic acid) or goitre prevention (addressed by iodizing salt) (Olson et al., 2021; Osendarp et al., 2018).

2.2.4 Food fortification

Food fortification, another intervention to control micronutrient deficiencies, is defined as 'the addition of one or more essential nutrients to food, whether it is normally contained in the food or not, to prevent or correct a demonstrated deficiency of one or more nutrients in the population or specific population group' (Johnson et al., 2004). Large-scale food fortification programs are generally divided into two categories: mandatory fortification, in which all producers of branded and packaged fortifiable foods must fortify the selected vehicles in accordance with national legislation standards, and voluntary fortification, in which producers may choose to fortify on their own accord, usually in accordance with a national voluntary fortification standard (Aaron et al., 2017). Various countries fortify commonly consumed foods such as edible oils, sugar, infant foods, cereals, milk, margarine, and flour (Cardoso et al., 2019).

Consumption of salt fortified with iodine led to a significant decrease in the prevalence of goitre in the Kenyan population, from 35% in 1999 to 6% in 2004 (Ministry of Health, 2018). Another study conducted by Mgamb et al. (2017)

reported low prevalence rates of folate deficiency (3%) among pregnant women attending Pumwani Maternity Hospital, attributed to consumption of fortified maize flour.

According to the Malawian National Micronutrient Survey (MNMS) data from 2009, vitamin A deficiency significantly decreased as a result of nearly half of the women using a brand of oil fortified with vitamin A when cooking (Williams et al., 2021). Similarly, in Guatemala, vitamin A deficiency reduced from 22% to 5% within one year, following fortification of sugar with vitamin A (Allen & Hurrell, 2006).

Fortification has several advantages, including being more affordable, easy to implement and more practical, and more effective in reaching a large segment of the population (Johnson et al., 2004). Food fortification is also a sustainable nutrition intervention hence its implementation in many low and middle-income countries (Allen et al., 2006). Despite the great potential of food fortification, there are a number of obstacles that make it difficult to establish the right conditions for a scale-up of food fortification around the world. These restrictions include lack of national regulations on food fortification and a low private-public partnership. Another disadvantage is that successful fortification is highly dependent on well-equipped laboratories for control and monitoring of fortified food quality (Olson et al., 2021).

2.3 Historical trends and success of food fortification

In 1910, margarine which was produced on large scale at an industrial level in Denmark, had to be fortified with vitamin A in order to curb the widespread vitamin A deficiency (xerophthalmia) (Nilson & Piza, 1998). Margarine was later fortified with Vitamin D. In 1923, Switzerland became the first country to fortify salt with iodine to avoid goitre and cretinism, which were common in the Alpine region. The United States later adopted the initiative in 1930. Rickets, caused by lack of vitamin D, was also previously prevalent in young children in the northern hemisphere due to a lack of sunlight during the winter months and low vitamin D intake.

In 1923, the United Kingdom and the United States began fortification of milk with vitamin D to avoid rickets. Iron was mostly used in the 1930s to fortify cereal flours and cereal foods for a large population, such as rice, soy sauce, and fish sauce in China, Vietnam, and the Philippines (Chadare et al., 2019). Niacin has been added to wheat flour in the United States since 1938 because bread is commonly consumed. Fortification of cereal products with thiamine, riboflavin, and niacin soon became a routine practice in the early 1940s (Kyritsi et al., 2011). In 1944, Canada as per the government directive began fortifying wheat flour with vitamins B_1 and B_2, niacin, and iron and margarine with vitamin A due to high rates of micronutrient deficiencies. It was a success as several clinical signs and indications of vitamin A and B deficiency were significantly decreased or eliminated, including skin follicular abnormalities, ocular hyperaemia, and magenta tongue. Beriberi was fully eliminated.

In 1974, Guatemala started fortifying sugar with vitamin A due to high prevalence of vitamin A deficiency in the population. Within two years, the prevalence of retinol deficiency in children was reduced from 3.3% to less than 0.2%. On the basis of evidence on the high prevalence of folic acid deficiency and its role in avoiding foetal neural tube abnormalities and some anaemias, Guatemala amended the fortification of wheat flour in 1994, adding folic acid in addition to the previously added vitamins B_1 and B_2, niacin, and iron (Nilson & Piza, 1998). Almost all Central American countries later adopted this policy in an effort against neural tube defects in new-borns.

In Kenya, voluntary iodine fortification of salt began in 1972 to combat goitre. In 1978, salt iodization was made mandatory (Ministry of Health, 2018). The prevalence of goitre in the general population has decreased significantly over time, from 35% in 1999 to 6% in 2004 (Ministry of Health, 2018). Some industries began voluntarily fortifying wheat and maize flour with vitamin A, vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, iron, and zinc after the success of mandatory salt iodization.

In 2005, the Kenya National Food Fortification Alliance (KNFFA) was established to plan, implement, and monitor food fortification initiatives (Ministry of Health,

2018). Kenya made fortification of wheat flour, maize flour, vegetable oils and fats mandatory in 2012, following the amendment to the Food, Drugs and Chemical Substances Act (CAP 254) of the laws of Kenya ((Ministry of Health, 2018). The law was amended again in July 2015 in CAP 254, Notice No. 157) to include fortification standards for maize, wheat, and vegetable oil. The number of industries fortifying the specified food vehicles has expanded in tandem with the proportion of fortified foods accessible during the last decade (Ministry of Health, 2018). According to most recent data, 24 wheat millers fortify about 80% of the market's wheat flour, 47 maize millers fortify 37% of the market's maize flour, 14 oil processors fortify 87% of the market's vegetable oil and fats, and three large-scale salt processors fortify 99.9% of the country's salt (Ministry of Health, 2018).

Fortification in Kenya has largely been successful, but there is still room for improvement. This includes enhancing the policy, leadership, and governance for food fortification, increasing the production of salt, maize flour, wheat flour, and vegetable oil and fats that are adequately fortified, strengthening regulatory oversight of fortified foods at the industry and market level, and monitoring and evaluating the performance of the food fortification programme at the industry, market and household levels (Ministry of Health, 2018).

Currently, over 140 countries have standards and regulations put in place for fortification programs (Olson et al., 2021). Nearly 140 countries are implementing national salt iodization programs, 102 being mandatory. About 85 countries have a requirement that at least one cereal grain (maize, rice, or wheat) be fortified with iron and folic acid. Additionally, over 40 countries require the fortification of edible oils, margarine, and/or sugar with vitamin A and/or vitamin D (Olson et al., 2021).

2.4 Fortification vehicles

The selection of suitable food vehicles to deliver micronutrients in the right amounts to a large population is very critical (WHO, 2009). The success of food fortification can be guaranteed if the food vehicles are staple foods consumed throughout the year by a large proportion of the vulnerable population (Nilson & Piza, 1998). Usually, fortificants are added to the selected food vehicle in the form of a micronutrient

premix (WHO, 2009). Fortificants are defined as the source of micronutrients, while micronutrient premixes are fortificants blends that are designed to provide specific and determinable amounts of micronutrients (East African Community, 2011). It is also important to select more than one food vehicle so as to reach various groups of population who have different diets.

There are several criteria that need to be met when selecting any staple food as a fortification vehicle. One of the criteria is that the food selected as a vehicle should be consumed widely by the population at risk (Ministry of Health, 2018). The added micronutrients should also not interfere with the metabolism of any other nutrient present in the food (Nilson & Piza, 1998). Additionally, the nutrients introduced should be sufficiently stable in the food under standard packaging, storage, distribution, and preparation. It is also necessary that the nutrients added should not modify the colour, taste, smell, texture, or cooking attributes of the food and should not significantly impair its shelf life. Another factor to consider is the additional cost to the consumer as a result of fortification, as well as the techniques for measuring, controlling, and enforcing the levels of the important nutrients supplied to food (Nilson & Piza, 1998).

Fortifying flour is significantly easier because nutrients in powdered form can be properly blended with the flour (Chadare et al., 2019). On this basis, wheat flour was proposed as a suitable fortification vehicle. Packaged wheat flour in Kenya is fortified with vitamin A, vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, iron, and zinc. Furthermore, salt is used as a fortification vehicle to deliver iodine in the diet, while vegetable fats and oils, such as cooking oils and margarine, are fortified with vitamin A. (Ministry of Health, 2018). Maize is one of the staple food crops in Kenya consumed by over 85% of the population (Aaron et al., 2017). For this reason, it is considered an efficient food fortification vehicle. Packaged maize flour is also readily available in retail markets. Maize flour has been used as a vehicle to increase the dietary intake of vitamin A, vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, iron, and zinc (WHO, 2009).

2.5 Specific fortificants and their stability

2.5.1 Fortification with vitamin A

Vitamin A, a fat-soluble vitamin, can be categorized into two based on the sources. That which is obtained from animal foods is called preformed vitamin A or retinol while that obtained from fruits and vegetables is referred to as provitamin A carotenoids (Randall et al., 2012).Vitamin A is an essential micronutrient. This means that it has to be obtained from dietary sources since it cannot be synthesized in the body (Pretorius & Schönfeldt, 2012). It is required for maintaining eye health and general maintenance of human health (Neidecker et al., 2007). Inadequate provision of Vitamin A results in increased risk of vitamin A deficiency (VAD) hence increased susceptibility to infections and impaired immune responses. However, excessive vitamin A consumption can have negative health consequences such as liver damage, joint pains, and bone abnormalities (Fiedler et al., 2014). Adults have a tolerable upper intake level of 3000 mg/day, while children have a lower level (Fiedler et al., 2014).

Regulations, technological considerations, and the properties of the food vehicle all influence the choice of vitamin A fortificant (Dunn et al., 2014). Vitamin A is a highly unstable compound in its ready-to-use form (retinol). It is thus esterified with the more stable corresponding esters in commercial preparations, typically with palmitic (retinyl palmitate) or acetic acid (retinyl acetate) (Allen et al., 2006). Retinyl acetate and retinyl palmitate are the two main commercial forms of vitamin A available for use in food fortification (Allen et al., 2006). The minimum and maximum regulatory levels for the most stable vitamin A (retinol) forms in premixes used to fortify maize flour is listed in Table 2.1. Vitamin A fortificant is usually added to flour after milling. When exposed to heat, light, or oxygen, vitamin A becomes unstable (Allen et al., 2006). As a result, antioxidants are added to all types of vitamin A, whether oily or dry, to extend their shelf life. The use of airtight packaging provides additional protection (Suri & Tanumihardjo, 2016). Packaging is an important factor in ensuring vitamin A stability (Ohanenye et al., 2021).

According to Wieringa et al. (2014), the retention of vitamin A in fortified rice was significantly affected by the cooking method, with retention ranging from 0% (excess water) to 80% (soaking), depending on the cooking method and producer of the rice premix. Pretorius and Schönfeldt (2012) also conducted research to determine the retinol content of fortified white maize meal and maize porridge in South Africa and reported 39.8% retinol retention in cooked maize porridge. Hemery et al. (2018) also reported that retinol showed storage losses of up to 90% at 40 ℃ and 75% relative humidity over a 12-month storage period

Table 2.1: Requirements for micronutrients in fortified milled maize products

Nutrient	Fortificant compound	Regulatory levels (mg/kg)	
		Minimum	Maximum
Vitamin A	Retinyl palmitate/retinyl acetate	0.5	1.4
Thiamine (B_1)	Thiamine mononitrate	3.0	9.4
Riboflavin (B_2)	Riboflavin	2	5.8
Niacin (B_3)	Niacinamide	14.9	43.4
Pyridoxine (B_6)	Pyridoxine hydrochloride	2	7.5
Folic acid (B_9)	Folic acid	0.6	1.7
Zinc	Zinc oxide	33	65
Iron	NaFeEDTA	10	30

Source: (East African Community, 2011)

2.5.2 Fortification with B-vitamins

The B-vitamins used in food fortification include folate/folic acid (vitamin B_9), thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), and pyridoxine (vitamin B_6). Almost all of these B-vitamins are lost during the milling process of cereal grains (Dunn et al., 2014). Fortification of maize flour and wheat flour is therefore practiced in many countries with an intent to restore the lost vitamins and even at higher levels to meet fortification standards (Dunn et al., 2014). B-vitamins

are added during milling and the most stable forms of B-complex vitamins used for fortification, including their regulatory levels are given in Table 2.1.

The loss of B-vitamins is mostly determined by various factors including food processing or preparation temperature, moisture content, pH, extrusion temperatures, storage temperature and relative humidity and the quality of the packaging (Suri & Tanumihardjo, 2016). The B-complex vitamins are relatively stable, with thiamin being the most labile to heat. Between 15% and 20% of thiamin is lost during the commercial baking of white bread. A portion of this loss is due to yeast fermentation, which can convert thiamin to co-carboxylase, a less stable form of the vitamin (Ottaway, 2010). The heat stability of folic acid is moderate, but it is susceptible to oxidizing and reducing agents (Khamila et al., 2020). Light on the other hand is the most critical factor influencing riboflavin stability, with light in the 420 to 560 nm range having the largest impact (Ottaway, 2010). Niacin is normally very stable in foods as it is stable to atmospheric oxygen, heat, and light however, since it is water-soluble most of it is lost through leaching during cooking (Dunn et al., 2014).

Past studies have indicated that significant losses of B-vitamins (B_1, B_2, B_3, B_6, B_9, and B_{12}) occur during processing, distribution, storage, and cooking (Dunn et al., 2014; Lee et al., 2018). The loss of micronutrients in fortified maize flour stored at 25 °C/60% RH and 35 °C/75% RH for six months was investigated by Khamila et al. (2020). In comparison to samples stored at 25 °C/60% RH, retention was found to be lower in samples stored at 35 °C/75% RH. In fortified corn tortillas, Rosado et al. (2005) reported cooking losses of thiamin ranging from 43% to 52%, and riboflavin losses of 54%. The retention of B-vitamins is significantly impacted by storage conditions, including temperature and relative humidity, and cooking according to these studies, hence the need for storage stability and cooking stability studies to determine the optimal conditions for storage and cooking for maximum micronutrient retention.

2.5.3 Fortification with iron

Iron is an important micronutrient in a diet. Some of the main functions of iron are delivery of oxygen to body tissues, immune system regulation, cognitive

development, prevention of iron deficiency anaemia (IDA), and prevention of maternal haemorrhage, which could lead to maternal deaths (Mannar & Hurrell, 2018). Bioavailability, fortification vehicle matrix, stability, impact on sensory quality i.e. colour and taste of the food, and cost are all factors considered in the selection of iron fortificants (Grimm et al., 2012). The three types of iron that are most frequently used for flour fortification are elemental iron, ferrous sulphate, and sodium iron ethylenediaminetetraacetate (NaFeEDTA)(Grimm et al., 2012). Due to its low cost, ferrous sulphate is preferred by many manufacturers; however, it is relatively unstable.

Table 2.1 shows the most stable form of iron utilized in fortification premixes, as well as the minimum and maximum regulatory levels. Iron fortificants are usually added to flour after milling (Grimm et al., 2012). Despite its high cost, NaFeEDTA has been used successfully for fortification due to its stability in maize and wheat flour, long shelf life, higher bioavailability than ferrous sulphate, and lack of inhibiting interactions with other nutrients (Randall et al., 2012). Additionally, NaFeEDTA does not impact sensory quality i.e. colour and taste of the food (Akhtar et al., 2005). Elemental iron is also more stable than ferrous sulphate, and there are no known harmful interactions with other nutrient fortifiers, despite the fact that its bioavailability is lower (Dunn et al., 2014).

