WATER USE OF MARULA (*SCLEROCARYA BIRREA*) TREES IN VARIOUS AGRO-ECOLOGICAL REGIONS AND POSTHARVEST UTILISATION OF ITS FRUIT AND BYPRODUCTS

Report to the

Water Research Commission

by

Shonisani E Ramashia[1], Mpho E Mashau[1], Tsietsi E Kgatla[1], Masiza S Mikasi[2], Mashudu V Makhado[3]; Sebinasi Dzikiti[4]

[1] Department of Food Science and Technology, University of Venda

[2] Department of Animal Science, University of Venda

[3]Department of Horticulture, University of Venda

[4] Department of Horticultural Science, Stellenbosch University

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Obtainable from
Water Research Commission
Private Bag X03
Gezina, 0031

hendrickm@wrc.org.za or download from www.wrc.org.za

The publication of this report emanates from a project entitled: *Water use and Marula (*Sclerocarya birrea*) tree crops in various agro-ecological regions and postharvest utilisation of its fruit and byproducts* (WRC Project No. C2019/2020-0025).

EXECUTIVE SUMMARY

MOTIVATION

Currently, food production in South Africa and elsewhere is dominated by exotic crops (i.e. cereals, fruit and vegetables). These require large amounts of inputs, management, and optimal growing conditions. However, many climate change models predict significant disruptions in future growing conditions in sub-Saharan Africa characterised by increases in the frequency and severity of droughts (Schulze, 2006; Jimènez *et al.,* 2020). This will negatively affect the production of conventional crops that require large amounts of water to survive. This will, in turn, endanger the food and nutritional security, especially of poor rural households (Everson *et al.,* 2008; Nkosi *et al.,* 2020). In addition to climate change, the rising cost of inputs, and increasing land degradation will render the production of conventional crops unsustainable in the future. There is, therefore, a need to identify alternative crops that can withstand harsh growing conditions to meet the increasing demand for food and nutrition.

South Africa is endowed with many indigenous species of trees and vegetables that produce nutrient-rich foods under harsh growing conditions. Yet these underutilised species have received limited research attention in favour of the exotic (papaya, passion and jackfruit) crop species. *Sclerocarya birrea* (A. Rich) Hochst. subsp. caffra (Sond.) *Kokwaro* (marula), a member of the *Anacardiaceae* family, is an example of an underutilised indigenous fruit tree species that grows naturally across large parts of sub-Saharan Africa. In South Africa, it is found in the tropical and subtropical regions in Limpopo, Mpumalanga, KwaZulu-Natal and in parts of the North West province. The economic and nutritional value of marula fruit and its byproducts is well documented (Wynberg *et al.,* 2002; Akinnifesi *et al.,* 2006). It is a great resource for alleviating poverty and diversifying rural livelihoods as it is a major source of income for both rural and commercial entrepreneurs. Besides being eaten raw, the fruit can be processed into a range of products. These include liquors, juices, jams, jellies, perfumes, skin care products etc. Today, marula products are marketed in over 160 countries, highlighting the global significance of this tree species.

Despite the clear economic benefits, most marula trees grow and are harvested in the wild unmanaged. Very little detailed research has been done on how marula trees interact with the environment in their natural habitats (Dye *et al.,* 2008; Dzikiti *et al.,* 2022). To some degree, this has contributed to its low levels of domestication and/or commercialisation. The first agronomic trials on the potential for cultivating marula commercially were done in the Negev desert in Israel in the 1980s using plant material imported from Botswana (Nerd and Mizrahi., 2000). More recently, trials have been done in China (Li *et al.,* 2015), Czechia and other countries outside the African continent. There is a need for local research to understand the environmental requirements of marula trees. Further research is also needed to improve both the quantity and quality of products from marula fruit and to increase the range of its uses.

AIMS AND OBJECTIVES

Overall aim

The overall aim of the study was to investigate how the water use, yield and growth of marula trees are influenced by rainfall and other climatic factors across South Africa. The second aim was to investigate the postharvest utilisation of marula fruit and its by-products.

Specific objectives

These were to:

- Quantify the diurnal and seasonal water use patterns of marula trees in different agroecological regions.
- Establish how fruit growth, yield, and water productivity of marula differ between agroecological regions.
- Establish how the quality of marula fruit (i.e. physicochemical, phytochemical, and nutritional properties) varies with environmental conditions and establish the safety of products like marula fruit juices and biscuits.
- Investigate the preservation methods and livestock feeding value of marula fruit by-products, and
- Develop and characterise a marula fruit-based carbonated soft drink.

MATERIALS AND METHODS

Annual rainfall varies greatly across South Africa, with high rainfall regions (>1000 mm/yr) along the east coast and more arid areas in the interior receiving less than 250 mm/yr. This rainfall distribution has, in turn, informed differences in the vegetation types across the country, with dense indigenous forests found on the eastern coastline and shrubby drought-tolerant species growing in the interior. Marula trees in South Africa grow under a range of rainfall regimes varying from around 250 mm in parts of the Limpopo Province to more than 800 mm along the KwaZulu-Natal coastline and in parts of the Mpumalanga Province. To investigate how variations in rainfall amounts influenced the performance of marula trees, data were collected in this study in three areas with contrasting rainfall patterns. These include: 1) the low rainfall Phalaborwa region in the Limpopo Province that receives less than 250 mm/yr, 2) the medium rainfall region (400 – 600 mm/yr) in the Moeketsi region near Tzaneen, and; 3) the high rainfall (> 800 mm/yr) Thulamahashe region in Mpumalanga.

Data were collected on the transpiration dynamics of four individual trees per site using the heat ratio method of monitoring sap flow. These data were collected hourly for periods ranging from two to three years per site. The leaf area index (m^2 of leaf area per m^2 of ground area) was also measured regularly at all the sites to monitor canopy development during the active growing season (October to May). The volumetric soil water content was measured at various depths in the root zone to give an estimate of the available soil moisture. The phenology and yield of the trees were also recorded at each site. These data were used to validate a marula-specific water use model that was initially developed by Dzikiti *et al.* (2022). The validated model was subsequently used to predict future changes in marula tree water requirements using six climate change models that follow the business-as-usual scenario. The model provided insights on how marula trees might respond to future changes in the period 1960 to 2099.

Developing new marula-based products and improving existing ones is an important research area aimed at adding value to the products from marula fruit. In this study, the Department of Food Science Technology at the University of Venda explored the possibility of developing two types of products. The first are wheat-marula composite biscuits and the second are biscuits

based on marula only. Both products were developed and evaluated using a specialist panel to assess the eating quality of the products. Thirdly, a lot of marula products, e.g. the peels and seeds, go to waste even causing environmental pollution in some instances. This study also explored the possibility of using processed marula peel as a stock feed for merino sheep. Detailed results are presented in the next sections.

RESULTS AND DISCUSSION

Tree size, and hence, the transpiring leaf area, varied considerably across the three sites. Trees in Thulamahashe had much bigger canopies, with an average leaf area index (LAI) of about 2.34. Phalaborwa and ZZ2 had similar leaf area indices of 1.26 although the canopies of the trees in Phalaborwa were more spread out. Mean transpiration of the trees in Thulamahashe peaked at about 226 litres per tree per day followed by Phalaborwa at approximately 160 litres per tree per day and lastly ZZ2 at 112 litres per tree per day (Table 1). To account for the differences in tree size between the sites, we normalized the transpiration data with the size of the canopy leaf area as summarized in Table 1.

Table 1. Comparison of transpiration rates across the three study sites.

