Interactive effect of maturity stages and fruit accessions on primary and secondary metabolites profile of *Mimusops zeyheri* Sond

By

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Declaration

I, Kamogelo Pollen Teffo, declare that neither I nor anyone else has ever submitted a dissertation for a degree to the University of Limpopo or any other institution titled "Interactive effect of maturity stages and fruit accessions on primary and secondary metabolites of *Mimusops zeyheri* Sond" for a Master of Agricultural Management (Plant Production). Also, this is my work in design and in execution, while related materials contained herein had been duly acknowledged.

_____ _____

Teffo K.P **Date**

Dedications

I dedicate this full dissertation to my past, present and future generations of Molamudi (Ditholo, Re batho babo nnanna wa kwapa wa mohonyoko) and Teffo (Bathokoa ba maseboko seboko a se bokwe baneng).

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In the name of the Father, and the Son, and the Holy Spirit

To: God, Supervisors, Family and Friends

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Conference contributions

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Teffo, K.P., Mpai, S. Mashela, P.W., and Ndhlala, A.R. 2023. Accessions variation and different fruit maturity stages affect mineral of *Mimusops zeyheri* Sond. Paper at the 48th South African Association of Botanists (SAAB) annual conference. The Ranch Protea Hotel, Polokwane, South Africa. 17-20 January 2023.

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Teffo, K.P., Mpai, S., Mashela, P.W., and Ndhlala, A.R. 2022. Impact of post-harvest maturity stages on quality and physicochemical attributes in five accessions of *Mimusops Zeyheri Sond*. Paper at the 12th Faculty of Science and Agriculture Postgraduate Research Day. 21-23 September 2022. Bolivia Lodge, Polokwane.

Teffo, K.P., Mpai, S., Mashela, P.W., and Ndhlala, A.R. 2022. Impact of post-harvest maturity stages on quality and physicochemical attributes in five accessions of *Mimusops Zeyheri Sond*. Paper at Indigenous Plants Use Forum (IPUF). 04-07 July 2022 Virtual platform.

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List of abbreviations

%	Percentage
°C	Degrees Celsius
µg	Microgram
µL	Microliter
µm	Micrometre
AAS	Amino Acid Score
Ala	Alanine
ANVISA	National Health Surveillance Agency
AOAC	Association of Official Analytical Chemists
Arg	Arginine
Asp	Asparagine
Ca	Calcium
Carb	Carbohydrates
CRD	Complete Randomized Design
DAFF	Department of Agriculture, Fishery and Forestry
DM	Dry matter
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl -1-picrylhydrazyl
DRI	Daily Recommended Intake
DSI	Department of Science and Innovation

EPR	Electron Paramagnetic Resonance
FAO	Food and Agricultural Organization
FCs	Flavonoids contents
Fe	Iron
Fib	Fibre content
FRAP	Ferric Reducing Power Assay
g	Grams
GAE	Gallic acid equivalents
GBRCE	Green Biotechnologies Research Centre of Excellence
GenStat	General Statistics
His	Histidine
HPLC	High Performance Liquid Chromatography
ICPE	Inductively Coupled Plasma Optical Emission Spectrometry
IFAD	International Fund for Agricultural Development
Ile	Isoleucine
K	Potassium
kg	Kilogram
L	Litre
LATS	Limpopo Agro-Food Technology Station
Leu	Leucine
Leu	Leucine

m	Meter
mm	Millimetre
m/z	Mass number
Mg	Magnesium
mg	Milligram
ml	Millilitres
Mn	Manganese
Moi	Moisture content
MS	Mass Spectrometer
N	Newton
Na	Sodium
NASEM	National Academic of Science, Engineering and Medicine
Nm	Nano meter
P	Phosphorus
PCA	Principal Component Analysis
PCs	Phenolic contents
PDA	Photodiode Array
Phe	Phenylalanine
Pro	Proline
Prot	Protein
PVA	Principle Component Analysis plot

QTOF	Quadrupole Time of Flight
RCD	Randomized Complete Design
Rpm	Revolution per minute
Rt	Retention time
SA	South Africa
SD	Standard deviation
SDG2	Sustainable Development Goal 2
SSA	Statistics of South Africa
STIs	Sexual Transmitted Infections
T	Titre volume
T1	Green fruit
T2	Breaker fruit
T3	Pale yellow fruit
T4	Yellow/ orange fruit
TA	Total Anthocyanins
TB	Tuberculosis
TE	Trolox equivalents
TFC	Total Flavonoids Content
Thr	Threonine
TPC	Total Phenolic Content
Trp	Tryptophan

Tyr	Tyrosine
UL	University of Limpopo
UNICEF	United Nations Children’s Fund
UPLC	Ultra-Performance Liquid Chromatograph
UPLC-MS	Ultra-Performance Liquid Chromatograph Mass Spectrometer
UV	Ultraviolet
V	Volume
Va	Aliquot volume
Val	Valine
Vf	Total volume
VSN	Victory Sport Network
WFP	World Food Programme
WHO	World Health Organization
Zn	Zinc

Abstract

Mimusops zeyheri Sond is a member of the Sapotaceae Family and it is an undervalued or unappreciated indigenous fruit tree consumed raw as a healthful snack in rural communities across Sub-Saharan Africa. It is known for its predominantly high vitamin C content. The objectives of this study were to investigate whether the interaction between different accessions and fruit maturity stages has effect on post-harvest quality, physicochemical attributes, primary and secondary metabolites in *M. zeyheri*. To achieve the objectives of this study, fruits of *M. zeyheri* were harvested from trees of five accessions namely, 6E, M7, 3E, HY, and 3L at four different maturity stages: dark green (T1), breaker (T2), pale yellow (T3), and yellow (T4). A 5 × 4 factorial experiment was established as an interaction between five accessions and four fruit maturity stages. The experiment was laid in a randomised complete design with four replication per accession and maturity stage. The mean separation was done using a Duncan's Multiple Range Test at the significance level of 5% using the GenStat 18th version.

Five accessions and four fruit maturity stages demonstrated significant variation ($p< 0.05$) on quality and physicochemical attributes. Accession 6E had the highest fruit length at T1 to T4 maturity stage (25.85-27.63 mm). The other accessions including 3E, HY, and 3L had similar moderate length however, these were higher than that of accession M7 which exhibited the least, irrespective of maturity stage. Fruit firmness declined as fruits ripened with the highest values recorded at T1 and lowest values at T4 fruit maturity stages, for all five accessions. Fruits of accession HY had the highest total soluble solids (32.80%) at T4 maturity stage while that of accession 3L had lowest total soluble solids (2.40%) at T1 maturity stage. Accession M7 had the highest total titratable acid (3.20%) at T1 maturity stage, whilst accession 6E had the lowest total titratable acidity (0.22%) at T4 maturity stage. The highest TSS/TA ratio values were reported in accession M7 (32.20%) at T4 maturity stage than all accessions. All the accessions at T1 to T4 stages demonstrated different values of colour change on Hunter a*. The Hunter L* values were lower at T1 and highest at T4 maturity stages. The H˚ angle were high at T1 and lowest at T4 maturity stages as fruit reached ripening period.

On the nutritional compositions, accession HY recorded highest values of moisture content at T1 to T4 maturity stages (93-97%) and protein content at T2 to T4 maturity stages (0.41-0.40%), whilst accession HY recorded at T4 maturity stage (0.43%). The moisture content was highest in accession 6E at T1 and T3 maturity stages (92 and 89%) and ash content (4.20-3.75%) at T1 to T3 maturity stages. The dry matter, ash and protein content were reduced from T1 to T4 maturity stages, while moisture content enhanced with advanced of fruit maturity stages. The mineral such as calcium, potassium, iron, magnesium, manganese, sodium, zinc and phosphorus were assessed. Accession M7 had highest values of calcium at T1 maturity stage (27.73 mg/L), magnesium (5.96 and 4.37 mg/L) at T1 and T2 maturity stages and sodium (5.63-3.64 mg/L) at T1 to T4 maturity stages. Accession 3E had highest values of potassium at T2 maturity stage (8.70 mg/L) and Manganese at T1 and T2 maturity stages (0.07 and 0.06 mg/L). Furthermore, accession HY had highest values of calcium at T2 to T4 maturity stages ranged from (21.50-17.17 mg/L), T1 to T3 maturity stages were recorded in iron (1.57-0.37 mg/L) and potassium (50.83-44.93 mg/L). Accession 3E had highest values of phosphorus at T1 to T3 maturity stages ranged from (9.39-6.83 mg/L), zinc ranged from (1.73-0.60 mg/L). The mineral compositions decreased with advanced of fruit maturity stages in all accessions. This study demonstrated that fruits of five accessions of *M. zeyheri* at T1 maturity stage should be used for the food fortification program due to high amount of mineral compositions and fruits at T4 maturity stage should be consumed as raw fruits for snacking, dried fruits and can also be processed to generate fermented juices, jellies and dried fruits to reduce the malnutrition and food insecurity.

Secondary metabolites, such polyphenolic and flavonoids were identified and quantified in five accessions of *M. zeyheri* at two different fruit maturity stages, which were T1 and T4. For untargeted secondary metabolites, methanol extracts were analysed using ultra-performance liquid chromatography mass spectrometer. In five accessions of *M. zeyheri*, exploratory principal component analysis plot revealed five major clusters based on the heterogeneity of metabolites. The highest value of 3,4-bis(acetyloxy)-5-acetamido-6-(3-nitrophenoxy)oxan-2-yl]methyl acetate was recorded in accession 3L at the T1 fruit maturity stage (719.90 mg/kg) including paeonoside (48.98 mg/kg) and T4 fruit maturity stage of 7-oxo-8,9-dihydroxy-4'-N-demethyl staurosporine (122.48 mg/kg). The highest value in accession HY was recorded at the

T4 fruit maturity stage (646.70 mg/kg). The three flavonoid metabolites found in *M. zeyheri* that were most abundant were quercetin, quercetin galactoside, and quercetin glucoside. The results discovered that *M. zeyheri* fruit possesses a high level of concentration at T1 and T4 fruit maturity stages which could contribute and serve as a food source, especially for vulnerable rural communities that need food security, and prevent chronic non-communicable diseases.

Keywords: Accession, Flavonoids, Maturity stage, *Mimusops Zeyheri*, Nutritional element, Phenolic compounds, Principal components analysis, Physiochemical.

CHAPTER 1

General introduction

1. Research problem

1.1 Background

Transvaal red milkwood (*Mimusops zeyheri* Sond.), belongs to the Family of Sapotaceae (Hankey, 2005). Its common names include Transvaal red milkwood (English), *Moepel* (Afrikaans), *Mmupudu* (Sepedi), *Umpushane* (isiZulu), *Mbubulu* (Venda), and *Mgamba kapu* (siSwati) (Lemmens, 2005). The tree is indigenous to southern Africa and people consume its fruits (Mngadi, 2017). In South Africa, it is distributed widely across the northern parts of the Limpopo province to the southern parts of the KwaZulu Natal province (Hyde *et al*., 2012). *Mimusops zeyheri* is a perennial evergreen tree that can grow up to 25 meters in height and yield oval-shaped fruits with pointed tips which turn from green to yellow or orange during ripening (Mashela and Mollel, 2001) as shown in Figure 1.1. It forms leaves during the winter season, and the inflorescence commences between April and September during the harvest of fruits (Hankey, 2005).

Figure 1.1: *Mimusops zeyheri* fruit tree

Some *M. zeyheri* trees possess a bushy structure with thick leaves however, they produce fewer fruits which makes them more suitable to be considered for shade provision (Mashela *et al*., 2013). By contrast, other accessions exhibit retarded growth but produce a higher fruit yield. In addition to the differences in yield and structure, various accessions of *M. zeyheri* also vary in the shape and taste of fruits (Mashela *et al*., 2013). Although different accessions of *M. zeyheri* are found and consumed in South Africa, there is less scientific information regarding the primary and secondary metabolites associated with each accession. Studies by Chivandi *et al*. (2015) and Mngadi (2017) indicated that fruits of *M. zeyheri* are a source of essential macro-and micronutrients, proteins, and carbohydrates.

During the fruit development stage, fruits of the plant undergo different stages of maturity which are set just after physiological maturity is achieved (Ndou *et al*., 2019). The stages involve the convention of the green pigmentation chlorophyll and starch to carotenoids and sugars, components that improve the taste of the fruits (Kopsell *et al*., 2005). In general, literature show that tree fruits undergo five main different maturity stages characterized by different colour of the exocarp skin and are: green (stage 1), breaker (stage 2), pink (stage 3), red (stage 4), and dark red (stage 5) (Ndou *et al*., 2019). Therefore, the gradual changes lead to the maturity stage. Metabolomic is defined as the study of metabolites within sample plant tissues (Kim *et al*., 2011). Untargeted metabolites are often carried out to create a holistic metabolome profile without being biased for certain metabolite compounds. On the other hand, targeted metabolites are performed in the case when concentration of a certain compound is assessed based on the reference standards (Koek *et al*., 2011).

1.2 Problem statement

Exotic and indigenous plant organs have been the main source of food for many rural populations, with agriculture serving as the foundation of food systems (Akinola *et al*., 2020). This has largely been due to profound primary and secondary metabolites contained in the food and are credited for the prevention of hidden hunger and non-communicable diseases (Tchuenchieu and Kesa, 2020). Providing access to sufficient, nutrient-rich, and affordable food that is produced sustainably is one of the most critical concerns faced in South Africa (Bobo, 2021). Statistics South Africa of

2018 demonstrated that 23.8% of individuals with around 20.2% of households lack regular access to food, whereby nearly 70% of the poorest households live in rural areas (Bobo, 2021). According to Gqaleni *et al*. (2007), 1.5 million children are malnourished and about 14 million individuals experience food insecurity. However, challenges facing rural and/or unemployed South Africans, like their counterparts in other developing nations, struggle with the inadequate nutritional consumption, food insecurity, and poor healthcare services and intriguingly, these have worsened following the outbreak of the COVID-19 pandemic (Mngadi *et al*., 2017).

In recent years, the world has experienced a decline in number of plant species consumed by humans. For example, the Food and Agriculture Organization (FAO *et al*., 2022) revealed that about 10 000 out of 300 000 known plant species have been used for human nourishment and only 150 to 200 of these have been grown commercially. Therefore, contribution of knowledge partly through the determination of the quality of wild fruits that are widely consumed is crucial. In fact, apart from knowledge on the quality of wild fruits, knowledge on how the maturity stages of wild fruits can be optimized to achieve the best 'ready to eat stage'. Furthermore, assess the effects of optimizing maturity stages on the concentration of primary and secondary metabolites as well as profile these key components therein. Scholarly studies have revealed information on untargeted and targeted metabolic compositions of common fruits of trees that are indigenous in South Africa, including 11 accessions of the Kei apple Mpai *et al*. (2018), and in five stages of maturity for Natal plum (Ndou *et al*., 2019). However, so far, there is rarely information on the interaction of accessions and fruit maturity stages on quality, physicochemical attributes and nutritional compositions, including secondary metabolites in *M. zeyheri*. Yet, such information is essential for promoting this indigenous fruit into food fortification program.

1.3 Rationale of the study

Mimusops zeyheri is a tree that is indigenous in South Africa and bear fruits that are well-known and edible and are harvested in rural communities in the Limpopo province (Mngadi, 2017). An earlier study by Garcia-Rios *et al*. (2022) revealed that the quality and physicochemical properties of indigenous fruits exhibit good potential for inclusion in the food fortification program. FAO (2022) indicated that signatories to the

Sustainable Development Goal 2 (SDG2) of zero hunger, have seven years left to end food insecurity, hunger, and all kind of various malnutrition. The FAO (2022) has asserted that in order to produce food and healthy diets that cost less, is safe, nutritious, and more accessible to everyone in a sustainable and inclusive manner, Agri-food systems must be reformed to reach the targets (SDG2) by 2030 (FAO, 2022). Furthermore, in order to eliminate undernutrition by 2030 (SDG2) in developing communities, countries need to adopt and implement initiatives that promote the intake of a balanced diet (Omotayo, 2020). Unfortunately, the potential of most indigenous edible fruits is largely neglected by scholars in the fields of horticulture, food science, botany, and economics (Omotayo, 2020). The objective of the South African National Policy on Food and Nutrition Security is to guarantee the accessibility, affordability, and availability of safe and nourishing food at the national and household levels (Mbhenyane, 2017). Edible indigenous fruits such as *M. zeyheri* can provide the primary metabolites needed in daily diets for food nutrition, decrease food insecurity, and be a source of income to rural communities where they are adapted and nutritious food may be unaffordable (Mashela and Mollel, 2001).

1.4 Purpose of the study

1.4.1 Aim

The purpose of the study was to develop scientific information on the interactive effect of maturity stages and fruit accessions variations on primary and secondary metabolites profile of *M. zeyheri*.

1.4.2 Objectives

I. To evaluate whether fruits from maturity stages of different accessions of *M. zeyheri* have different post-harvest quality and physicochemical attributes.
II. To assess whether different accessions and fruit maturity stages of *M. zeyheri* have different levels of primary metabolites.
III. To investigate whether different accessions and fruit maturity stages have different levels of untargeted secondary metabolites profile of *M. zeyheri*.

1.5 Reliability, validity, and objectivity

Reliability for the current study was determined by utilizing methods and data analysis methods such as GenStat 18th version (VSN International, Hempstead, UK) that are widely acceptable in the scientific community. Validity was achieved through replicating the treatments to increase the range of validity as well as control and repeat the experiments in time. The objectivity of the study was fulfilled by discussing the results based on empirical evidence as shown by statistical analyses, compared with results of other scholarly studies for similarities and differences with findings in other studies, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

The level of bias in this study was kept to a minimum by increasing the number of replications and randomizations for each accession and treatment (Leedy and Ormrod, 2005).

1.7 Scientific contribution

The findings of this research study will contribute new knowledge and scientific information on how maturity stages of fruits of *M. zeyheri* vary between accessions and how their maturity stages affect the quality, physico-chemical, nutrition, and metabolome profiles. The research will further contribute much needed information to local indigenous fruit farmers that could guide sustainable production and quality of fruits of *M. zeyheri* and such could benefit programs aimed at incorporating fruits of the plant into food fortification program aimed at alleviating food insecurity.

1.8 Structure of the dissertation

The structure of this dissertation is similar to that of research papers or articles. Chapter 1: Which is the introduction, outlines research problem including the background. The problem statement and rationale which include the aim and objectives are thoroughly outlined and finally the scientific contribution of the study.

Chapter 2: Consists of the work done on *M. zeyheri* and other fruit trees and work not yet done on the research problem, was outlined as a literature review.

Chapter 3: Consists of detailed materials and methods employed to carry out the qualities, physiochemical attributes and statistical analysis. The results, discussions, and conclusion regarding the qualities and physiochemical attributes are all recorded in this chapter.

Chapter 4: Comprise of the detailed materials and methods employed to carry out the nutritional composition experiments. The results, discussions and conclusion for nutritional composition are all recorded in this chapter.

Chapter 5: Include the materials and methods employed to conduct secondary metabolites' experiments. The methods of analysis are all outlined. The results, discussions and conclusion for secondary metabolites are all outlined and recorded in this chapter.

Chapter 6: The significance of the findings was summarized and integrated to provide their significance with recommendations for future research.

Chapters 3, 4 and 5 are structured in a research publication format. In text reference cited in every chapter are acknowledged at the end of that chapter, following the Harvard style of author alphabet as approved by the Senate of UL.

References

Akinola, R., Pereira, L.M., Mabhaudhi, T., De Bruin, F.M. and Rusch, L., 2020. A review of indigenous food crops in Africa and the implications for more sustainable and healthy food systems. *Sustainability*, 12(8): 3493.

Bobo, Z.N., 2021. Promoting Traditional and Indigenous Foods in South Africa: A Desktop Review (Masters Dissertation). *University of Stellenbosch*, South Africa.

Chivandi, E., Mukonowenzou, N., Nyakudya, T. and Erlwanger, K.H., 2015. Potential of indigenous fruit bearing trees to curb malnutrition, improve household food security, income and community health in Sub-Saharan Africa: A review. *Food Research International*, 76: 980-985.

FAO, IFAD, UNICEF, WFP and WHO. 2022. The State of Food Security and Nutrition in the World 2022. Repurposing food and agricultural policies to make healthy diets more affordable. *Rome, FAO*.

Garcia-Rios, D., Aguilar-Galvez, A., Chirinos, R., Pedreschi, R. and Campos, D., 2020. Relevant physicochemical properties and metabolites with functional properties of two commercial varieties of *Peruvian Pouteria* lucuma. *Journal of Food Processing and Preservation* 10(2): 251-257.

Gqaleni, N., Moodley, I., Kruger, H., Ntuli, A. and McLeod, H., 2007. Traditional and complementary medicine care delivery. *South African health review*, 2007(1): 175-188.

Hankey, A. 2005. *Mimusops* zeyheri Sond. Walter Sisulu Botanical garden. [Accessed 12 April 2012] from http://www. plantzafrica.com.

Hyde, M.A., Wursten, B.T. and Ballings, P., 2012. Flora of Zimbabwe: Species information: *Mimusops zeyheri*. [Accessed 12 April 2012] from http://www.zimbabweflora.co.zw.

Kim, J.M. and Shin, M.S., 2011. Characteristics of (*Rubus coreanus* Miq.) fruits at different ripening stages. *Korean Journal of Food Science and Technology*, 43(3): 341-347.

Koek, M.M., Jellema, R.H., van der Greef, J., Tas, A.C. and Hankemeier, T., 2011. Quantitative metabolomics based on gas chromatography mass spectrometry: status and perspectives. *Metabolomics*, 7(3): 307-328.

Kopsell, D.E., Kopsell, D.A., Lefsrud, M.G. and Curran-Celentano, J., 2005. Variability in elemental accumulations among leafy *Brassica oleracea* cultivars and selections. *Journal of Plant Nutrition*, 27(10): 1813-1826.

Leedy, P.D. and Ormrod, J.E., 2005. Practical Research: Planning and Design. Prentice hall, Upper Saddle River, New Jersey.

Mashela, P.W. and Mollel, N., 2001. Farmer identified indigenous fruit tree with suitable attributes for the semi-arid Northern Province of South Africa. *South African journal of Agricultural Extension*, 30: 1-12.

Mashela, W.P., Kgabo, M.P. and Bombiti, N., 2013. Responses of Mmupudu (*Mimusops zeyheri*) indigenous fruit tree to three soil types. *African Journal of Agricultural Research*, 8(12): 1066-1069.

Mbhenyane, X.G., 2017. Indigenous foods and their contribution to nutrient requirements. *South African Journal of Clinical Nutrition*, 30(4): 5-7.

Mngadi, S., Moodley, R. and Jonnalagadda, S.B., 2017. Elemental composition and nutritional value of the edible fruits of coastal red milkwood (*Mimusops caffra*) and impact of soil quality on their chemical characteristics. *Journal of Environmental Science and Health,* Part B, 52(7): 435-445.

Mngadi, S.V., 2017. *Elemental composition and nutritional value of the edible fruits of coastal red milkwood (Mimusops caffra) and (Mimusops zeyheri) and the impact of soil quality* (Doctoral Thesis, University of Kwa Zulu Natal, South Africa).

Mpai, S., Du Preez, R., Sultanbawa, Y. and Sivakumar, D., 2018. Phytochemicals and nutritional composition in accessions of Kei-apple (*Dovyalis caffra*): Southern African indigenous fruit. *Food Chemistry*, 253: 37-45.

Ndou, A., Tinyani, P.P., Slabbert, R.M., Sultanbawa, Y. and Sivakumar, D., 2019. An integrated approach for harvesting Natal plum (*Carissa macrocarpa*) for quality and functional compounds related to maturity stages. *Food Chemistry*, 293: 499-510.

Omotayo, A.O., Ijatuyi, E.J., Ogunniyi, A.I. and Aremu, A.O., 2020. Exploring the resource value of Transvaal red milk wood (*Mimusops zeyheri*) for food security and sustainability: An appraisal of existing evidence.

Tchuenchieu, A.K. and Kesa, H., 2020. Promotion of indigenous foods consumption for non-communicable diseases management: A challenge for South Africa in the 4th Industrial Revolution Context.

CHAPTER 2
Literature Review

2.1 Work done on *Mimusops zeyheri*

2.1.1 Morphology of *Mimusops zeyheri*

According to Janick and Paull (2008), flowers of *M. zeyheri* grow in sessile clusters along branches between the shoot tip and trunk. When the fruit is immature, the green leathery skin that surrounds the round to oval fruit (weighing 50-250 g) turns light yellowish (Figure 2.1). At maturity, each fruit contains 1-5 lustrous brown to black seeds and a sweet, flour-like fruit pulp that is white (Janick and Paull, 2008). While flowers bloom throughout the year, the main flowering season begins in September to November, when bees are expected to play a substantial role in pollination (Janick and Paull, 2008).

Figure 2.1: *Mimusops zeyheri* fruits at ripe fruit maturity stage, (Source: Janick and Paull, 2008).

2.1.2 The origin of *Mimusops zeyheri*

According to Omotayo *et al.* (2020), trees of *M. zeyheri* are native to several countries in Southern Africa including South Africa, Eswatini, Botswana, Lesotho, Namibia, Mozambique, and Zimbabwe and other parts of tropical Africa as shown in Figure 2.2.

The *M. zeyheri* grown naturally in the wild and are adapted to dry areas and bushveld woodland, in a variety of soil types and tropical or subtropical climates (Omotayo *et al.,* 2020). Therefore, it can be ideally cultivated in areas located in low to medium altitudes and that which receive summer rainfall with little or no frost (Hankey, 2005 and Omotayo *et al.,* 2020).

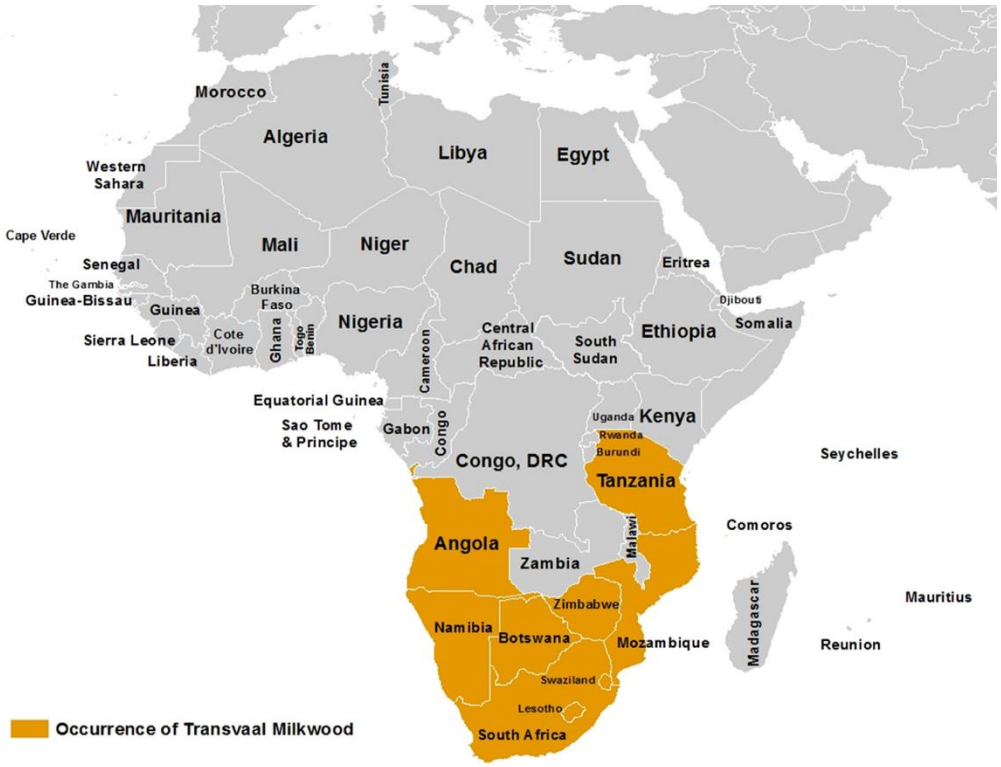

Figure 2.2: The region where *M. zeyheri* is growing (Source: Omotayo *et al*., 2020)

2.1.3 Traditional uses of *Mimusops zeyheri*

Various organs of *M. zeyheri* are harvested for various uses and largely, people use the aboveground organs. For example, extracts of the leaves and other aboveground plant parts have a variety of uses in conventional medicine. According to Lapea *et al*. (2014), the amaZulu of South Africa harvest and process the bark and utilize it to cure wounds and ulcers. Additionally, most tribes in southern Africa use the bark to treat sexual transmitted infections (STIs), particularly gonorrhoea (Dewet *et al*., 2012). The roots of *M. zeyheri* is processed into an infusion and is used to cure candidiasis, especially in the Kingdom of Eswatini (Swaziland) (Mngadi, 2017). Other uses of processed roots infusion include whitening teeth. Overall, the root infusion is reportedly effective in the treatment of inflammations, bleeding gums, tuberculosis, uterine

issues, candidiasis, TB, weight loss, and several sexually transmitted diseases (Mngadi, 2017; Omotayo *et al*., 2020).