Heat, light, oxidizing chemicals, and pH do not degrade minerals, unlike vitamins (Reddy & Love, 1999). Iron is a stable compound during storage and distribution, therefore major losses are unlikely (Dunn et al., 2014). Kuong et al. (2016) reported less than 10% losses of iron in fortified rice during storage at 40 ℃ and 75% relative humidity for 12 months. Some studies have however, reported mineral loss during normal heating (cooking) because they readily leach into hot cooking fluids (Gharibzahedi & Jafari, 2017; Ottaway, 2010). Cooking stability studies need to be conducted to assess iron retention in fortified foods.

2.5.4 Fortification with zinc

Zinc (Zn), a trace mineral, is a micronutrient because small quantities of it are needed in the human body (Johnson et al., 2004). A variety of minerals, including

zinc, can be found in fruits, vegetables, and animal-based food. Zinc is essential for taste and smell perception, wound healing, normal foetal development, sperm production, immune system health, making protein and genetic material, and improvement of digestion (Gharibzahedi & Jafari, 2017). Zinc deficiency can lead to adverse effects such as weight and hair loss, digestive problems (diarrhoea and appetite loss), weak immunity (delayed wound healing and high susceptibility to infections) (Gharibzahedi & Jafari, 2017).

Zinc fortificants are not readily soluble and can lead to unpleasant taste of food products (Ohanenye et al., 2021). The choice of the best zinc fortificant thus is based on its most soluble form and its effect on the alteration of food flavour and odour (Randall et al., 2012). Because of its stability, low cost, and minimal sensory effects, zinc oxide is recommended for the cereal flour fortification (Dunn et al., 2014). The predominant oxidation state is zinc oxide and is very stable (East African Community, 2011). Table 2.1 shows the most stable form of zinc utilized in premixes as well as the minimum and maximum regulatory levels.

Previous studies have indicated that minerals such as zinc, generally have a high retention capacity hence regarded as more stable (Abebe, 2012). Wieringa et al. (2014) investigated the stability and retention of micronutrients in fortified rice prepared using different cooking methods. The overall retention of zinc was between 75% and 100% and was unaffected by the cooking method (Wieringa et al., 2014). Another study conducted by Hemery et al. (2018), reported high retention capacity of zinc in fortified wheat flour stored at high temperatures (40°C) and high relative humidity (75%).

2.6 Challenges of food fortification

Food fortification has had a lot of success in combating micronutrient deficiencies, but there are still some challenges faced. One of the challenges is that socioeconomic factors hinder the practice of food fortification. National policies in most middle and low-income countries do not put enough emphasis on food fortification. It is difficult to access poor people and promote consumption of the manufactured fortified foods due to the costs involved (Chadare et al., 2019). Further, local food processing

industries lack access to free or subsidized micronutrient premixes used to fortify the locally processed foods. Increase of food prices continue to undermine poor people's food security and livelihoods. Despite various foreign aids, expensive fortified food products remain inaccessible since they are out of reach for underprivileged communities who often grow and process their own staple foods.

Apart from socioeconomical challenges, lack of awareness limits the food fortification practices. Efforts must be made to educate consumers about the existence and importance of fortified foods for their health (Pambo et al., 2011). The media is one of the most essential sources of nutrition and fortification information. However, credible information about food fortification is not generally available in some African countries (Chadare et al., 2019). Due to lack of a mandatory provision and low awareness levels about the benefits of fortification, nonfortified food has a pricing advantage and hence is mostly preferred by the majority of the population.

The practice of food fortification is also limited by the technical challenges. These technical fortification challenges are mostly due to inappropriate fortification procedures and protocols leading to nutrient loss, uncontrolled exposure of the fortified foods by retailers to chemical and physical factors which impact micronutrient stability and companies' irregular monitoring and unreliable quality control procedures. It is very important to ensure compliance through regulatory monitoring that aims to meet the national fortification standards for fortified foods (Method & Tulchinsky, 2015). Governments in developing nations may lack the resources to effectively monitor compliance, particularly when there are several small processing companies operating especially in the rural areas.

According to Luthringer et al. (2015), the efficiency of detecting and enforcing non-compliant and inadequately fortified products is significantly influenced by the financial inputs used for monitoring. Internal quality assurance and control procedures, as well as external government monitoring are included in regulatory monitoring of food fortification (Luthringer et al., 2015). Cooperation, therefore, between regulatory bodies and food producers will be an important strategy for

successful fortification schemes. Additionally, development of non-destructive and quick analytical techniques such as Fourier Transform Near Infrared Spectrophotometry will be extremely beneficial in monitoring fortification standards compliance.

2.7 Near-infrared (NIR) spectroscopy

2.7.1 Background and principle of NIR spectroscopy

There are numerous regions that make up the electromagnetic spectrum. The NIR region of the electromagnetic spectrum was the first region invisible to the eye to be discovered (Pasquini, 2018). William Hershel made the first discovery of the NIR region of the electromagnetic spectrum in 1800 while observing radiation that was outside the visible spectrum (Givens et al., 1997).

The visible and mid-infrared (MIR) radiation spectrum are separated by the NIR radiation region, which has a wavelength range of 780 to 2500 nm (wavenumbers: 12821 to 4000 cm^{-1}). In NIR spectroscopy, the product (sample) is exposed to NIR radiation, and the reflected or transmitted radiation is measured (Ketelaere et al., 2007). As the radiation passes through the product, its spectral properties vary due to wavelength dependent scattering and absorption. This variation is determined by the product's chemical composition as well as its light scattering characteristics, which are related to its microstructure (Ketelaere et al., 2007).

The absorbed near-infrared radiation causes specific vibrational frequencies in the molecules. The bending and stretching vibrations of the C-H, N-H, O-H, and S-H chemical bonds are what cause the majority of molecular overtones and combination bands seen in the NIR spectral area (Aenugu et al., 2011; Eldin, 2014). The NIR spectra are usually very similar. This similarity leads to the use of advanced multivariate statistical techniques, such as partial least squares regression to extract useful information from the typically complex NIR spectrum (Ketelaere et al., 2007).

NIR spectra contain both chemical and physical information and can be used to determine several qualities. Physical information can be used to identify properties

like crystalline shape and particle size, while chemical information can be used for qualitative and quantitative analysis (Pasquini, 2003). The radiation in the NIR region can deeply penetrate into a product. As a result, non-destructive analysis can be performed using a bigger sample path length or sample thickness with little to no sample preparation (Patel, 2017; Roggo et al., 2007). Early NIRS research focused on determining the moisture and fat content of meat, the moisture content of wheat flour, and the moisture content of intact peanuts (Pasquini, 2003).

2.7.2 FT-NIRS instrumentation

A basic Fourier transform near infrared (FT-NIR) spectrophotometer is made up of five basic sections namely: sample compartment, light source, light wave selection system (monochromator), detector/s, and a signal processor system (Spielbauer et al., 2009). Figure 2.1 is a schematic diagram of the main parts of the near-infrared spectroscopy system. In NIR systems, it is common to use open sample cups or sample cells confined by silica or quartz (materials transparent to NIR light) in laboratory instrumentation (Spielbauer et al., 2009).

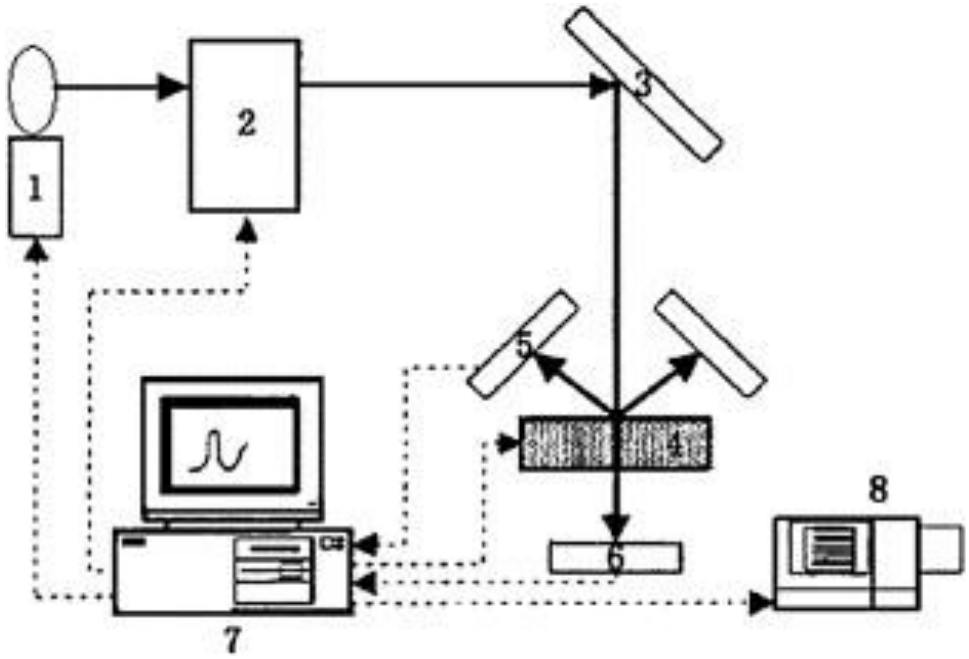

Figure 2.1: Sketch diagram of NIR spectrophotometer. 1-light source, 2-light wave selection system, 3-reflector, 4-sample compartment, 5-diffuse reflection detector, 6-transmission detector, 7-signal data processing system, 8-printer. Source: (Cen & He, 2007)**.**

For NIR spectroscopy, a single polychromatic thermal source is typically used (Patel, 2017). An inert solid, primarily tungsten (a small and rugged tungsten halogen lamp) electrically heated to 1500 - 2200K (Kelvins) irradiates uniformly in the infrared (IR) spectral range with wavelength emission ranges between 320 and 2500 nm (Agelet & Hurburgh, 2010). The wavelength chosen is determined by the solid-state properties of the sample for analysis as well as its fluorescence (Patel, 2017).

Before the radiation hits the sample, it is spilled by a monochromator (Pasquini, 2003). Monochromators are pre-dispersive instruments that scan a sample with grating mechanical motion. Polychromatic NIR light enters through an entrance slit and is then collimated (light rays are made parallel) by a mirror. The light hits the dispersion grating and later hits a focusing mirror, which reflects it to a second exit slit to either hit the sample (transmittance mode) or hit the single- channel detector

(reflectance mode) (Agelet & Hurburgh, 2010). Monochromators mostly use diffraction grating. A diffraction grating is made up of a large number of parallel lines or slits that are separated by a distance equal to the wavelength of light (Pasquini, 2003).

In NIRS, photon detectors are the most commonly used. Silicon, lead sulphide (PbS), and indium gallium arsenide (InGaAs) detectors are examples of detector materials (Patel, 2017). Most detectors gather light intensity from a wide wavelength range. Analysis necessitates the recording of signal values at specified wavelengths. Filtering the polychromic light beam yields discrete wavelength values (Agelet & Hurburgh, 2010). Basic filters, through absorption, absorb all light wavelengths except the one of interest (Agelet & Hurburgh, 2010). After wavelength separation, detectors are used to record the signal (Patel, 2017). The incident light energy is converted to an electric analogue signal via detectors. After that, the electrical signal is amplified and converted to digital, which can then be processed by the computer (Agelet & Hurburgh, 2010).

NIR instruments can function in different modes of measurement based on the arrangement of light source and detector used to obtain NIR spectra. The measurement modes that are typically used are transmittance, reflectance, or transflectance. In transmittance measurement, the light source, sample, and detector are all placed in a straight line to determine the fraction of light that is transmitted through the sample (Patel, 2017). Transmission analysis necessitates that the sample is partially transparent.

In reflectance measurement, the detector is placed on the same side of the sample as the source to record the signal reflected by the sample (Patel, 2017). The sample is assumed to be infinitely thick and incapable of transmitting light that reflects back to the detector. Generally, reflectance measurements are frequently performed on solids, powders, slurries, and opaque liquids.

Transflectance, on the other hand, is whereby the radiation that transmits through a sample, is reflected by a surface like a mirror or metal plate and is again transmitted back through the sample. This mode doubles the optical path length by

having the radiation beam traverse the sample twice (Pasquini, 2003). Similar to transmittance measurement, the light source, sample, and detector are all placed in a straight line (Patel, 2017).

2.7.3 Traditional dispersive NIR and FT-NIR spectroscopy

The majority of NIR spectrophotometers use grating monochromators to collect spectral data, which is measured using a single or diode array detector (Armstrong et al., 2006). Fourier-transform near-infrared reflectance (FT-NIR) technology is just one way that NIR instruments can acquire spectral data. FT-NIR hardware is generally more complex, although developments in circuits, techniques, and manufacturing have considerably improved detector sensitivities, resolution, and resistance to vibrational effects (Armstrong et al., 2006).

Fourier transform-NIR technology is an advance in the traditional dispersive NIR spectroscopy. The fourier transform technology offers advantage such as high signal to noise ratio, high light output due to absence of slits, fast measurement, instrument simplicity, high resolution and accuracy (Agelet & Hurburgh, 2010). In FT-NIR instrumentation, there are fewer optical elements to attenuate radiation which results in increased power reaching the detector and a higher signal-to-noise ratio (Armstrong et al., 2006). The resolution at specific wavelengths is also substantially higher, allowing for the detection of constituents that interact within very narrow bands. Furthermore, since all wavelengths are measured simultaneously in FT-NIRS, spectral collection is carried out considerably more quickly than with a dispersive NIRS (Armstrong et al., 2006).

In FT-NIR the incoming light/radiation is split by the beam splitter. One part of the beam is reflected by a fixed mirror, and the other part by a moving mirror. The reflected beams are recombined back in the beam splitter to generate the interferogram signal, which is a result of light interferences (Meyer et al., 2006).The interferograms must be fourier transformed into spectrum similar to the spectra obtained by any traditional spectrometer, but with the expectation of higher throughput and frequency accuracy (Manley et al., 2002). Figure 2.2 illustrates the difference between traditional dispersive NIR and FT-NIR spectroscopy.

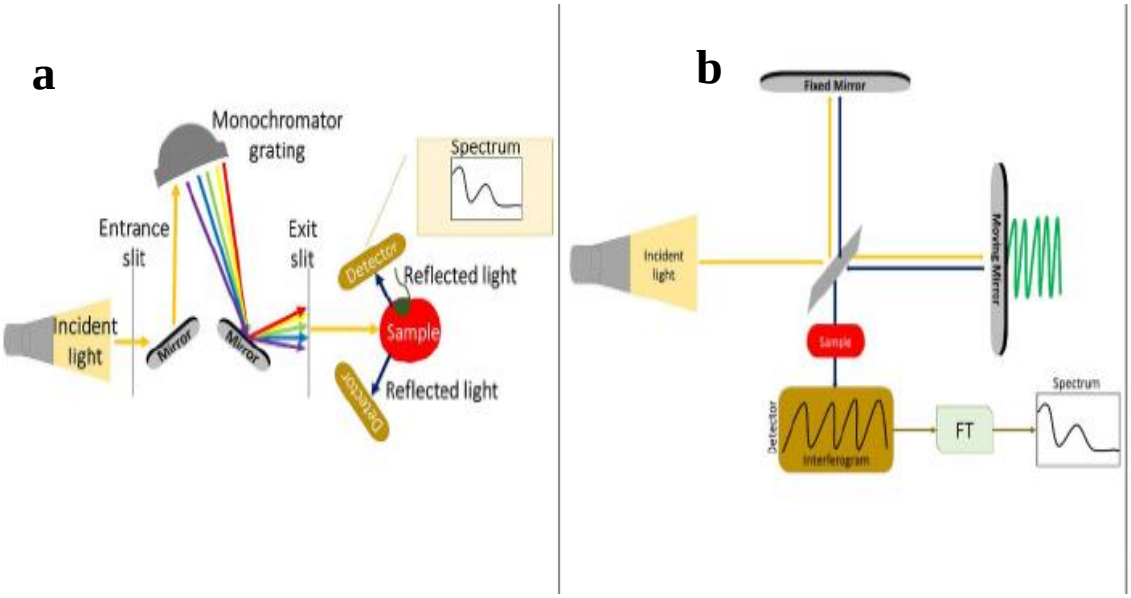

Figure 2.2: Schematic representation of mode of measurement for (a) Dispersive NIR instrument and (b) FT-NIR instrument. Adapted from Badaró et al. (2022).