Site	Maximum transpiration (L/tree/d)	LAI	BA (m^2)	Leaf area (m^2)	Normalised transpiration ($L/m^2/d$)	Soil water content at field capacity (cm^3/cm^3)
Thulamahashe	226	2.34	95	222	1.02	0.08
ZZ2	112	1.26	38	48	2.33	0.15
Phalaborwa	160	1.26	79	100	1.60	0.11

LAI is the mean leaf area index, BA is the mean basal area of the canopy of the four trees per site

Our data shows that trees at the ZZ2 site had the highest transpiration per unit leaf area peaking at about 2.33 litres per m^2 of leaf area per day. This was followed by trees at Phalaborwa that transpired about 1.60 litres per m^2 per day and lastly. Trees at Thulamahashe had the least transpiration per unit leaf area of about 1.02 litres per m^2 per day. These data suggest that rainfall amount is not the only factor that determines the water use by the marula trees at a given site. Rather it is more likely the combination of the amount of rainfall, its distribution and the soil type that determines the amount of water that is available to sustain the transpiration rates. For example, in Table 1, the moderate rainfall ZZ2 site which had heavier soils had the highest

transpiration per unit leaf area compared to the other two (2) sites. Thulamahashe had more sandier soils with a low water holding capacity and hence lower transpiration rates. These results are consistent with those reported by Dzikiti *et al.* (2022) who observed that transpiration by Marula trees growing on dunes in KZN was more strongly correlated with the available soil water than with climate variables.

The average annual transpiration of the trees at Thulamahashe was about 28 027 litres per tree with an average yield of about 28.9 kg/tree. At ZZ2, the average annual transpiration per tree was about 23 690 litres with a much higher yield of about 53.9 kg/tree. At Phalaborwa, the average annual transpiration of the trees was about 25 336 litres with an average yield of 23.4 kg/tree. These figures give water efficiency (WUE), defined as kg of fruit per m^3 of water transpired of 1.03 for Thulamahashe, 2.27 for ZZ2 and 0.92 kg/m^3 for Phalaborwa. These data suggest that the heavier soils at ZZ2 contributed to the substantially higher yield and hence higher WUE which was about double that at the other two (2) sites. These WUE values are quite low compared to those of exotic tree species. For example, Nel et al (2024) obtained a WUE of about 14 kg/m^3 for irrigated mango trees in the Malelane area of Mpumalanga. Dzikiti *et al.* (2018) recorded a WUE of up to 18.0 kg/m^3 for apple trees in the Western Cape. Therefore, it is probable that the yield of the marula trees can be substantially increased with supplementary irrigation, although further trials are required to confirm this. The response of indigenous plants to irrigation can be unpredictable. For example, rooibos, a species that is indigenous to the Western Cape has been found to perform poorly under irrigation. So further research is required to confirm whether irrigation can indeed benefit marula trees.

We used the environmental data and sap flow data collected in this study to validate a big leaf Penman-Monteith type model parameterized for marula trees. The results show that this model can satisfactorily predict the transpiration rate of marula trees under a range of growing conditions. Lastly, we then applied this model to predict future trends in tree transpiration using climate data from six (6) climate change models. The results show that while the atmospheric evaporative demand, depicted by reference evapotranspiration is expected to increase by between 10 and 12% in the Thulamahashe area for the period 1960 to 2099, daily tree transpiration is projected to increase by less than 5% over the same period. According to the

Penman-Monteith model, the reason for this is that future warmer and drier conditions are expected to increase the vapour pressure deficit (VPD) of the air. The increasing VPD on the other hand appears to cause stomatal closure which reduces the transpiration rates. The impact of the stomatal closure on yield is not known. However, the yield may probably remain unchanged given the fact that the water vapour gradient across the stomatal pore is much steeper than the CO_2 gradient. Therefore, a small reduction in stomatal conductance is likely to reduce transpiration more than CO_2 assimilation. This situation may even lead to increased WUE by the marula trees making them ideal crops for the future.

This chapter examined how oven drying (OD), microwave drying (MD), solar drying (SD), and shade drying (SHD) affect the phytochemical and antioxidant characteristics of marula peel flour in two particle sizes (250 and 106 µm). The flour was tested for the impact of drying procedures on ascorbic acid, phenolic compounds, antioxidants, and colour pigments (chlorophyll, carotene, and anthocyanins). The use of heat during 60°C oven drying resulted in a drop in chemical and bioactive qualities, notably total flavonoids (32.82 to 22.87 mg/g) and total phenolic compounds (8.35 to 6.79 mg/g), which decreased progressively after drying and continued to diminish as particle size decreased. The lower particle size (106 µm) led to a reduction in the measured parameters.

Marula fruit peel flour is the end product of grinding and milling cereals, beans, and other seeds. It adds structure to baked goods, including cakes, cookies, pastries, and bread. The goal of this study was to create flour from marula peels and assess its nutritional composition, antioxidant capabilities, functional qualities, and microbiological purity. The nutritional composition of Marula peel flour was determined (fat, ash, protein, and moisture), as well as antioxidant, FTIR, physicochemical, functional, and microbiological quality. It was established that marula peel flour is a good source of essential nutrients, such as proteins and phytochemicals, which aid in the prevention of heart disease, cancer, and other chronic disorders.

The wheat-marula biscuits were made with the following ratios: The ratios included 5, 10, 15, and 20% marula peel flour, with 100% wheat flour biscuits serving as a positive control. The wheat-marula (WM) peel composite biscuits were examined for their proximate composition,

physical qualities, antioxidant properties, and FTIR (Fourier-Transform Infrared Spectra). The experiment was repeated twice. All results collected from the analysis of each sample were carried out in triplicate. The acquired data was analysed with SPSS version 26 (IBM Chicago, USA), and the means were separated using the Duncan multiple range test. The candidate utilized a 95% confidence interval ($p<0.05$) to assess significance.

Several analyses were carried out to assess the chemical composition of dried marula fruit peel used as byproducts for feeding the pen lambs. A number of analysis were carried out, including DM, OM, CP, CF, ADF, NDF, and GE values. All data from this experiment were subjected to analysis of variance for a 2 x 2 factorial in a completely randomised design using Minitab 19's GLM technique (Minitab, 2019). Tukey's studentised multiple range test identified statistically significant variations between means.

The goal of this work was to produce and characterise carbonated marula juice through spontaneous fermentation. The physicochemical parameters and bioactive components were studied to assess the effect of fermentation. The results showed that fermentation maintained a higher ascorbic acid (vitamin C), carotene, total acidity, and volatile acids in carbonate marula juice. On the other hand, fermentation reduced the residual sugar, total soluble sugar, pH, and viscosity of carbonated marula juice. Fermentation altered the colour of carbonated marula juice, raising the L* value from 55.17 to 57.76 while decreasing the a* and b* values from 3.74 to -1.03 and 13.55 to 7.25, respectively. According to the findings, carbonated marula juice can spontaneously ferment into probiotic beverages.

The purpose of this study was to extract and identify the microbial composition of raw, fermented, and carbonated marula juice. Marula fruit samples were gathered in Phalaborwa. The marula fruit juice was made, fermented, and then carbonated. Microorganisms such as Enterobacteriaceae (Coliforms and *Escherichia coli*), aerobic count, lactic acid bacteria, yeast, and moulds were identified. Fermented marula juice had abundant amounts of coliforms, yeast, *E. coli,* lactic acid bacteria, and aerobic bacteria. Although fermented marula juice is not safe to consume, it is important to properly clean and sanitise all equipment and surfaces before

handling marula juice. This helps to prevent contamination from outside sources. In addition, further processing is necessary.

NEW KNOWLEDGE AND INNOVATION

This study has several innovative elements, and it has generated new knowledge. Firstly, no information existed until now on how the water used by marula trees varies across a rainfall gradient. This study suggests that while rainfall and distribution are important, the water-holding capacity of the soils is essential in sustaining high transpiration and high yield levels for Marula trees. Trees growing on heavier soil, but moderate rainfall (ZZ2 site) had higher WUE than trees growing in the higher rainfall (Thulamahashe site) but growing on sandy soils. Secondly, this study provides, for the first time, quantitative information on how the water requirements of the marula trees are likely to be influenced by future climate change. The team provides a plausible explanation of why the water use of the marula trees may not rise under climate change even if the atmospheric evaporative demand is projected to increase quite considerably in the study area.

Marula fruit peels were dried using different drying methods, however, research on the thermal characteristics and microbiological analysis of marula fruit peels. There is no need to conduct microbial analyses for dried products as they undergo the heating process. In addition, a study on the effects of freeze drying on antioxidant capacity and activity is needed to compare it to the solar, shade, microwave, and oven drying methods utilised in the study. The study emphasises the potential of marula peel flour as a desirable ingredient in nutritional and antioxidant-rich biscuits. According to the findings of this study, dried marula fruit peels can be added to lamb diets as a feed element to give an alternate energy source for the animals while preserving their growth and carcass characteristics. However, future trials with longer, higher amounts of marula fruit peels in diets might help to provide a more robust evaluation of the effects of dried marula fruit peels on lamb development performance. The marula fruit peel flour was used to make marula biscuits. The microbial analyses of fermented carbonated juice were enumerated.