2.1.4 Nutritional status of *Mimusops zeyheri*

Omotayo *et al*. (2020) have provided evidence that trees of *M*. *zeyheri* growing in different ecologies exhibit different nutritional status of chemical properties determined in fruits (Wilson and Downs, 2012). Also, scholars have shown 2.0% carbohydrates and ash content that ranges from 2.80 to 4.10% (Mngadi, 2017). Chivandi *et al*. (2011) revealed that about 91.1% of the organic matter of the fruits produce that constitute 88.3% of dry matter. When compared to the exotic fruit such as guava, which has 20 mg/g, vitamin C ranging between 50 to 80 mg/g of fresh fruit (Omotayo *et al*., 2020). Apart from the mineral nutrition of the fruits, the leaves of *M. zeyheri* contain essential nutrient elements including calcium, magnesium, potassium, nitrogen, and phosphorus (Omotayo *et al*., 2020). The seeds on the other hand exhibit amino acids: glutamic acid, lysine, phenylalanine, tryptophan, arginine, aspartic acid, glycine, histidine, isoleucine, leucine, valine, methionine, proline, threonine, alanine, serine, and cysteine (Chivandi *et al*., 2011), reported to account for 97% of the crude protein content (9.3%). Of the nutritional contents, the primary amino acid and glutamic acid can make up about (13.8%) of the crude protein.

2.1.5 Secondary metabolites properties of *Mimusops zeyheri*

The Sapotaceae Family, which includes *M. zeyheri*, is well known for its wide range of secondary metabolites, particularly saponins, flavonoids, and polyphenols (Baky *et al*., 2016). However, there is dearth of published information on biological activities of *Mimusops zeyheri*. Other species of the genus exhibit antifungal, gastroprotective, and antinociceptive qualities (Omotayo *et al*., 2020).

2.2. Work done on other fruit trees

2.2.1 Metabolites description

Metabolites are substances that are vital and contribute to the growth and development of organs of plants (Mediani *et al*., 2017). Metabolites profiling is an effective and efficient strategy that caters for a comprehensive metabolites analysis with different applications in crop sciences (Farog *et al*., 2016). These methods are used to control food quality, assess variation between plant accessions, and classification of maturity stages for food fortification program (Farog *et al*., 2016). The study of the method known as “metabolomics” entails a thorough examination of the entire metabolome under certain circumstances (Mediani *et al*., 2017). Therefore, a deeper understanding of fruit physiology can be obtained by examining the fruit metabolites that underlie fruit maturation and ripening (Mdnor *et al*., 2022). Metabolites are classified into two categories namely, primary and secondary metabolites.

Primary metabolites are small molecules which include sugars, amino acids, proteins, nucleic acids, and polysaccharides (Rehad *et al*., 2018). In addition, vitamins, minerals, and fibre make primary metabolites (Klee, 2010). While consuming plant food that contain metabolites improve the nutritional value of humans, their presence in plants increase the growth and development of plants (Guerriero *et al*., 2018). Secondary metabolites such as terpenoids, phenolic compounds, alkaloids and sulphur-containing compounds consist of various chemical compounds established by the plant cell through metabolic pathways obtained from the primary metabolic pathways (Rehab *et al*., 2018). Results of a study conducted by Nelson and Whitehead (2021) revealed that plant secondary metabolites are vital for determining biotic and abiotic interaction. In addition, colour, flavour, and odour of fruits are determined by plant secondary metabolites (Nevo and Ayasse, 2020).

2.2.2 Phenolic acids and flavonoids

Phenolic acids and flavonoids makeup the largest percentage of phenolic chemicals (Sehrawat *et al*., 2022). Phenolic acids have a C_6-C_3 structure and are the precursors to other phenolic compounds like lignin (Sehrawat *et al*., 2022). Hydroxycinnamic acid are converted into benzoic acid and its derivatives by losing two carbon atoms. To date, there are biotechnological methods that are used to produce phenolic acid at a bigger scale while, two benzene rings, A and B, are joined by a heterocycle pyrene

ring C that contains oxygen in flavonoids, which have a C_6-C_3-C_6 structure (Dias *et al*., 2021). Depending on how much the core heterocyclic ring is saturated, flavonoids can be categorized into two broad categories. Phenolic acids compounds are Protocatechuic acid, p-hydroxybenzoic acid, Vanillic acid, Caffeic acid, p-Coumaric acid, Ferulic acid, Syringic acid and Sinapinic acid (Sehrawat *et al*., 2022). The flavonoid compounds are Quercetin, Rutin, Macluraxanthone, and Genistein (Dias *et al*., 2021).

There are two classes of phenolic acids namely, hydroxybenzoic acid compounds which are primarily found as glucosides as well as hydroxycinnamic acid compounds which are largely simple esters that contain hydroxycarboxylic acids or glucose (Sehrawat *et al*., 2022). Flavonoids are the largest group of secondary metabolites with more than 6000 different types (Xlibris, 2014). They are categorized into sub-groups of flavones, flavonols, flavanones, flavanonols, anthocyanins, and chalcones (Ignat *et al.,* 2011). In addition, flavones are present in fruits, vegetables, grains, leaves, and flowers. Flavonols are the building block of proanthocyanins taking place abundantly in different fruits and vegetables (Iwashina, 2013). Flavonones provide a variety of health benefits and part of it is credited to their ability to scavenge free radicals and pigments called anthocyanins, which are mostly found in the outer cell layers of different fruits including cranberries, raspberries, strawberries, blueberries, and blackberries (Panche *et al.,* 2016).

The interest shown especially by researchers on antioxidants is because they have properties that link their consumption with the prevention of non-communicable chronic diseases (Haminiuk *et al.,* 2012). Phenolic compounds are the second most prevalent class of organic compounds in the plant kingdom and exhibit a variety of functions in plants including structural support, defence against ultraviolet (UV) solar radiation, and biotic or abiotic stress (Laura *et al.,* 2019). The concentration and quality of phenolic acids and flavonoids in fruits and vegetables are altered by factors including ambient temperature, soil properties, sun irradiation, irrigation, fertilization, harvest stage, and post-harvest (wounding, temperature, change of environment, and light irradiation) handling and technologies (Laura *et al.,* 2019).

2.2.3 Antioxidant activities

According to Shahidi and Zhong (2012), antioxidants are molecules that, when present in food or human body in very small concentrations, delay, inhibit, or prevent oxidative processes. Their presence in food can result in the degradation of food quality while their presence in a human body can trigger the onset and spread of degenerative diseases.

Various assays have been used to detect hydrogen atoms or electron transfer from putative antioxidants to free radicals. The antioxidant activities reported by these methods are typically linked to their ability to scavenge specific types of radical species, some of which may be synthetic and unrelated to biology (Shahidi and Zhong, 2015).

2.2.3.1 Antioxidant: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH is one of the assays that are widely used and is crucial in assessing the activity of antioxidants of radical scavenging (Prior *et al.,* 2005). The DPPH is a suitable chromogen radical with a deep purple colour and this assay relies on antioxidants donating electrons to counteract the DPPH radical, which results in a color change that can be seen at 517 nm (Prior *et al*., 2005). The DPPH assay is a straightforward procedure that just needs a UV spectrophotometer or an EPR (Electron Paramagnetic Resonance) spectrometer (Benzie and Strain, 1999). Furthermore, DPPH test is simple and reliable, but due to its sensitivity, it may necessitate to considering a number of variables, including the type and quantity of solvent employed, the presence and concentration of hydrogen and metal ions, and the freshness of the DPPH reagent (Dawidowicz *et al.,* 2012). In addition, DPPH radical scavenging assay has also been utilized in combination with other techniques such as High Performance Liquid Chromatography (HPLC) for rapid screening of a large number of antioxidant samples (Dawidowicz *et al.,* 2012).

2.2.3.2 Antioxidant: Free Reducing Power Assay (FRAP)

The FRAP is based on the idea that compounds with reduction potential combine with potassium ferricyanide to generate potassium ferrocyanide, which subsequently reacts

with ferric chloride to form a ferric-ferrous complex, which has an absorption maximum at 700 nm (Bhalodia *et al.,* 2013).

2.2.4 Effect of fruit maturity stages on secondary metabolites in different fruits

2.2.4.1 The secondary metabolites at unripe fruit maturity stage in different fruits

Secondary metabolites have been determined in various research and shown to vary among different types of fruits that are unripe (Table 2.1). For example, Zang *et al*. (2008) determined bioactive components and antioxidant capacity of Chinese berry (*Myrica rubra* Sieb.) at the unripe fruit maturity stage. They reported the presence of total phenolic content (139.81 mg/100 g), total flavonoids content (58.74 mg/100 g), total anthocyanins (11.81 mg/100 g) and antioxidant activity of FRAP (2.61 TE/100 g) and DPPH (3.59 TE/100 g). During the unripe fruit stage, the Natal plum (*Carissa macrocarpa*) revealed total phenolic content (906.00 mg/100 g), FRAP (6.17 GAE/100 g), and DPPH (0.164 GAE/100 g), respectively (Ndou *et al*., 2019). Compared across regions, Gull *et al*. (2012) recorded highest of total phenolic content (33.16 mg/100 g) in fruits of guava plants growing in the Bhakkar region as well as total flavonoid content (46.03 mg/100 g), FRAP (39.64 GAE/100 g), and DPPH (1.65 GAE/100 g) in that of plants from the Faisalabad region. The secondary metabolites are affected by a variety of factors such as plant species, region, climatic condition, fruit maturity stage, and harvesting time (Kondakova *et al*., 2009; Gull *et al*., 2012).

Table 2.1: Secondary metabolites at unripe fruit maturity stage on various fruits

Fruit names	Fruit maturity stage	Secondary metabolites (mg/ 100 g)					References
		Total Phenolics Content (TPC)	Total Flavonoids Content (TFC)	Total Anthocyanins (TA)	Ferric Reducing Antioxidant Power Assay (FRAP)	2,2-Diphenyl-1-picrylhydrazyl (DPPH)	
Chinese bayberry	Unripe	139.81	58.74	11.81	2.61	3.59	Zang *et al*., 2008
Natal plum	Unripe	906.0	-	-	6.17	0.164	Ndou *et al*., 2019
Guava 1	Unripe	24.81	28.82	-	34.06	1.18	Gull *et al*., 2012
Guava 2	Unripe	32.72	46.08	-	39.64	1.65	
Guava 3	Unripe	33.16	35.05	-	32.62	1.12	

2.2.4.2 The secondary metabolites at the ripe fruit maturity stage in different fruits

Scholarly research studies have revealed secondary metabolites to vary among different types of fruits and fruit maturity stage (Table 2.2). Fruits of the Chinese bayberry (*Myrica rubra* Sieb.) showed total phenolic (287.56 mg/100 g), total flavonoid content (100.30 mg/100 g), total anthocyanins (62.41 mg/100 g), FRAP (3.59 GAE/100 g) and DPPH (5.85 GAE/100 g) (Zang *et al*., 2008). Ndou *et al*. (2019) recorded total phenolic content (575.40 mg/100 g), FRAP (6.17 GAE/100 g) and DPPH (0.164 GAE/100 g) in Natal plum (*Carissa macrocarpa*). Gull *et al*. (2012) discovered highest of total phenolic content (30.22 mg/100 g), total flavonoid content (31.09 mg/100 g), FRAP (1.12 mg/100 g) in indigenous guava (*Psidium guajava* L.) fruits from the Faisalabad region and DPPH (31.84 mg/100 g) from Islamabad region.

Table 2.2: Secondary metabolites at ripe fruit maturity stage in different fruits

Fruit names	Fruit maturity stage	Secondary metabolites (mg/ 100 g)					References
		Total Phenolics Content	Total Flavonoids Content (TFC)	Total Anthocyanins (TA)	Ferric Reducing Antioxidant Power Assay (FRAP)	2,2-Diphenyl-1-picrylhydrazyl (DPPH)	
Chinese bayberry	Ripe	287.56	100.3	62.41	3.59	5.85	Zang *et al*., 2008
Natal plum	Ripe	575.4	-	-	8.01	0.22	Ndou *et al.,* 2019
Guava 1	Ripe	11.47	21.86	-	0.80	31.84	Gull *et al*., 2012
Guava 2	Ripe	30.22	31.22	-	1.12	21.56	
Guava 3	Ripe	20.54	20.54	-	0.94	23.88	

2.2.4.3 The accessions/cultivars difference on secondary metabolites in different fruits

The data shown in Table 2.3 show differences in secondary metabolites as determined in various accessions of difference fruits. Of selected Chinese bayberry (*Myrica rubra* Sieb.), Zang *et al*. (2008) discovered that accession Biqi had the highest total phenolic content (117.63 mg/100 g), total flavonoids content (256.93 mg/100 g), TA (76.24 mg/100 g), FRAP (3.69 TE/100 g) and DPPH (6.32 TE/100 g). Dyduch *et al*. (2015) reported that fruits of accession Baron among wild strawberry (*Fragaria vesca* L.) had the highest TPC (1.245 mg/100 g) and TFC (4.858 mg/100 g) and TA (444.25 mg/100 g). On the other hand, the other wild strawberry (*Fragaria vesca* L.) fruit from Turkey showed highest TPC (228 mg/100 g), TA (33.51 mg/100 g) and FRAP (49.11 TE/100 g) in accession FV-2, including DPPH (11.38 TE/100 g) in accession FV-10, respectively (Yildiz *et al*., 2014

Table 2.3: Secondary metabolites of accessions/ cultivars in different fruits

Fruit names	Cultivars/ Accessions	Secondary metabolites (mg/ 100 g)					References
		Total Phenolics Content	Total Flavonoids Contents	Total Anthocyanins (TA)	Ferric Reducing Antioxidant Power Assay (FRAP)	2,2-Diphenyl-1-picrylhydrazyl (DPPH)	
Chinese bayberry	Baizhong	13.61	61.65	-	1.08	2.95	Zang *et al*., 2008
	Fenhong	29.41	82.19	6.12	1.64	3.82	
	Wuzhong	62.75	147.20	26.62	2.37	4.74	
	Biqi	117.63	256.93	76.24	3.69	6.32	
Wild strawberry	Baron van	1.245	4.858	300.00	-	-	Dyduch *et al*., 2015
	Yellow wonder	1.178	4.483	214.61	-	-	
	Regina	1.20	4.787	444.25	-	-	

Wild strawberry	FV-1	193	-	33.01	8.18	37.05	Yildiz *et al*., 2014
	FV-2	228	-	33.51	7.91	49.11	
	FV-3	170	-	28.61	10.27	30.82	
	FV-4	210	-	47.66	7.96	47.18	
	FV-5	201	-	43.07	8.11	43.14	
	FV-6	190	-	25.11	9.31	39.05	
	FV-7	177	-	31.64	9.96	38.10	
	FV-8	214	-	51.78	8.00	47.62	
	FV-9	207	-	39.64	8.07	44.09	
	FV-10	187	-	29.66	11.38	33.15	
	FV-11	191	-	38.41	9.67	37.70	
	FV-12	217	-	49.07	7.94	46.10	
	FV-13	211	-	45.18	7.98	45.01	
	FV-14	195	-	45.64	8.40	43.18	
	FV-15	171	-	29.37	10.84	31.05	

2.2.5 Different fruit maturity stages

The fruit maturity stage is regarded is the main factor that affects carotenoid contents. In particular, the carotenoid contents are enhanced during the ripening process as result of the metabolic action of ethylene (Carrillo-Lopez and Yahia, 2009). Maturity stage is another important factor that influences the compositional quality of fruits. In fact, harvesting at the proper fruit maturity stage is essential for optimum quality and often for the maintenance of fruit quality after harvest and storage (Savikin *et al*., 2014). Fruit maturity is divided into two categories namely, horticultural and physiological maturity.

Horticultural fruit maturity refers to a plant growth stage where a plant possesses organs that are used by consumers or processors (Kader, 1999; Wills *et al*., 2013). On the other hand, physiological fruit maturity is considered the stage when fruits/ vegetables exhibit maximum growth. The latter growth stage is usually associated with full ripening in the fruits (Mkhathini *et al*., 2017). According to the regulatory systems underpinning the ripening process, fruits can be categorized into two types. The first type are fruits that are Climacteric such as tomato, apple, pear, and melon. These are characterized by an increase in respiration and ethylene production that occurs when the fruit ripens. The second are non-climacteric fruits such as orange, grape, and pineapple (Rooban *et al*., 2016). The difference is the absence of an ethylene associated with respiratory peak. Climacteric fruits exhibit an increase in respiration and a simultaneous burst of ethylene production at the start of ripening. Following the revelation that fruits can mature without any increase in climacteric respiration, the link between climacteric respiration and fruit ripening has received much attention from researchers.

Plant fruits can be climacteric or non-climacteric with the former normally harvested when fully matured because they ripen on the parent plant. Although smaller in size when matured, the respiration rate and ethylene production increase sharply to a climacteric peak at the start of ripening, and after ripening, they begin to drop. Once non-climacteric fruits are picked from trees, they do not reach the ripening stage (Rooban *et al*., 2016). Non-climacteric fruits exhibit a relatively low profile and a slow reduction in their respiration pattern and ethylene production throughout the ripening

process. They also produce very low levels of endogenous ethylene and do not respond to external ethylene treatment (Rooban *et al*., 2016).

Table 2.4: Illustrate indigenous fruits which are climacteric and non-climacteric.

Common names	**Species names**	**Fruit types**	**Maturity types**	**References**
Natal plum	*Carissa macrocarpa*	Non-climacteric fruit	Horticultural maturity	Mphaphuli *et al*., 2020
Wild date palm	*Phoenix reclinata*	Non-climacteric fruit	Horticultural maturity	Sebastian *et al*., 2023
Mobola plum	*Parinari curatellifolia*	Climacteric fruit	Physiological maturity	Ngadze *et al*., 2017
African mangosteen	*Garcinia livingstonei*	Non-climacteric fruit	Horticultural maturity	Dicsbalis, 2011
monkey orange	*Strychnos spinosa Lam*	Climacteric fruit	Physiological maturity	Sitrit *et al*., 2003
Sour fig	*Carpobrotus edulis*	Non-climacteric fruit	Horticultural maturity	Campoy *et al*., 2018
Purple passion	*Syzygium cordatum*	Climacteric fruit	Physiological maturity	Nxumalo and Fawole, 2022

2.2.6 Primary metabolites

2.2.6.1 Amino acids

According to Sibiya *et al.* (2021), amino acids constitute two organic substances that consist of amide and acid, both considered building blocks of proteins. Amino acids are vital for the human body given that they stimulate various biological activities improve the structure of cells as well as transport and store nutrients (Galli, 2007). There are 20 amino acids divided into two categories namely, essential and non-essential amino acids (Sibiya *et al.,* 2020). The essential amino acids (Isoleucine, leucine, valine, phenylalanine, tryptophan, histidine and methionine) are contained in food and cannot be synthesized by the human body (Gen *et al*., 2019). By contrast, non-essential amino acids (Alanine, glycine, proline, aspartic acid, glutamic acid, arginine, serine, cysteine, asparagine, tyrosine, and glutamine) are also sourced from food, however, they can be synthesized by humans (Gen *et al*., 2019). While sourced from food, non-essential amino acids can be produced by the human body (Ha and Zemel, 2003; Mpai *et al*., 2018).

2.2.6.1.1 The accessions/ cultivars different on amino acids of various fruits

Table 2.5 shows difference in essential amino acids such as isoleucine (Ile), leucine (Leu), valine (Val), phenylalanine (Phe), tryptophan (Trp) and histidine (His) determined in fruits of different plant accessions. Among cited literature, D'Angelo *et al*. (2019) reported that accessions of Andean tomato (*Solanum lycopersicum* L.) showed different concentrations of essential amino acids. The results revealed that either lle, Val, Phe and Trp decreased in the order: accession 572 > accession 549 > accession 3806 > accession 3836 > accession 3842. According to Spricigo *et al*. (2021), accession 8 had highest Ile (7.8 mg/100 g), Leu (2.8 mg/100 g) and Val (4.7 mg/100 g), accession 1 had highest (Ile of 4.1 mg/100 g) and Val (2.5 mg/100 g), accession 4 had highest of Ile (4.2 mg/100 g) and accession 6 had Ile (5.7 mg/100 g), Leu (2.2 mg/100 g) and Val (3.5 mg/100 g) in Cambuci fruit (*Campomanesia* phea).

Table 2.5: Amino acids composition on accessions/ cultivars in different fruits

Fruit names	Cultivars/ Accessions	Amino acids (mg/ 100 g)						References
		Isoleucine	Leucine	Valine	Phenylalanine	Tryptophan	Histidine	
Andean tomato	549	0.13	-	0.10	0.27	0.07	-	D'Angelo *et al*., 2019
	557	0.31	-	0.25	0.81	0.09	-	
	560	0.10	-	0.03	0.13	0.09	-	
	571	0.21	-	0.17	0.68	0.15	-	
	572	0.35	-	0.40	1.54	0.26	-	
	3806	0.40	-	0.27	1.22	0.18	-	
	3815	0.03	-	0.07	0.24	0.09	-	
	3836	0.39	-	0.26	1.23	0.23	-	
	3842	0.30	-	0.21	0.53	0.13	-	
Cambuci	1	4.1	1.5	2.5	-	-	-	Spricigo *et al*., 2021
	2	3.5	1.3	2.2	-	-	-	
	3	1.3	0.3	0.7	-	-	-	
	4	4.2	1.5	2.5	-	-	-	
	5	3.1	1.0	1.8	-	-	-	
	6	5.7	2.2	3.5	-	-	-	
	7	0.8	0.1	0.3	-	-	-	
	8	7.8	2.8	4.7	-	-	-	
	9	2.2	0.7	1.3	-	-	-	
	10	2.4	0.8	1.4	-	-	-	

2.2.7 The importance of mineral compositions

Mineral compositions is a term used to describe essential nutrient elements that occur in fruits and vegetables, and include micro-and macronutrients. The National Agency of Health Surveillance - ANVISA (BRASIL 2005) demonstrated that food with minimum of 15% of the Daily Recommended Intake (DRI) per 100 g of solid food is regarded as source of minerals, and food with minimum of 30% of the reference DRI per 100 g of solid food is regarded as rich in minerals (Guedes *et al*., 2017).

Of mineral compositions, potassium is the major intracellular cation that contributes to the metabolism and synthesis of proteins and glycogen (Shin *et al*., 2013). Potassium contributes to fight against bacteria and cleanses the digestive system, sodium takes part in the metabolism of water, promotes digestion, assimilation, osmosis and alkalizes the blood, and then calcium helps to strengthen the bones and magnesium assist in the assimilation of phosphorus (Guedes *et al*., 2017).

Other mineral components include magnesium, sulfur, copper, manganese, and iron are part of the chlorophyll molecule. On the other hand, magnesium is one of the main components of chlorophyll, while copper, manganese and iron are required for chlorophyll synthesis, being present in higher concentration in unripe fruits. Furthermore, as the fruit matures, they experience a degradation in chlorophyll, responsible for the green coloration of fruits, with consequent reduction of magnesium, sulfur, copper, manganese and iron molecules (Guedes *et al*., 2017).

Table 2.6: List of edible indigenous fruit mineral compositions present (Sibiya *et al*., 2020)

Species names	**Common names**	**Mineral Compositions**					
		Ca	**Fe**	**K**	**P**	**Mg**	**Zn**
Carpobrotus edulis L.	Sour figs	*	*	*	*	*	*
Ficus sycamorus L.	Sycamore fig	*		*	*	*	*
Ximenia americana L.	Blue sour plum	*	*	*		*	*
Vangueria infausta Burch.	Wild medlar		*	*	*	*	*
Sclerocarya birrea	Marula	*	*		*	*	*

Phoenix reclinata Jacq	Wild date palm	*	*	*		*	*
Uapaca kirkiana Mull.	Sugar plum	*	*	*		*	*
Bridelia mollis Hutch	Velvet sweet berry		*	*	*		*

2.2.7.1 The nutritional compositions of unripe fruit in different fruits

The difference with respect to nutritional compositions such as protein (Pro), fat content, moisture content (Moi), ash content, carbohydrates (Carb), Fibre content (Fib) and dry matter (DM) of different fruit trees at unripe fruit maturity stage are shown in Table 2.7. According to Vita *et al*. (2020), the nutritional value of fruits of *Sararanga sinuosa* at the unripe maturity stage included protein (0.56%), fat (0.49%), moisture content (87.44%), ash content (0.72 %), carbohydrates (10.72%), and fibre content (11.41%). Another study conducted by Chukwuka *et al*. (2013) reported that *Carica papaya* L. fruits had protein (1.46%), fat (0.55%), moisture content (81.39%), ash content (4.84%), carbohydrates (18.47%), and fibre content (11.62%) and dry matter (18.61%). A study by Khawas and Deka (2016) reported that culinary banana peel had protein (6.12%), fat (1.97%), moisture content (62.98%), ash content (6.24%), carbohydrates (23.30%), and fibre content (15.97%). Lastly, fruits of Ambarella (*Spondias Cytherea* Sonn.) exhibited protein (1.76%), fat (0.34%), moisture content (90.5%), and ash content (6.78%) (Ahmad *et al*., 2005).

Table 2.7: Nutritional compositions of unripe fruit maturity stage in different fruits

Fruit names	Fruit maturity stage	Nutritional compositions (%)							References
		Protein content	Fat content	Moisture content	Ash content	Carbohydrates content	Fibre content	Dry Matter	
Berries	Unripe	0.56	0.49	87.44	0.72	10.72	11.41	-	Vita *et al*., 2020
Papaya	Unripe	1.46	0.55	81.39	4.84	18.47	11.62	18.61	Chukwuka *et al*., 2013
Banana	Unripe	6.12	1.96	62.98	6.24	23.30	15.97	-	Khawas and Deka, 2016
Ambarella	Unripe	1.76	0.34	90.5	6.78	-	-	-	Ahmad *et al*., 2005

2.2.7.2 The nutritional compositions of ripe fruit maturity stage in different fruits

Table 2.8 shows differences in nutritional concentration of various fruits at ripen fruit maturity stage of the plant species, fruits of *Sararanga sinuosa* berries recorded protein (0.63%), fat (0.43%), moisture content (86.39%), ash content (0.57%), carbohydrates (11.97%), and fibre (1.54%) (Vita *et al*., 2020). Chukwuka *et al*. (2013) discovered protein (0.29%), fat (0.35%), moisture content (89.21%), ash content (2.83%), carbohydrates (9.65%), fibre (6.18%), and dry matter (10.79%) in fruits of *Carica papaya* L. According to Khawas and Deka (2016), banana peels had protein (7.03%), fat (3.94%), moisture content (58.40%), ash content (8.72%), carbohydrates (32.43%), and fibre (21.23%). Ahmad *et al*. (2005) reported levels of protein (2.33%), fat (0.53%), moisture content (90%), and ash content (6.23%) in fruits of Ambarella (*Spondias Cytherea* Sonn.). Tables 2.7 and 2.8 show increases in protein and carbohydrates of unripe to ripe fruits of berries as well as fat, moisture content, ash content, fibre decreased from unripe to ripen fruit maturity stages (Vita *et al*., 2020). A similar trend was observed in papaya fruits, their moisture content increased and protein, fat, ash content, carbohydrates and fibre decreased from unripe to ripen fruit maturity stages (Chukwuka *et al*., 2013). In banana peel, the protein, fat, ash content, carbohydrates, and fibre increased and moisture decreased from unripe to ripen fruit maturity stage (Khawas and Deka, 2016). Lastly, in fruits of ambarella revealed increased nutritional composition from unripe to ripen fruit maturity stage (Ahmad *et al*., 2005).