Armstrong et al. (2006) conducted a comparative study on dispersive and fourier transform near infrared spectrophotometers used to measure wheat flour and wheat grain attributes. The findings demonstrated that FT-NIR and NIR equipment had comparable prediction abilities, and none appeared to be better than the other. The FT-NIR and NIR instruments had about the same 1-minute scan times. However, a larger proportion of users reported that sample preparation and presentation for FT-NIR was easier than that of dispersive NIR, since the sample was simply put in a sample cup. FT-NIR instrument also used twice more sample as compared to dispersive NIR system.

2.7.4 Chemometrics

Chemometrics is the use of mathematical and statistical techniques to derive useful knowledge from analytical data, such as NIR spectral data (Rinnan et al., 2009). The near infrared spectrum is composed of molecular overtones and combination bands. This, combined with the complex chemical composition of typical fortified maize flour, results in a highly complex near infrared spectrum. Furthermore, wavelength dependant scattering effects, experimental noise, environmental influences, and other sources of variability may further complicate the spectrum (Ketelaere et al., 2007).

As a result, assigning specific absorption bands to specific functional groups, and chemical components is challenging. Chemometrics in NIRS analysis includes spectral pre-processing techniques and building calibration models for quantitative and qualitative analysis using regression techniques such as partial least squares regression (PLS), principal component regression (PCR), and multiple linear regression (MLR) (Cen & He, 2007; Naes, 1991).

2.7.4.1 Spectral pre-processing techniques

During the calibration process, after obtaining the raw NIR spectral data, several spectral pre-processing techniques might be applied. Spectral pre-processing is also known as data pre-treatment. Visual inspection of the spectra can identify abnormal and noisy spectra in instrumental data. A visual inspection is, however, frequently insufficient, and potential outliers may not be detected until the data has been pre-processed (Agelet & Hurburgh, 2010). Before being used for qualitative or quantitative purposes, NIR spectral data sets are usually pre-processed (Pasquini, 2003). These data pre-treatments are primarily used to overcome problems caused by radiation scattering by a solid sample, and other spectrum base-line-affecting phenomena. Pre-processing reduces the noise in the spectra (smoothing techniques) and increases the spectral signal (differentiation) (Pasquini, 2003). Generally, pre-processing is done to simplify the spectra, get rid of interferences, and make information extraction from NIR spectral data easier. Figure 2.3 illustrates the effect of pre-processing on a NIR spectra.

The first and second derivatives pre-processing techniques have very often been used. First derivative pre-processing is often applied to remove the baseline offset, whereas second derivative pre-processing corrects the signal terms that vary linearly across wavelengths (baseline slope) and improves spectral resolution (Agelet & Hurburgh, 2010).

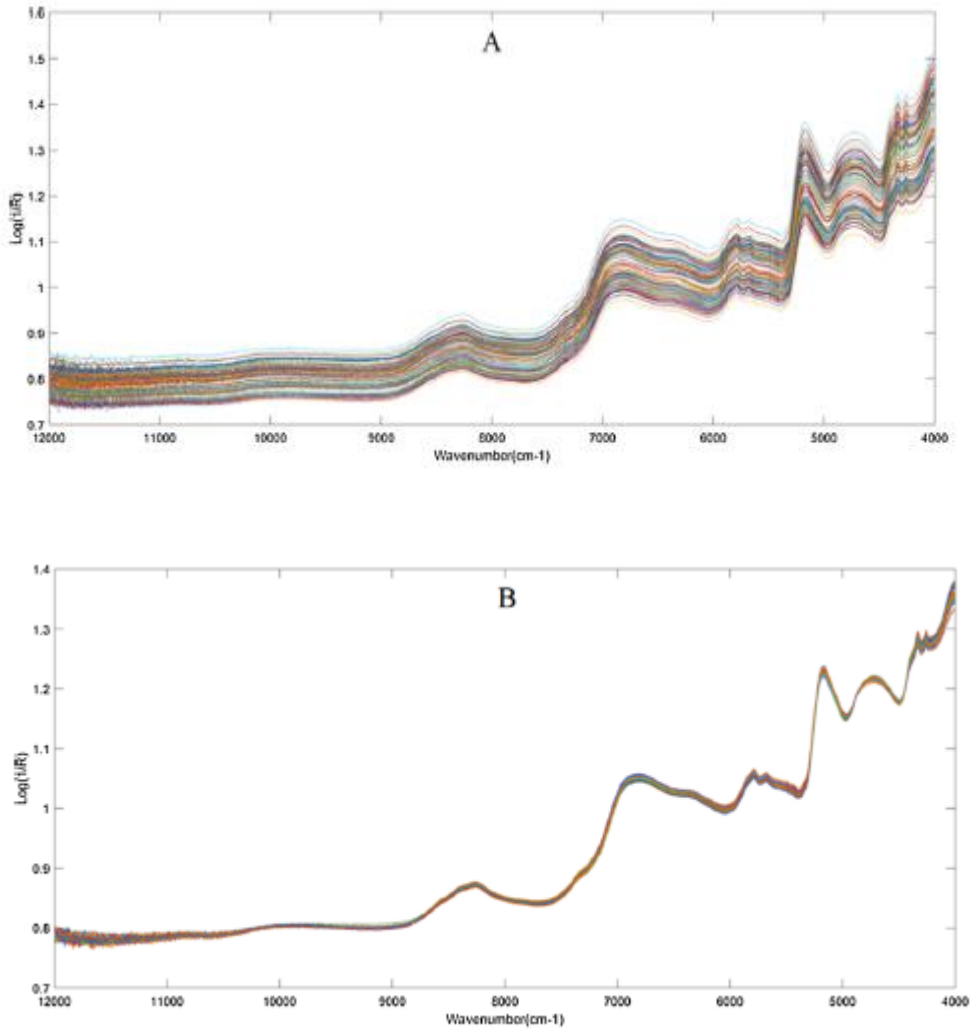

Figure 2.3: The raw NIR spectra (A), and the NIR spectra after pre-processing by multiplicative scatter correction (MSC) (B). Adapted from Zhu et al. (2021).

Standard normal variate (SNV), detrend, and multiplicative scatter correction (MSC) are also employed as pre-processing techniques. SNV is a scatter correction approach that is widely used to normalize spectra when the effective pathlength varies across samples in a data set (Rinnan et al., 2009). Pathlength variation can arise when measuring the spectra of granular or powdery materials due to sample presentation that is not fully consistent or particle size variation between samples (Roggo et al., 2007). Detrend on the other hand is a method for removing baseline offset, slope, or curvature from spectra (Feng & Sun, 2013; Rinnan et al., 2009).

MSC is a pre-processing technique based on a related set of spectra and is set-dependent. The mean spectrum in MSC is determined from all of the spectra in a specified data set. Individual spectra are effectively forced by MSC to behave as closely as possible to the mean spectrum (Rinnan et al., 2009).

Several NIR spectroscopy models to predict various parameters in food systems have been developed using different spectral pre-processing techniques. Bag et al. (2011) developed an FT-NIRS model for rapid estimation of moisture in bael pulp. The best calibration model was developed with min-max normalization spectral pre-processing. A combination of first derivative and vector normalization was the best pre-processing techniques for calibration models to predict sugar and acidic content in intact grape berries (Basile et al., 2020). In another study, Zhu et al. (2021) reported that a combination of orthogonal signal correction (OSC), standard normal variate (SNV), and multiplicative scatter correction (MSC) pretreatment techniques improved the model performance used for rapid analysis of lipid and protein content in green coffee beans.

2.7.4.2 NIRS calibration model development

Scanning of the samples through irradiation with near infrared light is the first step done for the generation of NIR spectra unique to the sample (Agelet & Hurburgh, 2010). The NIR spectra are then correlated with the sample characteristic/ concentration (reference value) usually determined using an existing analytical method (Baishya et al., 2021; Cao, 2013). NIR calibration models are developed through correlating either raw or pre-processed spectra with one or more chemical-physical information of a set of samples by the use of mathematical techniques such as Multiple Linear Regression (MLR), Principal Component Regression (PCR), and Partial Least Squares Regression (PLS-R) using specialized computer software (Jiao et al., 2020). MLR, PCR, and PLS-R are chemometric tools that assume a linear relationship between the spectral data and the concentration or other property value to be calculated (Roggo et al., 2007).

To build the calibration set, a suitable number of reference samples covering a wide range of analyte concentrations should be used (Cao, 2013). Depending on the nature

of the samples (heterogeneous or homogenous), the number of reference samples needed for feasibility studies and initial calibrations varies (Yu et al., 2021). Calibrations of homogeneous mixtures (i.e., pharmaceutical powders) may necessitate fewer calibration sets than calibrations of agricultural samples with high compositional complexity and heterogeneity, such as whole grains or forages (Agelet & Hurburgh, 2010).

An ideal calibration set should cover the chemical, spectral, and physical properties of the population to be analysed in order to avoid future extrapolations when predicting new samples (Fluvià, 2015). The quality of the reference data influences NIRS calibrations. A careful search for a suitable method and laboratory should therefore be carried out (Agelet & Hurburgh, 2010).

During NIR calibration model development, a number of statistical parameters are calculated and show the accuracy of the calibration equation, demonstrating its efficiency in predicting unknowns (Cao, 2013; Okazaki, 2012; Pasquini, 2018). The final statistics to consider when evaluating model performance and prediction potential are co-efficient of determination (R^2), root means square error of prediction (RMSEP), root mean square error of estimation (RMSEE), ratio of performance to deviation (RPD), and bias (Agelet & Hurburgh, 2010). Generally, models with high R^2, low RMSEE, low bias, and high RPD values are reliable and acceptable (Reddy et al., 2016; Egesel & Kahriman, 2012).

R^2 measures the correlation between the reference values and the spectral data. It shows the percentage of variance in the reference values that are replicated in cross-validation and prediction (Aenugu et al., 2011; Elfadl et al., 2010; Yu et al., 2021). Generally, a model with $R^2 > 0.83$ shows good robustness of prediction and an accurate calibration model (Elfadl et al., 2010). On the other hand, the calibration equation's ability to accurately replicate the constituent values of the spectra used to establish the calibration is measured by the root mean square error of estimation (RMSEE) (Elfadl et al., 2010). Additionally, bias otherwise known as the accuracy of the correlation determines the systematic disparity between the predicted values (y) and measured/reference values (x). Accurate calibration models exhibit bias

values close to 0 (Elfadl et al., 2010). RPD on the other hand tests the robustness of a model by assessing how well the developed model predicts the parameter analysed i.e. retinol in the validation set and the higher its value, the better the model's prediction capacity (De Girolamo et al., 2014; Xia et al., 2018; Ribeiro et al., 2021).

2.7.4.3 NIRS validation of models

Validation of the calibration models is a critical step in determining the model's suitability and accuracy to predict new samples. External validation and cross-validation are the commonly used approaches in NIRS (Sileoni et al., 2011). External validation, also known as test-set validation, is done using an independent sample set not used in building the calibration model. The validation set usually is composed of a third of the total samples (Sileoni et al., 2011). Usually, the best validation is done using randomized samples which were not previously used for calibrating (Agelet & Hurburgh, 2010).

Cross-validation is carried out by using the training set instead of validation using external samples. Often, cross-validation provides a basic assessment regarding calibration performance. The general idea of cross-validation is to keep one sample sub-set aside and use the remaining training samples to develop a calibration (Cao, 2013). The samples in the subset are then evaluated using this calibration as unknowns. The calibration developed is validated using the excluded samples, and the prediction values are recorded. This technique is repeated by returning the first sample to the training set and using another sample for validation until all of the samples have been predicted at least once (Cárdenas, 2012). The root means squared error of cross-validation (RMSECV) is a performance metric obtained during cross-validation that demonstrates the accuracy of the calibration in prediction (Feng & Sun, 2013). When cross validation is used, the RMSECV is defined as the prediction error of a calibration model, and when external validation is used, the RMSEP is defined as the prediction error (Mevik et al., 2004).

2.7.5 Application of NIR spectroscopy in food systems

Food and beverage industries have traditionally used NIR spectroscopy for quality inspection and process control. In the meat industry, NIR analysis is capable of rapid assessment of fat, water, protein, and other parameters simultaneously (Huang et al., 2008). The first on-line application of this technique was recorded for determining fat, moisture, and protein contents in ground beef on a conveyor using a diffuse NIR instrument set at the meat grinder's outlet, and the calibration tool was multiple linear regression (MLR) (Huang et al., 2008).

In addition, NIR spectroscopy has been used to sort fruits and vegetables based on their quality in terms of scale, colour, shape, and chemical composition variation (Lu et al., 2006). The use of conventional analytical techniques is very time-consuming and labour-intensive. Previously, NIR spectroscopy had been applied to determine sugar levels in intact peaches and mandarins (Huang et al., 2008).

The quality of grains and grain products is a crucial factor in setting prices based on factors like protein, starch, and hardness, among others. In the past, NIR technology has been utilized to measure several grain product components. The potential of NIR for determining the dry matter, crude protein, and starch contents of maize grain was investigated by Spielbauer et al. (2009). NIR spectra were gathered between 960 and 1690 nm. Modified partial least squares (MPLS) was used to calibrate the instruments. Furthermore, calibration models for the assessment of dry matter, soluble sugars, starch content, and in-vitro digestibility by cellulose in maize fodder have been developed (Lovett et al., 2004).

Yildiz et al. (2002) applied NIR spectroscopy for monitoring oxidation levels in soybean oils. In addition, the NIRS technique has been used in the quantification of peroxide value, conjugated diene value, and anisidine value in soybean oils. NIR spectroscopy has also been used for measuring degradation products in frying oils including free fatty acids (FFAs), which have a negative impact on the flavour and nutritional value of fried products (Weisshaar, 2014).

Moreover, the NIR technique has been used to determine constituents in alcoholic beverages such as fruit juices, teas, and soft drinks (Magwaza & Opara, 2015). Cozzolino et al. (2006) applied NIRS to monitor alcoholic content during alcoholic wine fermentation. In the baking industry, Sinelli et al. (2008) used an FT-NIR spectrophotometer to monitor the kinetics of dough proofing and bread staling. Reddy et al. (2016) used the non-destructive FT-NIR technique to predict vitamin C in citrus fruits and good prediction performance was obtained with a coefficient of determination (R^2) of 0.897. Rapid determination of vitamin C by NIRS has been widely exploited by many scientists (Magwaza & Opara, 2015). There is very little information from previous studies regarding FT-NIRS determination of retinol in maize flour samples.

2.7.6 Performance of different FT-NIRS calibration models for predicting various parameters in food

Several NIR spectroscopy models have been developed. Bag et al. (2011) developed an FT-NIRS model for rapid estimation of moisture in bael pulp. The best calibration model was developed with min-max normalization spectral pre-processing. This pre-processing method was found most suitable and the maximum coefficient of determination (R^2) value of 0.993 was obtained for the calibration model developed. The developed results indicated that FT-NIR spectroscopy could be used for rapid detection of moisture content in bael pulp samples without any sample destruction.

Chmielarz et al. (2019) also developed an FT-NIRS model for the rapid quantification of intracellular lipids in oleaginous yeasts. This was prompted by the fact that in most cases only the endpoint measurements of lipid accumulation are performed and kinetics of intracellular lipid accumulation is difficult to follow. The R^2 of the model, was 0.9, with RMSECV of 2.76 and RMSEP of 3.22, respectively.