CAPACITY BUILDING

1. **This study has supported capacity building of staff and students at the University of Venda as follows:**

a. Mrs Mashudu Makhado (Field technician Department of Horticulture)

Mashudu has registered for a PhD in Horticulture supported by this project. The title of her thesis is 'Investigating the Relationship between Water Use and yield of Marula (*Sclerocarya birrea*) Trees Growing along a Rainfall Gradient'. At the time of writing she was in her second year of the PhD and was expected to graduate in 2026.

b. MSc student 1 – Ms Rofhiwa Murovhi

Ms Rofhiwa Murovhi registered for her MSc in Animal Science in 2021. The title of her thesis was 'Effect of replacing maize meal with dried marula fruit skin on apparent digestibility, growth performance and carcass characteristics of South African mutton merino lambs'. She completed her thesis and graduated in September 2024.

c. BSc (Food Science and Technology) student – Ms Masinamela Christah

She registered her research BSc in Food Science and Technology in 2022. The title of her research project was 'Nutritional Composition, Functional, Antioxidant Properties and Microbial Quality of Marula Fruit Peel Flour'. She completed her research project and graduated in September 2023.

d. BSc (Food Science and Technology) student – Ms Elelwani Mafadza

Ms Elelwani Mafadza registered her BSc in Food Science and Technology in 2022. The title of her research project was 'Physicochemical, Antioxidant Properties and Structural Properties of Wheat-Marula Peel Composite Biscuits'. She completed her research project and graduated in September 2023.

e. BSc (Food Science and Technology) student – Mr Tharollo Makhubela

Mr Makhubela registered his BSc in Food Science and Technology in 2022. The title of his research project was 'The Effects of Different Drying Methods on Phytochemical and Antioxidant Properties of Marula Peels Flour with Different Particle Sizes'. He completed his research project and graduated in September 2024.

2. **Capacity building for non-degree purposes**

The following students contributed to various aspects of this project e.g. downloading data, measuring LAI etc:

a. Mr Kgaokgelo Edwin Ramatsetse
b. Ms Mashudu Makhado
c. Mr Khuthadzo Mathivha
d. Mr Masilo Mabotja

3. **Community capacity building assists in charging the sap flow batteries etc:**

a. Ms M. Seunane
b. Mr T. Matsipa
c. Mr C. Nxumalo

CONCLUSIONS

This study has provided insights into how environmental conditions influence the water use and yield of marula trees. There exists a significant relationship between transpiration and climatic factors e.g. solar radiation, vapour pressure deficit of the air and the available soil moisture. The soil water holding capacity, in addition to the rainfall, seemed to be the main drivers of the differences in tree response across the various sites. Modelling of future water requirements of marula trees under climate change suggests that water use by the trees will not increase significantly as the atmospheric evaporative demand increases. This is likely to lead to increased water use efficiency and to marula being an ideal alternative fruit tree crop as growing conditions get harsher.

Among all the drying methods used, shade drying is recommended for fruit peels since it keeps most of the bioactive compounds. The use of marula peel flour in biscuits improves ash content, total phenolic content, total flavonoid content, vitamin C, and antioxidant activity. In comparison to the control, the addition of marula peel flour greatly increases hardness while decreasing protein, fat, thickness, mass, spread ratio, and colour. More investigation into the effects of marula peel flour on mineral content, fatty acid profile, microbiological quality, and biscuit morphology is needed. This research has revealed that dried marula fruit peels can be used as

a potential energy source for lambs without negatively affecting the growth and carcass characteristics of lambs at up to 10% inclusion level.

RECOMMENDATIONS FOR FUTURE RESEARCH

While a significant effort has been made to quantify the response of marula trees to contrasting growing conditions, further research is needed to:

a. Study differences in the leaf morphology and leaf function at the different sites.
b. Quantify key eco-physiological responses that lead to differences in water use and yield e.g. stomatal conductance, leaf photosynthesis, etc;
c. Study the response of marula trees to supplementary irrigation to increase tree performance.
d. investigate the effect of spontaneous fermentation on the nutritional, structural properties and metabolites of marula peel.
e. Determine the impact of fermented marula peel on the nutritional, antioxidant properties and volatile compounds of wheat and gluten-free biscuits.
f. Extract beneficial probiotics in fresh and fermented marula juice.

EXTENT TO WHICH CONTRACT OBJECTIVES HAVE BEEN MET

The aims and objectives of the project as stipulated in the proposal have been met to a large extent. However, there are additional aspects that have been included in the project to generate more insights into how marula trees respond to environmental conditions. For instance, climate change modelling was not part of the original proposal. But this has been included, and it added significant insights into the possible role of marula as an alternative future crop requiring less water than exotic tree crops. Furthermore, the development of marula fruit peel flour, biscuits and fermented carbonated marula juice was another innovation of the project. The microbial safety of the fermented carbonated juices was enumerated.

ACKNOWLEDGEMENTS

Firstly, we acknowledge the Water Research Commission for initiating and funding this project (project no WRC C2019/2020-0025). Their support is sincerely appreciated. Secondly, we wish to thank the following entities for allowing us to undertake this project on their properties: Mafemane Secondary School in Thulamahashe, ZZ2 Farm in Moeketsi and Mokgale Wastewater Treatment Works in Phalaborwa. We are very grateful for their support. Lastly, we wish to thank the following members of the reference group for guiding this project:

Reference group members	**Location**
Prof N.S. Mpandeli	Water Research Commission
Dr L. Nhamo	Water Research Commission (Chairman)
Dr S.N. Hlophe-Ginindza	Water Research Commission
Dr T. Volschenk	Agricultural Research Council
Prof S. Walker	Agricultural Research Council
Dr R. Mulidzi	Agricultural Research Council
Dr P. Maponya	Agricultural Research Council
Mr M. Masevhe	University of Limpopo
Dr N.J. Taylor	University of Pretoria
Dr C. Madakadze	University of Pretoria
Mr N. Olivier	South African Forestry Company SOC Limited

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AWS	Automatic Weather Station
DWS	Department of Water and Sanitation
FAO	Food and Agriculture Organization of The United Nations
HRM	Heat Ratio Method
LAI	Leaf Area Index
PPFD	Photosynthetic Photon Flux Density ($\mu mol/m^2/s$)
R^2	Coefficient of Determination
RAW	Readily Available Water (mm)
RH	Relative Humidity (%)
SPAC	Soil-Plant-Atmosphere Continuum
TAW	Total Available Soil Water (mm)
VPD	Vapour Pressure Deficit (kPa)
WP	Water Productivity (kg/m^3)
WRC	Water Research Commission
WUE	Water Use Efficiency (kg/m^3 or kg/kg)

LIST OF SYMBOLS

A	Net carbon dioxide (CO_2) assimilation rate (µmol/m^2/s)
A_{max}	Maximum net carbon dioxide (CO_2) assimilation rate (µmol/m^2/s)
A_{area}	Surface area(m^2)
C_p	Specific heat capacity of air (J/kg/K)
C_s	Specific heat capacity of the sap (J/kg/°C)
C_w	Specific heat capacity of the wood matrix (J/kg/°C)
d	Zero plane displacement (m)
ETo	Reference evapotranspiration (mm/d)
g_a	Aerodynamic conductance (m/s)
g_c	Canopy conductance (m/s)
g_{max}	Maximum stomatal conductance (m/s)
g_s	Stomatal conductance (m/s)
h	Plant height during the midseason period (m)
J_{max}	Electron transport rate (µmol/m^2/s)
k	von Karman's constant
k_r	Parameter for solar radiation stress factor (W/m)
k_{vpd}	Vapour pressure deficit stress factor (kPa)
k_w	Thermal diffusivity of green (fresh) wood (cm^2/s)
P_a	Atmospheric pressure (Pa)
R	Solar irradiance
r_b	Boundary layer resistance (s/m)
r_l	Mean leaf resistance (s/m)
R_n	Net radiation (W/m^2 or MJ/m^2/day)
R_{so}	Solar radiation under clear sky conditions (MJ/m^2/day)
T_{air}	Air temperature at 2 m height (°C)
T_{leaf}	Leaf surface temperature (°C)
T_{max}	Maximim air temperature (°C)
T_{min}	Minimum temperature (°C)