Table 2.8: Nutritional compositions of ripe fruit maturity stage in different fruits

Fruit names	Fruit maturity stage	Nutritional compositions (%)							References
		Protein content	Fat content	Moisture content	Ash content	Carbohydrates content	Fibre content	Dry Matter	
Berries	Ripe	0.63	0.43	86.39	0.57	11.97	1.54	-	Vita *et al*., 2020
Papaya	Ripe	0.29	0.35	89.21	2.83	9.65	6.18	10.79	Chukwuka *et al*., 2013
Banana	Ripe	7.03	3.94	58.40	8.72	32.43	21.23	-	Khawas and deka, 2016
Ambarella	Ripe	2.33	0.53	90.0	6.23	-	-	-	Ahmad *et al*., 2005

2.2.7.3 The mineral composition of unripe fruit maturity stage in different fruits

When fruits determined at the maturity stage, the concentration of mineral compositions such as Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P) and Zinc (Zn) varies among different fruits at unripen fruit maturity stages are shown in Table 2.9. For example, fruits of the *Carica papaya* L. at unripen stage were reported to exhibit various minerals concentration such as Ca (58.78 mg), K (58.67 mg), Mg (12.80 mg), Na (25.68 mg), and P (9.46 mg) (Chukwuka *et al*., 2017). Gudes *et al*. (2017) demonstrated that during the maturity stage, unripe fruits of Cagaita (*Eugenia dysenterica* Dc.) contained Ca (44.52 mg), Fe (1.96 mg), K (896.50 mg), Mg (26.66 mg), P (82.96 mg), and Zn (0.77 mg). The concentration of K, Ca, Mg, Na, Fe, and Mn were reportedly higher in unripe fruits, which increases its potential for processing, since Cagaita fruits are usually processed unripe (Gudes *et al*., 2017). Four accessions of the Balkan indigenous apple (*Malus domestica*), including Kolacara, Budimka, Sumatovka, and Kozara were shown to vary in minerals concentration at unripe stage, and whereby accessions Sumatovka and Kozara had highest Ca (0.16 mg) while Fe (7.42 mg) was highest in accession Sumatovka. On the other hand, K (1.48 mg) was highest in accession Kolacara Mn (0.19 mg) and Mg (1.87 mg) was markedly increased in accession Sumatovka while Na (17.10 mg), P (0.41 mg), and Zn (0.96 mg) were greater in accession Kolacara (Savikin *et al*., 2014).

Table 2.9: Mineral compositions of unripe fruit maturity stage in different fruits

Fruit names	Fruit maturity Stage	Mineral compositions (mg/ 100g)								References
		Ca	Fe	K	Mg	Mn	Na	P	Zn	
Papaya	Unripe	58.78	-	88.67	12.80	-	25.68	9.48	-	Chukwuka *et al*., 2013
Cagaita	Unripe	44.52	1.96	896.5	26.66	0.61	-	82.96	0.77	Gudes *et al*., 2017
Kolacara	Unripe	0.12	5.31	1.48	0.17	1.45	17.10	0.41	0.96	Savikin *et al*., 2014
Budimka	Unripe	0.10	5.44	1.48	0.15	1.04	7.95	0.41	0.42	Savikin *et al*., 2014
Sumatovka	Unripe	0.16	7.42	1.32	0.19	1.87	4.48	0.22	0.53	Savikin *et al*., 2014
Kozara	Unripe	0.16	6.86	1.29	0.16	1.28	8.09	0.40	0.58	Savikin *et al*., 2014

2.2.7.4 The mineral compositions of ripe fruit maturity stage in different fruits

As expected, the mineral compositions of fruits at the maturity stage is affected by accession. Such variation is shown by the literature shown in Table 2.10. Among fruits that have revealed variation are that of papaya as reported by Chukwuka *et al*. (2013) that they had Ca (46.76 mg), K (56.27 mg), Mg (10.40 mg), Na (25.76 mg), and P (8.80 mg). Fruits of the Cagaita (*Eugenia dysenterica* Dc) had Ca (26.16 mg), Fe (0.91 mg), K (767.40 mg), Mg (20.37 mg), Mn (0.41 mg), P (75.27 mg), and Zn (0.76 mg) while ripe fruits exhibited 16.6% of the daily requirement, being source of nutrient, respectively (Gudes *et al*., 2015). In the study conducted by Savikin *et al*. (2014), it is evident that fruits of four accessions of indigenous Balkan apple (*Malus domestica*) varied in concentrations of minerals. In essence, accession Kolacara had highest Ca (0.31 mg/100 g), Fe (55.18 mg/100 g), Mg (0.29 mg/100 g), Mn (3.99 mg/100 g), Na (15.06 mg/100 g), P (0.51 mg/100 g), and Zn (1.10 mg/100 g). Accession Sumatovka had highest K (1.58 mg/100 g) at ripe fruit maturity stage. Alezandro *et al*. (2013) reported that accession Sabora fruit had highest Zn (2.9 mg), Mn (2.7 mg) while accession Paulista fruit had highest K (1320 mg) and Mg (120 mg).

Table 2.10: Mineral compositions of ripen fruit maturity stage in different fruits

Fruit names	Fruit maturity Stage	Mineral compositions (mg/ 100g)								References
		Ca	Fe	K	Mg	Mn	Na	P	Zn	
Papaya	Ripe	46.76	-	56.27	10.40	-	25.76	8.80	-	Chukwuka *et al*., 2013
Cagaita	Ripe	26.16	0.91	767.4	20.37	0.41	-	-	0.76	Gudes *et al*., 2017
Kolacara	Ripe	0.31	55.18	1.12	0.29	3.99	15.06	0.51	1.10	Savikin *et al*., 2014
Budimka	Ripe	0.12	18.50	1.25	0.14	1.36	6.02	0.32	0.46	Savikin *et al*., 2014
Sumatovka	Ripe	0.12	7.96	1.58	0.17	1.30	4.80	0.41	0.41	Savikin *et al*., 2014
Kozara	Ripe	0.17	9.05	1.04	0.21	2.30	6.98	0.28	0.76	Savikin *et al*., 2014

2.2.7.5 The nutritional compositions of accessions/ cultivars in different fruits

Table 2.11 depicts differences shown in some published studies on nutritional compositions in accessions of various fruits. The study by Yousaf *et al*. (2020) outlined nutritional compositions of fruits of eight accessions of indigenous guava (*Psidium guajava* L.) whereby accession Ghota gola had highest fat (0.93%), whilst accession Gola had highest moisture content (84.3%), accession Surahi had highest fibre content (3.46%), and accession Solabahar had highest ash content (0.68%). Al-Juhaimi *et al*. (2014) recorded nutritional compositions in fruits of seven accessions of date plum (*Phoenix dactylifera* L.) and, accession Soughi had highest crude protein (2.41%), accession Soukari had highest moisture content (4.82%), accession Soulage had highest ash content (2.22%), and accession Barhi had highest fibre content (3.90%). Fruits of the Chinese jujube (*Ziziphus jujuba* Mill.) recorded markedly increased nutritional compositions some accessions such as accession Yazao which had highest protein (6.86%) and ash content (7.18%), accession Sanbianhong revealed greater moisture content (22.52%) and carbohydrates (85.65%) (Lin *et al*., 2007).

Table 2.11: Nutritional compositions of accessions/ cultivars in different fruits

Fruit names	Cultivars/ Accessions	Nutritional compositions (%)							References
		Protein content	Fat content	Moisture content	Ash content	Fibre content	Carbohydrates content	Dry Matter	
Indigenous guava	Gola	2.90	0.90	84.3	0.65	3.40	-	-	Yousaf *et al*., 2020
	Ghota gola	2.30	0.86	83.1	0.60	3.35	-	-	
	Surahi	2.11	0.92	83.2	0.63	3.46	-	-	
	Chute surahi	2.04	0.85	84.3	0.61	3.34	-	-	
	Sufaida	2.06	0.93	82.9	0.66	3.45	-	-	
	Lal badshah	2.03	0.87	84.2	0.59	2.96	-	-	
	Sdabahar	1.99	0.87	83.1	0.68	3.32	-	-	
	Karela	2.02	0.85	83.7	0.67	3.44	-	-	
Date plum	Soukari	2.32	-	4.82	2.13	1.91	-	-	Juhaimi *et al*., 2014
	Soulage	1.51	-	2.11	2.22	2.89	-	-	
	Barhi	2.34	-	2.13	2.00	3.90	-	-	
	Khulas	2.10	-	1.92	1.68	3.22	-	-	
	Rozaiz	1.67	-	2.42	1.71	3.01	-	-	
	Soughi	2.41	-	2.06	2.15	3.00	-	-	
	Monaif	2.18	-	1.97	1.84	2.84	-	-	
Jujube	Yazao	6.18	-	20.98	2.78	7.18	80.86	-	Lin *et al*., 2007
	Jianzao	4.75	-	17.38	2.41	5.24	84.85	-	
	Junzao	6.43	-	21.09	3.01	5.83	82.17	-	
	Sanbianhong	6.60	-	22.52	2.56	5.56	85.63	-	
	Jinsixiaozano	5.10	-	18.99	2.26	6.11	81.62	-	

2.2.7.6 The mineral compositions of accessions/ cultivars in difference fruits

Table 2.12 depicts differences that are shown in literature on concentration of mineral nutrients of fruit of different plant accessions. Chen *et al*. (2007) discovered that fruits of pear accession Yali showed markedly greater Na (25 mg/100 g) while accession Dangshan had highest K (1688 mg/100 g). Also, the authors showed that accession Jingbai had a significantly higher Mg (92 mg/100 g), Fe (0.98 mg/100 g) and Mn (0.47 mg/100 g) while accession Ninomiyahak had highest Ca (46.50 mg/100 g), Zn (0.80 mg/100 g) and Cu (0.92 mg/100 g). Fruits of the black mulberry (*Morus nigra* L.) showed different content of mineral compositions as affected by genotypes. In particular, genotype M8 had highest K (1254 mg/100 g) while genotype M17 had highest Na (329 mg/100 g) and Mg (132 mg/100 g), and genotype M5 recorded increased Cu (0.58 mg/100 g). Finally, genotype M28 recorded the highest Ca (45 mg/100 g), Na (329 mg/100 g), Zn (4.36 mg/100 g), Fe (10.59 mg/100 g), and Mn (12.67 mg/100 g) (Koyuncu *et al*., 2014). Fruits of five accessions of Aonla (*Phyllanthus emblica* L.) revealed differences with respect to minerals concentration, whereby accession Kanchan had highest K (63.68 mg/100 g), accession Desi had highest of Ca (29.54 mg/100 g) and accession Chakaiya had highest Fe (3.19 mg/100 g) (Kumar and Khatkar, 2018). Therefore, variation of minerals concentration differ within genotype and accessions as the results of growing conditions, ecological factors and genetic factors (Koyuncu *et al*., 2014).

Table 2.12: Mineral compositions of accessions/ cultivars in different fruits

Fruit names	Cultivars/ Accessions	Mineral compositions (mg/ 100 g)								References
		K	Ca	Na	Mg	Zn	Fe	Mn	Cu	
Pears	Yali pear	897	20.5	25	35.5	0.15	0.48	0.11	0.31	Chen *et al*., 2007
	Kuerle	1085	22.5	19.5	94.5	0.11	0.79	0.18	0.41	
	Dangshan	1688	11.5	12.0	117.0	0.21	0.61	0.30	0.40	
	Nangwo	1068	31.0	12.5	68	0.46	0.51	0.32	0.39	
	Jingbai	3.50	44.50	1050	92	0.28	0.98	0.47	0.28	
	Ninomiyahak	1336	46.50	4.45	83	0.81	0.92	0.40	0.92	
	Nitaka	990	32.0	4.50	76	0.35	0.73	0.23	0.46	
	Wujiuxiang	1098	16.0	8.50	67	0.24	0.56	0.17	0.30	
Black mulberry	M-5	999	33	205	148	3.17	6.77	4.20	0.58	Koyuncu *et al*., 2014
	M8	1254	42	247	105	2.21	5.50	3.83	0.28	
	M11	818	31	309	136	3.09	4.47	3.93	0.34	
	M14	999	30	291	140	3.39	6.62	4.93	0.31	
	M17	1165	29	329	152	2.07	6.65	4.40	0.22	
	M18	1225	27	279	150	2.77	5.61	3.93	0.36	
	M22	1264	21	229	92	3.09	10.34	3.77	0.36	
	M28	599	45	329	99	4.36	10.59	12.67	0.31	
Aonla	Bonarasi	56.81	18.61	-	-	-	1.83	-	-	Kumari and khatkar, 2018
	Chakaiya	60.67	25.65	-	-	-	3.19	-	-	
	Desi	42.69	29.54	-	-	-	2.02	-	-	
	Kanchan	63.68	25.74	-	-	-	2.60	-	-	
	Na -7	62.08	22.33	-	-	-	2.79	-	-	

2.3 Work not done on *Mimusops zeyheri*

Various literature has been written on the effect of primary and secondary metabolites of fruits at different fruit maturity stages and accessions/ cultivars on indigenous and horticultural fruits, currently, there is no published literature on the primary metabolites and secondary metabolites profile of five accessions of *M. zeyheri* that are harvested at different fruit maturity stages at the time of writing this review.

References

Ahmad, H., Ishak, S. A., Ismail, N., and Noor, M. A. M. (2005). Some physical and chemical properties of ambarella (*Spondias Cytherea* Sonn.) at three different stages of maturity. *Journal of Food Composition and Analysis*, 18: 819-827.

Alezandro, M.R., Dubé, P., Desjardins, Y., Lajolo, F.M. and Genovese, M.I., 2013. Comparative study of chemical and phenolic of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg. *Food Research International*, 54(1): 468-477.

Al-Juhaimi, F., Ghafoor, K. and Özcan, M.M., 2014. Physicochemical properties and mineral contents of seven different date fruit (*Phoenix dactylifera* L.) varieties growing from Saudi Arabia. *Environmental Monitoring and Assessment*, 186(4): 2165-2170.

Baky, M.H., Kamal, A.M., Elgindi, M.R. and Haggag, E.G., 2016. A review on phenolic compounds from family Sapotaceae. *Journal of Pharmacognosy and Phytochemistry*, 5(2): 280-287.

Benzie, I.F. and Strain, J.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *In Methods in Enzymology*. 299: 15-27

Bhalodia, N.R., Nariya, P.B., Acharya, R.N. and Shukla, V.J., 2013. In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. *An International Quarterly Journal of Research in Ayurveda*, 34(2): 209.

Campoy, J.G., Acosta, A.T., Affre, L., Barreiro, R., Brundu, G., Buisson, E., González, L., Lema, M., Novoa, A., Retuerto, R. and Roiloa, S.R., 2018. Monographs of invasive plants in Europe: Carpobrotus. *Botany Letters*, 165(34): 440-475.

Carrillo-Lopez, A. and Yahia, E.M., 2009, April. Qualitative and quantitative changes in carotenoids and phenolic compounds in tomato fruit during ripening. *In VI International Postharvest Symposium* 877: 1303-1308.

Chen, J., Wang, Z., Wu, J., Wang, Q. and Hu, X., 2007. Chemical compositional characterization of eight pear cultivars grown in China. *Food Chemistry*, 104(1): 268-275.

Chivandi, E., Davidson, B., Pretorius, B. and Erlwanger, K., 2011. Proximate, mineral, amino acid, fatty acid, vitamin E, phytate phosphate and fibre composition of *Mimusops zeyheri* (Red Milkwood) seed. *International Journal of Food Science and Technology*, 46(3): 555-560.

Chukwuka, K.S., Iwuagwu, M. and Uka, U.N., 2017. Evaluation of nutritional components of *Carica papaya* L. at different stages of ripening. *IOSR Journal of Pharmacy and Biological Sciences*, 6(4): 13-16.

D'Angelo, M., Zanor, M.I., Burgos, E., Asprelli, P.D., Boggio, S.B., Carrari, F., Peralta, I.E. and Valle, E.M., 2019. Fruit metabolic and transcriptional programs differentiate among Andean tomato (*Solanum lycopersicum* L.) accessions. *Planta*, 250(6): 1927-1940.

Dawidowicz, A. L., Wianowska, D., and Olszowy, M., 2012. On practical problems in estimation of antioxidant activity of compounds by DPPH method (problems in estimation of antioxidant activity). *Food Chemistry*, 131: 1037-1043.

De Wet, H., Nzama, V.N. and Van Vuuren, S.F., 2012. Medicinal plants used for the treatment of sexually transmitted infections by lay people in northern Maputaland, KwaZulu Natal Province, South Africa. *South African Journal of Botany*, 78: 12-20.

Dias, M.C., Pinto, D.C. and Silva, A.M., 2021. Plant flavonoids: Chemical characteristics and biological activity. *Molecules*, 26(17): 5377.

Dicsbalis, Y., 2011. Effects of harvesting seasons and maturity stages on internal characteristics of the mangosteen having different surface properties. *International Journal of Food Science and Technology*, 46(4): 717-723.

Dyduch-Siemińska, M., Najda, A., Dyduch, J., Gantner, M. and Klimek, K., 2015. The content of secondary metabolites and antioxidant activity of wild strawberry fruit (*Fragaria vesca* L.). *Journal of Analytical Methods in Chemistry*, 2015.

Farag, M.A., Handoussa, H., Fekry, M.I. and Wessjohann, L.A., 2016. Metabolite profiling in 18 Saudi date palm fruit cultivars and their antioxidant potential via UPLC-qTOF-MS and multivariate data analyses. *Food and Function*, 7(2): 1077-1086.

Galli, F., 2007. Amino acid and protein modification by oxygen and nitrogen species. *Amino Acids*, 32: 497-499.

Gan, R.Y., Chan, C.L., Yang, Q.Q., Li, H.B., Zhang, D., Ge, Y.Y., Gunaratne, A., Ge, J. and Corke, H., 2019. Bioactive compounds and beneficial functions of sprouted grains. In Sprouted grains, AACC *International Press*: 191-246.

Gudes, M.N.S., Rufini, J.C.M., Marques, T.R., Melo, J.O.F., Ramos, M.C.P. and Viol, R.E., 2015. Minerals and phenolic compounds of Cagaita fruits at different maturation stages (*Eugenia dysenterica*). *Revista Brasileira de Fruticultura*: 39(1): 231-238.

Guerriero, G., Berni, R., Muñoz-Sanchez, J.A., Apone, F., Abdel-Salam, E.M., Qahtan, A.A., Alatar, A.A., Cantini, C., Cai, G., Hausman, J.F. and Siddiqui, K.S., 2018. Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. *Genes*, 9(6): 309.

Gull, J., Sultana, B., Anwar, F., Naseer, R., Ashraf, M. and Ashrafuzzaman, M., 2012. Variation in antioxidant attributes at three ripening stages of guava (*Psidium guajava* L.) fruit from different geographical regions of Pakistan. *Molecules*, 17(3): 3165-3180.

Ha, E. and Zemel, M.B., 2003. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. *The Journal of Nutritional Biochemistry*, 14(5): 251-258.

Haminiuk, C.W., Maciel, G.M., Plata-Oviedo, M.S. and Peralta, R.M., 2012. Phenolic compounds in fruits an overview. *International Journal of Food Science and Technology*, 47(10): 2023-2044.

Hankey, A., 2005. *Mimusops zeyheri* Sond. (Sapotaceae). *CABI Compendium*. https://doi.org/10.1079/cabicompendium.84857229.

Ignat, I., Volf, I. and Popa, V.I., 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126(4): 1821-1835.

Iwashina, T., 2013. Flavonoid properties of five families newly incorporated into the order Caryophyllales. *Bulletin of the National Museum of Nature and Science*, 39: 25-51.

Janick, J. and Paull, R.E., 2008. *Mimusops zeyheri* Transvaal red milkwood. The Encyclopaedia of Fruit and Nuts. https://doi.org/1079/97808551996387.000.

Kader, A. A. 1999. Fruit maturity, ripening, and quality relationships. *Acta Horticulture* 485: 203-208.

Khawas, P. and Deka, S.C., 2018. Comparative Nutritional, Functional, Morphological, and Diffract gram Study on Culinary Banana (Musa ABB) Peel at Various Stages of Development. *International Journal of Food Properties*, 19:12: 2832-2853.

Klee H.J., 2010. Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology. *New Phytologist* 187: 44-56.

Kondakova, V., Tsvetkov, I., Batchvarova, R., Badjakov, I., Dzhambazova, T.; Slavov, S., 2009. Phenol compounds qualitative index in small fruits. *Biotechnology* 23: 1444-1448.

Koyuncu, F., Çetinbas, M. and Erdal, I., 2014. Nutritional constituents of wild-grown black mulberry (*Morus nigra* L.). *Journal of Applied Botany and Food Quality*, 87.

Lapena, I., Turdieva, M.K., Lopez Noriega, I. and Ayad, W.G., 2014. Land Reform and Farm Restructuring in Central Asia: Progress and Challenges Ahead. Natural Resource Management and Policy, 131-170. https://doi.org/10.1007/0-387-29779-0_6.

Laura, A., Moreno-Escamilla, J.O., Rodrigo-García, J. and Alvarez-Parrilla, E., 2019. Phenolic compounds. *In Postharvest Physiology and Biochemistry of Fruits and Vegetables* 10(3): 253-271.

Lemmens, R.H.M.J., 2005. *Mimusops zeyheri* Sond. Record from PROTA4U. *PROTA* Wageningen, Netherlands (consulted April 2015): http://www. prota4u. Org/search. Asp.

Lin, J.W., Fan, L.P., Ding, S.D. and Ding, X.L., 2007. Nutritional composition of five cultivars of Chinese jujube. *Food Chemistry*, 103(2): 454-460.

Mdnor, S., Ding, P., Abas, F. and Mediani, A., 2022. 1H NMR Reveals Dynamic Changes of Primary Metabolites in Purple Passion Fruit (*Passiflora edulis* Sims) Juice during maturation and ripening. *Agriculture*, 12(2): 156.

Mediani, A., Abas, F., Maulidiani, M.; Khatib, A., Tan, C., Ismail, I.S., Shaari, K. and Ismail, A., 2017. Characterization of metabolite profile in *Phyllanthus niruri* and correlation with bioactivity elucidated by nuclear magnetic resonance based metabolomics. *Molecules*, *22*, 902.

Mkhathini, K.M., Magwaza, L.S., Workneh, T.S. and Mwithiga, G., 2017. Determinations of physical and chemical properties of KwaZulu-Natal's household white peach 'landrace in relation to extension services a case study of impendle local municipality. *South African Journal of Agricultural Extension*, 45(2): 95-109.

Mngadi, S.V., 2017. *Elemental composition and nutritional value of the edible fruits of coastal red milkwood (Mimusops caffra) and Transvaal red milkwood (Mimusops zeyheri) and the impact of soil quality* (Doctoral Thesis, Kwa Zulu Natal, University of Kwa Zulu Natal), South Africa.

Mpai, S., Du Preez, R., Sultanbawa, Y. and Sivakumar, D., 2018. Phytochemicals and nutritional composition in accessions of Kei-apple (*Dovyalis caffra*): Southern African indigenous fruit. *Food Chemistry*, 253: 37-45.

Mphaphuli, T., Slabbert, R.M. and Sivakumar, D., 2020. Storage temperature and time changes of phenolic compounds and antioxidant properties of Natal plum (Carissa macrocarpa). *Food Bioscience*, 38: 100772.

Ndou, A., Tinyani, P.P., Slabbert, R.M., Sultanbawa, Y. and Sivakumar, D., 2019. An integrated approach for harvesting Natal plum (*Carissa macrocarpa*) for quality and functional compounds related to maturity stages. *Food Chemistry*, 293: 499-510.

Nelson, A.S. and Whitehead, S.R., 2021. Fruit secondary metabolites shape seed dispersal effectiveness. *Trends in Ecology and Evolution*, 36(12): 1113-1123.

Nevo, O. and Ayasse, M., 2020. Fruit scent: biochemistry, ecological function, and evolution. *Co-evolution of Secondary Metabolites*. 15(1): 403-425.

Ngadze, R.T., Linnemann, A.R., Nyanga, L.K., Fogliano, V. and Verkerk, R., 2017. Local processing and nutritional composition of indigenous fruits: The case of monkey orange (*Strychnos* spp.) from Southern Africa. *Food Reviews International*, 33(2): 123-142.

Nxumalo, K.A. and Fawole, O.A., 2022. Effects of chitosan coatings fused with medicinal plant extracts on postharvest quality and storage stability of purple passion fruit (*Passiflora edulis var*. Ester). *Food Quality and Safety*, 6: 016.

Omotayo, A.O., Ijatuyi, E.J., Ogunniyi, A.I. and Aremu, A.O., 2020. Exploring the resource value of Transvaal red milk wood (*Mimusops zeyheri*) for food security and sustainability: An appraisal of existing evidence. *Plants*, 9(11): 1486.

Panche, A.N., Diwan, A.D. and Chandra, S.R., 2016. Flavonoids: an overview. J Nutrition Sci. Dec 29; 5:e47. Doi: 10.1017/jns.2016.41. PMID: 28620474; PMCID: PMC5465813.

Prior, R. L., Wu, X. and Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53: 4290-4302.

Šavikin, K., Živković, J., Zdunić, G., Gođevac, D., Dordević, B., Dojčinović, B. and Dordević, N., 2014. Phenolic and mineral profiles of four Balkan indigenous apple cultivars monitored at two different maturity stages. *Journal of Food Composition and Analysis*, 35(2): 101-111.

Sebastian, J.K., Nagella, P., Mukherjee, E., Dandin, V.S., Naik, P.M., Jain, S.M., Al-Khayri, J.M. and Johnson, D.V., 2023. Date Palm: Genomic Designing for Improved Nutritional Quality. In *Compendium of Crop Genome Designing for Nutraceuticals*, 25(3): 1-64.

Sehrawat, R., Rathee, P., Akkol, E.K., Khatkar, S., Lather, A., Redhu, N. and Khatkar, A., 2022. Phenolic acids-versatile natural moiety with numerous biological applications. *Current Topics in Medicinal Chemistry*, *22*(18): 1472-1484.

Shahidi, F., Zhong, Y. and Chandrasekara, G. A., 2012. Antioxidants and human health. In L.Yu, R. Tsao, and F. Shahidi. Cereals and Pulses: *Nutraceutical Properties and Health Benefits,* 33(2): 273-308.

Shin, H., Yoon, Y.S., Lee, Y., Kim, C.I. and Oh, S.W., 2013. Dairy product intake is inversely associated with metabolic syndrome in Korean adults: Anseong and Ansan cohort of the Korean Genome and Epidemiology Study. *Journal of Korean Medical Science*, 28(10): 1482-1488.

Sibiya, N.P., Kayitesi, E. and Moteetee, A., 2020. Mineral composition of selected indigenous wild southern African fruits. *South African Journal of Botany*, 132: 87-94.

Sibiya, N.P., Kayitesi, E. and Moteetee, A.N., 2021. Proximate analyses and amino acid composition of selected wild indigenous fruits of Southern Africa. *Plants*, 10(4): 721-735.

Sitrit, Y., Loison, S., Ninio, R., Dishon, E., Bar, E., Lewinsohn, E. and Mizrahi, Y., 2003. Characterization of monkey orange (*Strychnos spinosa* Lam.), a potential new crop for arid regions. *Journal of Agricultural and Food chemistry*, 51(21): 6256-6260.

Spricigo, P.C., Correia, B.S.B., Borba, K.R., Taver, I.B., Machado, G.D.O., Wilhelms, R.Z., Queiroz, L.H.K., Jacomino, A.P. and Colnago, L.A., 2021. Classical food

quality attributes and the metabolic profile of cambuci, a Native Brazilian Atlantic Rainforest Fruit. *Molecules*, 26(12): 3613-3628.

Wills, R.B.H., Mcglasson, W.B., Graham, D. and Joyce, D.C., 2013. Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals, 5th edition. CAB International, Oxfordshire, UK.

Wilson, A.L. and Downs, C.T., 2012. Fruit nutritional composition and non-nutritive traits of indigenous South African tree species. *South African Journal of Botany*, 78: 30-36.

Xlibris, 2014. Fascinating Facts about Phytonutrients in Spices and Healthy Food: Scientifically Proven Facts Paperback.

Yildiz, H., Ercisli, S., Hegedus, A., Akbulut, M., Topdas, E.F. and Aliman, J., 2014. Bioactive content and antioxidant characteristics of wild (*Fragaria vesca* L.) and cultivated strawberry (*Fragaria x ananassa* Duch.) fruits from Turkey. *Journal of Applied Botany and Food Quality*, 87: 274-278.

Yousaf, A.A., Abbasi, K.S., Ahmad, A., Hassan, I., Sohail, A., Qayyum, A. and Akram, M.A., 2020. Physico-chemical and Nutraceutical Characterization of Selected Indigenous Guava (*Psidium guajava* L.) Cultivars. *Food Science and Technology*, 41: 47-58.