Another study was carried out by Kahrıman et al. (2020) to determine whether it is possible to detect secondary biochemical components in maize flour samples by near-infrared spectroscopy. Calibration models were developed for six different secondary biochemical components, namely amylose, amylopectin, lysine, tryptophan, zein, and phytic acid. Results showed that NIR spectroscopy could be

used to detect secondary quality components in maize. The most successful prediction model was for amylose content (R^2: 0.96, RMSEP: 1.784 & RPD: 3.09). Models for the other traits (amylopectin, zein, lysine, tryptophan, phytic acid) gave acceptable results (RPD > 2) for material screening purposes.

NIR spectroscopy was used successfully to monitor oxidation levels (peroxide value) in corn oil samples. The calibration models developed were satisfactory as they showed performance metrics of a correlation coefficient of 0.99, RMSEC of 0.93 and RMSEP of 1.25. There are, however, limited studies done on the application of NIR spectroscopy to predict retinol in maize flour. Based on this information, the development of prediction models to predict retinol amounts in fortified maize flour will be of great importance.

2.7.7 Advantages and disadvantages of NIR techniques in food analysis

Results from NIR systems are more stable and reproducible (Yang and Irudayaraj, 2001). Furthermore, NIR spectroscopy requires minimal sample preparation and can be used on-line for monitoring procedures in industries (Huang et al., 2008). NIR spectroscopy is also fast (one minute or less per sample), non-destructive, and non-invasive (Pasquini, 2003). FT-NIR is the best system for quality control applications in industries since a single spectrum could give a lot of information concerning a high number of samples (Pasquini, 2003). Developments in computer science and chemometrics have led to the employment of NIR technique by many food researchers across the world.

Despite the low operational costs of near-infrared spectroscopy, the FT-NIR spectrophotometer itself is very expensive (Huang et al., 2008). This has really limited its practical application. Furthermore, building robust calibration models is very tedious and takes a long time. Some calibration models are not reliable and stable enough when used practically (Huang et al., 2008). For this reason, proper chemometrics needs to be chosen by researchers to build robust models. In some cases, conventional methods of analysis may not give accurate reference data to calibrate the NIR system. This makes it even harder to develop reliable calibration models.

Additionally, NIR technique is not sensitive to mineral content, since there is no absorption of minerals in the NIR spectrum region (Cozzolino et al., 2006). A combination of different detection techniques with NIR spectroscopy, such as x-ray fluorescence spectroscopy and UV light has been used to solve this problem efficiently (Huang et al., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Storage and cooking stability studies

3.1.1 Study design and sample collection

The study was conducted using a factorial design. Fresh samples of fortified maize flours from two brands, coded XX1 and XY2, were sampled from different large-scale commercial millers in Kiambu County, at the point of production, on the same day that the fortificants were added. The fortificant premixes used to fortify these two brands were different. The samples were thereafter brought to the laboratory for analysis.

3.1.2 Sample preparation for storage stability studies

Samples from brands XX1 and XY2 were first conditioned for 72 hours at 25 °C to equilibrate their moisture content. 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 labelled 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 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 contents of retinol, folate, niacin, riboflavin, and thiamine in the flour samples were analysed immediately after sampling.

3.1.3 Sample preparation for cooking stability studies

One fortified maize flour brand (XX1), sampled randomly from a large-scale commercial miller at the point of production, was used to evaluate the cooking stability of micronutrients. The amounts of retinol, B-vitamins, iron, and zinc were first determined before cooking. Traditional *'ugali'* was then prepared according to the recipe described by Wanjala et al. (2016) where 470 ml of distilled water was heated to boiling point in an aluminium cooking pot, approximately 270 g of fortified maize flour was then added gradually, and stirred thoroughly for approximately 6 minutes to a commonly preferred texture and consistency. The heat was turned down and the *'ugali'* was left to cook further for about 2 minutes. The total cooking time was approximately 8 minutes. Preparation of *'ugali'* was done in triplicate. The *ugali* samples were then left to cool to room temperature (25°C) before analysis of the vitamins and minerals.

3.1.4 Determination of moisture content

The AOAC (1995) method was used to assess the moisture content of uncooked fortified maize flour and ugali samples. This was done to correct for moisture differences between the uncooked fortified flour and the *ugali* samples. Clean and dry moisture dishes were first weighed. Five grams of the samples were then weighed into the moisture dishes and oven dried at 105°C for 4 hours to constant weight. The samples were then cooled in a desiccator and the final weight recorded. The percentage moisture content was calculated using the following formulae:

$$\% \text{ Moisture content} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

3.1.5 Determination of retinol

Extraction and quantification of retinol in fortified maize flour (for both stability and FT-NIR model development studies), and *ugali* samples were carried out according

to the method described by Zahar & Smith (1990) with slight modifications. Two grams of flour was weighed into a centrifuge tube. This was followed by adding 15 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 15 ml of hexane containing 0.01% butylated hydroxytoluene (BHT) (wt/vol) was added. The contents of the tubes were properly mixed for one minute using a vortex mixer, then left to stand for two minutes before being thoroughly mixed for an additional minute. Two ml of cold water (1ºC) was added to each centrifuge tube and then the tubes were inverted 10 times. The samples were centrifuged at 1000 rpm for 10 min. Afterward, the upper-organic layer was pipetted into a round-bottomed flask 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 324 nm. Concentrations of retinol were calculated using peak areas of the samples and the standard curves of the retinol standards.

3.1.6 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) in fortified maize flour and *ugali* samples was carried out according to Ekinci and Kadakal (2005) with slight modifications. To 5g of sample, 20 ml of deionized water was added followed by vortexing at medium speed for 1 minute. The mixture was then centrifuged for 10 minutes at 1000 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 MeOH 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 234 nm, 266 nm, 324 nm, 281 nm and 261 nm respectively. 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.

3.1.7 Determination of iron and zinc

Zinc and iron contents in uncooked fortified maize flour and *ugali* samples were determined according to the method described by (AOAC, 1995). First, two grams of flour was weighed in triplicate into crucibles and charred using a hot plate followed by ashing in a muffle furnace (Advantec KL-420) at 550 ℃ for 5 hours. The resulting ash was then diluted to 100 ml with 0.5M HNO_3 and filtered through Whatman's No. 1 filter paper before being analysed using an Atomic Absorption Spectrophotometer (Shimadzu AA-7000, Japan with ASC-7000 autosampler). Iron and zinc were identified at 248.3 nm and 213.9 nm respectively.

3.1.8 Determination of cooking retention

Cooking retention of micronutrients was assessed by determining the micronutrient amounts in uncooked and cooked (*ugali*) fortified maize flour on dry basis. The percentage retention of micronutrients (retinol, thiamine, riboflavin, niacin, pyridoxine, and folate) was calculated using the following formulae described by Bengtsson et al. (2008):

$$\%\ \text{Retention} = \frac{\text{micronutrient content of ugali (dry basis)}}{\text{micronutrient content of uncooked maize flour (dry basis)}} \times 100$$

3.1.9 Data analysis

The means and standard deviations for all parameters were computed in Microsoft Excel. The mean values for the storage stability studies were then analysed by analysis of variance (ANOVA) using GenStat statistical tool (19th Edition, 2018) to

assess the effect of storage conditions on micronutrient stability over the 6-month storage period. Tests were conducted at 95% confidence level, with $p \leq 0.05$ considered statistically significant. Duncan's mean separation test was done to determine significant differences between the means. Cooking stability data were subjected to Student *t*-test using the GenStat statistical package (19^{th} Edition, 2018), and the differences between micronutrient content of the samples before and after cooking were considered statistically significant at $p \leq 0.05$.

3.2 FT-NIRS model development studies

3.2.1 Sample collection and preparation

This study included 150 samples of randomly selected packaged fortified maize flours. The samples were collected from retail outlets, wholesale shops and supermarkets in ten counties in Kenya. These counties included: Nairobi (n-21), Kiambu (n-18), Uasin Gishu (n-16), Nakuru (n-17), Elgeyo-Marakwet (n-12), Kwale (n-11), Kilifi (n-10), Kisumu (n-15), Busia (n-13) and Mombasa (n-17) (Table 4.4). This was done to adequately represent millers from the Central, Eastern, Western and Coastal regions of Kenya. To avoid duplication, fortified maize flours from the same region under the same commercial brand names with similar batch numbers (identification number assigned to products made in the same manufacturing run) were pooled together. Thus, the maize flours included in the study were those belonging to different commercial brand names and those with different batch numbers within a brand. This resulted in a total of 150 samples. The different samples were mixed thoroughly using a commercial blender (Omniblend 1-TM-767) to ensure homogeneity. The homogenized fortified maize flour samples were then transferred into airtight zip-lock bags and stored in carton boxes at room temperature until the actual day of analysis. Storing samples in airtight zip-lock bags and carton boxes was done to reduce the adverse effects of environmental factors such as oxygen and light respectively.

3.2.2 NIR spectral data acquisition

Acquisition of NIR spectral data was done according to the method described by Wafula et al. (2020) with little modifications. Samples were thoroughly mixed prior to each scan. Three (3) sub-samples were drawn from each sample then transferred to a plastic cup of 10 cm diameter and put to a depth of about 3 cm. The sub-samples were scanned over a range of 12000 cm^{-1} to 4000 cm^{-1} using Bruker MPA (Multi-Purpose Analyzer) FT-NIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a semi-conductor lead sulphide detector (RT-PbS). Scanning was done in reflectance mode using the integrating sphere at 30 ℃ with a resolution of 16 cm^{-1}. For each sample, three independent spectra (as determined during preliminary research) were recorded. Each spectrum obtained was an average of 64 scans. Based on visual assessment of the spectra, the wavelength range was narrowed down to a range of 9000 cm^{-1} to 4000 cm^{-1} to remove noisy regions.

3.2.3 NIRS spectral pre-processing

Spectral pre-processing was done using OPUS software 7.8 (Bruker Optik GmbH, Ettlingen, Germany), according to the method adapted from Bag et al. (2011). This was done to reduce the prediction errors associated with spectral noise and the influences of temperature changes, particle size differences, light diffusion and baseline shifts on the NIR spectra while increasing signal from chemical information (de Girolamo et al., 2014; Jiao et al., 2020). Before calibration, the raw spectra were treated to a variety of pre-processing techniques, including the first and second derivatives of Savitzky-Golay and the standard normal variate (SNV). To address spectral baseline shifts, the first derivative pre-processing technique was applied, while the second derivative was used to enhance spectral resolution. Standard normal variate (SNV) pre-processing technique was also employed to correct scatter in the spectra caused by pathlength variation occurring during scanning due to particle size difference between the samples. The most optimal pre-processing technique combination was first derivative + SNV. Additionally, prior to calibration, all spectral data were mean-centred. Mean centering (MC) was the basic pre-treatment used that reduced bias noise from all NIR spectra.

3.2.4 Selection of samples for calibration and validation sets

The most ideal outcome of this study would have been the successful development of a single model capable of predicting retinol levels in fortified maize flour samples over a wide range of concentrations. However, a single calibration model for predicting retinol in fortified maize flour had a poor predictive capability hence the decision to develop two separate models. The fortified maize samples were grouped into two, retinol < 1.0 mg/kg and retinol ≥ 1.0 mg/kg (retinol values from HPLC). This resulted in two data sets used in developing two (2) separate models. Each data set was divided into calibration and validation sets. The calibration and validation sets were randomly selected in a way that they covered the whole range of the reference data. Calibration for model I was developed using 63 samples in the range of concentrations of 0 to 0.62 mg/kg retinol, whereas calibration for model II was developed using 37 samples in the range of concentrations of 1.0 to 3.32 mg/kg retinol (Table 3.1). Validation set of 31 samples and 19 samples were used for external validation of calibration for model I and model II respectively (Table 3.1).

Table 3.1: Range, mean and standard deviation of calibration and validation data set

	Calibration set				Validation set			
	n	**Range (mg/kg)**	**Mean (mg/kg)**	**SD**	**n**	**Range (mg/kg)**	**Mean (mg/kg)**	**SD**
< 1.0 mg/kg	63	0-0.62	0.34	0.18	31	0-0.6	0.28	0.18
≥ 1.0 mg/kg	37	1.0-3.32	1.47	0.53	19	1.0-2.89	1.34	0.43

3.2.5 PLS-R model development and validation

PLS-R model development and model validation was done according to the method adapted from Wafula et al. (2020), as illustrated in Figure 3.1. Calibration models I and II were generated using OPUS software 7.8 (Bruker Optik GmbH, Ettlingen, Germany). The calibrations were derived by performing partial least squares regression (PLS-R) which correlated retinol reference values (y) with the pre-

processed NIR spectra (x), meaning PLS-R was used to predict retinol (y) from the NIR spectra (x). The OPUS software program was used to select the best model using the default optimization command, which was based on a combination of the number of PLS factors, wavenumber ranges, and pre-treatment methods. In this study, only 5% outlier samples with high errors were excluded from the calibration set. External validation was also done using OPUS software 7.8 (Bruker Optik GmbH, Ettlingen, Germany) to test the reliability of the models. For model validation, the samples used were independent of the calibration set.

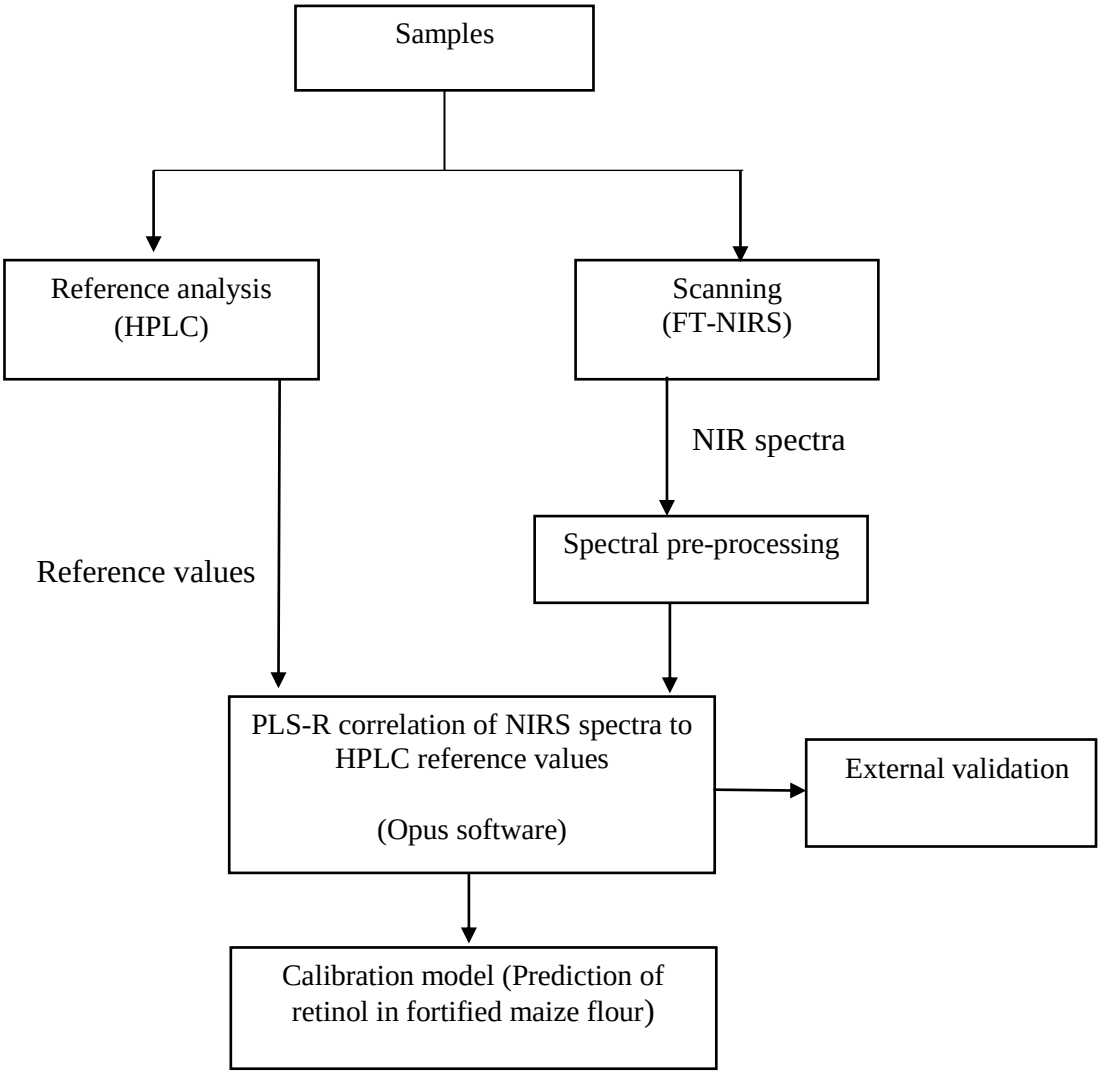

Figure 3.1: Process flowchart for calibration model development and validation.