T_{opt}	Optimal temperature for tree growth (°C)
u_2	Wind speed at 2 m height (m/s)
z_o	Roughness length (m)
Z_r	Soil sample depth (m)
γ	Psychrometric constant (kPa/K)
Δ	Slope of the saturation vapour pressure vs air temperature curve (kPa/K)
θ	Volumetric soil water content (cm^3/cm^3)
θ_{FC}	Volumetric soil water content at field capacity (cm^3/cm^3)
θ_{PWP}	Volumetric soil water content at wilting point (cm^3/cm^3)
λ	Latent heat of vaporization (J/kg)
ρ_a	Density of dry air (kg/m^3)
ρ_b	Density of wood or soil (g/cm^3)
ρw	Density of water (kg/m^3)
ψ_{leaf}	Leaf water potential (MPa)
ψ_{soil}	Soil water potential (MPa)

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Most climate change models predict future increases in the frequency and severity of droughts in sub-Saharan Africa (Schulze, 2006; Jimènez *et al.,* 2020). This will have deleterious effects on the production of conventional crops such as cereals (wheat, rice), vegetables, and fruit crops thereby endangering the food and nutritional security, especially of poor rural households (Everson *et al.,* 2008; Nkosi *et al.,* 2020). There is, therefore, a need to identify alternative crops that can withstand harsh growing conditions to meet the increasing demandfor food and nutrition. The cultivation and utilisation of underutilised food sources, including indigenous edible species, have become a subject of intense research interest in climate change adaptation studies in recent years (Padulosi *et al.,* 2011; Ebert, 2014; Mabhaudhi *et al* 2017). According to Mabhaudhi *et al.* (2017), indigenous crop species are "*crops which naturally grow within a specific geographic location, and they are often characterised by limiteddevelopment relative to their potential*".

Sclerocarya birrea (A. Rich) Hochst. subsp. caffra (Sond.) *Kokwaro* (marula), a member of the *Anacardiaceae* family, is an example of an underutilised indigenous fruit tree species that grows naturally across large parts of sub-Saharan Africa. In South Africa, it is found in the tropical and subtropical regions in Limpopo, Mpumalanga, KwaZulu-Natal and parts of the North West Province. The economic value of marula fruit and its byproducts is well documented (Wynberg *et al.,* 2002; Akinnifesi *et al.,* 2006). It is a great resource for alleviating poverty and diversifying rural livelihoods as it is a major source of income for both rural and commercial entrepreneurs. Besides being eaten raw, the fruit can be processed into a range of products. These include liquors, juices, jams, jellies, perfumes, skin care products etc. Today, marula products are marketed in over 160 countries highlighting the global significance of this tree species.

Despite the clear economic benefits, most marula trees, grow and are harvested in the wild unmanaged. Very little detailed research has been done on how marula trees interact with the environment in their natural habitats (Dye *et al.,* 2008; Dzikiti *et al.,* 2022). To some degree, this has contributed to its low levels of domestication and/or commercialisation. Although marula trees have been planted in backyard gardens or farmer fields, albeit on a very small scale, there is a need to increase the cultivated area to increase this species' footprint on the country's economy. Increasing marula's contribution to the economic wellbeing, especially of rural households, is a major strategic objective of various provincial governments in the country.

For example, there are initiatives to establish marula processing hubs in Limpopo and KwaZulu-Natal provinces, respectively. The purpose of these hubs is to add value to the Marula products through various beneficiation activities. In this way, it is envisaged that incomes for poor communities and commercial entrepreneurs can be increased thereby tackling the triple challenges of hunger, unemployment and food insecurity. However, the sustainability of these initiatives requires a consistent supply of marula fruit for processing. This can only be achieved through large-scale cultivation and management to reduce yield losses caused by adverse environmental conditions.

The first agronomic trials on the potential for cultivating marula commercially were done in the Negev desert in Israel in the 1980s using plant material imported from Botswana (Nerd and Mizrahi, 2000). More recently, trials have been done in China (Li *et al.,* 2015) and in Czechia. The purpose of the trials was to identify alternative horticultural fruit tree species that are better adapted to harsh growing conditions. In South Africa, most funding for eco-physiological and agronomic research has been channelled towards commercial exotic fruit tree crops. Examples include citrus and nectarines (Gush et al., 2019; Taylor *et al.,* 2014; Taylor and Vahrmeier, 2018), apples (Voschenk *et al.,* 2003; Dzikiti *et al.,* 2018a, b), peaches and plums (Dzikiti and Schachtschneider, 2015), among others. Very little attention has been given to the so-called neglected underutilised tree species such as marula.

Indigenous trees like marula are known to thrive under harsh climatic conditions and in nutrient-poor soils (Mabhaudhi *et al.,* 2017; Nkosi *et al.,* 2020). They can tolerate droughts,

and they are resistant to most pests and diseases that afflict exotic species (Du Preez pers. comm.). They also require limited management skills, thereby bringing resource-poor rural households into mainstream agriculture in line with government policies such as the National Development Plan 2030, the New Growth Path, and the United Nations' Sustainable Development Goals, especially goals number 1 to 3. Before domestication and commercialisation of marula can be implemented on a large scale,numerous pre- and post-harvest research gaps need to be addressed. These include the need to remove the many unknowns around the performance of marula in different agroecological zones and to establish the limits of the species' adaptability to climates and soils. Research is also needed to select marula plant material with desirable traits for cultivation. The Agricultural Research Council's Institute for Tropical and Subtropical Crops in Nelspruit, for example, is involved in this kind of research. However, they lack the support to sustainthe long-term research needs for such a programme. Desirable traits include larger fruit size withsmaller stone and thinner skins, improved precocity, higher yields, less alternate bearing, andgreater tolerance to droughts, pests, and diseases.

Further research is also required to document the phenology of marula trees to better understand the timing of leafing, blossoming, fruit drop, and other seasonal features. The Water Research Commission has funded other research projects on marula and other indigenous tree species (Ntshidi *et al.,* 2022). But data were not collectedin different agro-ecological regions given the large number of indigenous fruit tree species studied.-Related to the marula phenology research, there is a need to identify maleplants that produce pollen when superior females are blossoming for pollination and to increase fruit set.

On the postharvest side, there is a need to expand the marula product range, and to add value to the products to increase income. This is one of the goals of the marula processing hubs whose goals are, among others, to support research on the beneficiation of marula products. There is a need to invent new innovative marula-based products such as carbonatedsoft drinks, developing ways of storing and using marula waste (e.g. skins and shells) which pose environmental pollution. The waste can, for example, be converted into stock feed long after the marula season has ended etc. This study, being done by a multidisciplinary team of

researchers, therefore attempts to strike a balance between selected pre – and postharvest research needs to provide information that can be used to increase benefits from marula products.

To close some of the information gaps highlighted above, this study seeks to address the following research questions:

a. How do the water use patterns of marula trees vary in different agro-ecological zones?
b. How does the marula water use, tree and fruit growth and yield respond to water deficitsand climate extremes?
c. What are the main drivers of water use and yield variations in different agro-ecological regions?
d. How does the water use efficiency of marula trees vary between agro-ecological zones?
e. How do the environmental conditions in various agro-ecological zones influence the physicochemical, phytochemical and nutritional properties of marula fruit?
f. Is it technically and economically feasible to manufacture products such as carbonated softdrinks from marula fruit, and stock feed from marula waste?

To address these questions, our research activities entailed a combination of fieldwork using state-of-the-art measurement and modelling techniques and lab work. Data were collected from marula trees growing in different agro-ecological regions in the Limpopo and Mpumalanga Provinces, respectively.

1.2 Aims and objectives

1.2.1 Overall aim

The overall aim of the study was to investigate the factors influencing differences in water use, yield and growth of marula trees in different agro-ecological regions across South Africa. The second aim was to investigate the postharvest utilisation of marula fruit and its byproducts.