Zhang, W., Li, X., Zheng, J., Wang, G., Sun, C., Ferguson, I.B. and Chen, K., 2008. Bioactive components and antioxidant capacity of Chinese bayberry (*Myrica rubra Sieb*. and Zucc.) fruit in relation to fruit maturity and postharvest storage. *European Food Research and Technology*, 227(4): 1091-1097.

CHAPTER 3

Interaction effect between accessions and fruit maturity stages on the quality and physicochemical attributes of *Mimusops zeyheri* Sond.

Abstract

Mimusops zeyheri Sond. is a member of the Sapotaceae Family and is indigenous in South Africa. Fruits of the wild tree species are harvested for consumption as a healthful snack, especially in rural communities across the country. The objective of the study was to assess whether there is a significant interaction between accessions and fruit maturity stages as affected by post-harvest quality and physicochemical attributes in *M. zeyheri.* Data was collected and analysed using GenStat 18th version. Fruits were harvested from five accessions of *M. zeyheri* trees; 6E, M7, 3E, HY and 3L at different fruit maturity stages; T1 (green), T2 (breaker), T3 (pale yellow), and T4 (dark yellow). Post-harvest quality attributes (fruit length, width, and firmness) and physicochemical attributes (total soluble solids, total titratable acidity, TSS / TA ratio and color change) were determined. Fruits of accession 6E were longest (27.62 mm) during the T1 maturity stage while that of accession HY were widest (20.05 mm) during T4 maturity stage, compared to the other accessions. Across the accessions and maturity stages, the firmness of the fruits declined as they ripened, however, the highest value was recorded at T1 maturity stage and lowest at T4 maturity stage, in all five accessions. Accession HY had highest total soluble solids (32.80 brix˚) at T4 maturity stage compared to accession 3L, which revealed the lowest total soluble solids (2.40 brix˚) at T1 maturity stage. Accession M7 had highest total titratable acidity (3.20%) at T1 maturity stage whilst accession 6E had lowest total titratable acidity (0.22%) at T4 maturity stage. The highest TSS/TA ratio value was shown in accession M7 (32.20%) at T4 maturity stage relative to its counterparts. During fruit maturity stages T1 to T4, all the accessions demonstrated variation, as shown by a change of colour on Hunter a*. The Hunter L* values were lower at T1 and highest at T4 maturity stages. The H˚ angle were high at T1 and lowest at T4 maturity stages as fruit reached ripening period. Therefore, fruits of *M. zeyheri* have the potential to be marketable as fresh based on their quality and physicochemical attributes in functional food program.

Keywords: Accessions, Fruit maturity, Indigenous fruit, Physicochemical, Sapotaceae.

3.1 Introduction

According to Ndou *at el*. (2019), indigenous fruits, including Natal plum (*Carissa macrocarpa*) undergo different stages of maturity and through each stage, the exocarp skin colour differ; green, breaker, pink, red, and dark-red. Mpai *et al.*, (2018) showed that different indigenous fruit accessions show marked differences in primary and secondary metabolites. While the aforementioned studies contribute knowledge on indigenous fruits, currently, there rarely are studies that have assessed an interaction effect between accessions variation and fruit maturity stages on post-harvest quality and physicochemical components of *M. zeyheri* and it remains unknown. In South Africa, indigenous fruits constitute an important part in the food basket of the rural and/or farming households (Mkhathini *et al*., 2017). Therefore, knowledge on the quality, as affected by accession and fruit maturity stage would improve knowledge. Information on this is crucial given that fruits of *M. zeyheri* accessions differ with respect to shape, colour, texture, flavour and aroma of fruits.

The quality of edible fruits is regarded as a crucial factor in determining their shelf life as well as consumer purchase decisions (Mngadi *et al*., 2017). Without a doubt, practices used before the harvest of food products affect both the post-harvest quality and storage life and therefore have a substantial impact on fruit quality (Mousavi and Motesharezadeh, 2020). Fruit quality is the degree to which a produce is considered excellent, and the concept encompasses aspects such as flavor, flesh skin, size, and nutritional value in addition to storage qualities. The quality and acceptance of fruits go hand in hand, therefore, it is critical to check quality in order to assure both food safety and that consumers are likely to be satisfied and ultimately choose a certain produce (Singham *et al*., 2015). The concept of fruit quality is divided into two evaluations namely, objective and subjective. An objective evaluation of fruit quality is pivotal in the food industry given that it involves routine monitoring of quality and to ensure that food is acceptable to consumers (Moussaoui and Varela, 2010). Subjective evaluation on the other hand can be considered as a link between consumer and marketing research, with a focus on customers' behaviour and psychology, as well as research and development, with emphasis made on technical elements of food (Moussaoui and Varela, 2010). Objective quality includes attributes

such as appearance, flavor, texture, and aroma. These are assessed in order to protect food safety and to reduce fruit losses between harvest and consumption.

Maturity refers to a development stage that gives minimum satisfactory quality to ultimate consumer (Sudheer *et al*., 2007). There are indices that are used to determine maturity of a particular commodity. These indices are essential for the trade guideline, marketing policy, and ensuring that fruits are harvested at the right maturity stage to provide some marketing elasticity and to ensure the attainment of acceptable consumption quality to the consumer (Verma *et al*., 2000). Fruits picked prior or after the correct stage of maturity may develop physiological disorders when stored and may exhibit poor desert quality. Maturity indices that are currently used are based on a compromise between indices that would ensure the best eating quality to the consumer and those that offer the needed of elasticity in marketing (Sivakumar *et al*., 2011).

According to Crisosto and Tiwari (2013), maturity indices parameters such as weight, size, and flesh colour, firmness, skin colour, total soluble solids, and titrable acidity. Together, these are used to determine ripeness of various fruits and as shown in literature, the parameters vary between cultivars and therefore, it is crucial that they be assessed in each cultivar because this information is critical for commercialisation of fruits (Mkhathini *et al*., 2017). This chapter presents the objective: interaction effect of five accessions of *M. zeyheri* harvested at four different fruit maturity stages on quality and physiochemical attributes.

3.2 Materials and methods

3.2.1 Study location

Fruits of *M. zeyheri* were harvested from domesticated trees at the Green Biotechnologies Research Centre of Excellence (GBRCE). The area receives minimum average rainfall of 500 mm and the site consists of hutton soil type. Moreover, summer temperatures range from 27 °C to 30 °C while winter temperature range from 5 °C to 17 °C.

3.2.2 Research design and treatments

The collected fruits were harvested randomly from trees of five accessions namely; 6E, M7, 3E, HY and 3L. A 5 × 4 factorial experiment was established as an interaction between five accessions (6E, M7, 3E, HY and 3L) and four fruit maturity stages [dark green (T1), breaker (T2), and pale yellow (T3) and dark yellow (T4)]. The experiment was laid in a randomised complete design (RCD) with four replications. Each accession consist of four fruit maturity stages, whereby 3 replication consist of 20 fruits per replication were taken on each maturity stages to be accessed of quality and physicochemical attributes.

3.2.2.1 Procedures and practices

3.2.2.1.1 Harvesting and maturity stages determination procedures

Fruits were harvested from the month of September to November in 2022. Stages of fruit maturity were determined according to Ndou *et al.* (2019) whereby visual skin colour was used as a key index for classification. Four fruit maturity stages were considered: dark green (T1), breaker (T2), pale yellow (T3), and dark yellow/ orange (T4), as shown in Figure 3.1.

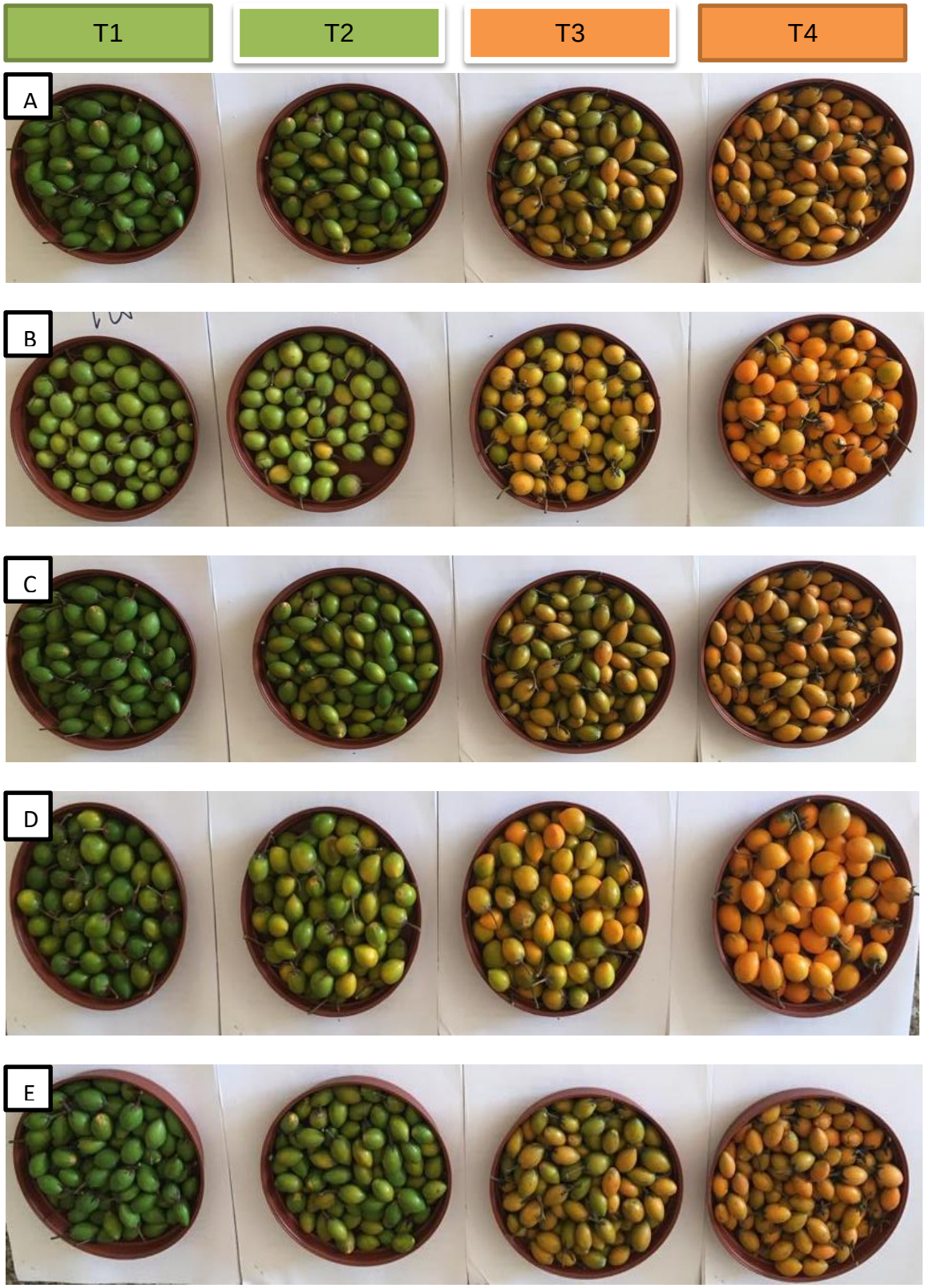

Figure 3.1: Illustrate the schematic version of five accessions *M. zeyheri*, represented by letters (A: 6E; B: M7; C: 3E; D: HY and E: 3L) at four fruit maturity stages (T1: Green; T2: Breaker; T3: Pale yellow and T4: Yellow fruit).

3.2.3 Data collection

3.2.3.1 Quality attributes

I. Measurement of fruit size

The length and width of each fruit, for each accession and fruit maturity stage, were measured using a vernier calliper (Mitutoyo 500, United Kingdom) and expressed in mm.

II. Determination of fruit firmness

Fruit firmness, for each accession and fruit maturity stage, was determined using a hand-held firmness penetrometer (Effigi 11 mm Prob, T.R. Turoni Srl. Italy). Two opposite readings were taken at the equatorial region of the fruit in a scale of 100 to 0, where 100 represented hard and unripe fruit and, 0 represented soft and overripe fruit. The decrease in scale from 100 showed loss of firmness as fruit ripened and expressed as newton (N) (Hanif *et al*., 2010).

3.2.3.2 Physicochemical attributes

I. The determination of Total Soluble solid (TSS)

The fruits were crushed using pestle and mortar and the collected juice was tested for TSS using a digital refractometer (121, Yagami International Ltd, Tokyo, Japan). Few drops were placed on the prism of the refractometer to allow for reading measurements (Pila *et al*., 2010). Total soluble solids of the fruits were expressed in Brix˚.

II. The determination of titratable acidity (TA)

The fruits were peeled, 10 g of the pulp, for each accession and fruit maturity stage, was weighed and homogenised with 40 ml of distilled water using a stirrer. Thereafter, the mixture was centrifuged at 6000 rpm for one minute and the supernatant was recovered through filtering with glass wool. 10 ml of the filtrates were added into conical flasks, separately. Two drops of phenolphthalein indicator were titrated against 0.1 N sodium hydroxide (NaOH) until a pink colour was observed. The volume of NaOH titrated was recorded. Titratable acidity (TA) was calculated using the following formula:

% malic acid = v * N * equivalent factor/ ml

Where v represent volume titrated, N indicate NaOH normality and ml represent millilitres of juice. Equivalent factor of the predominant malic acid was 0.067.

III. The determination of TSS/ TA ratio

TSS/ TA ratio was calculated by dividing the TSS value of each fruit juice sample by that of the percentage of TA (Brix˚/ Acid), respectively (Rooban *et al*., 2016).

IV. The determination of colour change coordinates

The colour of the fruits was analysed using a Minolta CR-400 Chroma meter (Minolta, Osaka, Japan), which uses the Munsell colour system specified for three dimensions such as lightness, hue angle and chromaticity. The colour value L* indicates (0 = black and 100 = white), a* represent redness and b* indicates yellowness of the fruit and hue angle were displayed automatically and recorded (Chepngeno *et al*., 2016). Three readings were taken at the central region of fruits of each accession and fruit maturity stage.

3.3 Statistical analysis

Data were subjected to statistical analysis using the GenStat 18th version statistical package (VSN International, Hempstead, UK). Where significant, treatment means were separated through using the Duncan's Multiple Range Test, at the significance level of 5%.

3.4 Results and discussions

3.4.1 Variation of five accessions and four fruit maturity stages on fruit size

Fruits of the selected five accessions of *M. zeyheri* harvested at different maturity stages revealed significant ($p > 0.05$) variation in length and width, which slightly reduced from T1 to T4 maturity stages in all accessions (Figures 3.2 and 3.3). The selected accessions showed significant variations whereby 6E had the highest fruit length at T1 to T4 maturity stages (25.85-27.63 mm) (Figure 3.2). The fruit length of the other accessions including 3E, HY and 3L was similar and intermediate however, their values were higher than that of accession M7 which exhibited the lowest

irrespective of their maturity stage. On the other hand, the width of the fruits was similar for between the accessions 6E, M7, HY, and 3E, however, these were markedly higher compared to that of accession 3L, irrespective of the maturity stage. Results showing variations in length and width of different accessions have also been observed in other studies including that by Ndou *et al*. (2019) and Mothapo (2014). These results could suggest that there is heterogeneity in the size and maturity stages of fruits of the same Genus. Figures 3.2 and 3.3 show results on fruit length and width, and these are among morphology attributes determined in the selected fruits. The results highlight a pattern of accumulative growth during the fruit set and development. The fruit longitudinal and transverse with respect to fruit length and width showed variation from T1 to T4 maturity stage in all accessions. Also, during T1 maturity stage, fruits recorded significantly enhanced length and width compared to that of T3 and T4 maturity stages, and a similar trend was shown in the study of Muiruri *et al*. (2022) which assessed deciduous fruits such as mango (*Mangifera indica*). In addition to genetic variability, the differences in width and length could have been as a result of the division and expansion of cells, which occur in fruits during the first and second growth and development (Nieuwenhuizen *et al*., 2007). Lastly, this result confirm that fruit length and width can be utilized to estimate the extent to which a fruit has grown, because it increases with fruit maturity stage and can be also incorporated as a maturity index for fruits of *M. zeyheri*.

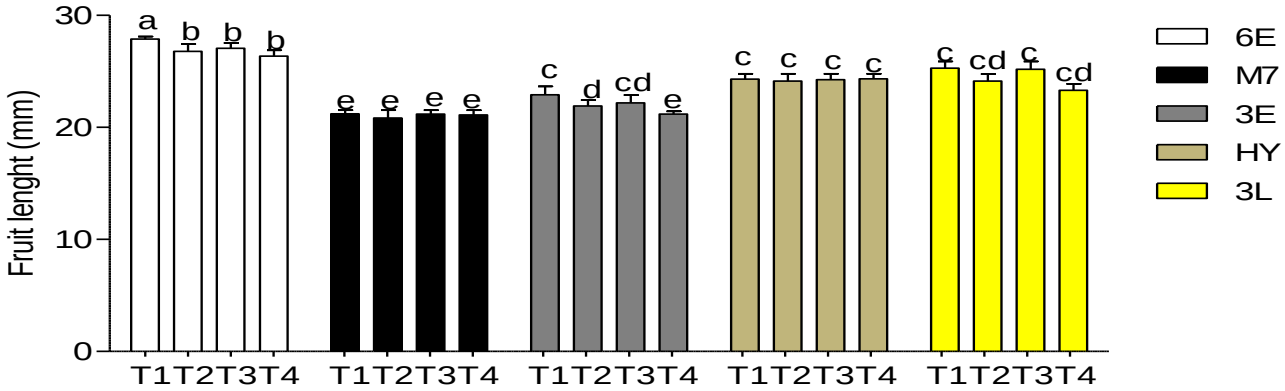

Figure 3.2: The variation of five accessions of *M*. *zeyheri* harvest at different fruit maturity stages (T1-T4) of fruit length. Fruit maturity stages (T1: Green; T2: breaker;

T3 pale yellow and T4: Yellow fruits). Bars (Mean ± SD) with different letters are significantly different (p> 0.05) according to Duncan's Multiple Range Test.

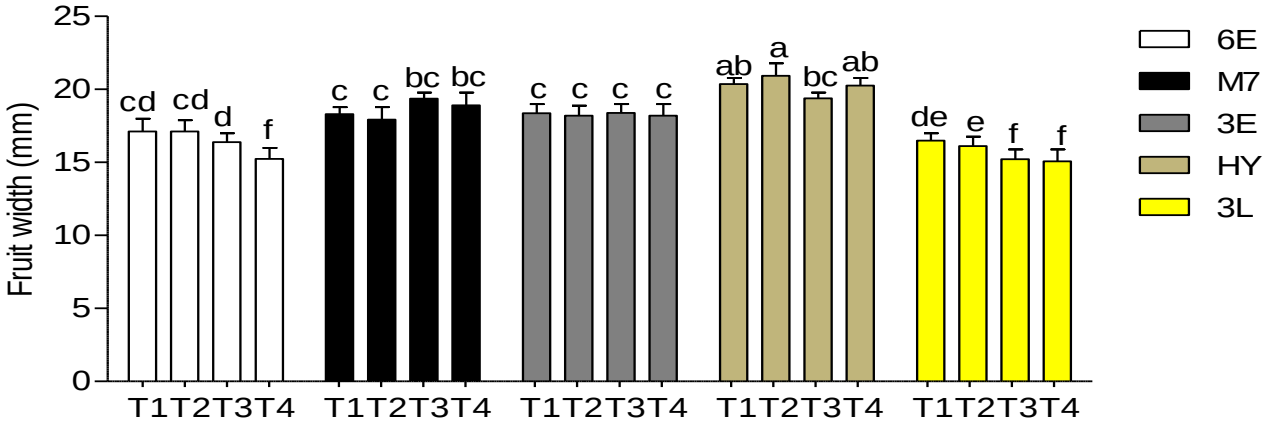

Figure 3.3: The variation of five accessions of *M. zeyheri* harvest at different fruit maturity stages (T1-T4) of fruit width. Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Bars (Mean ± SD) with different letters are significantly different (p > 0.05) according to Duncan's multiple range test.

3.4.2 Variation in fruit firmness between accessions and maturity stages

The selected five accessions of *M. zeyheri* that were harvested at the selected different fruit maturity stages had significant variations (p > 0.05) on fruit firmness. The highest mean values of fruit firmness were recorded at the T1 maturity stage and the lowest mean values were observed during the T4 maturity stage, in all accessions, as shown in Figure 3.4. Of the accessions, accession 3L had the significantly highest fruit firmness (6.40 kg) while accession M7 had the markedly lowest fruit firmness (0.83 kg) (Figure 3.4). Significant differences in the firmness of fruits have also been reported in other scholarly studies including that by Rooban *et al*. (2016); Ndou *et al*. (2019). Results of this study revealed that fruit firmness was reduced as the result of advancement in fruit maturity stages, from T1 to T4 (Figure 3.4). The reduction in fruit firmness is one of main attributes that hinder quality and post-harvest life of fruits (Oluwaseun *et al*., 2013). According to Ndou *et al*. (2019), a decline in fruit firmness is associated with softening of fruits, which occur during ripening as result of physiological changes in the cell wall structure by means of pectolytic enzymes and solubilisation action of pepic polysaccharides. Moreover, fruit firmness play an essential and major activity in post-harvest and is affected by how fruits are packed,

sorted, and transported (Oluwaseun *et al*., 2013). The parameter is directly related to flesh firmness of fruits, which is an essential in parameter processing (Muiruri *et al*., 2022) because when fruits are firmer, they are more suited and conducive for use in processing products such as jams and other preserves (Muiruri *et al*., 2022). Therefore, when fruits are softer during the T4 maturity stage, they are considered suitable to make products such as fruit juices and dried fruits. Without a doubt, fruit firmness is a vital maturity index for *M. zeyheri* fruit. The results observed from this study were congruent with that of Mkhathini *et al*. (2017) and Ndou *et al*. (2019), and given such congruency with literature, the results confirm and elaborate that fruit firmness is an ideal tool to exercise in determining the ripeness fruit maturity stage in *M. zeyheri* fruit.

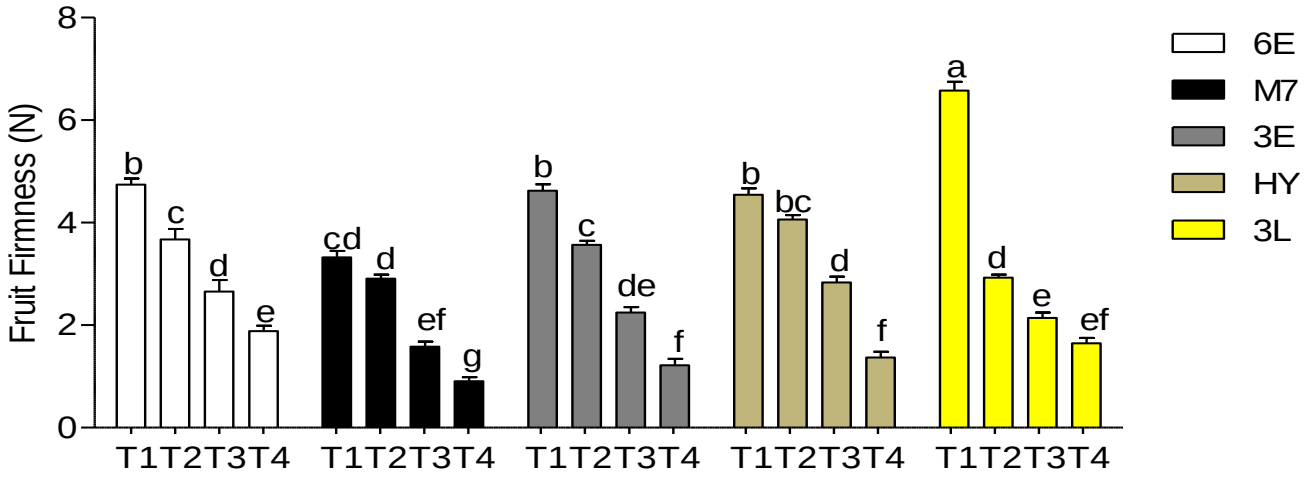

Figure 3.4: The variation of fruit firmness on different fruit maturity stages (T1 –T4) in five accessions of M. zeyheri. Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Bars (± SD) with different letters are significantly different (p> 0.05) according to Duncan's multiple range test.

3.4.3 Variation in fruit Total Soluble Solids (TSS) response to accessions and maturity stages

The TSS of fruits *M. zeyheri* accessions harvested at different fruit maturity stages had significant variations (p> 0.05). Overall, the TSS increased from T1 to T4 maturity stages, across the accessions (Figure 3.5). The highest mean values of TSS content at T1 to T4 maturity stages (24.40-32.60 Brix˚) were observed in accession HY while

the least mean values at T1 to T4 maturity stages (2.40-7.60 Brix˚) were recorded in accession 3L, as shown in Figure 3.5. The study conducted by Wu *et al*. (2005) reported that different types of fruits exhibited an increase in TSS during fruit ripening. Rooban *et al*. (2016) and Ndou *et al*. (2019) showed that the TSS content of deciduous and indigenous fruits increased as fruits reached ripening and ready-to-eat stage. According to Kusumiyati *et al*. (2022), the TSS content is an essential attribute of fruit physiochemical attribute because it determines the concentration of soluble solids in liquids, which affects the taste and show the level of sweetness of the fruit.

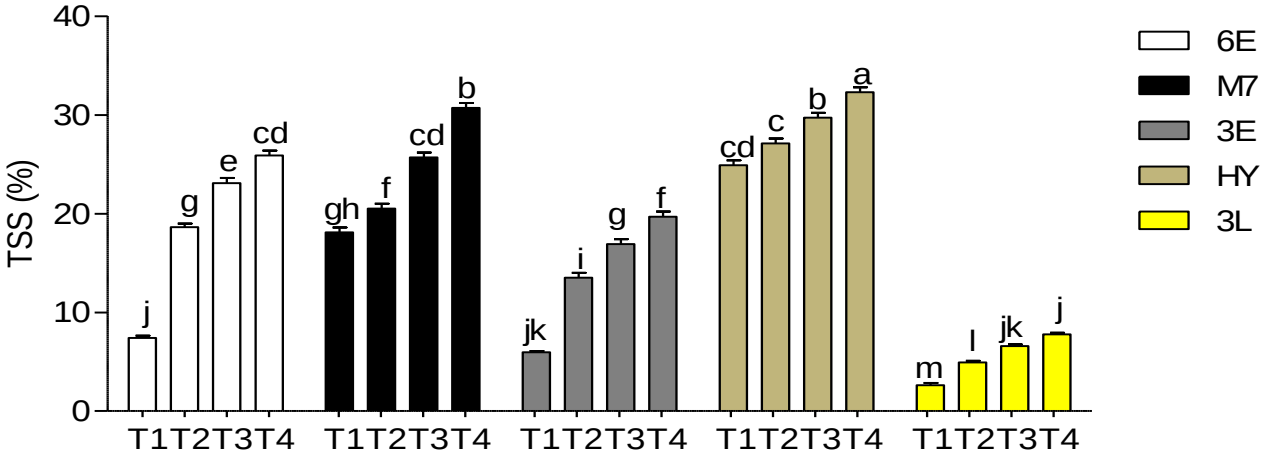

Figure 3.5: The variation of Total Soluble Solids (TSS) on different fruit maturity stages (T1-T4) in five accessions of *M. zeyheri*. Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Bars (± SD) with different letters are significantly different (p> 0.05) according to Duncan's multiple range test.

3.4.4 Effect of accessions and maturity stages on Titratable Acidity (TA)

The titratable acidity (TA) of fruits picked from the selected accessions of *M. zeyheri* at different fruit maturity stages showed marked variation (p> 0.05). In general, there was a gradual decreased in TA as the fruits reached ripening period, as shown in Figure 3.6. In this study, accession M7 exhibited the highest TA at T1 maturity stages (3.88) in comparison to all other accessions while accession 3L had the lowest values of TA T1 to T4 maturity stages (0.94-0.64%) (Figure 3.7). On the other hand, during the T1 maturity stage, the fruits revealed higher TA relative to that shown during T4 maturity stage. Between the accessions, 6E at T4 maturity stage had the lowest mean value for TA (0.22%) compared to its counterparts. According to Hwang *et al*. (2022),

the decrease in TA content of fruits seem common as shown by published literature including that in blueberries (*Vaccinium*) fruit reaching ripeness stage. Rooban *et al*. (2016) also reported decreased of TA of mango (*Mangifera indica*) and cashew apple (*Anacardium occidentale* L.) fruit, which was high at T1 (unripe) and low at T4 (ripe) stage. TA concern the concentration of acid present in fruits (Dadzie and Orchard, 1997). Ndou *et al*. (2019) emphasised that a decline of TA during the ripening stage signifies conversion of starch hydrolysis, leading to an increase in total sugar and reduced in acidity. Also, the TA content decreases as a result of the presence of organic acids which utilize TA as substrate during respiration (Zhang *et al*., 2021). Furthermore, the reduction of TA could be the result of susceptibility of critic acid to oxidative destruction as caused by the ripening area (Aina, 1990). The decreases in TA observed in the results of this study could be attributed to reduced sourness taste of fruits, which in turn enhance the sweet taste, as was evident in *M. zeyheri* fruit in this study (Figure 3.6).