3.2.6 Statistical evaluation

The retinol results from HPLC were entered into Microsoft Office Excel 2019 and the means and standard deviations calculated. The prediction potential of the developed FT-NIRS calibration models was evaluated by statistical terms of coefficient of determination for calibration (R^2_C), and the root mean square error of estimation (RMSEE). The reliability of the generated models was assessed according to the coefficient of determination of validation (R^2_V), root mean square error of prediction (RMSEP), ratio of performance to deviation (RPD), and bias values.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Storage stability of vitamins in fortified maize flour

4.1.1 Vitamin A (Retinol)

At the beginning of storage, retinol concentration in brands XX1 and XY2 was at 0.63 mg/kg (100%) and 0.51 mg/kg (100%), respectively, which was within the recommended levels. There was a significant difference ($p < 0.05$) in the amounts of retinol in the samples after 6 months of storage at 25 ℃/ 75% RH, and 35 ℃/ 83% RH for both brands XX1 and XY2 (Tables 4.1 & 4.2). Retention of retinol after six months of storage at 25 ℃/ 75% RH was 66.4% and 73.3% for brands XX1 and XY2 respectively (Figure 4.1). The amount of retinol retained after a 6-month storage period at 35 ℃/ 83% RH was 54.3% and 61.9% for brands XX1 and XY2 respectively (Figure 4.2).

These results were different from those reported by Dunn et al. (2014) of 75% vitamin A retention in cornmeal flour stored at 27 ℃ for six months. Higher retention values of 83.8% and 75.7 % were reported by Khamila et al. (2020) after storage of fortified maize flour samples at 25 ℃ / 60% relative humidity and 35 ℃ / 75% relative humidity respectively for six months. For both brands at each monthly analysis, retention of retinol was higher for samples stored at 25 ℃/ 75% RH as compared to those stored at 35 ℃/ 83% RH. Overall, the results show significantly higher losses of retinol at high temperatures and high relative humidity.

Table 4.1: Storage retention of vitamins in fortified maize flour (Brand XX1)

	Folate		Niacin		Retinol		Riboflavin		Thiamine	
Storage conditions/ Months	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**
0	1.56± 0.06^{a}	1.56± 0.06^{a}	21.53± 0.30^{a}	21.53±0.30 ^{a}	0.64 ± 0.02^{a}	0.64± 0.02^{a}	2.41± 0.15 ^{a}	2.41± 0.15 ^{a}	2.91± 0.03^{a}	2.91± 0.03^{a}
1	1.55 ± 0.04^{a}	1.53± 0.08^{a}	21.26±0.41^{ab}	20.01±0.47^{b}	0.63± 0.02^{a}	0.62± 0.03^{a}	2.39± 0.04^{ab}	2.38± 0.01^{a}	2.82± 0.03^{b}	2.70± 0.18^{b}
2	1.52± 0.06^{a}	1.51± 0.02^{ab}	20.57±0.58^{bc}	19.85±0.32^{b}	0.59± 0.05^{ab}	0.56± 0.01^{b}	2.32± 0.01^{ab}	2.31± 0.02^{ab}	2.66± 0.06 ^{c}	2.43± 0.03^{c}
3	1.51± 0.01^{ab}	1.48± 0.04^{ab}	20.30±0.60^{cd}	19.00±0.22^{bc}	0.57± 0.04^{bc}	0.52± 0.03^{c}	2.28± 0.03^{bc}	2.26± 0.06^{bc}	2.61± 0.01^{d}	2.30± 0.06^{cd}
4	1.45± 0.03^{bc}	1.43± 0.04^{bc}	19.59±0.33^{d}	18.48±0.12^{cd}	0.51± 0.01^{cd}	0.43± 0.04^{d}	2.18± 0.05^{cd}	2.16± 0.02^{c}	2.47± 0.03^{e}	2.23± 0.04^{d}
5	1.41± 0.02^{cd}	1.38± 0.03^{c}	19.53±0.31^{de}	17.72±0.20^{de}	0.46± 0.03^{de}	0.39± 0.02^{e}	2.12± 0.07^{de}	1.98± 0.04^{d}	2.24± 0.01^{f}	2.01± 0.02^{e}
6	1.38± 0.02^{d}	1.21± 0.01^{d}	19.29±0.40^{e}	17.15±0.30^{e}	0.42± 0.03^{e}	0.35± 0.03^{f}	2.02± 0.01^{e}	1.74± 0.03^{e}	2.16± 0.02^{g}	1.81± 0.01^{f}
P-values	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

Values are means ± standard deviation. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Retinol is more susceptible to losses in hot and humid surroundings than in cold and dry conditions (Kuong et al., 2016). Temperature may influence the oxidation rate of fat-soluble vitamins such as retinol, resulting in decreased vitamin stability (Saensukjaroenphon et al., 2020). Vitamin A is among the most unstable vitamins, with retinol being less stable than retinyl esters (Ottaway, 2010). The relative amount of retinol retained was also brand-dependent (Figures 4.1 and 4.2), 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 (WHO, 2009). Fortificants are defined as the source of micronutrients, while micronutrient premixes are a blend of different fortificants developed to deliver specific and determinable levels of micronutrients (East African Community, 2011).

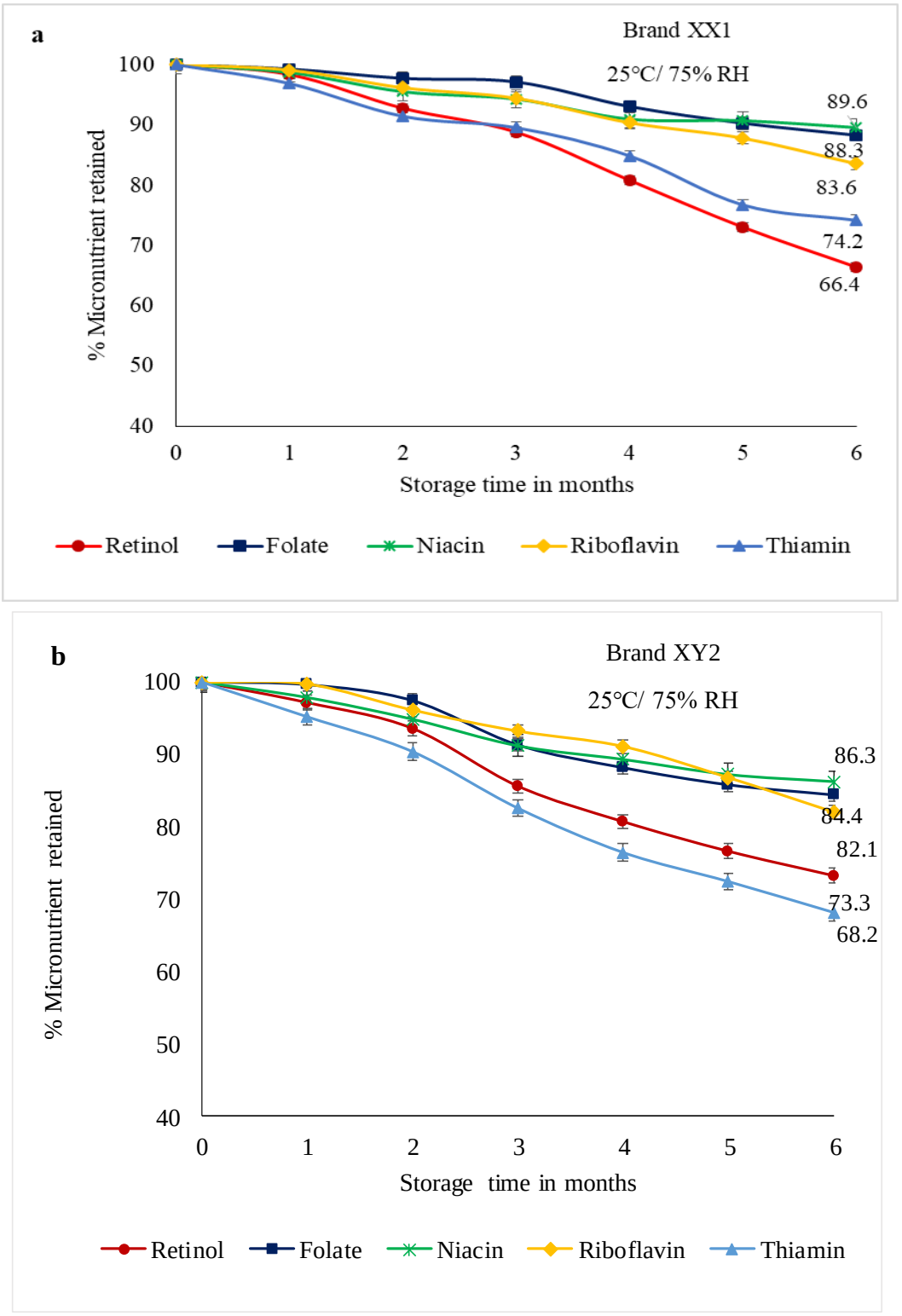

Figure 4.1: Retention of micronutrients in fortified maize flour from (a) brand XX1 and (b) brand XY2 stored at 25 °C/ 75% RH.

Fortification premixes vary in the type of encapsulates and antioxidants used in their formulation, which may affect the stability of retinol across brands (Johnson et al., 2004). 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 (Johnson et al., 2004). The protected form of retinyl palmitate, which is specially coated is the most stable and is usually preferred for use in flour fortification (Dunn et al., 2014).

4.1.2 Vitamin B1 (Thiamine)

The amount of thiamine in brands XX1 and XY2 was 2.91 mg/kg (100%), and 2.77 mg/kg (100%), respectively, at the start of storage, which was slightly below the recommended range of 3.0mg/kg - 9.4mg/kg. As shown in Figure 4.1, 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. Similar to retinol, samples stored at 35 ℃/ 83% RH for six months showed lower retention values of 62.1% and 57.6% for brand XX1 and brand XY2 respectively (Figure 4.2). At the end of the storage period, for both storage conditions and brands, the thiamine content in fortified maize flour were significantly different ($p < 0.05$) (Tables 4.1 & 4.2). 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 (Whitfield et al., 2021). If the makeup ingredients are not stable, the premix may not be stable (Florence & Tola, 2016). 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 (Tuncil, 2018). In the absence of light and moisture, however, both thiamine hydrochloride and thiamine mononitrate are relatively stable to atmospheric oxygen and highly stable when used in dry products with light and moisture-resistant packaging (Ottaway, 2010).

Thiamine hydrochloride, despite being sold in a crystalline state, also exists in an amorphous form, particularly in fortificants, which is more labile (Tuncil et al., 2020). Thiamine degradation rate increases with increased relative humidity (Voelker et al., 2021b).

Table 4.2: Storage retention of vitamins in fortified maize flour (Brand XY2)

	Folate		**Niacin**		**Retinol**		**Riboflavin**		**Thiamine**	
Storage conditions/Months	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**	**25^{0}C/ 75%RH**	**35^{0}C/ 83%RH**
0	1.40± 0.02^{a}	1.40 ± 0.02^{a}	17.20 ±0.26^{a}	17.20±0.26 ^{a}	0.51± 0.01 ^{a}	0.51± 0.01 ^{a}	2.34± 0.06 ^{a}	2.34± 0.06 ^{a}	2.77± 0.09 ^{a}	2.77± 0.09 ^{a}
1	1.37± 0.05 ^{a}	1.38 ± 0.02 ^{a}	16.83±0.17^{ab}	16.38± 0.23^{ab}	0.49 ± 0.01^{a}	0.48± 0.01^{ab}	2.33±0.06 ^{a}	2.33± 0.05 ^{a}	2.64± 0.01 ^{ab}	2.52± 0.19 ^{b}
2	1.37± 0.06^{a}	1.35± 0.07 ^{a}	16.26±0.08^{bc}	15.96± 0.56^{b}	0.48±0.02^{ab}	0.46± 0.01^{b}	2.25± 0.05^{ab}	2.23± 0.06 ^{b}	2.51± 0.17 ^{b}	2.30± 0.10 ^{c}
3	1.28 ± 0.01^{b}	1.26± 0.01 ^{b}	15.68±0.66^{cd}	15.45± 0.46^{bc}	0.44±0.06^{bc}	0.43± 0.03^{c}	2.23± 0.02 ^{b}	2.07± 0.03^{c}	2.29± 0.13 ^{c}	2.13± 0.04^{cd}
4	1.24± 0.02^{bc}	1.21± 0.03 ^{b}	15.36±0.27^{de}	15.27± 0.31^{bc}	0.40±0.02^{cd}	0.38± 0.01^{d}	2.20± 0.04 ^{b}	2.04± 0.01^{c}	2.12± 0.19^{cd}	2.08 ± 0.07^{d}
5	1.20± 0.05^{bc}	1.13± 0.02 ^{c}	15.00±0.29^{e}	14.40± 0.36^{cd}	0.37±0.01^{de}	0.32± 0.01 ^{e}	2.17± 0.02 ^{b}	1.88± 0.03 ^{d}	2.09± 0.04^{cd}	2.02± 0.06 ^{d}
6	1.18± 0.07^{c}	1.06 ± 0.01^{d}	14.83±0.30 ^{e}	13.26± 0.22^{d}	0.35± 0.01^{e}	0.29± 0.01 ^{e}	1.92± 0.01 ^{c}	1.7± 0.05 ^{e}	2.03± 0.01^{d}	1.72± 0.02 ^{e}
P-values	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

Values are means ± standard deviation. Means with different superscript letters in the same column are significantly different at $p \leq 0.05$.