1.2.2 Specific objectives

These were to:

- Quantify the diurnal and seasonal water use patterns of marula trees in different agro-ecological regions.
- Establish how fruit growth, yield, and water productivity of marula differ between agro-ecological regions.
- Establish how the quality of marula fruit (i.e. physicochemical, phytochemical, and nutritional properties) varies with environmental conditions and establish the safety of products like marula fruit juices and jams.
- Investigate the preservation methods and livestock feeding value of marula fruit byproducts.
- Develop and characterise a marula fruit-based carbonated soft drink.

CHAPTER 2

KNOWLEDGE REVIEW

2.1 Ecology and uses of marula

Marula trees (Fig. 2.1) have been precious plants to people on the African continent for many centuries (Helm and Witkowski, 2012; Gouwakinnou *et al.,* 2009). It grows in open woodland, and it is widespread right through the semi-arid and deciduous savannas of sub-Saharan Africa covering South Africa, its neighbouring countries as well as Madagascar (Enweremadu and Rutto, 2015; Hamidou *et al.,* 2014; Shackleton *et al.,* 2002). The *Sclerocarya* genus consists of only two species, which are *birrea* and *gillettii*. However, *birrea* has three subspecies, namely *Birrea*, *Caffra* and *Multifoliolat*a (Shackleton *et al.,* 2001). *Sclerocarya birrea* is found naturally or ploughed in the Sahel, East and southern Africa on the margins of the moist forest zone (Orwa *et al.,* 2009).

The height of the tree can reach up to 18 m while the trunk diameter can be as much as 120 cm (Orwa *et al.,* 2009; von Teichman, 1982). The tree grows well in clay or sandy loam soils and is widespread in areas that receive annual rainfall of between 200 and 1 370 mm. The most common subspecies of *birrea* is *caffra* and it is cultivated in Southern, East and West Africa and Madagascar (Ngorima, 2006; Shackleton *et al.,* 2002). Subspecies *gillettii* is found only in areas south of the equator and is indigenous to a small area in eastern Kenya (Hall et al., 2000). Subspecies *multifoliolata* is reported to be indigenous to Tanzania and is found in combined wooded grassland and deciduous woodland (Shackleton *et al.,* 2008; Shackleton *et al.,* 2002).

Marula trees grow in a wide range of altitudes varying from sea level up to 1 800 m above sea level (Bandeira *et al.,* 1999; Jacobs and Biggs, 2002; Ngorima, 2006). Temperatures varying from as low as 10°C are favourable for the growth of marula trees in high altitudes whereas temperatures from 40°C occur in low-lying areas (Hall *et al.,* 2000). Warm and frost-free climate is desirable for the growth of marula trees which are also tolerant to salt (du Plessis, 2002). High-altitude areas with short and intermittent frosts also support marula

growth although they are also common in warm, frost-free places, such as the Lowveld region of South Africa (Ngorima, 2006; Neumann and Hirsch, 2000). Marula is very sensitive to frost and this is a critical environmental requirement which restricts its distribution. The species is moderately resistant to drought. Wide temperature ranges are good for the germination of marula seeds, which usually takes place at temperatures between 27 and 37°C (Hamidou *et al.,* 2014; Bandeira *et al.,* 1999; Lawes *et al.,* 2004; Lewis, 1987).

Fig 2.1. A fully grown marula tree with the insert showing unripe fruit.

The highest density of marula trees is found in dry areas that receive annual rainfall in the range of 250 to 800 mm. It is also found in areas with humid to sub-humid climatic conditions on a wide range of soil types. The subspecies *gillettii* commonly grows in areas with seasonal rainfall which vary in amounts from 200 to 1 500 mm per annum (Hall *et al.,* 2002; McCullum, 2000). This makes the *gillettii* subspecies common in hot areas since it is relatively tolerant to drought and quite common in areas receiving below 200 mm annual rainfall. Higher marula populations in South Africa are found in the rainfall range of 400 to 1,000 mm per annum (Hamidou *et al.,* 2014; Hall *et al.*, 2002).

2.2 Contribution of marula to ecosystem functioning

Marula plays a vital role in ecosystems since it provides many goods and services to both humans and animals. It is a keystone tree species that also plays an important part in the ecology of other plants and animals, and is often regarded as a community-dominant species (Kgomoamagodi, 2008; Ngorima, 2006). It provides shade and food to different animal species, including birds, mammals and insects (Hal, 2014; Jacobs and Biggs, 2002; Palgrave, 2002). Given its substantial canopy size (see Fig. 2.1), marula forms a big umbrella with a cool sub-canopy environment – thus providing a good habitat for other flora and fauna. Domestic animals and wildlife, such as zebra, kudu and impala, browse marula leaves. Various butterfly and moth species use marula as their host plant. The larvae of most of the moth species are a popular food in southern Africa (Pegg, 2014; Hal, 2013). For example, the larvae of the mopane worm (*Imbrasia belina*) are found on marula trees. This means that intercropping marula with mopane trees can reduce the defoliation pressure due to the mopane moth.

The bark of the marula tree is grey. It is consumed by rhinos, elephants, warthogs, primates, and domestic animals such as goats and cattle. Marula fruit is very rich in vitamin C as will be detailed later in this report (Pegg, 2014; Helm and Witkowski, 2013). On the negative side, mosquitoes utilise the water-filled holes in the trunks of marula trees as breeding grounds. These interconnections are also reported to control the population characteristics of marula (Hamidou *et al.,* 2014; Kgomoamagodi, 2008). Over-browsing disrupts the population composition (Gadd, 2002) and this contributes to the often-non-availability of immature trees. Nevertheless, there is little or no evidence of successful regeneration and recruitment of marula. This demonstrates the influence of browsing by animals in the control of marula seedlings (Lawes et al., 2004). The seedlings of marula are also vulnerable to fire which also has a negative influence on its regeneration prospect (Jacobs and Biggs, 2002).

2.3 Yield patterns of marula trees

The phenology of marula trees varies widely with growing conditions and with species or genotypes. On average, the trees come into leaf in late September to early October followed by flowering, depending on environmental conditions. Fruit set is from around early to mid-November and fruit growth continues with maturity/ harvest between February and June. The number of fruits that a single mature marula tree can produce varies from a few hundred to as many as 7000 in one season (Nyoka *et al.,* 2015) depending on environmental conditions. The yield variations also depend on whether it is an on or off year as alternate bearing is quite pronounced. In some years completely no fruit grows on the trees.

In a comprehensive study on 64 marula trees by Shackleton (2002), the fruit yield of mature trees fluctuated from 21 to 56 kg per tree with a mean of 36.8 kg per tree corresponding to approximately 1 753 fruits per tree. In another study, data from 122 marula trees by Todd (2001) collected in the 1999 to 2000 season produced a mean yield of around 17.4 kg of fruits per single mature tree. In the study by Dzikiti *et al.* (2022), the average yield of a single mature marula tree was less than 5.0 kg in a single season on a site with deep sandy dune-type soils in northern KwaZulu-Natal. Similar size trees in Mpumalanga on the other hand, growing on heavy clayey loam soils yielded up to 70 kg of fruit per tree per season (Ntshidi *et al.,* 2022). Therefore,environmental conditions, mostly root zone soil water availability likely affect the yield of this species. Marula trees produce male and female plants as separate entities since they are dioecious (Dlamini, 2011; Morris *et al.,* 2006). So, members of the community usually deliberately chop the male trees since they do not produce fruits (Nghitoolwa *et al.,* 2003; Ngorima, 2006). Yet they are essential for pollination, which influences the yield of the female trees.

A study conducted in Namibia by Nghitoolwa *et al.* (2003) on adult marula trees demonstrated that female trees were generally more abundant than male ones, possibly because of the chopping of male trees. This has negative consequences on the natural flow of pollen, especially when the trees are far apart. This results in a reduction in fruit yield. The distribution of male vs female plants is a significant problem for marula trees growing under management on farmland, mostly related to the production and distribution of pollen to female trees

(Nghitoolwa *et al.,* 2003). For example, in commercial apple orchards, usually one in every eight to ten trees is a male pollinator located within a short distance of the female plants. Therefore, establishing the right balance between male and female trees is critical in marula's domestication and commercialisation programmes. At present, the ideal male-female ratios for optimal production are not known, and this topic warrants further investigation in carefully designed agronomic trials. The present study will, to some extent, provide useful insights by investigating and quantifying how the marula yield is influenced by soil and climate conditions for trees growing in their natural habitat.