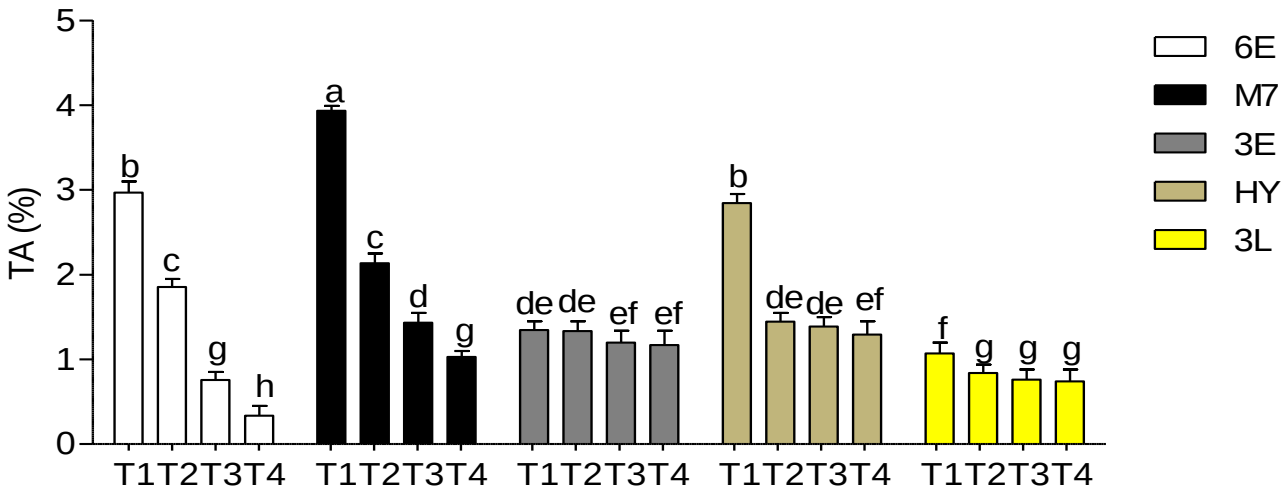

Figure 3.6: The effect of Total Acidity (TA) on different fruit maturity stages (T1 –T4) in five accessions of *M. zeyheri*. Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Bars (± SD) with different letters are significantly different (p> 0.05) according to Duncan's multiple range test.

3.4.5 Variation in TSS/TA ratio across accessions and maturity stages

Fruits of *M*. *zeyheri* collected cross five accessions and during different fruit maturity stages had significant variation (p> 0.05) in TSS/TA ratio. The TSS/TA ratio significantly increased with the fruit maturity stages and the markedly highest mean value was observed in T4 maturity stage, for all accessions, as shown in Figure 3.7. The results revealed that accession HY had the highest mean values of TSS/TA ratio at T1 to T3 maturity stages (9.27-23.61%). An increase in the TSS/TA ratio of fruits sampled across different accessions and maturity stages, was reported by Mkhathini *et al*. (2017). On the other hand, the TSS/TA ratio of accession 3L was the lowest when determined at T1 to T4 maturity stages (2.55-12.67%). When determined at maturity stage T4, accession 6E had the highest TSS/TA ratio (56.78%), an observation also made by Ndou *et al*. (2019). Magwaza *et al*. (2013) emphasised that TSS and TA and their ratio reveal considerable variation during maturation period of fruits. In this study, the results showed an increased trend of TSS/TA ratio as dependent on increased TSS values, which increased with advancement of fruit maturity, while TA values decrease with ripeness from T1 to T4 maturity stages (Figure 3.7). According to Ndou *et al*. (2019), an increase in TSS/TA ratio during maturation period is as a result of gluconeogenesis, hydrolysis of polysaccharides including starch and reduced acidity and present of sugar and organic acids. Therefore, TSS/TA ratio can be utilized to determine fruit maturity stages of different accessions.

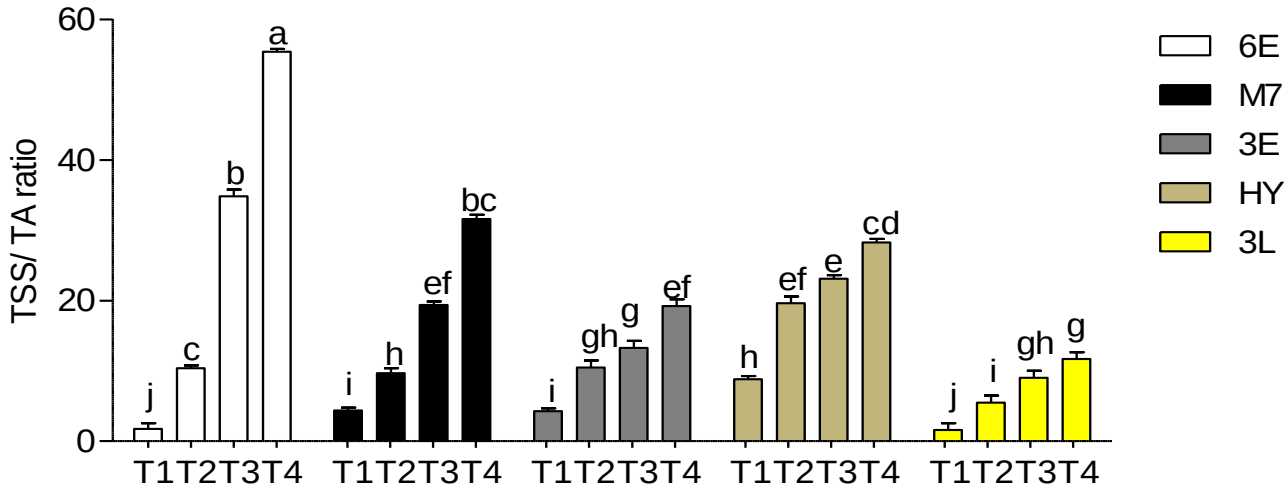

Figure 3.7: The effect of TSS/ TA ratio on different fruit maturity stages (T1-T4) in five accessions of *M. zeyheri*. Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow

and T4: Yellow fruits). Bars (± SD) with different letters are significantly different (p> 0.05) according to Duncan's multiple range test.

3.4.6 Variation of five accessions and four fruit maturity stages on colour change

Five accessions of *M. zeyheri* harvested at different fruit maturity stages had significant variation (p> 0.05) on Hunters a*, L* and h˚ angle. Mean values of Hunter a* at T1 to T4 maturity stages varied across accessions (6E, M7, 3E and HY), whilst accession 3L values enhanced from T1 to T4 maturity stages (Table 3.1). Accession HY had highest value of Hunter a* at T1 maturity stage (4.91), accession 3E had highest value at T2 maturity stage (4.06), and accession M7 had highest values at T3 and T4 maturity stages (5.36 and 5.49). According to Nambi *et al*. (2016), a change in fruit colour from green to yellowish/orange lead to increase of Hunter a* and such occur as the result of the breakdown of chlorophyll. Moreover, fruits lose their green colour as they reach ripening and when undergoing the development of carotenoids on the peel of flesh.

Mean values of Hunter L* were higher at T1 and lowest at T4 maturity stages, in all accessions. Of the accessions, 6E had highest values at T1 and T3 maturity stages (63.00 and 62.02). On the other hand, accession M7 had highest values at T2 and T4 maturity stages (62.97 and 58.91) when compared to other accessions (Table 3.1).

The h˚ angle showed significantly increased mean values at T1 maturity stage and least at T4 maturity stage as the fruit reaches ripen stage. Accession 6E had highest value at T1 maturity stage (73.25), whilst accession 3L had highest values at T2 and T3 maturity stages (71.59 and 70.54) and accession 3E had highest value at T4 maturity stage (62.78) in Table 3.1.

The pulp of *M. zeyheri* fruit developed a yellowish to orange color with decreasing Hunter L* and increasing Hunter a* and b* values at T4 maturity stage. A similar observation was shown in papaya (*Carica papaya* L.) conducted by Zuhair *et al*. (2013). Moreover, changes in Hunter a* and b* values showed that the fruit color

changed from green to yellowish/ orange with the advancement of fruit maturity stages (Kim *et al*., 2013).

Table 3.1: List of colour change coordinates of five accessions of *Mimusops zeyheri* fruits at four fruit maturity stages

Accessions	**Fruit maturity stages**	**Colour change coordinates**		
		a*	**L***	**h˚ angle**
6E	T1	3.35±0.28^{ij}	63.00±1.09^{a}	73.25±1.31^{a}
	T2	3.28±0.06^{i}	57.82±0.52^{cd}	65.29±1.01^{e}
	T3	4.11±0.05^{f}	62.02±0.35^{ab}	70.25±0.57^{abc}
	T4	5.05±0.48^{c}	57.55±2.07^{d}	58.92±3.28^{g}
M7	T1	4.15±0.57^{f}	59.77±1.77^{cd}	61.84±2.10^{ef}
	T2	3.87±0.60^{hi}	62.97±3.28^{ab}	70.08±1.05^{c}
	T3	5.36±0.32^{b}	57.36±2.93^{de}	57.97±3.62^{h}
	T4	5.49±0.40^{a}	58.91±3.37^{d}	59.72±4.10^{def}
3E	T1	3.45±0.33^{def}	60.27±2.15^{ab}	67.94±4.45^{d}
	T2	4.06±0.14^{fg}	59.69±0.62^{c}	67.37±1.25^{e}
	T3	4.53±0.34^{de}	61.12±2.79^{ab}	67.60±2.28^{de}
	T4	4.54±0.54^{de}	57.82±2.79^{cd}	62.78±3.72^{ef}
HY	T1	4.91±0.56^{d}	59.96±1.61^{bc}	60.91±1.17^{f}
	T2	3.97±0.36^{h}	56.08±4.58^{ab}	58.24±1.97^{fg}
	T3	4.86±0.49^{de}	55.83±2.80^{ef}	53.82±5.76^{gh}
	T4	4.50±0.32^{de}	58.40±1.32^{d}	62.36±1.75^{ef}
3L	T1	2.95±0.32^{f}	56.24±2.72^{de}	67.64±3.54^{de}
	T2	3.84±0.06^{hi}	59.75±0.64^{ab}	71.59±1.02^{ab}
	T3	4.56±0.20^{ef}	61.13±1.10^{ab}	70.54±0.63^{bc}
	T4	5.12±0.93^{bc}	55.01±2.66^{f}	54.46±6.25^{h}
P-Values		0.03**	0.02**	0.05*

Values are expressed as mean ± standard deviation (n= 3). For all the values within a column, different letter superscripts mean significant differences (p> 0.05) according to Duncan's multiple range test. Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). *: p> 0.05; **p> 0.01; ***: p> 0.001.

3.5 Conclusion

Observations of this study confirm existence of variations between accessions and different fruit maturity stages on quality and physicochemical attributes. The variations between accessions could be caused by among other factors, genetic make-up. The weight, length and width of fruits of *M. zeyheri* showed statistically no significant differences during fruit ripeness from green to yellow. However, TSS increased as the

fruit ripened. The ratio of fruit TSS/TA increased, which could indicate that the ripeness of the accessions. Fruit firmness showed a clear and steady decrease as the fruit ripened, from green to yellow. Therefore, it is vital to promote the cultivation and marketing of fruits of *M. zeyheri*, both as fresh fruit for snacking or freeze-dried powder for food supplementation programs. Furthermore, it has the potential to provide an accessible, available and affordable enriched functional food with valuable health benefits. Accessions 6E and HY at T4 fruit maturity stage is recommended.

References

Aina, J.O., 1990. Physicochemical changes in African mango (*Irvingia gabonensis*) during normal storage ripening. *Food Chemistry*, 36 (3): 205-212.

Chepngeno, J., Owino, W.O., Kinyuru, J. and Nenguwo, N., 2016. Effect of calcium chloride and hydrocooling on postharvest quality of selected vegetables. *Journal of Food Research*, 5(2): 23.

Crisosto, C.H. and Tiwari, G., 2013. Non-destructive determination of flesh color in clingstone peaches. *Journal of Food Engineering*, 116(4): 920-925.

Dadzie, B.K. and Orchard, J.E., 1997. Post-harvest criteria and methods for routine screening of banana and plantain hybrids. *Plant Breeding* 116(5): 487-491.

Hanif, M., Hafeez, S. and Riaz, A., 2010. Factors affecting customer satisfaction. *International Research Journal of Finance and Economics*, 60(1): 44-52.

Hwang, I.S. and Kim, M.K., 2022. Determination of Aroma Characteristics of Commercial Garlic Powders Distributed in Korea via Instrumental and Descriptive Sensory Analyses. *Preventive Nutrition and Food Science*, 27(4): 464.

Kim, S.M., Hsu, A., Lee, Y.H., Dresselhaus, M., Palacios, T., Kim, K.K. and Kong, J., 2013. The effect of copper pre-cleaning on graphene synthesis. *Nanotechnology*, 24(36): 365602.

Kusumiyati, K., Syifa, R.J. and Farida, F., 2022. Effect of various varieties and dosage of potassium fertilizer on growth, yield, and quality of red chili (*Capsicum annuum* L.). *Open Agriculture*, 7(1): 948-961.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S. and Terry, L.A., 2013. Canopy position affects rind biochemical profile of 'Nules Clementine'mandarin fruit during postharvest storage. *Postharvest Biology and Technology*, 86: 300-308.

Mkhathini, K.M., Magwaza, L.S., Workneh, T.S. and Mwithiga, G., 2017. Determinations of physical and chemical properties of KwaZulu Natal household white peach 'landrace'in relation to extension services a case study of impendle local municipality. *South African Journal of Agricultural Extension*, 45(2): 95-109.

Mngadi, S., Moodley, R. and Jonnalagadda, S.B., 2017. Elemental composition and nutritional value of the edible fruits of coastal red-milkwood (*Mimusops caffra*) and impact of soil quality on their chemical characteristics. *Journal of Environmental Science and Health, Part B*, 52(7): 435-445.

Mpai, S., Du Preez, R., Sultanbawa, Y. and Sivakumar, D., 2018. Phytochemicals and nutritional composition in accessions of Kei-apple (*Dovyalis caffra*): Southern African indigenous fruit. *Food chemistry*, 253: 37-45.

Mothapo, M.J., 2014. *Physicochemical properties and selected nutritional components of Wild Medlar (Vangueria Infausta) fruit harvested at two harvesting time* (Masters Dissertation, University of Limpopo, South Africa).

Mousavi, S.M. and Motesharezadeh, B., 2020. Boron deficiency in fruit crops. *Fruit Crops*. 191 -209.

Moussaoui, K.A. and Varela, P., 2010. Exploring consumer product profiling techniques and their linkage to a quantitative descriptive analysis. *Food Quality and Preference*, 21(8): 1088-1099.

Muiruri, J.K., Yeo, J.C.C., Zhu, Q., Ye, E., Loh, X.J. and Li, Z., 2022. Poly (hydroxyalkanoates): Production, applications and end of life strategies life cycle assessment nexus. *ACS Sustainable Chemistry and Engineering*, 10(11): 3387-3406.

Nambi, V.E., Thangavel, K., Rajeswari, K.A., Manickavasagan, A. and Geetha, V., 2016. Texture and rheological changes of Indian mango cultivars during ripening. *Postharvest Biology and Technology*, 117: 152-160.

Ndou, A., Tinyani, P.P., Slabbert, R.M., Sultanbawa, Y. and Sivakumar, D., 2019. An integrated approach for harvesting Natal plum (*Carissa macrocarpa*) for quality and functional compounds related to maturity stages. *Food Chemistry*, 293: 499-510.

Nieuwenhuizen, N.J., Beuning, L.L., Sutherland, P.W., Sharma, N.N., Cooney, J.M., Bieleski, L.R., Schroder, R., Macrae, E.A. and Atkinson, R.G., 2007. Identification and characterisation of acidic and novel basic forms of actinides, the highly abundant cysteine protease from kiwifruit. *Functional Plant Biology*, 34(10): 946-961.

Oluwaseun, A., David, F.O. and Issa, A.A., 2013. The role of religion in modern society: Masses opium or tool for development: A Case Study of Saw-Mill Area, Ilorin, and Kwara State, Nigeria. *International Journal of Sociology and Anthropology*, 9(6) 1-15.

Pila, N., Gol, N.B. and Rao, T.R., 2010. Effect of post-harvest treatments on physicochemical characteristics and shelf life of tomato (*Lycopersicon esculentum* Mill.) fruits during storage. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 9(5): 470-479.

Rooban, R., Shanmugam, M., Venkatesan, T. and Tamilmani, C., 2016. Physiochemical changes during different stages of fruit ripening of climacteric fruit of mango (*Mangifera indica* L.) and non-climacteric of fruit cashew apple (*Anacardium occidentale* L.). *Journal of Advanced Research*, (2): 53-8.

Singham, P., Birwal, P. and Yadav, B.K., 2015. Importance of objective and subjective measurement of food quality and their inter-relationship. *Journal of Food Processing and Technology*, 6(9): 1.

Sivakumar, D., Jiang, Y. and Yahia, E.M., 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. *Food Research International*, 44(5): 1254-1263.

Sudheer, K.P., Srivastav, R.K. and Chaubey, I., 2007. A simplified approach to quantifying predictive and parametric uncertainty in artificial neural network hydrologic models. *Water Resources Research*, 43(10): 245-254.

Verma, R., Chen, S., Feldman, R., Schieltz, D., Yates, J., Dohmen, J. and Deshaies, R.J., 2000. Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. *Molecular Biology of the Cell*, 11(10), pp.3425-3439.

Wu, B.H., Quilot, B., Génard, M., Kervella, J. and Li, S.H., 2005. Changes in sugar and organic acid concentrations during fruit maturation in peaches, P. davidiana and hybrids as analysed by principal component analysis. *Scientia Horticulturae*, 103(4): 429-439.

Zhang, W., Jiang, H., Cao, J. and Jiang, W., 2021. Advances in biochemical mechanisms and control technologies to treat chilling injury in postharvest fruits and vegetables. *Trends in Food Science and Technology*, 113: 355-365.

Zuhair, R.A., Aminah, A., Sahilah, A.M. and Eqbal, D., 2013. Antioxidant activity and physicochemical properties changes of papaya (*Carica papaya* L. cv. Hongkong) during different ripening stage. *International Food Research Journal*, 20(4): 117-121.

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CHAPTER 4

Interaction effect between accessions and fruit maturity stages on the nutritional compositions of *Mimusops zeyheri* Sond.

Abstract

Mimusops zeyheri is a well-known edible indigenous fruit found primarily in rural communities of the Limpopo province, among other provinces of South Africa. This study was aimed at profiling analytical-based information on nutritional properties including amino acids and mineral compositions of fruits of five accessions (6E, M7, 3E, HY and 3L) sampled at different maturity stages (T1, T2, T3 and T4). Data, including that on moisture, dry matter as well as ash and protein content was analysed using GenStat 18th version. Accession HY recorded highest moisture content at T1 to T4 maturity stages (93-97%) and protein content at T1 and T2 maturity stages (0.40-0.41%) of 3L accession whilst accession HY recorded highest protein content at T1 maturity stage (0.42%). The moisture content was highest in accession 6E at T1 and T3 maturity stages (92 and 89%) and ash content (4.20-3.75%) at T1 to T3 maturity stages. The fruits revealed markedly reduced dry matter, ash and protein content from T1 to T4 maturity stages, while their moisture content was increased during the advanced fruit maturity stages. Of essential nutrient elements, only concentration of calcium, potassium, iron, magnesium, manganese, sodium, zinc and phosphorus were assessed between the accessions and maturity stages. For example, accession M7 exhibited the highest concentration of calcium at T1 maturity stage (27.73 8 mg/L), magnesium at maturity stage T1 (5.96 mg/L) and maturity stage T2 (4.37 mg/L), and sodium at T1 to T4 maturity stages (5.63 to 3.64 mg/L). Accession 3E accumulated the highest concentration of potassium at T2 maturity stage (48. 70 mg/L) and manganese at T1 and T2 maturity stages (0.07 and 0.06 mg/L). Furthermore, accession HY recorded markedly increased calcium at maturity stages T2 to T4 (21.50-17.17 mg/L), iron (1.57, 0.62 and 0.37 mg/L) during maturity stages T1 to T3, and potassium (50.83, 50.00 and 44.93 mg/L). 3E revealed greater phosphorus during T1 to T3 maturity stages, which ranged from (9.39-6.83 mg/L) as well as zinc which ranged from (1.73-0.60 mg/L). The concentration of the determined mineral nutrients decreased with advancement of the fruit maturity stages, in all the selected accessions. Amino acids were also analysed due to less concentration of protein present in the fruits. This study recommends that fruits of the selected accessions are most suitable for consumption during the T4 maturity stage. This is informed by the

results which showed markedly increased concentrations of mineral nutrients and amino acids. The fruits at T4 maturity stage can be consumed as raw fruits for snacking to reduce the malnutrition and food insecurity.
Keywords: Accessions, Fruit maturity, Food fortification program, Indigenous fruit.

4.1 Introduction

Most governments in Sub-Saharan Africa have grappled to address the serious public health problem of food and nutrition insecurity and it is coupled with malnutrition such as under nutrition and micronutrient deficiencies (FAO *et al*., 2017). The FAO *et al*. (2017) reported that in the year 2016, while approximately 815 million individuals had insufficient food and were affected by hunger, majority of them resided in developing countries. Researcher suggests that the recognition and incorporation of indigenous fruits into mainstream food system could possibly contribute to the reduction and/or eradication of food poverty (Sibiya *et al*., 2021). In light of this, indigenous fruits are seen to can contribute mitigate such problems due to their adaptability to marginal growth conditions, ease of accessibility, and their high nutritional value.

Assessment of nutritional compositions of fruits is considered an important and suitable tool for determination of whether a population's nutritional needs are met effectively (Nieman and Lee 2019). Therefore, analysis of the nutritional content of edible indigenous fruits especially during different fruit maturity stages should provide with quality, quantities and evidence-based information. Knowledge on such information contributes to the commercialization, utilization, promotion and future research which, all together, would decrease the rate of hunger, poverty and related malnutrition in Sub-Saharan Africa (Omotayo *et al*., 2020). According to AOAC (2005), proximate technique is a system that analyses food for content of ash, moisture, protein, fat, and carbohydrates. In addition, indigenous fruits are highly and widely recommended given that are sources of mineral nutrients and amino acids, which confer health benefits and reduce risks of diseases risks (Ngadze, 2018). Benefits associated with mineral nutrients in fruits include their essentiality in supporting physiological roles in cognitive skills, learning abilities, body growth and bone formation, and essential for proper development of infants and children (Ngadze,

2018). Despite the well-documented ethno-botanical literature in SA (Omotayo *et al*., 2020), published literature show very little information on the nutritional compositions of *M. zeyheri* at different fruit maturity stage not is available.

This chapter presents the results on research that assessed the nutritional composition of five accessions of *M. zeyheri* harvested at four different fruit maturity stages.

4.2 The objective of the study

The objective of this chapter was to evaluate whether fruits of *M. zeyheri* picked from different accessions and maturity stages show different levels of nutritional compositions.

4.3 Materials and methods

4.3.1 Study location

The analysis of nutritional compositions including protein, ash, and minerals were conducted at the Limpopo Agro-Food Technology Station (LATS), University of Limpopo, South Africa.

4.3.2 Research design and treatments

The design and treatments used in this chapter are described in 3.3.2.

4.3.3 Procedures and practices

The pulp and peel of the indigenous fruits, which are edible, were carefully separated from the seed and mixed using a mortar and pestle. The samples were oven-dried at 40 °C until a constant weight was achieved and grounded into powder prior the analysis.

4.3.4 Data collection

4.3.4.1 Nutritional attributes

I. The determination of moisture content

Moisture content was determined by comparing the weight of fresh sample and oven dried sample at 30 °C for 72 hours according to the AOAC standard method (AOAC, 2000).

II. The determination of dry matter

To determine the dry matter content (%), fruits were heated in an oven (30 °C, 72 hours) until a constant weight was obtained and the weight loss was used to calculate the moisture and dry matter content in fruit.

III. The determination ash content

The AACC 08 - 01 (1999) method was used to calculate the ash content, which is the substance left over after all the organic stuff in food has been burned by oxidation. Prior to being weighed, crucibles were dried for five hours at 100 °C in an oven before being allowed to cool. Each sample was weighed into the crucibles at a rate of 2 g. After that, the samples in the crucibles were mounted on a tripod and heated until they were burned. The samples were then placed in a muffle furnace and cooked for 5 hours at 550 °C. Therefore, samples were taken out, cooled to room temperature in a desiccator, and weighed.

Following are the calculations for percentage ash:

$$\text{Ash \%} = \frac{(Mass\ food + crucible) - (Mass\ crucible)}{(Mass\ food + crucible) - (Mass\ crucible)} \times 100$$

IV. The determination protein content

The Association of Official Analytical Chemists (AOAC, 1990) micro Kjeldahl method was implement, whereby in a heating tube, 10 ml of concentrated sulphuric acid (H_2SO_4) was added to 2 g of each sample. The tube was filled with one selenium catalyst tablet, and the mixture was heated within a fume closet. The digest was poured into a volumetric flask measuring 100 ml, which was then filled with distilled water. A portion of the digest containing 10 ml was combined with an equal volume of 45% NaOH solution and then placed into a Kjeldahl distillation device. The combination was distilled, and the distillate was then put into a solution of 4% boric

acid with three drops of an indicator. Additionally, 50 ml of distillate were gathered and titrated in total. A second sample was used, and the average value was calculated. Using a protein conversion factor of 6. 25, the nitrogen content was computed and converted to a percentage of protein.

The formula of nitrogen % as follows:

Nitrogen % = (100 x W x N x 14 x VF) T/ 100 x VA

Note: W = Weight of the sample, N = Normality of the titrate (0.1N), VF = Total volume of the digest = 100 ml, T = Titre value and VA = Aliquot volume distilled.

V. The determination amino acids

Following the procedures outlined by Mpai *et al.,* 2018, the dried peel and pulp samples were processed for analysis of amino acids using an Amino Acid Analysis Application Solution (AccQ Tag) derivatization kit. The analysis of essential amino acids, including Histidine (His), Threonine (Thr), Lysine (Lys), Valine (Val), Leucine (Leu), Isoleucine (Ile) Phenylalanine (Phe), and non-essential amino acids including Arginine (Arg), Serine (Ser), Glycine (Gly), Aspartic acid (Asp), Glutamine acid (Glu), Alanine (Ala), Proline (Pro) and Tyrosine (Tyr) was performed using the ultra-performance liquid chromatography analysis following the methods described by Mokgalabone *et al*. (2023).

VI. Mineral attributes

A total of 10 g of dried peel and pulp of fruit samples were digested in 40 ml of 4% nitric acid (HNO_3) before the mixture was completely wetted by spinning the container in a vortex. After the samples had been magnetically agitated, they were placed in a 95°C. After a 90 minute water bath, the samples was cooled to ambient temperature, filtered, and decanted into 50 ml tubes that were foil wrapped before being subjected to an analysis of a few nutrient components using inductively coupled plasma optical emission spectrometry (ICPE-9000). The analysis's circumstances and the growth of the mineral standard curve mirrored those mentioned by Mokgalabone *et al*. (2023).

4.3.5 Statistical analysis

Data were subjected to statistical analysis using GenStat 18th version statistical package (VSN International, Hempstead, UK). The mean separation for significant variation was achieved using a Duncan at the significance level of 5%.