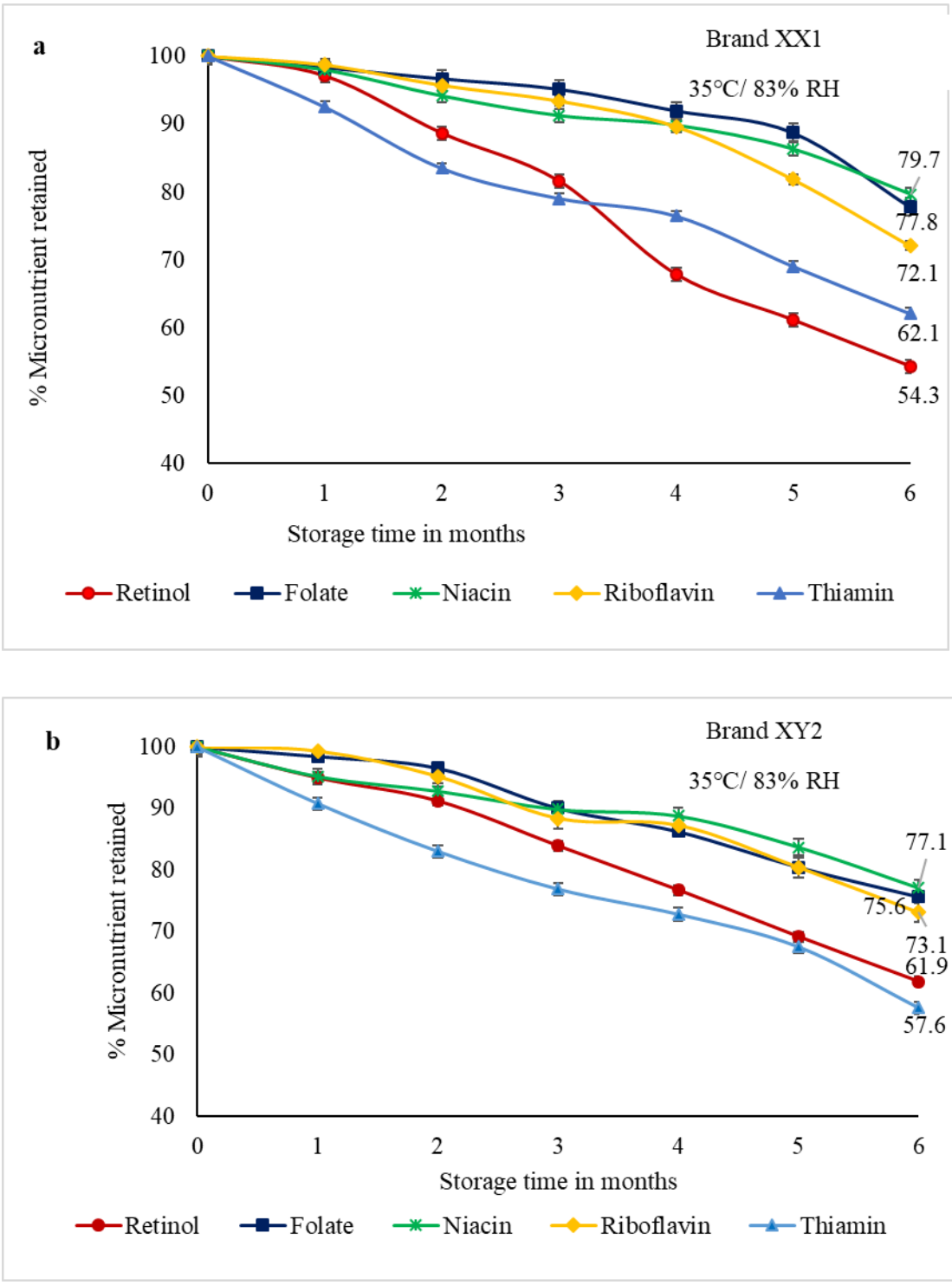

Figure 4.2: Retention of micronutrients in fortified maize flour from (a) brand XX1 and (b) brand XY2 stored at 35 °C/ 83% RH.

4.1.3 Vitamin B_2 (Riboflavin)

Riboflavin was relatively stable amongst the vitamins assessed. Riboflavin concentration was within the recommended range at the beginning of storage for brands XX1 and XY2 at 2.41 mg/kg (100%), and 2.34 mg/kg (100%), respectively. There was a significant loss ($p < 0.05$) of riboflavin in the brand XX1 flour samples after 2 months of storage at 25 °C/RH 75%. The loss in brand XX1 flour samples, however, was significant after 3 months of storage at 35 °C/RH 83% (Table 4.1). Figure 4.1 illustrates that at 25 ℃/ 75% RH, retention of riboflavin after six months of storage was 83.6% and 82.1% for brands XX1 and XY2 respectively. At 35 ℃/ 83% RH, the amount of riboflavin retained after a 6-month storage period was 72.1% and 73.1% for brands XX1 and XY2 respectively (Figure 4.2). Under the tested storage conditions after every month, retention of riboflavin was higher at 25 ℃/ 75% RH compared to 35 ℃/ 83% RH, for both brands. These values were different from those reported by Khamila et al. (2020) where fortified maize flour samples stored at 25 ℃/ 60% RH and 35 ℃/ 75% RH, 66% and 54.4% of riboflavin were retained respectively. Higher retention values of 94% after storage for 24 months at 30 ℃ have been reported by Coad & Bui, (2020). According to Ottaway, (2010), riboflavin is relatively stable during thermal processing, storage, and food preparation. However, it degrades when exposed to light. A combination of light and high temperatures makes riboflavin generally unstable in food products (Sheraz et al., 2014). Riboflavin is degraded through cleavage of the isoalloxazine ring, yielding a number of unstable compounds (Sheraz et al., 2014).

4.1.4 Vitamin B3 (Niacin)

When compared to other vitamins analysed, niacin was the most stable in both brands and both storage conditions. At the beginning of storage, the amount of niacin in brands XX1 and XY2 was 21.53 mg/kg (100%), and 17.20 mg/kg (100%) respectively, which was within the recommended regulatory levels. Niacin retention for flour samples stored at higher temperatures and relative humidity was lower than those stored in lower temperatures and relative humidity (Figures 4.1 and 4.2). At 35 ℃/ 83% RH, 79.7% and 77.1% niacin content were retained for brands XX1 and

XY2 respectively at the end of the storage period (Figure 4.2). Higher retention values of 89.6% and 86.3% for brands XX1 and XY2 respectively were observed for flour samples stored at 25 ℃/ 75% RH for six months (Figure 4.1). From this study, it was observed that at the end of the storage period, for both storage conditions and brands, the niacin content in fortified maize flour were significantly different ($p < 0.05$) (Tables 4.1 & 4.2). Niacin retention values observed in this study were consistent with those reported by Khamila et al. (2020) of 87.7% and 75.6% for flour stored for six months at 25 ℃/ 60% RH and 35 ℃/ 75% RH respectively. Beizadea, (2009) reported higher niacin retention values of 94% when fortified spaghetti was stored in the dark at 25 ℃ and 60% relative humidity for three months. Niacin exists as nicotinamide in fortified foods, and in both aqueous and solid systems, it is usually stable to oxygen, heat, and light (Ottaway, 2010). Although considered the most stable vitamin, niacin losses are mainly attributed to leaching into cooking water (Yusufali et al., 2012). Generally, maize has low niacin content.

4.1.5 Vitamin B9 (Folate)

At the start of storage, brands XX1 and XY2, met the recommended folate regulatory levels at 1.56 mg/kg (100%), and 1.40 mg/kg (100%) respectively. The results of this study show that there was a significant loss ($p < 0.05$) of folate in brand XY2 flour samples after 3 months of storage at both 25 °C/RH 75% and 35 ℃/ 83% RH (Table 4.2). As shown in Figure 4.1, there was 88.3% and 84.4% retention in samples stored at 25 ℃/ 75% RH for brands XX1 and XY2 respectively after a 6-month storage period. On the other hand, brand XX1 and brand XY2 flour samples stored at 35 ℃/ 83% RH retained 77.8% and 75.6% amounts of folate by the end of the 6-month storage period (Figure 4.2). These values compare favourably with the findings of Khamila et al. (2020) who reported 87.3% and 75.9% retention of vitamin B_9 for flour stored at 25 ℃/ 60% RH and 35 ℃/ 75% RH respectively. Compared to other B-vitamins assessed, folate was relatively stable. Another study by Hemery et al. (2020) reported a retention range of 81- 83% after 3 months when flour samples were stored at 65% RH in paper bags, irrespective of storage temperature, which was fairly consistent with the findings of this study. Folate is relatively stable to humidity and heat (Beizadea, 2009). Light, temperature, oxygen, and pH are all environmental

factors that can cause interconversion or degradation of folates, resulting in irreversible loss of activity (Liang, 2020). Folic acid is an essential micronutrient in the diet (Rosenberg & Selhub, 2018). The mechanism of folate degradation is determined by the vitamin's structure and the chemical environment (Bailey et al., 2015). Folate degradation typically involves alterations to the bond structures, the pteridine ring system, or both (Liang, 2020). In the presence of oxidants or reductants, folic acid can be cleaved and inactivated (Liang, 2020). A study conducted by Scientific Advisory Committee on Nutrition, (2017) 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%.

4.2 Cooking stability of vitamins and minerals in fortified maize flour

4.2.1 Vitamin A (Retinol)

Cooking of maize flour before consumption has an impact on the retention of micronutrients added during fortification. The amounts of retinol in cooked and uncooked fortified maize flour were significantly different ($p < 0.05$) (Table 4.3). In comparison with other micronutrients analysed, retinol was the least stable (43.2% retention) while the minerals were fairly stable (> 90% retention) (Figure 4.3). According to Lešková et al. (2006), retinol is more stable under an inert atmosphere and is rapidly lost when heated in the presence of oxygen. These results are in alignment with the findings of Pretorius and Schönfeldt (2012) that reported a 39.8% retention of retinol in maize meal porridge. Another study conducted by Wieringa et al. (2014) on the effect of cooking on fortified rice reported overall cooking retention of vitamin A to be 43%. The degree of heating and the properties of the food matrix influence stability of vitamins (Silveira et al., 2017). This is because heating above the critical temperature leads to the destabilization of hydrogen bonds (Turgeon & Rioux, 2011).

Table 4.3: Cooking retention of vitamins and minerals in fortified maize flour

Treatment	Micronutrient content (mg/kg)							
	Retinol	**Thiamine**	**Niacin**	**Pyridoxine**	**Folate**	**Riboflavin**	**Zinc**	**Iron**
Uncooked	0.33±0.02	2.81±0.10	20.33±0.61	1.90±0.01	1.41±0.01	2.33±0.02	26.83±0.97	25.79±0.60
Cooked	0.14±0.01	1.41±0.03	12.42±0.58	1.14±0.04	0.79±0.02	1.27±0.02	25.66±1.33	23.56±0.48
P-values	0.001	0.001	0.001	0.001	0.001	0.001	0.068	0.071

Values are means ± standard deviation

4.2.2. Vitamin B1 (Thiamine)

Thiamine content differed significantly ($p < 0.05$) between cooked and uncooked fortified maize flour (Table 4.3). Approximately half (50.3%) of the amount of thiamine in fortified maize flour was retained after cooking (Figure 4.3). Thiamine was the least stable B-vitamin. This is expected since heating causes the chemical breakdown of thiamine molecules (Bui & Small, 2007; Voelker et al., 2021a). Most importantly, among the B-vitamins, thiamine is the most heat-labile (Yaman et al., 2021). Thiamine is sensitive to heat above 70℃ hence its severe degradation during cooking (Verma & Verma, 2018). When foods are exposed to high levels of heat, light, and/or oxygen during preparation, most nutrient losses occur (Korus, 2020). A study on fortified wheat flour, reported 45-55% thiamine retention after cooking instant noodles, which corroborates the findings of this study (Bronder et al., 2017). Similarly, Lan & Small (2008) reported a loss of more than half (> 50%) of the thiamine content in fortified cooked noodles.

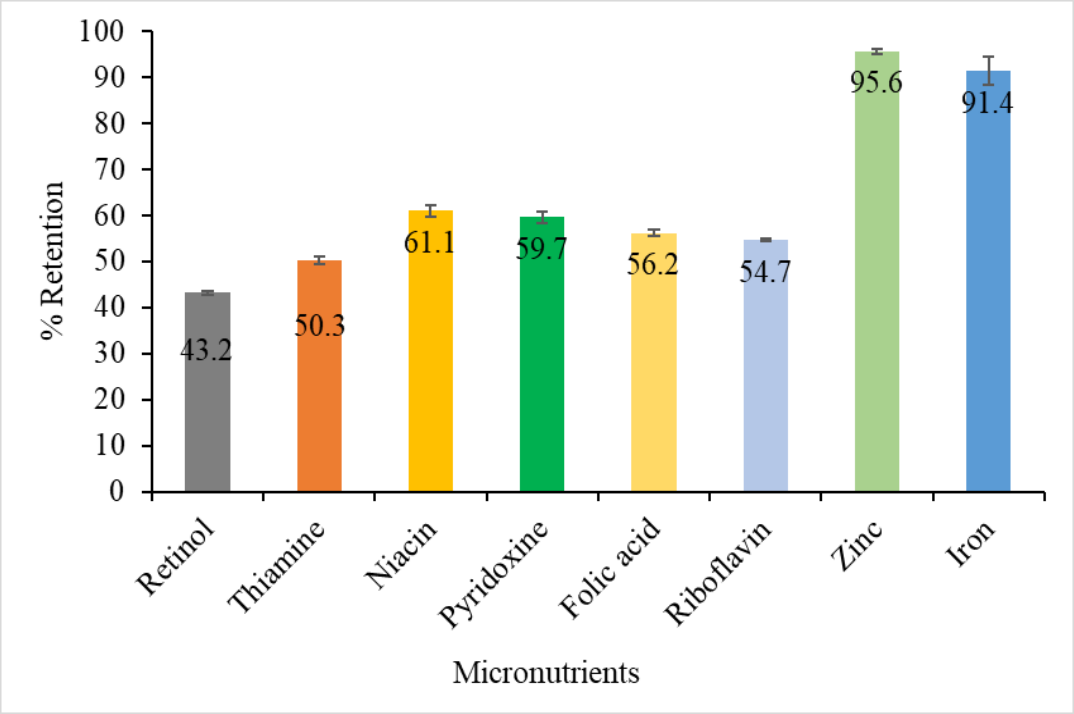

Figure 4.3: The retention of vitamins and minerals in fortified maize flour after cooking

4.2.3 Vitamin B2 (Riboflavin)

A significant difference ($p < 0.05$) in riboflavin concentration was observed between cooked and uncooked maize flour samples (Table 4.3). Cooking resulted in 54.7% retention of riboflavin (Figure 4.3). This was different from the findings of Bronder et al. (2017) who reported 85% retention of riboflavin after cooking instant noodles. Higher cooking time (8 minutes) may explain the lower retention values observed in this study as compared to the lower cooking time (5 minutes) used in the aforementioned study which resulted in higher riboflavin retention. According to Beizadea (2009), riboflavin is one of the most stable vitamins during thermal processing, storage, and food preparation. Exposure to light however makes it susceptible to degradation.

4.2.4 Vitamin B3 (Niacin)

Niacin amounts in cooked and uncooked fortified maize flour varied significantly ($p < 0.05$) (Table 4.3). With 61.1% retention after cooking, niacin was the most stable

of the B-vitamins analysed (Figure 4.3). According to Lešková et al. (2006), the most stable water-soluble vitamin is niacin since it is not degraded during processing or cooking. Although fairly heat stable, the water-soluble property of niacin makes it more prone to losses during cooking (Dunn et al., 2014). A majority of niacin is lost through leaching into cooking water (Beizadea, 2009). Although niacin is found in maize, most of it is bound to starch making it less bioavailable (Siyuan et al., 2018). Cooking as a processing technique helps in releasing the niacin bound to starch making it more bioavailable (Suri & Tanumihardjo, 2016).

4.2.5 Vitamin B6 (Pyridoxine)

There was a significant variation ($p < 0.05$) in pyridoxine content in fortified maize flour before and after cooking (Table 4.3). Pyridoxine was relatively stable compared to other micronutrients analysed, with retention of 59.7% (Figure 4.3). In contrast, a study conducted by Bronder et al. (2017) on the cooking of instant noodles, a product from fortified wheat flour, reported higher pyridoxine retention values of 77%. Pyridoxine is generally stable in the presence of oxygen and heat. It is however light-sensitive, especially in alkaline and neutral solutions. Additionally, metal ions, present in the cooking water, catalyse the decomposition process leading to vitamin B_6 losses (Ottaway, 2010). Furthermore, the type of thermal processing and cooking temperature influences vitamin B_6 (pyridoxine) losses (Beizadea, 2009).