2.3 Effect of harvesting marula fruit on the trees

Harvesting of marula fruit is an essential part of rural livelihoods in the Limpopo, Mpumalanga, KwaZulu-Natal and the Northwest provinces of South Africa. It contributes to both household consumption of the fruit and its products and income generation (Dlamini, 2011). Mostly women harvest marula by picking fruit from the ground where it ripens as illustrated in Fig. 2.2 (Dlamini, 2011; Neumann and Hirsch, 2000). Usually, marula fruit drops to the ground just before it ripens. The ripening process completes when the fruit has been detached from the trees for unclear reasons. Different studies have indicated that marula seeds germinate easily on the ground and vegetative propagation via utilisation of cuttings is used to grow new trees (Hamidou *et al.,* 2014; Maruzane *et al.,* 2002).

However, excessive picking of the fruit can have negative effects on the marula population dynamics in each area since the seeds are dispersed by animals which affect their distribution and germination and the sustainability of the species (Helm *et al.,* 2011; Lombard*et al.,* 2000). Authors such as Shackleton *et al.* (2001) and Shackleton et al. (2002) also argued that the collection of marula fruit for human utilisation might negatively affect the regeneration rates. Bishop and Scoones (2007) demonstrated the influence of technological advancement and increased harvesting of marula products on the tree in Botswana. The study showed how the level of resource consumption was influenced by the harvesting method.

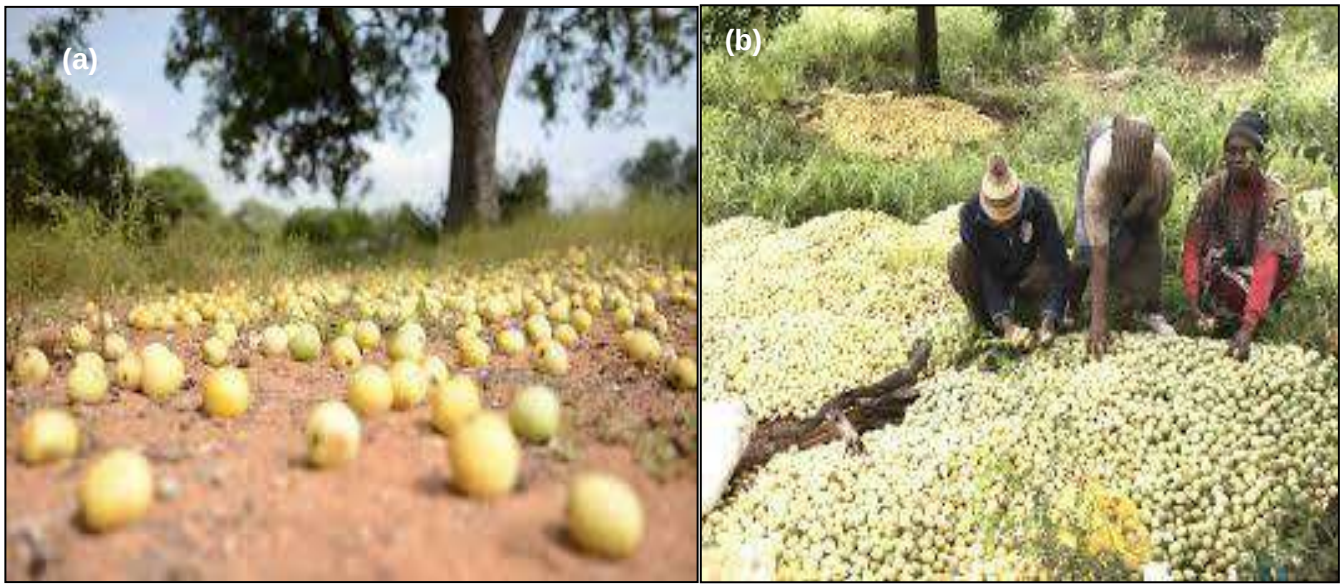


Fig 2.2. (a) Ripe marula fruit on the ground ready for picking. **(b)** Harvested fruit is mostly picked by women.

2.4 Pests and diseases that afflict marula trees and fruit

One challenge in the propagation of marula is that the tree is susceptible to different types of pests and diseases, such as insects and pathogenic fungi. The marula trees are prone to sap-stain fungi as well as other dangerous fungi. Moreover, pests such as *Ascomycetes psyllid* are a major problem that affects both nursery stock and wild trees. Termites often attack the roots and stem tissues (Orwa *et al.,* 2009). Marula fruit is also susceptible to the Natal and Mediterranean fruit flies (Graaf, 2007; Magagula and Ntonifor, 2014). According to Orwa *et al.* (2009), excessive infections have been discovered on wild trees although there were no signs that yields were affected. The same authors also indicated that white flies, aphids and thrips can also pose problems in the nursery, although they claim that utilisation of dichlorophos or malathion can manage these pests. Other possible problems in the nursery seem to emerge from the vulnerability of marula to ongoing disturbance of the root and mortality due to uncontrolled pruning of root at shallow depth. Powdery mildew can be endemic in humid conditions and can rapidly proliferate to all seedlings in a nursery unless sprayed with copper oxychloride.

2.5 Uses of different parts of marula trees

Marula trees are referred to as miracle trees in some communities because of their multiple uses. Almost every part of the tree, from the leaves, bark, wood, roots, fruit etc. has some use as we demonstrate in this section.

2.5.1. Marula fruit

Marula fruit is either eaten fresh or fermented for marula beer brewing (Hamidou *et al.,* 2014; Maroyi, 2013). The fruit has powerful alcoholic properties, and this is partly attributed to the twenty-nine different yeasts that are present in the skin (SyBille *et al*., 2012). Marula fruit is also good for collagen protein production, which is necessary for building and maintaining the health ofcartilage, joints, skin and blood vessels. It also acts as an anti-ageing agent (Hall, 2002). The mesocarp of the fleshy juice is pleasantly acidic, sour-taste, tart, thirst-quenching, refreshing,and energy-boosting (Bille *et al.,* 2013; Mn'gomba *et al.,* 2012). Marula juice is said to improve the utilisation of nutrients in the gut and acts as an aphrodisiac as it boosts sperm production (Marula Natural Products, 2012; Shackleton *et al.,* 2002).

The skin of marula fruit can be boiled and drank or burnt and utilised to substitute coffee. The fruit could be used to produce chutneys and pie fillings (Hal, 2013; Mawoza *et al.,* 2010). Marula plant material also has medicinal properties. The stem and bark contain antihistamines and can be steeped in boiling water and the steam inhaled for cleansing purposes (Ojewole, 2003; Mawoza *et al.,* 2010). The bark can also be crushed into powder, added to milk, millet or sorghum water, and drank for fever reduction (Hall *et al.,* 2000). A piece of the bark can be crushed to produce pulp, added to cold water and drunk to treat dysentery, diarrhoea, insect bites, burns and other disorders (Hall *et al.,* 2000). Marula fruit contains important bioactive compounds such as polyphenols, flavonoids, tannins, triterpenoids and phytosterols (Todorov and Dicks, 2009; Bille *et al.,* 2013). Moreover, different pharmacological studies have demonstrated that marula has functional properties such as anti-microbial, anti-diabetic, anti-diarrhoeal, anti-inflammatory, anti-hypertensive and antioxidant properties (Halet *al.,* 2014; Mawoza *et al.,* 2010).

2.5.2 Marula seeds

Marula seeds are rich in minerals and vitamins, such as iron, magnesium, calcium, zinc, phosphorus, nicotinic acid and thiamine (Abdalbasit and Ibrahim, 2012). They can also be eaten fresh, dried or milled and/or added to vegetables, soups and meat, making them reputable in providing flavours in foods (Maroyi, 2013; Sybille *et al.,* 2012). The fresh seeds are also added to boiled meat and consumed or added to porridge to enhance flavour (Kgomoamagodi, 2008). Marula seeds are also ground and shaped into meat-like cakes among Venda communities which can then be stored as “Venda biltong” for future use (Shone, 1979). Moreover, marula seeds can be turned into oil or snacks in Namibia. The oil is also used as a meat preservative among the Venda people (Duke, 1989; Maroyi, 2013).