4.4 Results and discussions

4.4.1 Variation of five accessions and four fruit maturity stages on moisture content

Fruits of *M. zeyheri* were harvested from five accessions and at different fruit maturity stages, had significant variations (p> 0.05) on moisture content. The results shown in Table 4.1 revealed that the fruits revealed an increase in moisture content from T1 to T4 maturity stages in all accessions. The marked increase in the moisture content across accessions has been observed in other studies including that by Haggag *et al*. (2013); Famuyini *et al*. (2020) and Jia *et al*. (2022). Of the five accessions, a markedly highest moisture content was shown by HY at T1 to T4 maturity stages (93-97% respectively), whilst that of 6E was highest at T2 maturity stage (94%) and that of M7 was increased at T4 maturity stage (96%) in Table 4.1. The results on moisture content shown in this study are in contrast with that by Nayab *et al*. (2020). They documented less moisture content ranged from (76.39-89.87%) in fruits of mulberry (*Morus alba* L.) of different varieties at different fruit maturity stages. In another study, Maisarah *et al*. (2014) recorded that during the early fruit maturity stage, papaya fruits exhibited more than threefold (44.50%) dry matter content compared to that reported during the ripe stage. According to Imran *et al*. (2010), moisture content is a vital attribute that is used to determine the quality, shelf life, and suitability of certain commodity for consumption either fresh, dried or processed. Moreover, it outlines the level of freshen in the fruits. The increase is the moisture content recorded with the advancement of fruit maturity stages are as the results of increased in juice content of fruits as they reach the ripening stage (Mahmood *et al*., 2012). Of noteworthy, other fruits such as jujube (*Ziziphus jujuba Mill*.), picked from eight cultivars across different fruit maturity stages, revealed decreased moisture content from unripe to ripe stages, in all cultivars and this was attributed to the fruits having lost water through evaporation (Yan *et al*., 2022).

4.4.2 Variation in fruit dry weight response to accessions and maturity stages

Table 4.1 show results on *M*. *zeyheri* fruits dry weight as affected by accessions and maturity stage, and there were significant variations (p> 0.05). Accession 6E had highest dry weight at T1, T3, and T4 maturity stages (92, 89, and 90%) (Table 4.1). On the other hand, the fruit dry weight of accession M7 was significant enhanced at

T2 stage (91%) compared to that of the other accessions, as shown in Table 4.1. The trend seen in this study on fruits dry weight is in agreement with results reported by Diba *et al*. (2017). The results shown in Table 4.1 illustrate a variation in dry weights of the fruits determined at different maturity stages, in all accessions. Napier and Combrink. (2005) emphasise that dry matter is a significant parameter that outline the carbon incorporation at different fruit maturity stages. However, the observed trend of reduction in dry weight as the fruits reached the ripening stage were mainly due to the indirect relations with moisture content that increased with advancement progress of ripening in fruits.

4.4.3 Variation of five accessions and four fruit maturity stages on ash content

The fruits of the five accessions of *M. zeyheri* harvested at different fruit maturity stages had significant variation ($p > 0.05$) on ash content. Results shown in Table 4.1 demonstrate a decrease in ash content as the fruits reached ripening stages from T1 to T4 maturity stages, in all accessions. The highest ash content at T1 to T3 maturity stages (4.20, 3.90 and 3.75%) were recorded in accession 6E whereas highest value at T4 maturity stage (3.99%) was observed in accession 3E, as shown in Table 4.1. The mean values of ash content shown in this study were higher compared to that reported in results of other studies including that by Mahmood *et al*. (2012), Famuyini *et al*. (2020); and Sibiya *et al*. (2021).

4.4.4 Effect of accession and maturity stage on protein content

Of the selected accessions and maturity stages of *M. zeyheri*, the fruits revealed significant variation ($p > 0.05$) in protein content. The protein content of accession HY was significantly highest at T1 maturity stage (0.42%) as shown in Table 4.1. On the other hand, accession 3L recorded the highest protein content at T2, T3, and T4 maturity stages which ranged from (0.41, 0.31 and 0.20%) (Table 4.1). During maturity stage T1, the protein content was highest at T1 maturity stage however, it deceased gradually from T2 to T4 maturity stages as the fruit reached the ripening stage, across the studied accessions. Accession 6E accumulated the least protein content at T1 to T4 (0.08, 0.07, 0.05 and 0.04%) maturity stages (Table 4.1). The results on protein

content shown in this study are in agreement with that in studies conducted by Hussain *et al*. (2010); Nayab *et al*. (2020) and Amarteifio and Mosase. (2006) in which they showed a decrease in protein content with the advancement of fruit maturity stages in fruits of jujube (*Ziziphus mauritiana Lamk*. and *Ziziphus spina-christi* L.), Loquat (*Eriobotrya japonica* L.) and Mulberry (*Morus alba* L.). The decreasing trend in protein content in fruit maturity or ripening stages could have been due to catabolic effects on proteins (Diba *et al*., 2017). Therefore, the protein content determined in fruits of the selected accessions of *M. zeyheri* demonstrated that they may not regarded as high accumulators of protein. The nutritional quality and quantity of fruits of the selected accessions of *M. zeyheri* at different fruit maturity stages, concentration of selected amino acids was analysed.

Table 4.1: Nutritional compositions of five accessions of *M. zeyheri* fruits at four fruit maturity stages.

Accessions	Fruit maturity stage	Nutritional compositions (%)			
		Moisture	Dry matter	Ash	Protein
6E	T1	93±0.02^{e}	91±0.00^{a}	4.20±0.00^{a}	0.08±0.00^{h}
	T2	94±0.03^{d}	89±0.00^{cd}	3.94±0.03^{b}	0.07±0.00^{j}
	T3	94±0.03^{d}	89±0.00^{ab}	3.75±0.00^{d}	0.05±0.00^{k}
	T4	95±0.04^{c}	90±0.00^{ab}	3.42±0.00^{g}	0.03±0.00^{k}
M7	T1	92±0.01^{f}	89±0.00^{cd}	4.19±0.00^{a}	0.17±0.01^{e}
	T2	94±0.03^{d}	90±0.00^{b}	3.82±0.00^{c}	0.07±0.00^{hi}
	T3	95±0.04^{c}	87±0.00^{d}	3.59±0.00^{e}	0.06±0.00^{i}
	T4	96±0.05^{b}	88±0.00^{cd}	3.54±0.00^{f}	0.04±0.00^{j}
3E	T1	89±0.00^{i}	88±0.02^{cd}	3.75±0.00^{d}	0.16±0.00^{f}
	T2	90±0.00^{h}	88±0.00^{cd}	3.26±0.00^{i}	0.16±0.00^{f}
	T3	91±0.01^{g}	87±0.00^{de}	3.00±0.00^{k}	0.11±0.00^{g}
	T4	93±0.02^{e}	86±0.00^{ef}	3.99±0.00^{l}	0.04±0.00^{j}
HY	T1	93±0.02^{e}	90±0.01^{abc}	2.97±0.00^{l}	0.42±0.00^{a}
	T2	94±0.03^{d}	87±0.01^{d}	2.81±0.00^{m}	0.03±0.00^{k}
	T3	96±0.05^{b}	89±0.00^{ab}	2.78±0.00^{n}	0.01±0.00^{l}
	T4	97±0.06^{a}	90±0.00^{b}	2.53±0.00^{o}	0.02±0.00^{l}
3L	T1	91±0.01^{g}	87±0.03^{d}	3.95±0.00^{b}	0.40±0.00^{b}
	T2	93±0.02^{e}	88±0.00^{de}	3.53±0.00^{f}	0.41±0.00^{b}
	T3	94±0.03^{d}	87±0.00^{d}	3.33±0.00^{h}	0.31±0.00^{c}
	T4	96±0.05^{b}	89±0.00^{cd}	3.21±0.00^{j}	0.20±0.00^{d}
P-Values		0.00***	0.00***	0.00***	0.00***

Values are expressed as mean ± standard deviation (n= 3). For all the values within a column, different letter superscripts mean significant differences (p> 0.05). Fruit maturity stages: T1: Green; T2: breaker; T3 pale yellow and T4: yellow fruits).*: p> 0.05; **p> 0.01; ***: p> 0.001.

4.4.5 Variation of five accessions and four fruit maturity stages on amino acids profile

Selected essential and non-essential amino acids of five accessions of *M. zeyheri* harvested at different fruit maturity stages are shown in Table 4.2. Seven out of nine essentials Histidine (His), Threonine (Thr), Lysine (Lys), Valine (Val), Leucine (Leu), Isoleucine (Ile) Phenylalanine (Phe), and eight out of eleven non-essential amino acids Arginine (Arg), Serine (Ser), Glycine (Gly), Aspartic acid (Asp), Glutamine acid (Glu), Alanine (Ala), Proline (Pro) and Tyrosine (Tyr) were determined in this study. The difference in the expression pattern of amino acid metabolizing enzymes occurs during

fruit maturity stages process (Boggio *et al*., 2000). The changes in amino acids content during fruit maturity stages seem to be dependent on accessions (Monti *et al*., 2016). The content of histidine was markedly increased in accession HY at T1 and T4 maturity stages (0.14 and 0.11 g/100 g), while at T3 maturity stage, accession 3E had the highest histidine (0.11 g/100 g). Accession M7revealed significantly higher histidine at T2 maturity stage of (0.12 g/100 g) compared to other accessions (Table 4.2). According to Dunstan *et al*. (2017), histidine is used for growth and repair of damaged tissues, including the protection of nerve cells. Threonine was highest in 3L at T1 and T2 maturity stages (0.21 and 0.24 g/100 g). On the other hand, that determined during maturity stages T3 and T4 was greater in 3E (0.17 and 0.18 g/100 g), as shown in Table 4.2. Threonine is an indispensable amino acid that participate in lipid metabolism and protein synthesis (Edgar, 2002). As shown in Table 4.2, lysine was undetectable in accessions HY, 3E, M7 and 6E, by contrast, it was detectable in accession 3L at T1 to T4 maturity stages are ranged from (0.07, 0.07, 0.04, and 0.02 g/100 g). As displayed in Table 4.2, mean values of phenylalanine and valine determined during T1 to T4 maturity stages were highest in accessions HY and 3L. Variation in the content of essential amino acids between plant accessions and maturity stage have been reported in various studies including Emaga *et al*. (2007) and Sensory *et al*. (2015). The isoleucine and leucine were detectable at T1 and T4 stages in all accessions whereas accession 3L had highest of isoleucine and leucine at T1 maturity stage (0.15 and 0.25 g/100 g) (Table 4.2).

Interestingly, tyrosine ranked as the most abundant amino acids in accession HY compared to other accessions at T1 to T4 maturity stages and they ranged from (0.28, 0.34, 0.39 and 0.41 g/100 g), respectively (Table 4.3). Koide and Sidhu (2009) emphasise that tyrosine helps to produce the melanin pigment which is responsible for the promotion of hair and skin colour. Accession HY outperformed the other accessions at T1 and T4 maturity stages (0.19 and 0.18 g/100 g) as it accumulation the highest content of arginine, whereas accession 3L had the highest mean values at T2 and T3 maturity stages (0.16 and 0.14 g/100 g). Famuyini *et al*. (2020) state that arginine plays an essential role in the removal of ammonia within the body. Contents of serine, glycine, aspartic acid, and glutamine were significantly increased in accession HY at T4 maturity stage with mean values of (0.13, 0.13, 0.39 and 0.25 g/100 g), respectively. Accession 3L recorded the highest serine (0.16, 0.12 and 0.10

g/100 g), glycine (0.21, 0.19 and 0.17 g/100 g), and glutamine (0.28, 0.24 and 0.17 g/100 g) during the T1 and T3 maturity stages. The trend revealed by the results on the content of essential amino acids presented in Table 4.3 is in agreement with results of Kassim *et al*. (2017). Alanine recorded the highest mean value in accession HY at T1 maturity stage (0.27 g/100 g), whilst accession 6E at T2 maturity stage (0.18 g/100 g) and accession 3E at T4 maturity stage (0.26 g/100 g). Of noteworthy, in all accessions at T3 maturity stage, alanine was not detectable. Proline recorded the highest mean values in accession 6E at T1, T2 and T4 maturity stages (0.30, 0.29 and 0.25 g/100 g), whereas accession 3E had highest mean value at T3 maturity stage (0.19 g/100 g).

Table 4.2: List of essential amino acids of five accessions of *Mimusops zeyheri* fruits at four fruit maturity stages

Accessions	Fruit maturity stages	Essential amino acids (g/100 g)						
		His	**Thr**	**Lys**	**Phe**	**Val**	**Ile**	**Leu**
6E	T1	0.07±0.01^{ef}	0.18±0.01^{cd}	nd	0.23±0.01^{d}	0.12±0.01^{e}	0.11±0.01^{bc}	0.17±0.01^{c}
	T2	nd	0.17±0.01^{def}	nd	nd	nd	nd	nd
	T3	nd	0.17±0.01^{def}	nd	nd	nd	nd	nd
	T4	0.08±0.01^{e}	0.15±0.01^{ef}	nd	0.21±0.01^{f}	0.15±0.01^{c}	0.07±0.01^{f}	0.15±0.01^{d}
M7	T1	0.13±0.01^{b}	0.15±0.01^{fg}	nd	0.23±0.01^{d}	0.14±0.01^{cd}	0.12±0.01^{b}	0.21±0.01^{b}
	T2	0.12±0.01^{bc}	0.15±0.01^{fg}	nd	nd	nd	nd	nd
	T3	nd	0.14±0.01^{gh}	nd	nd	nd	nd	nd
	T4	0.10±0.01^{cde}	0.12±0.01^{h}	nd	0.21±0.01^{f}	0.11±0.01^{ef}	0.11±0.01^{bc}	0.12±0.01^{e}
3E	T1	0.12±0.01^{cde}	0.16±0.01^{ef}	0.04±0.01^{b}	0.18±0.01^{g}	0.12±0.01^{e}	0.11±0.01^{d}	0.15±0.01^{d}
	T2	0.11±0.01^{cde}	0.17±0.01^{ef}	nd	nd	nd	nd	nd
	T3	0.11±0.01^{cde}	0.18±0.01^{def}	nd	nd	nd	nd	nd
	T4	0.08±0.01^{e}	0.18±0.01^{cd}	nd	0.17±0.01^{gh}	0.12±0.01^{e}	0.12±0.01^{e}	0.18±0.01^{c}
HY	T1	0.14±0.01^{a}	0.12±0.01^{h}	nd	0.23±0.01^{e}	0.14±0.01^{b}	0.08±0.01^{e}	0.17±0.01^{c}
	T2	nd	nd	nd	0.24±0.01^{bc}	0.16±0.01^{b}	nd	nd
	T3	nd	nd	nd	0.24±0.01^{bc}	0.16±0.01^{b}	nd	nd
	T4	0.11±0.01^{cde}	0.21±0.01^{b}	nd	0.26±0.01^{b}	0.16±0.01^{b}	0.11±0.01^{d}	0.17±0.01^{c}
3L	T1	0.11±0.01^{cde}	0.24±0.01^{a}	0.07±0.01^{a}	0.32±0.01^{a}	0.21±0.01^{a}	0.15±0.01^{a}	0.25±0.01^{a}
	T2	nd	0.20±0.01^{c}	0.07±0.01^{a}	nd	nd	nd	nd
	T3	0.08±0.01^{e}	0.15±0.01^{fg}	0.04±0.01^{b}	nd	nd	nd	nd
	T4	0.08±0.01^{e}	0.08±0.01^{i}	0.01±0.01^{c}	0.17±0.01^{h}	0.11±0.01^{ef}	0.11±0.01^{bc}	0.12±0.01^{e}
P-Values		0.00***	0.04**	0.12*	0.00***	0.00***	0.00***	0.00***

Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Histidine (His), Threonine (Thr), Lysine (Lys), Valine (Val), Leucine (Leu), Isoleucine (Ile) Phenylalanine (Phe). nd = Not Detected. For all the values within a column, different letter superscripts mean ± standard deviation (n= 3) significant differences (p> 0.05). *: p> 0.05; **p> 0.01; ***: p> 0.001.

Table 4.3: List of non-essential amino acids of five accessions of *Mimusops zeyheri* fruits at four fruit maturity stages

Accessions	Fruit maturity stages	Non-essential amino acids (g/100 g)							
		Arg	**Ser**	**Gly**	**Asp**	**Glu**	**Ala**	**Pro**	**Tyr**
6E	T1	0.15±0.01^{de}	0.08±0.01^{ef}	nd	0.18±0.01^{g}	0.17±0.01^{ef}	0.34±0.01^{a}	0.30±0.01^{a}	0.23±0.01^{f}
	T2	0.15±0.01^{e}	0.08±0.01^{ef}	nd	0.18±0.01^{g}	0.17±0.01^{ef}	0.18±0.01^{c}	0.29±0.01^{ab}	0.18±0.01^{gh}
	T3	0.12±0.01^{f}	Nd	nd	nd	nd	nd	nd	0.18±0.01^{gh}
	T4	0.11±0.01^{f}	0.08±0.01^{ef}	nd	0.21±0.01^{f}	0.17±0.01^{ef}	0.17±0.01^{d}	0.29±0.01^{ab}	0.17±0.01^{h}
M7	T1	0.11±0.01^{f}	0.08±0.01^{ef}	nd	0.15±0.01^{i}	0.15±0.01^{fg}	0.12±0.01^{f}	0.24±0.01^{g}	0.27±0.01^{de}
	T2	nd	0.08±0.01^{ef}	nd	0.17±0.01^{h}	0.15±0.01^{gh}	nd	nd	nd
	T3	nd	0.07±0.01^{g}	nd	0.17±0.01^{h}	0.15±0.01^{fg}	nd	nd	nd
	T4	0.12±0.01^{f}	0.07±0.01^{g}	nd	0.17±0.01^{h}	0.14±0.01^{i}	0.15±0.01^{e}	0.27±0.01^{d}	0.28±0.01^{d}
3E	T1	0.12±0.01^{f}	0.08±0.01^{ef}	0.08±0.01^{f}	0.23±0.01^{e}	0.15±0.01^{fg}	0.17±0.01^{d}	0.18±0.01^{hi}	0.17±0.01^{h}
	T2	nd	Nd	nd	0.23±0.01^{e}	nd	nd	0.17±0.01^{i}	0.17±0.01^{h}
	T3	nd	Nd	nd	nd	nd	nd	0.18±0.01^{hi}	nd
	T4	0.12±0.01^{f}	0.08±0.01^{ef}	0.11±0.01^{e}	0.21±0.01^{f}	0.21±0.01^{c}	0.18±0.01^{c}	0.23±0.01^{g}	0.20±0.01^{g}
HY	T1	0.18±0.01^{ab}	0.10±0.01^{d}	0.11±0.01^{e}	0.36±0.01^{b}	0.21±0.01^{c}	0.27±0.01^{b}	0.26±0.01^{e}	0.28±0.01^{d}
	T2	nd	Nd	nd	nd	nd	nd	nd	0.34±0.01^{c}
	T3	nd	Nd	nd	nd	nd	nd	nd	0.39±0.01^{b}
	T4	0.19±0.01^{a}	0.14±0.01^{b}	0.14±0.01^{d}	0.40±0.01^{a}	0.26±0.01^{b}	0.26±0.01^{b}	0.21±0.01^{f}	0.41±0.01^{a}
3L	T1	0.17±0.01^{bc}	0.15±0.01^{a}	0.21±0.01^{a}	0. 32±0.01^{c}	0.28±0.01^{a}	0.26±0.01^{b}	0.28±0.01^{c}	0.26±0.01^{e}
	T2	0.17±0.01^{cd}	0.12±0.01^{c}	0.20±0.01^{b}	0. 25±0.01^{d}	0.24±0.01^{b}	nd	0.26±0.01^{e}	0.20±0.01^{g}
	T3	0.15±0.01^{e}	0.10±0.01^{e}	0.17±0.01^{c}	0.18±0.01^{h}	0.18±0.01^{d}	nd	0.19±0.01^{h}	0.17±0.01^{h}
	T4	0.12±0.01^{f}	0.07±0.01^{g}	nd	0.12±0.01^{k}	0.15±0.01^{gh}	0.27±0.01^{b}	0.15±0.01^{j}	0.15±0.01^{i}
P-Values		0.001***	0.06*	0.01**	0.001***	0.02**	0.00***	0.00***	0.00***

Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Arginine (Arg), Serine (Ser), Glycine (Gly), Aspartic acid (Asp), Glutamine acid (Glu), Alanine (Ala), Proline (Pro) and Tyrosine (Tyr). nd = Not Detected. For all the values within a column, different letter superscripts mean ± standard deviation (n= 3) significant differences (p> 0.05). *: p> 0.05; **p> 0.01; ***: p> 0.001.

4.4.6 Effect of accessions and maturity stage on concentration of macro-and micronutrients in fruits of *Mimusops zeyheri*

The concentration of selected macro-and micronutrients across that five accessions of *M. zeyheri* harvested at different fruit maturity stages were markedly varied ($p > 0.05$). The particular nutrients were: phosphorus (P), potassium (K), calcium (Ca), magnesium (mg), sodium (Na), Iron (Fe), manganese (Mn) and zinc (Zn). Accession 3E revealed the highest concentration of P at T1 to T3 maturity stages (9.39, 9.39 and 6.83 mg/L), whilst accession 6E accumulated the highest P at T4 maturity stage (2.73 mg/L), as shown in Table 4.4. The mean values on the concentration of P shown in this study were lower when compared to that in other published studies including that by Mahmood *et al*. (2011) on strawberry (*Fragaria x ananassa*) as well as that by Hussain *et al*. (2010) on pomegranate at ripen stage and was higher in all fruit maturity stages. While P plays a multiple crucial roles, one of these is the development of bones and teeth. Also, it has a significant impact on how the body utilizes fats and carbohydrates. Lastly, it is needed for the body to produce proteins needed for the growth, maintenance, and repair of cells and tissues (Ndhlala and Tshabalala, 2023).

In this study, it was observed that accession 6E had highest concentration of K at T1 and T4 maturity stages (50.83 and 44.93 mg/L) compared to that in the other accessions. By contrast, accession HY recorded lowest K at T1 to T4 maturity stages ranging from (38.67, 38.47, 36.57 and 36.40 mg/L), respectively (Table 4.4). The concentration of K in fruits of the selected five accessions of *M. zeyheri* at T4 maturity stage was relatively lower as compared to that revealed in commercial and indigenous fruits such as banana which had mean K values (3580 mg/L) and *S. birrea* which revealed mean K values (2183 mg/100 g) Amarteifio and Mosase (2006) and USDA (2018). Kassim *et al*., (2017) reported that during the ripening stage, plants use potassium within the fruit to increase sugar levels to sweeten fruit so it is full of flavour. According to Marieb (2015), potassium is essential for ensuring proper functioning of the muscles and nerves including for synthesising protein and maintain a well-functioning metabolism. The National Academic of Science, Engineering and Medicine (NASEM) outlined that a daily intake of potassium from 2020-2025 for males between ages of 2-19 years is 2.4 mg/L and 1.9 mg/L for females of age of 2 to 19 years,

whereas in male adults aged 20 years and above, it is 3.016 mg/L and for women, it is 2.320 mg/L (Miltra *et al*., 2022). Fruits of *M. zeyheri* could be considered among solutions with regards to contributing towards the reduction of deficiencies of potassium.

The results presented in table 4.4, outline the concentration of Ca that was analysed at four fruit maturity stages at T1 to T4. Accession M7 had highest mean Ca (27.73 mg/L) at T1 maturity stage whilst that of accession 6E was increased at T2 and T3 maturity stages (21.50 and 17.17 mg/L) compared to other accessions (Table 4.4). Therefore, the concentration of Ca in the five accessions demonstrated a decrease from T1 to T4 maturity stages, and this trend was also reported in a study conducted by Chukwuka *et al*. (2013) using fruits of papaya (*Carica papaya* L.) at different stages of ripening. Moreover, accession HY exhibited the least Ca (11.60 mg/L) during the T1 maturity stage. According to Chukwuka *et al*. (2013), calcium plays an essential role in the bone structure including teeth development and functions. Fawole and Opara (2013) reported decreases in Ca in fruits of pomegranate as they reached the ripening stages and the decrease was attributed to the translocation of Ca into the seeds (sinks of Ca). Children between the ages of 4 and 8 require about 1000 mg/d of Ca due to their crucial stage of growth and development, because this mineral is a building block for healthy bones and teeth (FAO, 2004). Regular consumption of wild indigenous fruits could bridge gap of minerals deficiencies especially in rural communities (Sibiya *et al*., 2021). According to Napier and Combrink (2005), calcium is an essential element for fruit quality. In addition, calcium is necessary for cell division, elongation, and fruit growth with respect to activating enzymes (Torres *et al*., 2018). It contributes to stabilization of the cell wall, including making the cell wall permeable, which guards against enzymes breaking it down (Torres *et al*., 2018). Napier and Combrink (2005) state that high calcium content in fruits at unripe stage results in them becoming firmer which make their skin and flesh is less susceptible to breakdown disorders with reduced leakage through cell wall membranes, while good calcium content supply delays ripening and increases storability of fruits.

Accession M7 outperformed the rest of the accessions at T1 and T2 maturity stages as it recorded the highest Mg (5.96 and 4.37 mg/L), followed by accession 6E at T3 and T4 maturity stages of Mg (4.01 and 3.29 mg/L) respectively (Table 4.4). The

concentration of Mg shown in these results show differences compared to that by Seyhan *et al*. (2007); Alezandro *et al*. (2013) and Fawole and Opara (2013). Magnesium is essential for numerous bodily functions, including the production of protein, bone, and DNA as well as the control of blood pressure, blood sugar levels, and muscle and neuron function (Sojinu *et al*., 2021). Accession M7 revealed a markedly higher concentration of Na at T1, T2 and T4 maturity stages (5.63, 4.23 and 3.64 mg/L), while 6E accession had highest mean value (5.33 mg/L) at T4 maturity stage in Table 4.4. The concentration of Na decreased as the fruits reached the ripen stage, which was observed in the studies of Gudes *et al*. (2017) of Cagaita (*Eugenia dysenterica*) and Vita *et al*. (2020) of berries fruits.

Of the micronutrients, accession 6E accumulated the highest Fe at T1 to T3 maturity stages (1.57, 0.62 and 0.37 mg/L) compared to other accession (Table 4.5). Greater Fe in fruits has also been revealed in other scholarly studies, including that by Amarteifio and Mosase (2006). On the other hand, accession 3E had increased Fe at T4 maturity stage (0.49 mg/L). If the iron in the fruits were 100% bioavailable, one would need to consume 386.3 g of early stage or 300 g of late stage per day to acquire 18 mg of iron, whereas pregnant women would need to consume 579 g or 450 g of early stage or late stage per day (Diba *et al*., 2017). Trumbo *et al*. (2001) reported that the recommended daily intake of Fe for children between ages of 4-8 years should be less than 10 mg/d, meaning, fruits of *M. zeyheri* could address the need for iron in children. Despite being a micronutrient, iron is needed by the human body for the growth and development, generation of haemoglobin as well as protein in red blood cells that comes oxygen from the lungs to all parts of the body (Kermanshah *et al*., 2014 and Sibiya *et al*., 2021). Additionally, it makes it easier for proteins, carbohydrates, and fat to control body weight, which is a key factor in diabetes (Moses *et al*., 2012).

The concentration of Mn was significantly increased in accession 6E at T1 to T3 maturity stages and ranged from (0.22, 0.20, 0.17 and 0.14 mg/L), respectively (Table 4.5). On the other hand, fruits of accession 3L had lowest Mn at T1 and T2 maturity stages (0.07 and 0.06 mg/L), followed by that of accession 3E at T3 and T4 maturity stages, which had a similar mean value (0.01 mg/L). Significant differences in Mn between fruits of different plant accessions have been reported in literature, however,

the mean values shown in this study are not in agreement with that of results by Savikin *et al*. (2014) and Chawafambira *et al*. (2020). According to Marieb (2015), manganese is important for normal brain functions. The concentration of the other micronutrient, Zn, was markedly highest in accession 3E at T1 to T3 maturity stages (1.73, 1.10 and 0.60 mg/L), whereas that in accession 6E was increased at T4 maturity stage (0.41 mg/L) than other accessions (Table 4.5). The decrease in the concentration of Zn as fruits reach the ripening stage was also shown by Savikin *et al*. (2014). According Khawas and Deka (2016), zinc is required during rapid growth such as childhood, adolescence and pregnancy. It plays major role in DNA creation and supporting healthy immune system. Also, the micronutrient is essential and required for many cellular functions such as healthy growth, brain development, behavioural response, bone production and wound healing (Gudes *et al*., 2017). Fruits of the selected accession of *M. zeyheri* can contribute towards improving the recommended dietary allowance for zinc but to a lesser extent. The recommended daily intake of Zn ranges from 8-12 mg per day.