4.2.6 Vitamin B9 (Folic acid)

Folic acid content differed significantly ($p < 0.05$) in cooked and uncooked fortified maize flour (Table 4.3). Cooking resulted in a 43.8% loss in folic acid, an equivalent of 56.2% retention (Figure 4.3). Folic acid is relatively stable to heat, however, its water-soluble property contributes to the losses mostly due to leaching into the cooking water in food preparations (De Paiva Azevedo et al., 2020). The loss of folic acid during cooking and preparation is mostly attributed to vitamin leaching into the cooking water (Silveira et al., 2017). The findings of this study compared favourably with those of De Paiva Azevedo et al. (2020) who showed that cooking using the moist-heat method resulted in lower retention of folic acid (57%) as compared to using the dry-heat method (80 - 87%). Porasuphatana et al. (2008) on the other hand,

found higher folic acid retention values (92%) in boiled fortified rice. In this case, since cooking water was not drained, the loss of folic acid, just like the other B-vitamins, in the '*ugali*' is more likely to be due to hydrolysis (the chemical breakdown of a compound caused by interaction with water) of the vitamin in the cooked food matrix rather than leaching, because the water remained incorporated into the food during the cooking process and even after it was finished.

4.2.7 Iron and zinc

The amounts of iron and zinc in cooked and uncooked fortified maize flour were not significantly different ($p > 0.05$) (Table 4.3). After cooking, the flour samples retained 95.6% of zinc and 91.4% of iron (Figure 4.3). In comparison with other micronutrients assessed, iron and zinc were the most stable during cooking. This was expected since minerals such as iron and zinc are generally heat stable (Dunn et al., 2014; Hemery et al., 2018). Leaching (extraction of minerals by water) is a common way in which minerals are lost during processing and cooking. According to Andang'o (2007), NaFeEDTA, a form of iron fortificant has the advantage of stability during food preparation since it is water-insoluble which explains the high retention values observed in this study. These values compared well with the range reported by Mohibbe et al. (2021) of 96.0 - 99.7% retention for iron and 98.8% retention for zinc in cooked fortified rice. Wieringa et al. (2014) reported 100% iron retention and 89% zinc retention in fortified rice regardless of the producer or cooking method, which was consistent with the results of this study. Fortification of food with zinc is crucial since zinc deficiency is common in several countries, especially among infants and children. Iron is important in reducing the prevalence of iron-deficiency anaemia (IDA) which affects infants, children, and women of child-bearing age.

4.3 FT-NIRS model development and prediction of retinol in fortified maize flour

4.3.1 Retinol content of the fortified maize flours

Retinol content of fortified maize flours measured using HPLC method are shown in Table 4.4. The total retinol content ranged between 0 mg/kg ± 0.00 (not detectable) to 4.03 ± 0.82 mg/kg. Only 26.9 % of the analysed samples complied with the set standards for retinol (0.5-1.4 mg/kg), meaning that quite a number of samples did not meet the fortification requirements. This is somewhat comparable to the report of Khamila et al. (2020) which showed that retinol in fortified maize flour samples ranged from non-detectable to 1.2 mg/kg, with a mean of 0.4 ± 0.3 mg/kg and 33.3% of the samples analysed complied to the recommended standards. Samples with retinol concentrations lower than 0.499 mg/kg were considered under-fortified. Whereas samples with retinol levels above 1.4 mg/kg were termed over-fortified, based on the minimum and maximum regulatory levels outlined by East African Community, (2011) of 0.5 mg/kg and 1.4 mg/kg, respectively. Both of these sets of samples did not meet the regulatory requirements for compliance.

Retinol, in the form of retinyl palmitate/retinyl acetate, is one of the micronutrients added to maize flour as a fortificant (EAC, 2011; Allen et al., 2006; Grimm et al., 2012). Generally, the precision with which the chemical composition of the samples is determined using reliable and accepted reference procedures has a large impact on the performance of the calibrations developed. (Pasquini, 2003).

HPLC is a conventional method that is used to determine the amounts of retinol in fortified maize flour as a way of monitoring compliance to fortification standards. However, due to the lengthy extraction operations, HPLC as a monitoring approach is time-consuming and tedious especially when a significant number of samples are involved (Yang and Irudayaraj, 2001). Additionally, use of HPLC for quantification of retinol in samples is highly prone to oxidation and many interfering compounds which is a source of likely errors in the method (Zhang et al., 2018). FT-NIR spectroscopy on the other hand as a technique for retinol determination is quick (one minute or less turn-around per sample), and requires minimal sample preparation

hence non-destructive (Huang et al., 2008;Pasquini, 2003). Moreover, the results from FT-NIR systems are more stable and reproducible hence increased accuracy (Yang and Irudayaraj, 2001).

Table 4.4: HPLC retinol data used for NIR spectroscopy modelling

County	Sample size (n)	Range (mg/kg)	Mean (mg/kg)	SD
Nairobi	21	0.00 - 4.03	0.96	0.94
Kiambu	18	0.06 - 1.92	0.65	0.42
Uasin Gishu	16	0.02 - 1.40	0.46	0.33
Nakuru	17	0.00 - 1.67	0.45	0.53
Elgeyo-Marakwet	12	0.00 - 0.75	0.19	0.25
Kwale	11	0.00 - 0.76	0.27	0.28
Kilifi	10	0.00 - 0.72	0.17	0.22
Kisumu	15	0.01 - 0.64	0.23	0.22
Busia	13	0.02 - 1.17	0.30	0.36
Mombasa	17	0.00 - 0.72	0.30	0.24

4.3.2 NIR spectra of fortified maize flour

The characteristic raw/unprocessed spectra of the fortified maize flour samples are shown in Figure 4.4. This visual representation shows that, while all spectra had relatively similar shapes, there was variation in absorbance. NIR spectroscopy studies on various parameters of maize flour samples reported by Plumier (2013), Chen et al. (2017), Egesel and Kahriman (2012) obtained similar general shapes of spectral data as in the samples in this study. The fortified maize flour spectra generally resembled those of other agricultural products such as wheat (de Girolamo et al., 2014) and common beans (Wafula et al., 2020).

Absorption bands were linked to functional groups and compounds present in fortified maize flour samples. The dominant absorption bands included: 9000 cm^{-1} - 8000 cm^{-1} (second overtone C-H stretching), 6800 cm^{-1} - 6600 cm^{-1} (first overtone N-H stretching; first overtone O-H stretching), 5300 cm^{-1} - 5200 cm^{-1} (combination C-H stretching), 5000 cm^{-1} - 4700 cm^{-1} (combination N-H stretching; combination O-H stretching), and 4545 cm^{-1} - 4065 cm^{-1} (combination C-H stretching). These

peaks in the raw spectra could be attributed to the relatively high quantities of carbohydrates, proteins, and an appreciable amount of moisture and vitamins in maize flour (Gwirtz & Garcia-Casal, 2014; Qamar et al., 2017). The absorption bands in 5000 cm^{-1} - 4700 cm^{-1} are associated with protein and moisture content in the samples while absorption at 5300 - 5200 cm^{-1} is associated with polysaccharides such as amylose and amylopectin. Retinol, a minor parameter, mainly consists of C-H-O molecular bonds (Stefan, 2006). The absorption bands ranging from 4762 cm^{-1} - 4386 cm^{-1} are proposed to be related to C-H-O structures such as retinol (Kusumiyati et al., 2021).

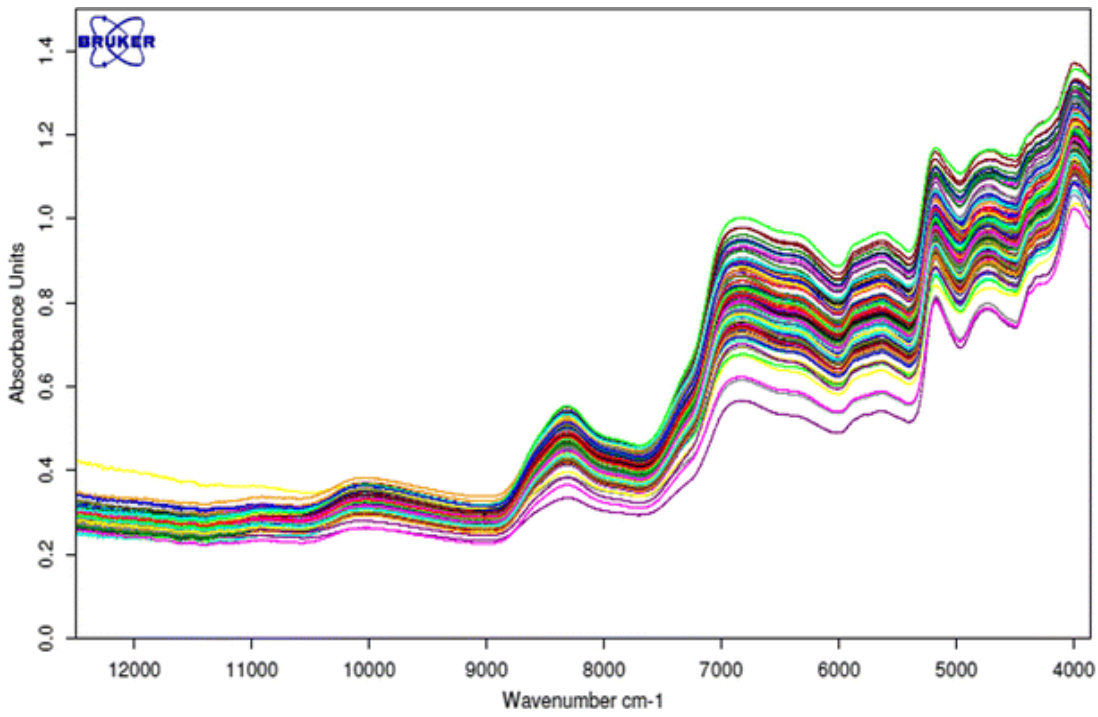

Figure 4.4: Raw NIR spectra of fortified maize flour

4.3.3 PLS-R models predicting retinol in fortified maize flours

Successful building of a single partial least squares regression (PLS-R) model that has the capacity to predict retinol levels in fortified maize flour samples over a wide range of concentrations would have been the most ideal outcome of this study. However, as illustrated in Figure 4.5 a single calibration model to predict retinol in

fortified maize flour had a poor predictive capability as demonstrated by low R^2 (R^2c = 0.20; R^2_V = 0.18), low RPD (1.11, 0.92) and high RMSE (RMSEE = 0.70; RMSEP = 0.81) values. An acceptable model should have a high R^2, a low RMSE and an RPD higher than 2.5 (Manley, 2014). There are some practical calibration situations where it is difficult to construct a single universal calibration equation for the entire population of interest hence lack of an adequate model for all objects (Agelet & Hurburgh, 2010). Various approaches to address non-linearity such as use of new pre-treatment methods, eliminating wavelengths, adding extra principal components/ latent variables to the model, or splitting the data into subsets or groups can be used (Agelet & Hurburgh, 2010). Based on this observation and knowledge, two separate models were developed in order to improve the model performance.

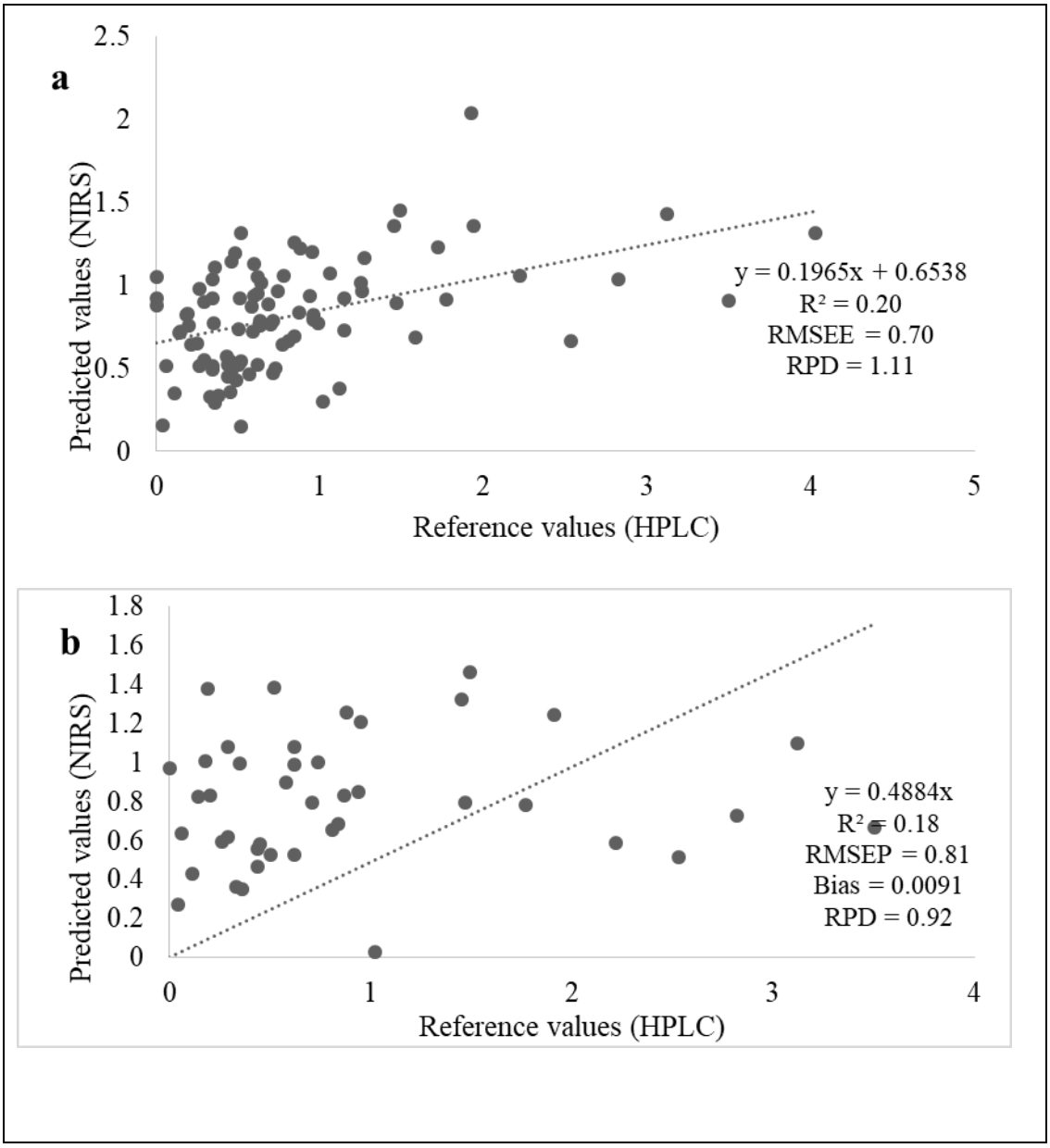

Figure 4.5: Calibration (a) and validation (b) of the correlation between reference values and FT-NIR predicted values of retinol in fortified maize flour (single model).

R^2: coefficient of determination; RMSEE: root mean square error of estimation; RMSEP: root mean square error of prediction; RPD: ratio of performance to deviation.

It was observed that splitting the data into two groups, retinol < 1.0 mg/kg and retinol ≥ 1.0 mg/kg, resulted in models I and II, respectively that showed better

prediction performance (Figures 4.6 and 4.7). The performance metrics for the single calibration model, models I and II are summarized in Table 4.5. From this table, it can be observed that there was a reduction in RMSEE and RMSEP values and an increase in R^2 values of model I and II compared to those of the single calibration model.