2.5.3 Marula leaves and wood

The larvae of mopane worms (*Imbrasia belina*) hatch on marula trees and are collected and eaten by humans. Marula Natural Products (2012) hypothesise that large saturnalia caterpillars, as well as larvae of the cerambycid wood-boring beetle, are collected from marula trees for boiling or roasting by certain tribes in southern African countries. Moreover, two parasitic mistletoe species ecologically use marula trees as their host, thus accounting for the infection of *Erianthemum dregei* and *Pedistylis galpinii* (Dzerefos *et al.,* 1999; Dzerefros, 1996). The leaves can be used for making compost and as animal feed together with stems or branches (Hall *et al.,* 2000; Shackleton *et al.,* 2002). Marula tree wood is quite often carved into different products, such as spoons, plates, and decorative animal figures (Shackleton *et al.,* 2008). However, wood has been traditionally used to make charcoal since it does not burn well (Hall, 2002). The inner bark (cambium) can be used in making ropes and the whole bark is utilised in making a light brown dye and bitter tincture (Marula Natural Products, 2012).

2.5.4 Marula oil

Marula seeds contain non-drying oil, which is a rich source of protein and is utilised in combating stretch marks because of its anti-ageing properties (Abdalbasit and Ibrahim, 2012; Mawoza *et al.,* 2010). Marula oil possesses a high amount of mono-unsaturated oleic acid, which is a useful natural alternative to sunflower oil to produce biodiesel (Elijah *et al.,* 2012).

However, marula oil has a lower vitamin E content than other nut oils since it is low in B-tocopherol (Eromosele and Paschal, 2003). The soft kernel of the marula fruit can be processed into high-stability lipid and oleic acid (Marula Natural Products, 2012). Marula oil has also been utilised in making various medicines, insecticides and cosmetic and skin care products and this is attributed to its chemical stability and slow oxidising effect (Hal, 2013). Marula oil is very stable to lipid oxidation and communities in southern Africa use it to preserve meat and its products (Elijah *et al.,* 2012; Enweremadu and Rutto, 2015). Marula oil is said to be ten times more resistant to lipid oxidation than olive oil, making it one of the most stable natural oils (Elijah *et al.,* 2012).

2.6 Role of marula in sustaining livelihoods

2.6.1 Rural livelihoods

Marula fruit benefits rural communities in many ways. They sell the fruit and kernels to generate income. Children and adults mostly consume the fruit fresh, and it has massive nutritional benefits. The vitamin C content in marula fruit varies from about 62 mg/100 g to over 400 mg/100 g (Hal, 2014) which is at least four times higher than that of an orange. The flesh typically contains about 180 mg of vitamin C/100 g, but it can be even higher (Bille *et al.,* 2013).

Boiled marula juice is used to sweeten porridge in West Africa. However, the juice can also be used as food additives in various forms of porridges cooked from maize, millet and sorghum in southern Africa (Maroyi, 2013). In northern KwaZulu-Natal, parts of Mozambique, Zimbabwe, and Inhaca Island for example, unpublished data shows that the fruit has been used for domestic or subsistence purposes (Maroyi, 2013). About 59 – 77% of households in the Bushbuckridge area were found to consume marula fruit four to five times a week during the marula season (Shackleton *et al.,* 2002; Sinthumule and Mzamani; 2019). On average, approximately US$30 of marula fruit is harvested per household in a year in the Bushbuckridge area. About 40% of households were observed to have either retained or planted marula trees in their fields or backyards, albeit on a small scale. Two percent of the households in the Bushbuckridge area sell fruit products, such as beer and kernels.

By comparison, approximately 36 kg of marula fruit is harvested per household per season on average in the Makua village in Sekhukhune (Shackleton *et al.,* 2002). Each household in Manganeng village consumes on average 4.5 kg of marula fruit, mostly harvested from the bush. In the Kavango region in Namibia, marula fresh fruit and its kernels are not used for human consumption (Shackleton *et al.,* 2002). Instead, they brew traditional non-alcoholic beverages (*oshiwa*) which are very sweet and are produced especially for children. People also produce ready-to-drink (*omagongo*) which is normally served to the senior headman or the King during the one-day marula festival which occurs once a year (Misihairabgwi and Cheikhyoussef, 2017). The ethnobotanical benefits of marula to rural communities across different countries are summarised in Table 2.1.

Decoctions, infusions, or steam from boiled roots are used to cure heavy menstruation, bilharzia, coughs, weakness, sore eyes, heart pains and as an antiemetic. The marula leaves are essential in the provision of a remedy for abscesses, spider bites, and burns.

Table 2.1. Ethno-medical utilisation of marula tree in selected African countries.

Country of origin	Medicinal use	Part used	Application method
South Africa	Arthritis, cholera, dysentery, diarrhoea	Stem, bark	Decoction oral take
	Fracture	Bark	-
	Anti-ageing (body massage), moisturising, soothing, therapeutic	Seeds	Soothing, therapeutic Seeds crush and boil seeds, skim off oil, then external application
	Asthma, diabetes mellitus, epilepsy,fever, toothache, urinary tract infections	Stem, bark	-
South Africa, East Africa	Malaria	Bark	Infused in brandy and used as both prophylacticand treatment
Botswana, South Africa	Influence on the gender of the unborn child	Bark powdered	The infusion made from male

			or female plant
South Africa, Swaziland	Arthritis, cramps, kidney pain, rheumatism, sciatic	Bark	Grounded, mixed with *Schotia brachypetala* and warm water to induce vomiting, in the steam bath.
South Africa, Kenya	Burns, boils, dysentery, diarrhoea, fever, rheumatism, ulcers	Bark	Decoction orally taken
South Africa, East Africa	Malaria	Bark	Infused in brandy and used as both prophylactic and treatment
Uganda	Cuts, kill lice on hair, wounds	Black gum developing on plant	Rubbed on affected body parts
	Abdominal and stomach pains, colic, gastric and stomach ulcers	Bark	Boiled and orally taken Occasionally
Tanzania	Wounds Gonorrhoea Bilharzia	Bark Leaves Roots	Extracts used for cleaning, juice orally taken -
Niger	Bite by venomous animals Haemorrhoids, piles, Gonorrhoea, syphilis	Bark Bark Bark maceration	Decoction is orally taken Powdered and used as enema. Orally taken

(Mokgolodi *et al.,* 2011).

The leaves are also utilised as a sedative. Both bark and leaves are said to have antiseptic and astringent characteristics. The VhaVenda-speaking people in Limpopo province use the ground bark combined with soft porridge to make weaning foods that strengthen babies.

2.6.2 Commercial utilisation of marula products and byproducts

There are five (5) commercial market channels for the marula industry as summarised in Table 2.2, with the first four being the most important. The independent marula fruit traders that supply fruits to the Distell marula pulp factory in Phalaborwa make a better profit than traders of kernels and marula fruit to the Mhala Development Centre (MDC) in Thulamahashe

(Shackleton *et al.,* 2009). Nevertheless, traders that opt to sell home-brewed beer earn higher than average income, but the value-adding does lead to higher profits than those trading raw fruits to Distell. In 2002, Distell invested R1.5 million in the local economy by constructing the Marula Pulp Processing Plant in Phalaborwa and MDC invested around R700 000 in the local economy in 2001. In total, the contribution made by the formal marula industry in South Africa was estimated to be R2.2 million within the local economy between 2001 and 2002, with 50% collected from rural traders. At an industry level, the supply of marula fruit exceeds demand, and this makes traders of fruit and kernels price takers with no leverage to increase the price. Traders are therefore exploited by the pricing methods of the marula product producers. There is currently no significant consumer demand for beer and kernels outside local markets. Therefore, there is a need to develop the market irrespective of the extensive knowledge of the marula fruit.

Table 2.2. Commercial marula industry market channels.