Table 4.4: List of mineral compositions of five accessions of *Mimusops zeyheri* fruits at four fruit maturity stages

Accessions	Fruit maturity stages	Macro mineral compositions (mg/L)				
		P	K	Ca	Mg	Na
6E	T1	6.44±1.78^{bc}	50.83±070^{a}	21.80±0.20^{b}	4.37±0.12^{b}	5.46±2.20^{ab}
	T2	5.50±1.01^{bcd}	50.00±783^{a}	21.50±0.14^{b}	4.25±0.15^{b}	5.33±1.36^{ab}
	T3	5.40±0.08^{bcd}	47.13±0.57^{abc}	19.63±0.47^{c}	4.01±0.04^{c}	3.92±0.34^{abcd}
	T4	2.73±1.16^{fghi}	44.93±0.28^{bcd}	17.17±0.15^{e}	3.29±0.09^{de}	3.33±0.07^{cdefg}
M7	T1	4.58±1.32^{de}	48.60±0.56^{ab}	27.73±0.50^{a}	5.96±0.06^{a}	5.63±0.60^{a}
	T2	4.47±0.55^{def}	45.87±0.40^{bc}	18.47±0.32^{d}	4.37±0.12^{b}	4.72±2.38^{abc}
	T3	2.95±2.59^{efghi}	45.77±0.12^{bc}	17.23±0.57^{e}	3.14±0.09^{ef}	4.23±1.41^{abcd}
	T4	1.19±0.70^{ij}	43.93±0.44^{cde}	16.47±0.58^{ef}	3.13±0.23^{efg}	3.64±1.11^{bcdef}
3E	T1	9.39±0.00^{a}	54.97±0.42^{bc}	15.60±0.36^{g}	2.99±0.13^{fg}	5.20±2.20^{abc}
	T2	9.39±0.00^{a}	45.60±0.17^{bc}	14.63±0.38^{h}	2.52±0.02^{h}	3.78±0.54^{abcdef}
	T3	6.83±3.21^{b}	40.70±0.70^{ef}	13.93±0.30^{hi}	2.40±0.26^{hi}	2.47±1.68^{defg}
	T4	2.60±0.24^{ghi}	39.80±0.11^{fg}	11.70±0.62^{l}	0.99±0.15^{k}	1.74±1.30^{g}
HY	T1	2.64±0.26^{fghi}	38.67±0.35^{fg}	14.00±0.44^{hi}	2.44±0.05^{hi}	2.44±0.19^{defg}
	T2	2.54±0.37^{hi}	38.47±0.49^{fg}	12.73±0.31^{jk}	2.38±0.04^{hi}	2.36±0.41^{defg}
	T3	2.36±0.30^{i}	36.57±0.40^{g}	12.93±0.31^{jk}	2.33±0.12^{i}	1.98±0.12^{fg}
	T4	0.19±0.08^{j}	36.40±0.46^{g}	11.60±0.30^{l}	2.05±0.08^{j}	1.57±0.32^{g}
3L	T1	4.89±0.78^{cd}	50.73±0.74^{a}	16.20±0.20^{fg}	3.34±0.09^{d}	5.17±1.38^{abc}
	T2	4.38±0.51^{defg}	48.70±0.66^{a}	15.60±0.36^{g}	3.20±0.06^{de}	3.78±0.91^{abcde}
	T3	4.20±0.51^{defgh}	41.70±0.44^{def}	13.63±0.96^{ij}	2.94±0.22^{g}	2.71±0.81^{defg}
	T4	2.59±0.46^{ghi}	40.20±5.98^{efg}	12.27±0.15^{kj}	2.46±0.40^{hi}	2.13±0.33^{efg}
P-Values		0.00***	0.00***	0.00***	0.00***	0.001***

Values are expressed as mean ± standard deviation (n= 3). For all the values within a column, different letter superscripts mean significant differences (p> 0.05). Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). P: Potassium, K: Phosphorus, Ca: Calcium, Mg: Magnesium, Na: Sodium. *: p> 0.05; **p> 0.01; ***: p> 0.001.

Table 4.5: List of mineral compositions of five accessions of *Mimusops zeyheri* fruits at four fruit maturity stages

Accessions	Fruit maturity stages	Micro mineral compositions (Mg/L)		
		Fe	Mn	Zn
6E	T1	1.57±0.07^{a}	0.22±0.01^{a}	0.66±0.03^{c}
	T2	0.62±0.16^{bc}	0.20±0.01^{ab}	0. 6±0.03^{c}
	T3	0.40±0.01^{efgh}	0.17±0.01^{bc}	0.18±0.10^{ef}
	T4	0.37±0.04^{fghi}	0.14±0.01^{d}	0.41±0.20^{d}
M7	T1	0.66±0.01^{b}	0.19±0.01^{abc}	0.38±0. 09^{d}
	T2	0.48±0.13^{def}	0.11±0.01^{de}	0.17±0. 04^{ef}
	T3	0.47±0.07^{def}	0.09±0.03^{fg}	0.16±0.18^{f}
	T4	0.44±0.04^{defg}	0.08±0.02^{fg}	0.06±0.18^{g}
3E	T1	0.55±0.06^{cd}	0.18±0.01^{bc}	1.73±0.19^{a}
	T2	0.51±0.15^{cd}	0.09±0. 03^{ef}	1.10±0.00^{b}
	T3	0.49±0.07^{def}	0.01±0.01^{k}	0.60±0.20^{c}
	T4	0.28±0.02^{ij}	0.01±0.01k	0.15±0.06^{f}
HY	T1	0.37±0.02^{ghij}	0.08±0.01^{fgh}	0.27±0.09^{h}
	T2	0.30±0.04^{hij}	0.07±0.01^{fgh}	0.16±0.03^{ef}
	T3	0.25±0.01^{j}	0.06±0.03^{fgh}	0.13±0.10^{f}
	T4	0.24±0.06^{j}	0.06±0.01^{fgh}	0.05±0.04^{g}
3L	T1	0.51±0.07^{de}	0.07±0.01^{ghi}	0.34±0.14^{de}
	T2	0.49±0.05^{de}	0.06±0.01^{hi}	0.24±0.04^{def}
	T3	0.35±0.02^{ghij}	0.04±0.01^{ij}	0.24±0.07^{def}
	T4	0.33±0.02^{ghij}	0.02±0.00^{jk}	0.17±0.09^{ef}
P-Values		0.00***	0.00***	0.00***

Values are expressed as mean ± standard deviation (n= 3). For all the values within a column, different letter superscripts mean significant differences (p> 0.05). Fruit maturity stages (T1: Green; T2: breaker; T3 pale yellow and T4: Yellow fruits). Fe: Iron, Mn: Manganese, Zn: Zinc. *: p> 0.05; **p> 0.01; ***: p> 0.001.

4.5 Conclusion

In this study, the results revealed that all five accessions of *M. zeyheri* fruits contained detectable amounts of nutritional, amino acids, and mineral compositions. In the main, the content and concentration of the determined nutritional parameters were significantly different between accessions and maturity stage ($p > 0.05$) of the selected nutritional and mineral compositions, the highest mean values were reported at T1 maturity stages and lowest at T4 maturity stages. The results on amino acids revealed variations in essential and non-essential attributes of five accessions at different fruit maturity stages. Therefore, accessions (6E, M7, 3E, HY and 3L) exhibited highest mean values of nutritional compared to the other accessions. The concentration of mineral nutrients was highest in accession (6E, 3E and M7). Of noteworthy, T1 to T3 maturity stages are unripe, quarter and semi-ripe fruits, intriguingly, the fruits exhibited the highest mean values of nutritional and mineral composition. The fruits can be recommended for food fortification program including agro-processing due to their merit to be utilized as food ingredients products such as jams, fermented juices, jellies and other products. The processing and preservation methods employed at the household level need to be improved in order to increase the availability of this food outside of its producing season. Based on these results, it is suggested that their inclusion could enhance the production of indigenous fruits as a long-term remedy for malnutrition in rural areas of developing nations. On the other hand, the T4 maturity stage involve fruits at the fully ripe stage and should be consumed as raw, dried, processed fruit for snacking. According to Omotayo and Aremu (2020), indigenous fruits that are native to the continent of Africa are key to the future of food security and reduced various deficiencies that may lie in underutilized of indigenous fruits. Lastly, in order to partake in the UN Sustainable Development Goals (UN SDG) 2030, the *M. zeyheri* as one of indigenous fruits could be excellent commencing point.

References

AACC, 1999. *Approved methods of the American Association of Cereal Chemists. Method 44- 15A. St*. Paul: AACC International.

Alezandro, M.R., Dubé, P., Desjardins, Y., Lajolo, F.M. and Genovese, M.I., 2013. Comparative study of chemical and phenolic of two species of *jaboticaba: Myrciaria jaboticaba* (Vell.) Berg and Myrciaria cauliflora (Mart.) O. Berg. *Food Research International*, 54(1): 468-477.

Amarteifio, J.O. and Mosase, M.O., 2006. The chemical composition of selected indigenous fruits of Botswana. *Journal of Applied Sciences and Environmental Management*, 10(2): 43-47.

AOAC, 1990. *Official methods of analysis (15th edition) Association of Official Analytical, Chemists.* Washington: AOAC International.

AOAC, 2005. *Official Methods of Analysis (18th edition) Association of Official Analytical, Chemists.* Maryland: AOAC International.

Boggio, S.B., Palatnik, J.F., Heldt, H.W. and Valle, E.M., 2000. Changes in amino acid composition and nitrogen metabolizing enzymes in ripening fruits of *Lycopersicon esculentum* Mill. *Plant Science*, 159(1): 125-133.

Chawafambira, A., Sedibe, M.M., Mpofu, A. and Achilonu, M., 2020. Uapaca kirkiana, an indigenous fruit tree in sub-Saharan Africa: A comprehensive review. *Cogent Food and Agriculture*, 6(1): 1766735.

Chukwuka, K.S., Iwuagwu, M. and Uka, U.N., 2013. Evaluation of nutritional components of Carica papaya L. at different stages of ripening. *IOSR Journal of Pharmacy and Biological Sciences*, 6(4): 13-16.

Diba, D., Bultosa, G. and Tolesa, G.N., 2017. Effect of maturity stages on nutritive quality and sensory properties of Fig fruits. *The Future of Biology* 10(18): 57-68.

Dunstan, R.H., Sparkes, D.L., Macdonald, M.M., De Jonge, X.J., Dascombe, B.J., Gottfries, J., Gottfries, C.G. and Roberts, T.K., 2017. Diverse characteristics of the urinary excretion of amino acids in humans and the use of amino acid

supplementation to reduce fatigue and sub-health in adults. *Nutrition Journal*, 16(1): 1-11.

Edgar, A.J., 2002. The human L-threonine 3-dehydrogenase gene is an expressed pseudogene. *BMC Genetics*, 3(1): 1-13.

Emaga, T.H., Andrianaivo, R.H., Wathelet, B., Tchango, J.T. and Paquot, M., 2007. Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. *Food Chemistry*, 103(2): 590-600.

Famuyini, J., Patrick, O.A. and Sedara, A., 2020. Effect of maturity stage on quality and shelf life of tomato (*Lycopersicum esculentum* Mill) using refrigerator storage system. *Eurasian Journal of Agricultural Research*, 4(1): 23-44.

FAO, 2004. Regional Overview of Food Security and Nutrition in Africa 2016. The challenges of building resilience to shocks and stresses.

FAO, IFAD, UNICEF, WFP, and WHO, 2017. State of food insecurity in the world. Building resilience for peace and food security. *FAO, Rome.*

Fawole, O.A. and Opara, U.L., 2013. Developmental changes in maturity indices of pomegranate fruit: A descriptive review. *Scientia Horticulturae*, 159: 152-161.

Gudes, M.N.S., Rufini, J.C.M., Marques, T.R., Melo, J.O.F., Ramos, M.C.P. and Viol, R.E., 2017. Minerals and phenolic compounds of cagaita fruits at different maturation stages (*Eugenia dysenterica*). *Revista Brasileira de Fruticultura*, 39(1). 1568-1576.

Haggag, H., Hossny, M., Nahavandi, S. and Creighton, D., 2013, April. Real time ergonomic assessment for assembly operations using kinect. In *2013 UKSim 15th International Conference on Computer Modelling and Simulation*: 495-500.

Hussain, J., Rehman, N.U., Khan, A.L., Hamayun, M., Hussain, S.M. and Shinwari, Z.K., 2010. Proximate and essential nutrients evaluation of selected vegetables

species from Kohat region, Pakistan. *Pakistan Journal of Botany*, 42(4): 2847-2855.

Imran, M., Khan, H., Shah, M., Khan, R. and Khan, F., 2010. Chemical composition and antioxidant activity of certain Morus species. *Journal of Zhejiang University Science B*, 11: 973-980.

Jia, T., Feng, C., Zou, S. and Gao, P., 2022. The Main Physicochemical Characteristics and Nutrient Composition during fruit ripening of *Stauntonia obovatifoliola* Subsp. Urophylla (Lardizabalaceae). *Horticultural*, 9(1): 29.

Kassim, N., Hambali, K. and Amir, A., 2017. Nutritional composition of fruits selected by long-tailed macaques (*Macaca fascicularis*) in Kuala Selangor, Malaysia. *Tropical Life Sciences Research*, 28(1): 91-101.

Kermanshah, A., Ziarati, P., Asgarpanah, J. and Qomi, M., 2014. Food values of two endemic wild almond species from Iran. *International Journal of Plant, Animal and Environmental Sciences*, 4(3): 380-388.

Khawas, P. and Deka, S.C., 2016. Isolation and characterization of cellulose nanofibers from culinary banana peel using high-intensity ultra-sonication combined with chemical treatment. *Carbohydrate polymers*, 137: 608-616.

Koide, S. and Sidhu, S.S., 2009. The importance of being tyrosine: lessons in molecular recognition from minimalist synthetic binding proteins. *ACS Chemical Biology*, 4(5): 325-334.

Koyuncu, F., Çetinbas, M. and Erdal, İ., 2014. Nutritional constituents of wild grown black mulberry (*Morus nigra* L.). *Journal of Applied Botany and Food Quality*, 87.

Kumari, P. and Khatkar, B.S., 2018. Nutritional composition and drying kinetics of aonla fruits. *Journal of Food Science and Technology*, 55: 135-3143.

Mahmood, T., Anwar, F., Abbas, M., Boyce, M.C. and Saari, N., 2012. Compositional variation in sugars and organic acids at different maturity stages in selected small fruits from Pakistan. *International Journal of Molecular Sciences*, 13(2): 1380-1392.

Maisarah, A.M., Asmah, R. and Fauziah, O., 2014. Proximate analysis, antioxidant and anti-proliferative activities of different parts of Carica papaya. *Journal of Tissue Science and Engineering*, 5(1): 1.

Marieb, E.N., 2015. Essentials of human anatomy and physiology. *British Journal of Nursing*, 31(17): 886-892.

Mitra, S., Paul, S., Roy, S., Sutradhar, H., Bin Emran, T., Nainu, F., Khandaker, M.U., Almalki, M., Wilairatana, P. and Mubarak, M.S., 2022. Exploring the immune-boosting functions of vitamins and minerals as nutritional food bioactive compounds: a comprehensive review. *Molecules*, 27(2): 555.

Mokgalabone, T.T., Mpai, S. and Ndhlala, A.R., 2023. Organic Medium Enclosed Trough Growing Technique Improves *Abelmoschus esculentus* (Okra) Growth, Yield and Some Nutritional Components. *Applied Sciences*, 13(9): 5645.

Monti, L.L., Bustamante, C.A., Osorio, S., Gabilondo, J., Borsani, J., Lauxmann, M.A., Maulión, E., Valentini, G., Budde, C.O., Fernie, A.R. and Lara, M.V., 2016. Metabolic profiling of a range of peach fruit varieties reveals high metabolic diversity and commonalities and differences during ripening. *Food Chemistry*, 190: 879-888.

Moses, A.G., Maobe, M.A., Gatebe, E., Gitu, L. and Rotich, H., 2012. Profile of heavy metals in selected medicinal plants used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya. *Global Journal of Pharmacology*, 6(3): 245-251.

Mpai, S., Du Preez, R., Sultanbawa, Y. and Sivakumar, D., 2018. Phytochemicals and nutritional composition in accessions of Kei-apple (*Dovyalis caffra*): Southern African indigenous fruit. *Food Chemistry*, 253: 37-45.

Napier, D.R. and Combrink, N.J.J., 2005, April. Aspects of calcium nutrition to limit plant physiological disorders. In *V International Pineapple Symposium* 702: 107-116.

Nayab, S., Razzaq, K., Ullah, S., Rajwana, I.A., Amin, M., Faried, H.N., Akhtar, G., Khan, A.S., Asghar, Z., Hassan, H. and Naz, A., 2020. Genotypes and harvest maturity influence the nutritional fruit quality of mulberry. *Scientia Horticulturae*, 266: 109311.

Ndhlala, A.R. and Tshabalala, T., 2023. Diversity in the Nutritional values of some Moringa *oleifera* Lam. Cultivars. *Diversity*, 15(7): 834.

Ngadze, R.T., 2018. *Value addition of Southern African monkey orange (Strychnos spp.): composition, utilization and quality* (Doctoral dissertation, Wageningen University and Research).

Ngadze, R.T., Linnemann, A.R., Nyanga, L.K., Fogliano, V. and Verkerk, R., 2017. Local processing and nutritional composition of indigenous fruits: The case of monkey orange (*Strychnos* spp.) from Southern Africa. *Food Reviews International*, 33(2): 123-142.

Nieman, D.C. and Lee, R., 2019. *Nutritional assessment*. United States of America: McGraw-Hill Education.

Omotayo, A.O. and Aremu, A.O., 2020. Underutilized African indigenous fruit trees and food–nutrition security: Opportunities, challenges, and prospects. *Food and Energy Security*, 9(3): 220.

Šavikin, K., Živković, J., Zdunić, G., Gođevac, D., Đorđević, B., Dojčinović, B. and Đorđević, N., 2014. Phenolic and mineral profiles of four Balkan indigenous

apple cultivars monitored at two different maturity stages. *Journal of Food Composition and Analysis*, 35(2): 101-111.

Sensoy, R.I.G., 2015. Determination of organic acids, sugars, and macro-micro nutrient contents of must in some grape (*Vitis vinifera* L.) cultivars. *JAPS: Journal of Animal and Plant Sciences*, 25(3).

Sereno, A.B., Bampi, M., dos Santos, I.E., Ferreira, S.M.R., Bertin, R.L. and Krüger, C.C.H., 2018. Mineral profile, carotenoids and composition of cocona (*Solanum sessiliflorum Dunal*), a wild Brazilian fruit. *Journal of Food Composition and Analysis*, 72: 32-38.

Seyhan, F., Ozay, G., Saklar, S., Ertaş, E., Satır, G. and Alasalvar, C., 2007. Chemical changes of three native Turkish hazelnut varieties (*Corylus avellana* L.) during fruit development. *Food Chemistry*, 105(2): 590-596.

Sibiya, N.P., Kayitesi, E. and Moteetee, A.N., 2021. Proximate analyses and amino acid composition of selected wild indigenous fruits of Southern Africa. *Plants*, 10(4): 721.

Sojinu, O.S., Biliaminu, N.T., Mosaku, A.M., Makinde, K.O., Adeniji, T.H. and Adeboye, B.M., 2021. The implications of ripening agents on chemical of plantain (*Musa paradisiaca*). *Heliyon*, 7(6): 07123.

Torres-León, C., Ramírez-Guzman, N., Londoño-Hernandez, L., Martinez-Medina, G.A., Diaz-Herrera, R., Navarro-Macias, V., Alvarez-Pérez, O.B., Picazo, B., Villarreal-Vázquez, M., Ascacio-Valdes, J. and Aguilar, C.N., 2018. Food waste and by products: An opportunity to minimize malnutrition and hunger in developing countries. *Frontiers in Sustainable Food Systems*, *2*: 52.

Trumbo, P., Yates, A.A., Schlicker, S. and Poos, M., 2001. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *Journal of the Academy of Nutrition and Dietetics*, 101(3): 294-301.

USDA, 2018. National Food and Nutrient Analysis Program Wave. Beltsville: Nutrient Data Laboratory.

Yan-hui, C., Hui-fang, G., Sa, W.A.N.G., Xian-yan, L., Qing-xia, H., Zai-hai, J., Ran, W., Jin-hui, S. and Jiang-li, S., 2022. Comprehensive evaluation of 20 pomegranate (*Punica granatum* L.) cultivars in China. *Journal of Integrative Agriculture*, 21(2): 434.

CHAPTER 5

Interaction effect between accessions and fruit maturity stages on untargeted secondary metabolites of *Mimusops zeyheri* Sond.

Abstract

Mimusops zeyheri is one of the most important indigenous fruits however, it has been neglected and undervalued in Sub-Saharan Africa. A wide variety of edible wild indigenous fruits can be found in Southern Africa where are used as sources of food by communities. Currently, literature rarely has studies showing comprehensive information about the metabolites profile of *M. zeyheri* fruits. The objective of this study was to investigate whether accessions and fruit maturity stages affect untargeted secondary metabolites profile of *M. zeyheri*. Ultra-performance liquid chromatography quadrupole time of flight mass spectrometer was used for the analysis of methanol extracts. In the phenolic and flavonoids chemical classes, 22 polar analyses were identified using the Ultra-performance liquid chromatography mass spectrometer untargeted secondary metabolites profile. A principal components analysis plot revealed fruits of *M. zeyheri* had five major clusters based on the heterogeneity of metabolites. The highest mean value of 3,4-bis(acetyloxy)-5-acetamido-6-(3-nitrophenoxy)oxan-2-yl]methyl acetate was recorded in accession 3L at the T1 stage (719.90 mg/kg) while Paeonoside was 48.98 mg/kg at the T4 maturity stage of 7-oxo-8,9-dihydroxy-4'-N-demethyl staurosporine (122.48 mg/kg). The highest mean value was shown by accession HY at the T4 maturity stage (646.70 mg/kg) of 3,4-bis(acetyloxy) acetate. The three most abundant flavonoids metabolites found in *M. zeyheri* were quercetin, quercetin galactoside, and quercetin glucoside. The results revealed that *M. zeyheri* fruit possess high level of concentration at T1 maturity stage could contribute to food fortification, while T4 maturity stage which could contribute and serve as food source especially for vulnerable rural communities that need food secured.

Keywords: Accessions, Flavonoids, Food security, Fruit maturity stage, Metabolites.

5.1 Introduction

In rural Sub-Saharan Africa, indigenous fruits are harvested from the wild, consumed and they contribute to the nutrition of rural communities (Pfukwa *et al*., 2020). Literature show that in the rest of developed communities, the potential of indigenous fruits remain untapped on the societal and economic fringes and therefore their commercial market sector remain unknown (Mabhaudhi *et al*., 2019). According to Omotayo *et al*. (2020), there is sufficient evidence that numerous African countries are hosts to a wealth of plant biodiversity, offering great opportunities for both dietary diversity and economic growth, however, the majority of these resources are not fully explored. Therefore, exploring these underutilized resources is crucial for both food and nutritional security as well as economic development, and one such resource is *M. zeyheri* (Omotayo *et al*., 2020). The search for beneficial secondary metabolites molecules, such as polyphenolic chemicals such as (phenolic acids, flavonoids, hydroxycinnamic acids, hydroxybenzoic acids, and stilbenes) has garnered more attention in recent decades with regard to human nutrition (Granato *et al*., 2020). Moreover, consuming polyphenol sources regularly promotes a balanced diet and offers health benefits like antioxidant, cardiovascular, antithrombotic, anti-inflammatory, and anticancer properties (Granato *et al*., 2020). Sources of polyphenol include seafood, plants, fruits, vegetables, juices, and drinks (Kharbach *et al*., 2022). According to Granato *et al*. (2018), specific chemical metabolites, including polyphenolic compounds, have an impact on the characteristics, color, taste, and nutritional value of food.

Various epidemiological studies indicate that diet that is high in secondary metabolites including antioxidants plays preventative functions in both health and diseases (Fawole *et al*., 2012). Furthermore, natural antioxidants may be vital in fight against oxidative stress, which is linked to degenerative illness such as cancer, cardiovascular disease, diabetes and aging (Fawole *et al*., 2012). The health benefits associated with the consumption of fruits and vegetables are from the secondary metabolites such as carotenoids and wide range of poly phenolics (Zhang *et al*., 2008). According to Fawole *et al*. (2012), fruits and vegetables that exhibit high phenolic compounds have markedly higher antioxidants properties which enables them to scavenge radicals. The identification and quantification of small-molecule metabolites in the metabolome are

the main goals of a niche are referred to as metabolomics (Xiao *et al*., 2012). The approach makes it easier to comprehend how complicated systems' biological and biochemical processes work (Bowen and Northen, 2010). Current metabolomics investigations can be divided into two category: targeted and untargeted. The targeted method concentrates on the examination of a particular class of molecules, a set of metabolites connected to a particular metabolic pathway, or both, while untargeted metabolites approach is worldwide analysis of metabolic changes brought on by disease, environmental changes, or genetic modifications. Typically, the untargeted technique is used to generate hypotheses, followed by targeted profiling for more certain quantification of pertinent metabolites (Xiao *et al*., 2012). Note that in this study focuses on the untargeted secondary metabolites.

It is critical to recognize the functional chemicals and comprehend their functions in order to support the medical, dietary, and food value of *M. zeyheri* fruits. In this context, it ought to emphasize the identification of phyto-constituents from diverse analyses, taking into account the beneficial or harmful effects of metabolite-metabolite interactions for specific bioactivities (Lim *et al*., 2020). The techniques required for analysing and maybe identifying all the metabolites with bioactive qualities are provided by plant metabolomics (Lim *et al*., 2020). The Sapotaceae family is well known for their wide range of phytochemicals presents in fruit such as saponins, flavonoids and poly phenolics (Chivandi, 2013). This chapter presents the results of untargeted secondary metabolites of five accessions of *M. zeyheri* harvested at two different fruit maturity stages.

5.2 Objective of the study

The objective of this chapter was to investigate whether fruits of *M. zeyheri* harvested from different accessions and picked at different maturity stages show untargeted and semi-targeted secondary metabolites.

5.3 Materials and methods

5.3.1 Study location

The Study location is described in section 3.3.1. Analysis of metabolites were performed as CAF at the University of Stellenbosch.

5.3.2 Research design and treatments

The research design and treatments are described in 3.3.2 and sample preparation were similar to those described in section 4.3.3

5.3.4 Data collection

5.3.4.1 Extraction of untargeted metabolites for UPLC analysis

By weighing a 2 g sample into a 50 ml centrifuge tube with a screw-cap, polar untargeted metabolites were recovered. The tubes were firmly closed after adding 15 ml of a mixture of 50% methanol and 1% formic acid. The samples were then vortexed for 1 minute, then extraction in an ultrasonic bath took place for 1 hour. After that, a 2 ml sample was taken out and centrifuged for 5 minutes at 14,000 rpm. The crystal clear supernatant was then put into 1.5 ml glass vials for evaluation.

5.3.4.2 Determination of untargeted secondary metabolites

In order to conduct high-resolution UPLC-MS analysis of untargeted metabolites, a Waters Synapt G2 Quadrupole Time of Flight (QTOF) Mass Spectrometer (MS) was connected to a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) (Waters, Milford, MA, USA). In order to gather UV and MS spectra simultaneously, column eluate was first routed through a Photodiode Array (PDA) detector before being sent to the mass spectrometer. The rest of the MS settings were optimized for the optimum resolution and sensitivity. Electrospray ionization was employed in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, and desolvation gas at 650 L/h. Data were collected by scanning in resolution mode and MSE mode between 150 and 1500 m/z. Two channels of MS data were captured in MSE mode, the first at low collision energy (4 V), and the second at a collision energy ramp (40-100 V), which also allowed for the acquisition of fragmentation data. For precise mass measurement, leucine enkephalin was employed as the lock mass

(reference mass), and sodium formate was used to calibrate the equipment. On a Waters HSS T3, 2.1 100 mm, 1.7 m column, separation was accomplished. The mobile phase contained acetonitrile with 0.1% formic acid as solvent B and 0.1% formic acid as solvent A in a 2 l injection volume. The gradient varied linearly from 100% solvent A to 28% B during the course of 22 minutes. It then transitioned to 40% B over 50 s, a wash phase of 1.5 min at 100% B, and 4 min of re-calibration to baseline settings. The column temperature was kept at 55 °C and the flow rate was 0.3 ml/min. The filtered material was injected onto a Brownlee Analytical C18 column (100 4.6 mm; 5 m particle size) using a 10 L volume (Perkin Elmer, Bridgeport Avenue, Shelton, USA). The mobile phase, which was a solution of water, acetonitrile, and acetic acid (78:20:2, v/v/v) that had been filtered under vacuum through a 0.45 m membrane prior to use at a flow rate of 1.0 ml/min at 30 ˚C, was eluted using an isocratic method. 10 ml of the mobile phase were used to re-calibrate the column in between injections of the sample. A wavelength of 280 nm was used for the detection process. Using a calibration curve created by infusing a range of catechin standards between 0.5 and 100 mg/l catechin, compounds were measured relative to that curve.