Table 4.5: Calibration and external validation statistics for prediction of retinol in fortified maize flour according to the models developed

	Single model		**Model I Retinol (< 1.0 mg/kg)**		**Model II Retinol (≥ 1.0 mg/kg)**	
Statistics	**Calibration**	**Validation**	**Calibration**	**Validation**	**Calibration**	**Validation**
R^2	0.20	0.18	0.81	0.82	0.93	0.81
RMSEE	0.70	-	0.08	-	0.16	-
RMSEP	-	0.81	-	0.09	-	0.22
Bias	-	0.0091	-	-0.0008	-	-0.0046
RPD	1.11	0.92	2.29	2.07	3.58	2.43
Rank	8	8	7	7	8	8

R^2: coefficient of determination; RMSEE: root mean square error of estimation; RMSEP: root mean square error of prediction; RPD: ratio of performance to deviation

The R^2c, RMSEE, and RPD before data splitting were 0.20, 0.70, and 1.11 respectively. Model I on the other hand achieved R^2c, RMSEE, and RPD of 0.81, 0.08, and 2.29 respectively while model II showed equally good results with R^2c, RMSEE, and RPD of 0.93, 0.16 and 3.58 respectively (Table 4.5 and Figures 4.6 and 4.7). When the models were applied to predict the retinol contents of the validation sets, the prediction results for model I were: R^2v = 0.82, RMSEP= 0.09, bias = -0.00088, and RPD = 2.07 (Table 4.5 and Figure 4.6) while the prediction results for model II were: R^2v = 0.81, RMSEP= 0.22, bias = -0.0046 and RPD = 2.43 (Table 4.5 and Figure 4.7). The reliability of the generated models was assessed according to the coefficient of determination of validation (R^2v), root mean square error of prediction (RMSEP), ratio of performance to deviation (RPD), and bias values. The models generally depicted high R^2 values, low RMSEP values, and low bias values hence reliable for application (Reddy et al., 2016; Egesel & Kahriman, 2012).

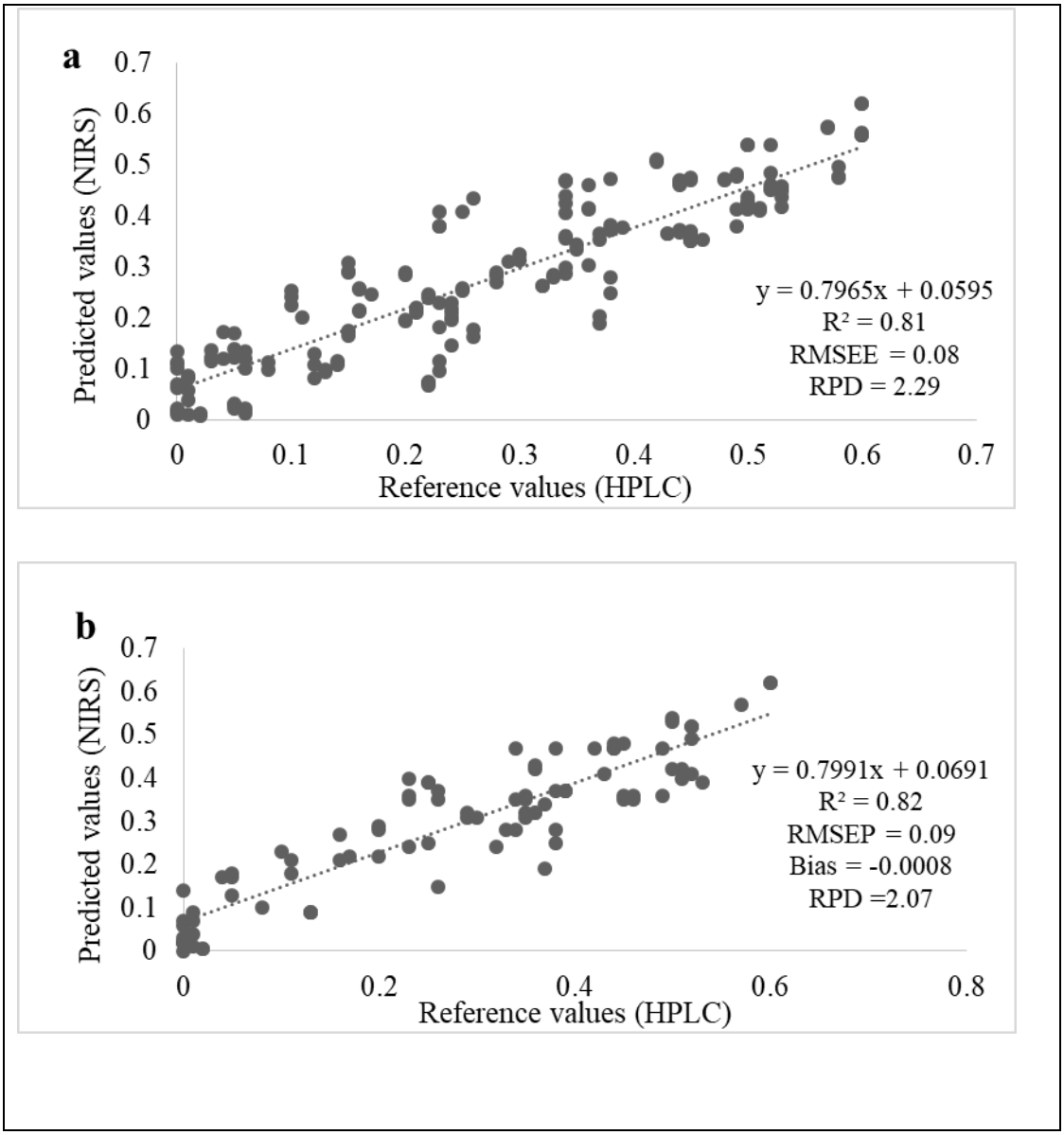

Figure 4.6: Calibration (a) and validation (b) of the correlation between reference values and FT-NIR predicted values of retinol (< 1.0 mg/kg) in fortified maize flour

R^2: coefficient of determination; RMSEE: root mean square error of estimation; RMSEP: root mean square error of prediction; RPD: ratio of performance to deviation.

Comparing the two models, external validation indicated that the prediction errors (RMSEP) for model II were slightly higher (0.22) than those of model I (0.09). Model II had a fewer number of samples and this is probably why it had slightly

higher errors for the RMSEP. Further, retinol estimates were negatively biased (bias = - 0.00088 and bias = -0.0046) for models I and II respectively (Figures 4.6 and 4.7). The bias value for an accurate calibration model should be close to 0 (Elfadl et al., 2010). Bias is the average difference between the NIRS predicted values (y) and measured/reference values (x). A positive value means that, on average, the model is overestimating the composition by this amount whilst a negative value represents an underestimation (Zhou et al., 2017). Although the models created would underestimate retinol amounts in fortified maize flour, the values were close to 0, indicating that the models developed were quite accurate and adequate, and hence can be adopted in food analysis.

Models I and II had R^2v of above 0.8 which implies that these models were acceptable and usable for screening and some approximate calibrations according to de Girolamo et al. (2014). The RPD values, 2.07 (model I) and 2.43 (model II), according to Xia et al. (2018) indicate that model I had a slightly lower robustness. When the prediction accuracy of calibration models is relatively insensitive to unknown changes of external factors, the model is said to be robust (Ketelaere et al., 2007). Temperature fluctuations, shifts in wavelength, and changes in detector stability over time are some of the factors that could have impacted model performance. The RPD tests the robustness of a model by assessing how well the developed model predicts the retinol in the validation set, and the higher its value, the better the model's prediction capacity (De Girolamo et al., 2014; Xia et al., 2018 ; Ribeiro et al., 2021). Regression models with RPD values ≥ 6.5 are excellent and suitable for process control or any application (Xia et al., 2018). Chang et al. (2001) however contrasts by mentioning that calibration models with RPD > 2 are considered satisfactory.

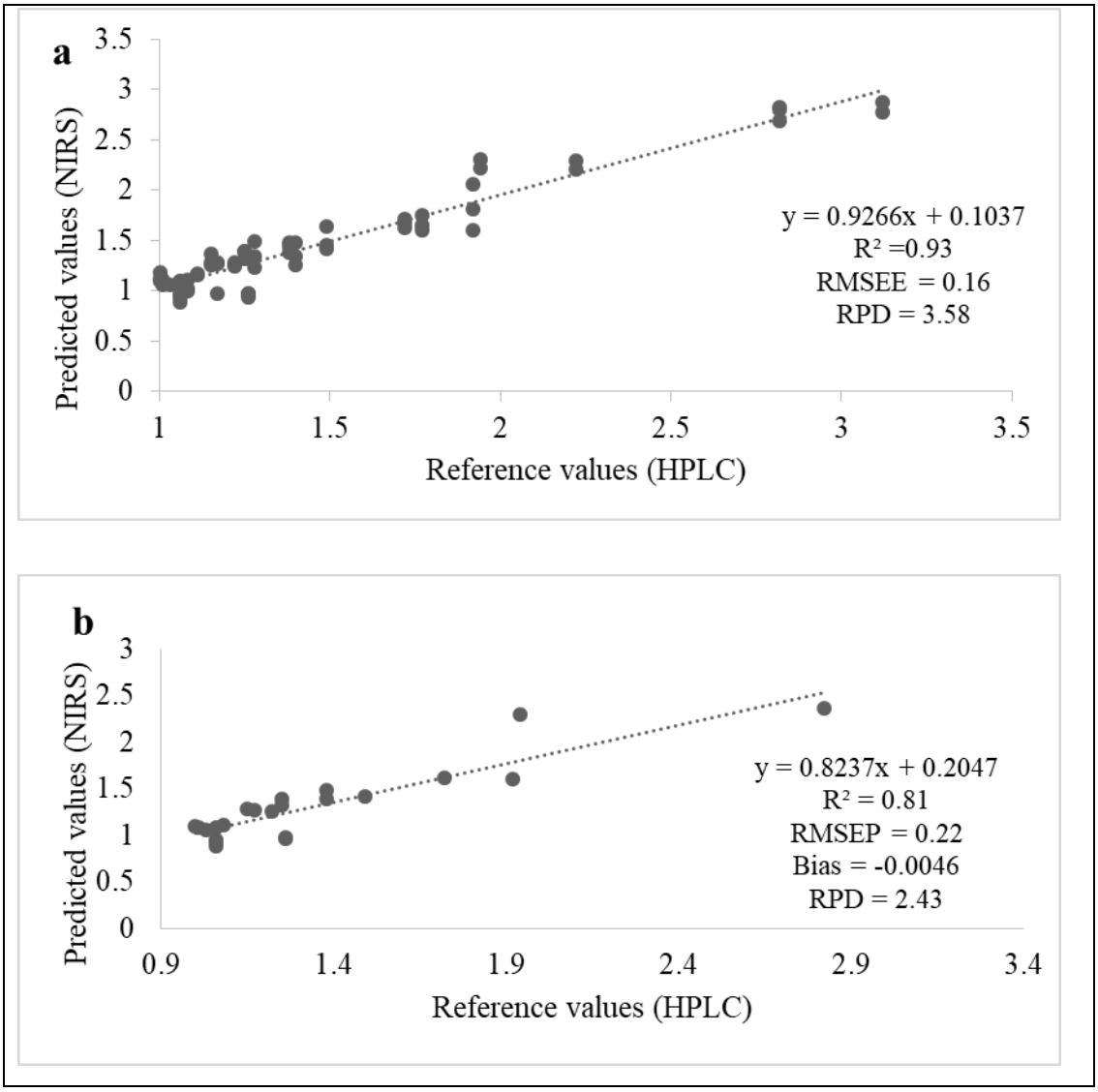

Figure 4.7: Calibration (a) and validation (b) of the correlation between reference values and FT-NIR predicted values of retinol (≥ 1.0 mg/kg) in fortified maize flour

R^2: coefficient of determination; RMSEE: root mean square error of estimation; RMSEP: root mean square error of prediction; RPD: ratio of performance to deviation.

There are limited studies done on the application of NIR spectroscopy to predict retinol in maize flour. For this reason, the discussion of this study's findings was mostly limited to NIR studies on the prediction of retinol in various food matrices and prediction of other parameters in maize flour. The work of Kahrıman et al.

(2019) obtained the following prediction performance of fat soluble secondary metabolites in maize flour, carotenoids (R^2v = 0.721; RMSECV = 3.069) and tocopherol (R^2v = 0.515; RMSECV = 2.0943). The models presented in the current study showed higher R^2v values and lower prediction errors than those of Kahrıman et al. (2019) that applied NIR spectroscopy to predict carotenoids and tocopherol in maize flour. Furthermore, Soulat et al. (2020) reported lower coefficient of determination (R^2v = 0.34) for the prediction of retinol from cow's milk. The prediction errors reported by Soulat et al. (2020) was slightly higher (RMSEP = 0.15). than those of model I (RMSEP = 0.09), and slightly lower than those of model II (RMSEP = 0.22). Altogether, the results of this study and those of the studies discussed here demonstrate that there is potential to successfully use NIR spectroscopy to rapidly assess retinol from different food matrices.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Storage and cooking stability studies

With regards to storage stability tests, retinol was the least stable vitamin for brand XXI after 6 months storage at both 25 °C/75% RH and 35 °C/83% RH, followed by thiamine, riboflavin, folate, and niacin. However, brand XY2 showed that after 6 months storage 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) had higher vitamins content retained for both brands XX1 and XY2. Generally, storage conditions (temperature and relative humidity) had a significant effect ($p < 0.05$) on the retention of retinol, and B-vitamins in selected commercial fortified maize flour.

Vitamins were more susceptible to cooking losses than minerals. Heat processing led to a significant reduction ($p < 0.05$) in the percentage retinol, and B-vitamins retention (i.e., losses ranging from 39% - 57%). Iron and zinc were highly stable during cooking, and there was no significant difference ($p > 0.05$) between cooked and uncooked fortified maize flour. This study's findings conclusively show that substantial vitamin losses in fortified maize flour occur during storage and cooking.

5.1.2 FT-NIRS model development studies

The range of retinol values used as reference values in this study (0 mg/kg ± 0.00 (not detectable) to 4.03 ± 0.82 mg/kg) comprised the retinol levels that may be routinely encountered in commercial fortified maize flour samples. Only 26.9 % of the analysed samples met the retinol fortification standards (0.5-1.4 mg/kg), indicating that a significant proportion of samples did not meet the fortification requirements. Initially, a single calibration model showed poor predictive performance. Improvement in the model predictive performance was achieved by

splitting the dataset hence developing two separate models. The model developed for predicting retinol ≥ 1.0 mg/kg illustrated slightly better prediction performance than the model for predicting retinol < 1.0 mg/kg. The models developed in this study had high R^2 values and low errors. FT-NIR spectroscopy can thus be used to adequately predict retinol in fortified maize flour. NIRS, by replacing time-consuming and laborious wet chemistry laboratory procedures, has the potential to be used for rapid regulatory monitoring of fortification compliance for a large number of samples.

5.2 Recommendations

More research is needed to determine the bio-accessibility of the vitamins and minerals 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. Additionally, given the observed trends in this study on micronutrient stability during storage and cooking, it is recommended that the legislated food fortification program be amended and adjusted to account for the projected micronutrient losses. It is also important to conduct studies to investigate the combined effect of storage and cooking on micronutrient retention in fortified maize flour. Moreover, premix manufacturers may explore adopting technologies such as microencapsulation of fortificants to increase micronutrient stability and, as a result, promote fortification success and efficacy.

The robustness of the models can also be improved in the future by addition of more calibration data. Furthermore, linear discriminant analysis (LDA) studies should be done to generate an algorithm that will be used to differentiate the two prediction models developed (model I used to predict retinol < 1.0 mg/kg and model II used to predict retinol ≥ 1.0 mg/kg). This will help determine which of the two models is to be employed for predicting the amount of retinol in a specific unknown sample. FT-NIR-based models for prediction of B-vitamins in fortified maize flour should also be developed so as to allow quick analysis.

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