5.3.5 Statistical analysis

Untargeted metabolites data were processed for five accessions at two different fruit maturity stages with complete randomized design (CRD) using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) 1,2 SWATH-MS/MS and DIA-MS: MS-DIAL: data independent MS/MS deconvolution for comprehensive metabolome analysis. Nature Methods 12.523-526.2015 identifying metabolites by combining mass spectrometry cheminformatics with metabolome databases. Exploratory principle analysis (PCA) was used to analyse Nature Methods, 15.53-56.2018. Statistical analysis of biochemical data was performed using GenStat 18th Edition (VSN International, Hempstead, UK). Using a Duncan at the significance level of 5%, the mean separation for relevant treatments was obtained.

5.4 Results and discussions

5.4.1 Untargeted secondary metabolites identification and multivariate analysis in five accessions *of Mimusops zeyheri* at two different fruit maturity stages

After the analysis of the quality and nutritional composition, their analysis showed no significant variation between T1 and T2 which were all at unripe stages. On the other hand, a similar trend of results was observed for T3 and T4 ripe fruit. Therefore, only T1 and T4 maturity stages were analysed for secondary metabolites to observe a clear trend. 22 different m/z signals were detected and the detected metabolites were within the polyphenolics and flavonoids compounds as shown in Table 5.1.

An organic heterooctacyclic compound and a member of phenols identified was 7-oxo-8,9-dihydroxy-4'-N-demethyl staurosporine at rt:10.114 and m/z of 497.14825 substituted by hydroxy groups, whilst 6-Hydroxysandoricin was detected at rt of 16.745 and m/z of 557.2443. The identified compounds included [3,4-bis(acetyloxy)-5-acetamido-6-(3-nitrophenoxy)oxan-2-yl]methyl acetate; 7-oxo-8,9-dihydroxy-4'-N-demethyl staurosporine; unknown sulfate; Paeonoside; unknown alkaloid; sulfate compound; 6-Hydroxysandoricin; Acetovanillone Apocynin; Myricitrin; Quercetin galactoside; Quercetin glucoside. In the current study, major phenolic identified and detected was acetovanillone; apocynin at retention time (rt) of 15.612 and mass time (m/z) of 165.05455. On the other hand, the selected fruits exhibited flavonoids including Quercetin galactoside at rt of 17.852 and m/z of 463.08307; Quercetin glucoside at rt of 18.152 and m/z of 463.08774 and Quercitrin at rt of 19.946 and m/z of 447.09332 (Table 5.1). Furthermore, other phenolic compounds that were identified and detected were Paeonoside at rt of 13.139 and m/z of 327.108 and glycosyloxyflavone at rt of 17.621 and m/z of 463.08521 identified as Myricitrin. The identified and detected metabolites were present in all five accessions at two different fruit maturity stages. Also, there were differences among the detected compounds, the method of extraction and the equipment used may be identified as the major attributes. Gas Chromatography Mass Spectrophotometry (GC-MS) was used to separate and identify phenolic compounds in the Beer (2006) and Loots *et al*. (2006). However, phenolic compound detection by using GC-MS was limited due to the low volatility of these compounds (Robbins, 2003).

Table 5.1: Tentative peak assignment of the phenolic metabolites detected in five accessions of *Mimusops zeyheri* harvested at two different fruit maturity stages

Molecule no.	Mass to charge (m/z)	Retention time (rt)	Molecular formula	Fragment ion	Compound names
1	467.1305	8.451	$C_{20}H_{24}N_2O_{11}$	425.351.303.231	[3,4-bis(acetyloxy)-5-acetamido-6-(3-nitrophenoxy)oxan-2-yl]methyl acetate
2	467.1343	9.514	$C_{20}H_{24}N_2O_{11}$	425.351.303.231	[3,4-bis(acetyloxy)-5-acetamido-6-(3-nitrophenoxy)oxan-2-yl]methyl acetate
3	497.14825	10.114	$C_{21}H_{26}N_2O_{12}$	439.241	7-oxo-8,9-dihydroxy-4'-N-demethyl staurosporine
4	481.14581	11.252	$C_{30}H_{26}O_4S$	451.351.231.189.97	unknown sulfate
5	481.14993	12.413	$C_{30}H_{26}O_4S$	449.351.287.231.189.97	unknown sulfate
6	327.108	13.139	$C_{15}H_{20}O_8$	205.147.85	Paeonoside
7	401.1494	13.244	$C_{24}H_{22}N_2O_4$	269.161.101	unknown alkaloid
8	481.1027	13.772	$C_{18}H_{26}O_{13}S$	387.336.292.153	sulfate compound
9	165.05455	15.612	$C_9H_{10}O_3$	147.135.127	Acetovanillone;Apocynin
10	495.1165	16.156	$C_{15}H_{28}O_{18}$	173.316.479.495	Unknown
11	557.2443	16.745	$C_{23}H_{42}O_{15}$	425.161.89	6-Hydroxysandoricin
12	473.08423	16.917	$C_{23}H_{22}O_7S_2$	149.179.293.311	Sulfate
13	425.20673	17.202	$C_{18}H_{34}O_{11}$	311.263.161.101	unknown
14	505.15631	17.542	$C_{18}H_{34}O_{14}S$	316.241.15.97	unknown sulfate
15	463.08521	17.621	$C_{21}H_{20}O_{12}$	316.179	Myricitrin
16	463.08307	17.852	$C_{21}H_{20}O_{12}$	300.271	Quercetin galactoside
17	463.08774	18.152	$C_{21}H_{20}O_{12}$	300.271	Quercetin glucoside
18	321.15381	18.211	$C_{14}H_{26}O_8$	265.191.179	Butyl (S)-3-hydroxybutyrate glucoside
19	447.09332	19.946	$C_{21}H_{20}O_{11}$	301.300.271	Quercitrin
20	431.10229	21.949	$C_{21}H_{20}O_{10}$	285.255.111	Kaempferol rhamnoside
21	347.24515	23.919	$C_{18}H_{36}O_6$	347	Sativic acid
22	476.27628	24.4	$C_{30}H_{39}NO_4$	279.153	unknown

5.4.2 Variation of untargeted secondary metabolites in five *Mimusops zeyheri* accessions harvested at two fruit maturity stages

Of the selected accessions harvested at two fruit maturity stages, there were significant variations ($p > 0.05$) in the untargeted secondary metabolites, as shown in Table 5.2. However, the quantities of metabolites present within the fruits differed in terms various accessions and two different fruit maturity stage T1: Green fruit that are unripen and T4: Yellow fruit that are fully ripen. Therefore, to capture variation of visual metabolites presents, to spot the movement of metabolomics change induced by five accessions of *M. zeyheri* and two different fruit maturity stages, multivariate analyses were performed. In order to generate multidimensional data that illustrated the biochemical changes pertaining to five accessions and two fruit maturity stages, statistical analyses were performed (Figure 5.1). Five accessions and fruit maturity stages were analysed using unsupervised principal component analysis (PCA), resulting in a dimensional exploratory non-biased overview (Figure 5.1). Based on the five accessions and fruit development stages, the results exhibited five clusters (Figure 5.1). The PCA plot (Figure 5.1) demonstrated clear groupings of the samples according to the accessions but there was no clear clustering pattern related to two different fruit maturity stages. This was demonstrated by the first cluster which showed commonalities in metabolites across all fruit development stages in the accession 6E, and the second cluster in the accession 3E. Regardless of the phases of fruit development, the third cluster clearly demonstrated metabolite similarities between accessions 3E, HY, and M7. Accession 3L had highest mean value of 3,4-bis(acetyloxy)-5-acetamido-6-(3-nitrophenoxy)oxan-2-yl]methyl acetate at T1 maturity stage (719.90 mg/kg), whilst accession HY had highest value at T4 maturity stage (646.70 mg/kg). The content of 7-oxo-8,9-dihydroxy-4'-N-demethyl staurosporine recorded was highest in accession 6E at T1 maturity stage (129.06 mg/kg) and accession 3L at T4 maturity stage (122.48 mg/kg), respectively (Table 5.2). Therefore, accession 3L harvested at T1 maturity stage showed a higher mean value of Paeonoside (48.98 mg/kg). On the other hand, accession 3E depicted markedly increased higher value at T4 maturity stage (268.59 mg/kg) compared to the other accessions. Moreover, the sulfate compound showed significantly greater mean values at T1 and T4 maturity stages (9158.43 and 159.86 mg/kg), respectively. Furthermore, the content of Myricitrin was the most abundant in the accession 3L

when compared to other accessions at T1 and T4 maturity stages (771.08 and 741.45 mg/kg) in Table 5.2. On the other hand, quercetin galactoside showed highest mean values in 3L accession at T1 and T4 maturity stages (2207 and 3899.50 mg/kg).

Lastly, the findings indicated that Quercitrin, Quercetin galactoside, and Quercetin glucoside were the most prevalent flavonoids metabolites discovered in *M. zeyheri*. However, all of the polyphenol metabolites varied significantly between the T1 and T4 maturity stages in five accessions of *M. zeyheri*. According to Claude *et al*. (2021), state plant secondary metabolites are a class of organic molecules that help protect plants from biotic and abiotic stress. Although they are not necessary for plant growth, plant secondary metabolites play a critical role in how plants react to stressful conditions. Recent research on the metabolism of individual plant tissues revealed interdependencies between some tissues that contain particular metabolite groups (Darwish *et al*., 2021). Since metabolites are the end products of interactions between the genetic system and the environment, they are also a part of the biological systems control (Moing *et al*., 2020). The results of this study have demonstrated the variation of five accessions and two different fruit maturity stages in phenolic compounds metabolites. The metabolites form part of the phosphoenolpyruvate from the glycolysis pathway and erythrose 4-phosphate (E4- P) from the non-oxidative branch of the pentose phosphate pathway are transformed into chorismate through the shikimate pathway, also known as the chorismate biosynthesis pathway (Tzin and Galili, 2010). Moreover, this study is the first to report untargeted secondary metabolites at two different fruit maturity stages. Similar metabolites have been detected and quantified in five accessions of *M. zeyheri* such as myricitrin, quercetin galactoside, quercetin glucoside and quercitrin in a study by Mpai *et al*. (2018), accessions of Kei-apple (*Dovyalis caffra*), and Bowen-Forbes *et al*. (2010) of Blackberries, raspberries. Therefore, this study reveals that *M. zeyheri* accessions are rich source of untargeted secondary metabolites which are vital for the health benefits of human-being.

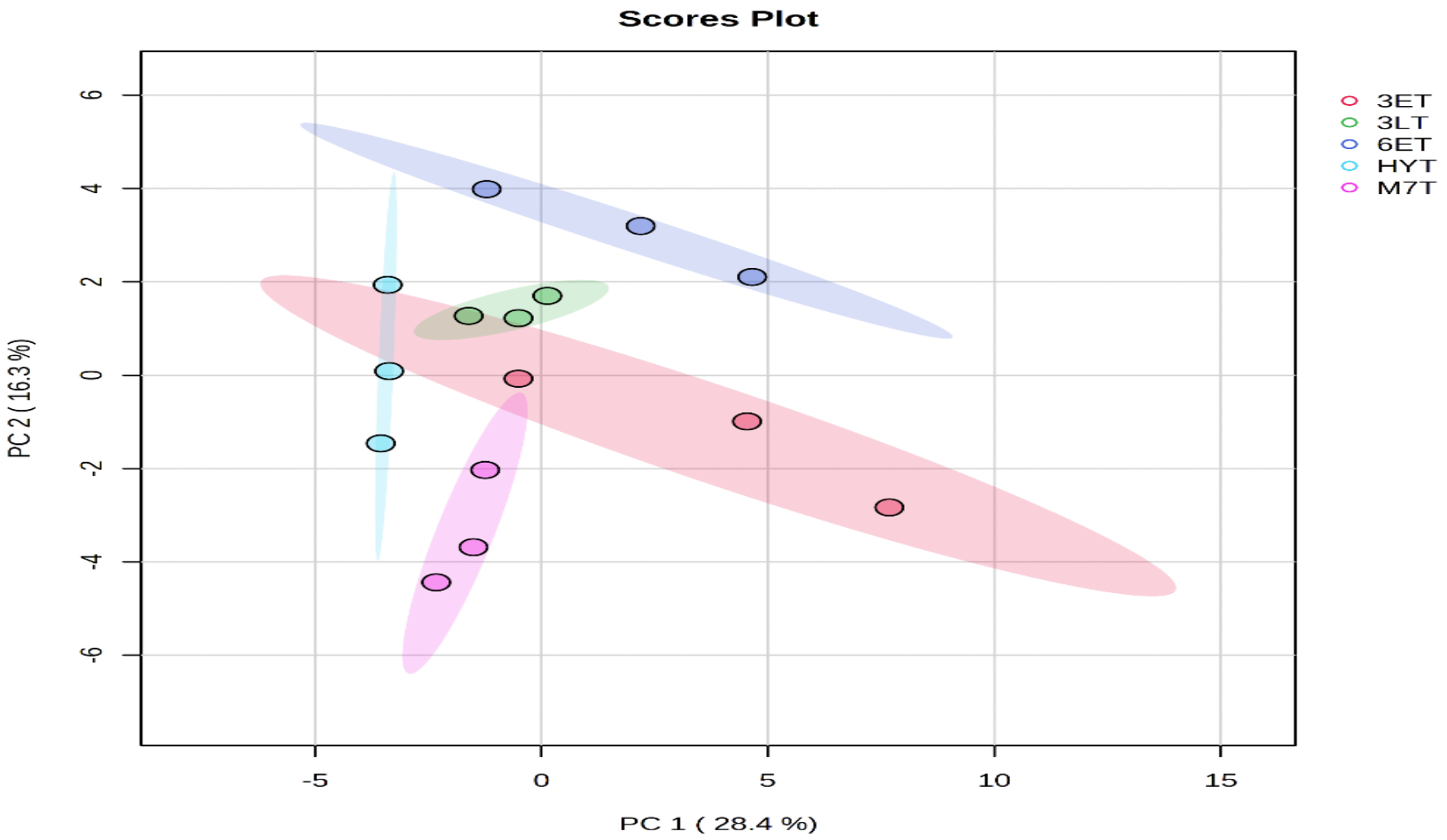

Figure 5.1: Principal Component Analysis plot.

Table 5.2: Quantification of the phenolic metabolites present in five accessions of *Mimusops zeyheri* harvested at two different fruit maturity stages

Compounds (mg/ kg)	Accessions									
	HY		3E		M7		6E		3L	
	T1	T4	T1	T4	T1	T4	T1	T4	T1	T4
3,4-bis(acetyloxy) acetate	708.03±7.07^{b}	646.70±0.71^{c}	590.24±0.71^{e}	258.03±0.71^{i}	614.00±0.71^{d}	565.86±0.71^{f}	708.22±2.12^{b}	372.90±0.71^{h}	719.90±1.41^{a}	501.48±0.71^{g}
7-oxo-demethyl staurosporine	76.04±0.72^{e}	51.79±0.71^{g}	71.30±1.41^{f}	125.18±1.41^{b}	103.05±1.41^{d}	70.81±0.71^{f}	31.02±1.41^{i}	129.06±0.71^{a}	122.48±2.12^{c}	47.41±0.71^{h}
Paeonoside	23.18±0.72^{f}	21.17±1.41^{f}	37.69±2.21^{d}	268.59±1.41^{a}	34.75±1.41^{d}	65.39±0.71^{b}	28.75±1.41^{e}	24.79±1.41^{ef}	48.98±1.41^{c}	28.04±0.71^{e}
Alkaloid derivative	94.67±0.72^{c}	108.26±0.71	36.88±0.71^{h}	32.88±1.41^{j}	34.85±0.71^{i}	102.30±1.41^{b}	52.97±1.41^{e}	54.49±1.41^{d}	42.53±0.71^{g}	48.36±0.71^{h}
Acetovanillone	518±0.72^{d}	386±1.41^{g}	488±1.41^{f}	896±1.41^{a}	498±1.41^{e}	542±1.41^{c}	278±0.71^{i}	124±1.41^{j}	666.50±0.71^{b}	294.50±0.71^{h}
6-Hydroxysandoricin	3.13±0.71^{f}	4.15±0.71^{f}	3.14±0.71^{f}	396.66±2.82^{a}	3.92±0.71^{f}	0.05±0.71^{g}	7.24±0.71^{e}	140.07±1.41^{b}	45.94±0.71^{c}	28.23±0.71^{d}
Sulfate	76.44±0.71^{a}	48.52±0.71^{b}	37.82±0.71^{e}	7.08±0.71^{h}	40.88±0.71^{c}	39.55±1.41^{d}	41.69±0.71^{c}	17.10±0.71^{g}	22.84±0.71^{f}	17.71±1.41^{g}
Sulfate derivative	5.97±0.71^{h}	32.58±0.71^{f}	6.34±0.71^{h}	242.08±2.12^{b}	12.35±0.71^{g}	1.84±0.14^{i}	68.82±0.71^{e}	285.05±1.41^{a}	108.18±0.71^{d}	129. 64±0. 71^{b}
Myricitrin	443.59±0.71^{d}	414.96±1.41^{e}	304.51±0.71^{i}	476.28±1.41^{c}	374.34±0.71^{f}	336.28±1.41^{h}	350.98±0.71^{g}	111.91±0.71^{j}	771.08±0.71^{a}	741.45±1.41^{b}
Quercetin galactoside	803.50±0.71^{h}	996.00±1.41^{g}	1614.00±1.41^{f}	394.00±1.41^{j}	1862.00±1.41^{c}	1732.00±1.41^{e}	1822.00±1.41^{d}	604.50±0.71^{i}	2207±1.41^{b}	3898.50±0.71^{a}
Quercetin glucoside	652.50±0.71^{f}	609.50±0.71^{g}	498.00±0.71^{h}	755.00±1.41^{e}	1342.50±0.71^{b}	1076.50±0.71^{c}	808.50±0.71^{d}	337.50±0.71^{i}	607.50±0.71^{g}	1482.50±0.71^{a}
Butyl (S)-3-hydroxybutyrate glucoside	10.02±0.71^{g}	3.57±0.71^{h}	11.05±0.71^{g}	230.62±1.41^{b}	10.34±0.71^{g}	448.32±0.71a	46.11±0.71^{f}	175.12±1.41^{c}	54.79±0.71^{e}	102.86±0.71^{d}
Quercitrin	1684.60±0.71^{d}	1373.90±1.41^{h}	1389.40±0.71^{g}	1673.60±2.12^{e}	1821.40±1.41^{a}	1558.70±0.71^{f}	1388.70±1.41^{g}	1154.90±1.41^{i}	1768.70±0.71^{b}	1753.50±1.41^{c}
Kaempferol rhamnoside	35.22±1.41^{h}	39.30±0.71^{g}	5.78±0.71^{i}	763.01±1.41^{a}	56.75±0.71^{e}	140.35±0.71^{c}	57.97±1.41^{e}	47.72±0.71^{f}	659.38±0.71^{b}	108.18±0.71^{d}
Sativic acid	264.06±1.41^{a}	193.17±0.71^{d}	112.64±0.71^{f}	197.04±2.12^{d}	238.75±0.71^{b}	196.97±3.54^{d}	195.31±2.12^{d}	175.73±0.71^{e}	215.20±0.71^{c}	109.13±0.71^{f}

Values are expressed as mean ± standard deviation (n= 3), column with different alphabetic letters a-j is significantly different (p≤ 0.05). Two fruit maturity stages: T1: green fruits and T4: yellow fruits.

5.5 Conclusion

The untargeted secondary metabolites profiling of five *M. zeyheri* accessions at two different fruit maturity stages were investigated as guided by untargeted metabolomics combined with multivariate analysis. In this study, 22 identified untargeted metabolites of several functional chemical groups, including polyphenolics, flavonoids and others. Statistical analysis revealed significant variations among the five accessions and two different fruit maturity stages. The quantification and comparison of the selected indigenous fruits, from different accessions of *M. zeyheri*, with the frequently consumed local fruits show that it is significant for dietary diversification or food supplementation and it will be helpful to encourage the cultivation of suitable accessions *M. zeyheri* fruits as a source of secondary metabolites for dietary supplement and potential for added value.

References

Beer, J.S., John, O.P., Scabini, D. and Knight, R.T., 2006. Orbitofrontal cortex and social behaviour: integrating self-monitoring and emotion-cognition interactions. *Journal of Cognitive Neuroscience*, *18*(6): 871-879.

Bowen, B.P. and Northen, T.R., 2010. Dealing with the unknown: metabolomics and metabolite atlases. *Journal of the American Society for Mass Spectrometry*, 21: 1471-1476.

Bowen-Forbes, C.S., Zhang, Y. and Nair, M.G., 2010. Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Journal of Food Composition and Analysis*, 23(6): 554-560.

Chivandi, E., 2013. *In vitro and in vivo chemical characteristics of kigelia Africana, Mimusops zeyheri, Terminalia serica and Ximenia caffra nuts and nuts meals* (Doctoral Thesis, University of Witwatersrand, South Africa).

Claude, S.J., Park, S. and Park, S.J., 2021. Transcriptome wide identification and quantification of caffeoylquinic acid biosynthesis pathway and prediction of its

putative BAHDs gene complex in A. spathulifolius. *International Journal of Molecular Sciences*, 22(12): 6333.

Darwish, A.G., Das, P.R., Ismail, A., Gajjar, P., Balasubramani, S.P., Sheikh, M.B., Tsolova, V., Sherif, S.M. and El-Sharkawy, I., 2021. Untargeted metabolomics and antioxidant capacities of muscadine grape genotypes during berry development. *Antioxidants*, 10(6): 914.

Fawole, O.A., Makunga, N.P. and Opara, U.L., 2012. Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complementary and Alternative Medicine*, 12(1): 1-11.

Granato, D., Mocan, A. and Câmara, J.S., 2020. Is a higher ingestion of phenolic compounds the best dietary strategy? A scientific opinion on the deleterious effects of polyphenols in vivo. *Trends in Food Science and Technology*, 98: 162-166.

Granato, D., Santos, J.S., Maciel, L.G. and Nunes, D.S., 2016. Chemical perspective and criticism on selected analytical methods used to estimate the total content of phenolic compounds in food matrices. *TrAC Trends in Analytical Chemistry*, 80: 266-279.

Kharbach, M., Viaene, J., Yu, H., Kamal, R., Marmouzi, I., Bouklouze, A. and Vander Heyden, Y., 2022. Secondary-metabolites fingerprinting of Argania spinosa kernels using liquid chromatography–mass spectrometry and chemometrics, for metabolite identification and quantification as well as for geographic classification. *Journal of Chromatography A*, 1670: 462972.

Lim, V., Gorji, S.G., Daygon, V.D. and Fitzgerald, M., 2020. Untargeted and targeted metabolomic profiling of Australian indigenous fruits. *Metabolites*, 10(3): 114.

Loots, D.T., van der Westhuizen, F.H. and Jerling, J., 2006. Polyphenol composition and antioxidant activity of Kei-apple (Dovyalis caffra) juice. *Journal of Agricultural and Food Chemistry*, 54(4): 1271-1276.

Mabhaudhi, T., Chimonyo, V.G.P., Hlahla, S., Massawe, F., Mayes, S., Nhamo, L. and Modi, A.T., 2019. Prospects of orphan crops in climate change. *Planta*, 250: 695-708.

Moing, A., Pétriacq, P. and Osorio, S., 2020. Special Issue on “fruit metabolism and metabolomics”. *Métabolites*, 10(6): 230.

Mpai, S., Du Preez, R., Sultanbawa, Y. and Sivakumar, D., 2018. Phytochemicals and nutritional composition in accessions of Kei-apple (Dovyalis caffra): Southern African indigenous fruit. *Food Chemistry*, 253: 37-45.

Omotayo, A.O. and Aremu, A.O., 2020. Underutilized African indigenous fruit trees and food–nutrition security: Opportunities, challenges, and prospects. *Food and Energy Security*, 9(3), 220.

Pfukwa, T.M., Chikwanha, O.C., Katiyatiya, C.L., Fawole, O.A., Manley, M. and Mapiye, C., 2020. Southern African indigenous fruits and their byproducts: Prospects as food antioxidants. *Journal of Functional Foods*, 75: 104220.

Robbins, R.J., 2003. Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, 51(10): 2866-2887.

Tzin, V. and Galili, G., 2010. The biosynthetic pathways for shikimate and aromatic amino acids in Arabidopsis thaliana. *The Arabidopsis book American Society of Plant Biologists*, 8(1): 222-231.

Xiao, J.F., Zhou, B. and Ressom, H.W., 2012. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. *TrAC Trends in Analytical Chemistry*, 32(1): 1-14.

Zhang, W., Li, X., Zheng, J., Wang, G., Sun, C., Ferguson, I.B. and Chen, K., 2008. Bioactive components and antioxidant capacity of Chinese bayberry (*Myrica rubra Sieb*. and Zucc.) fruit in relation to fruit maturity and postharvest storage. *European Food Research and Technology*, 227: 1091-1097.

CHAPTER 6

Summary of findings, significance, recommendations and conclusions

6.1 Summary of findings

The purpose of this study was to document analytical information on five accessions of *M. zeyheri* harvested at different fruit maturity stages. Fruits of *M. zeyheri* are harvested from the wild and consumed especially by rural populations and make a significant contribution to food and nutrition security. However, recent literature does not clearly indicate the post-harvest quality and physicochemical attributes including primary and secondary metabolites. The results of this study demonstrated variations among five accessions and four fruit maturity stages on quality attributes such as fruit weight (g), fruit width (mm), fruit length (mm) and fruit firmness (N) and physicochemical attributes such as TSS (Brix ˚), TA (%), TSS/TA ratio (%) and colour change coordinates (a*, b*, L* and H ˚angle). Furthermore, the five accessions of *M. zeyheri* showed significant differences on primary metabolites which are nutritional, amino acids and mineral compositions to possess different concentrations at four fruit maturity stages. The nutritional compositions of five accessions at four fruit maturity stages showed decreased protein and ash content from T1 to T4 maturity stages, while mineral compositions depicted high concentration at T1 and lower at T4 maturity stages. Furthermore, the study highlighted that the five accessions of *M. zeyheri* had different concentration of these metabolites. On the other hand, secondary untargeted metabolites such as polyphenolics and flavonoids were identified and demonstrated high concentration at two fruit maturity stages which were T1 and T4 maturity stages in five accessions of *M. zeyheri*.

6.2 Significance

The results from this study is evidence that fruits of *M. zeyheri* are good sources of primary and secondary metabolites. However, currently, South Africa, like other developing countries, is encountering demerit of food insecurity which is bolted by malnourishing and undernutrition. Therefore, it must be improved with immediate effect. The fruits of *M. zeyheri* have the potential and ability to contribute and decrease the percentage of food insecurity in urban and local regions of South Africa. The fruits

possess nutritional, amino acids and minerals compositions needed by the body to function effectively and efficiently.

6.3 Recommendations

It was evident from this research project that the five accessions of *M. zeyheri* exhibit different mineral compositions at four fruit maturity stages and metabolites profile at two fruit maturity stages. Future research on the fruits could focus on how to commercialize, and farm with indigenous fruits at small-scale and commercial scale to increase their consumption worldwide due to benefits the fruits is containing. The promotion of these indigenous fruits is essential to be in the formal market basket.

6.4 Conclusions

Fruits of *M. zeyheri* are edible, rich in vitamin C, exhibit higher concentrations of nutrients and other mineral compositions, and contain secondary metabolites. The results of this research project demonstrated that the five accessions at four fruit maturity stages had significant variation in the quality, physiochemical, and mineral compositions, whilst two fruit maturity stages, that is, the green and yellow fruits, showed higher concentrations of untargeted secondary metabolites with merit of reducing non-communicable diseases.

In addition, the results of the present study revealed the numerous mineral nutritional values and importance of *M. zeyheri* as well as its beneficial role in the enhancement of human health and nutrition. Therefore, incorporation of *M. zeyheri* into food fortifications program and in diet may contribute in improving its quality and subsequently reduce the risk for the development of food insecurity.

Lastly, the variation in untargeted secondary metabolites found in the present study is another breakthrough and revelation that the medicinal attributes of *M. zeyheri* fruit are advantageous to human diseases and several ailments. Apart from the above highlighted nutritional and medical characteristics of *M. zeyheri*, the untargeted secondary metabolites detected in this work is another scientific point towards its

earlier use in many herbal formulations for the cure of various ailments in particular the regulation of blood pressure, diabetes, disorders and ulcer.