Marula product	Responsible company	Status
Amarula cream	Distell (Pty) Ltd	The community trust and Distell own a marula pulping factory in Phalaborwa and a distillation factory in Stellenbosch. Amarula cream is South Africa's biggest and most generally distributed alcoholic beverage marketed nationally and internationally in more than 160 countries.
Juice	Mhala Development Centre	Marula juice produced in Thulamahashe, under the Mine Workers' Development Agency Marula Project. Pulping halted due to low productivity.
Oil	Mhala Development Centre	Oil is traded to Body Shop, an international environmentally conscious and fair-trade cosmetics retailer. The French company Aldiva S.A. is also involved in the commercial development of the oil. Low productivity of oil.
Beer	Rural Communities	Traditional marula beer produced by rural communities Actively traded within producer communities
Marula kernels/ traditional cooking oil	Mirma Kernel	Kernels are sold at informal and pension markets in South Africa and on a request basis from people's homes. Kernels and oil are traded locally and within the main towns in Namibia.

(Mander *et al.*, 2002; Shackleton *et al.,* 2002)

2.7. Commercial marula products

The most common processed marula products include jams, jellies, juice, and traditional/ commercial wines (Shackleton *et al.,* 2002, Saka *et al.,* 2008, Rampedi and Olivier, 2013). Figure 2.3 shows a summary of these products. Fresh fruit pulp or puree or a mixture of fruit juice concentrates are ingredients for the manufacture of dehydrated fruit rolls known as fruit leathers (Diamante *et al.,* 2013). Marula kernel extracts both seed oil and nut's hard-shell oil which are widely used as ingredients for cosmetics in skin care products, soaps, shampoos etc. These appear to be beneficial for oily, acne-prone, dry and ageing skin. It is also effective at keeping hair soft, moisturised, deodorants, mosquito repellent and applied in meat preservatives. Since marula oil is high in antioxidants, essential fatty acids and amino acids are also used to treat leather. Marula juice is processed into different alcoholic fermented products, such as marula gin, marula cream liqueur, and wine which are commercially available in different brands the world over.

2.8 Segmentation of the marula market

The marula industry has diverse segments ranging from food and drinks, cosmetics/essential oils (kernel oil), and pharmaceuticals as indicated in Fig 2.3. The marula pharmaceuticals sector is less commercialised, comprising mainly informal herbal medicine traders in rural areas and cities. The marula oil market is segmented based on the area of source, end uses, sales channel, and region. Marula oil is segmented into seed and nutshell. The oil is extracted using the decortications process and the global marula oil is segmented into industrial, commercial and household. The oil is refined, has high nutritional value, heat resistance and stability and is widely used in the baking, confectionary and sauces industries. The commercial segment is subdivided into hotels, restaurants, cafes and institutional food sectors. There is direct and indirect sales channel segmentation, while the indirect sales channel is subdivided into hypermarkets, stores, and online retail. Africa, the Middle East, North America, Latin America, Europe, Asia, and the Pacific are the main markets for marula products.

The industrialisation strategies of marula as an emerging industry in South Africa follow the unequal dualistic economy, and it is not adequately structured as shown in Fig 2.4. There are

no institutional arrangements, value chain actors, standardisation and regulation, and customer-accessible systems. The marula suppliers (individual households and communal marula resource owners) are central to the marula supply chain as illustrated by Fig 2.5. Value chain actors engage in upstream marula tree production and add value as informal traders andsmall-scale processors. Through their traditional knowledge of seed nurturing, and harvesting practices, supply and sell their marula products (beer brewing, jam, achaar and dried nuts production) directly to the public market. Moreover, they are capable of building social networks and links with unfair practice industry due to insufficient government support and regulation.

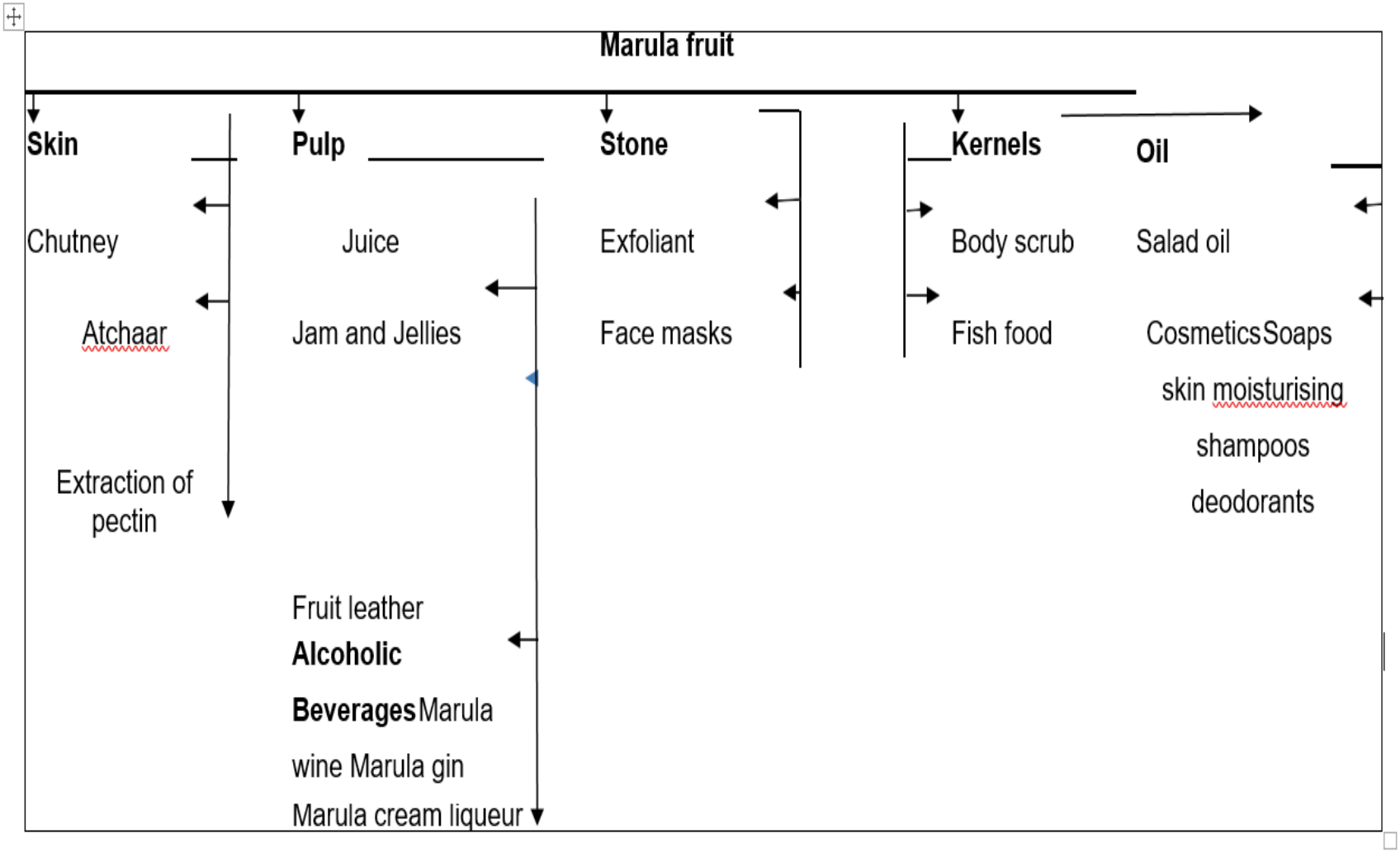


Fig 2.3. Schematic diagram showing marula fruit products

Table 2.3 The segment and sub-segment of commercial marula products which include food, drinks, cosmetics including for pharmaceuticals. African people traditionally consume marula as fresh and processed into juice, beer, jam achaar and oil.

Table 2.3. Commercialised products and their key players.

Marula Sectors	**Marula Fruit**	**Enterprise**
Food and Drinks Marula Fruit, Pulp and Kernel	Juice	Natural Products (MNP)
	Beer, Jam, Fruit leathers and Jellies	Self-Initiatives and Community informal trading
	Amarula Pulp	Mirma/Distell JV, Phalaborwa, Limpopo
	AMarula Cream	Distell (Stellenbosch Winery)
Marula Fruit, Pulp, Kernel	skincare Products soaps and	Marula Manufacturing (Pty) Ltd,
Oils/Cosmetics and Kernel-based	Shampoos	Metista, African Botanics, Lonza Groups, LLC, ROK stars, PLC, ACURE Organic
Pharmaceuticals Herbal Meds(whole tree) Leaves, Bark and Kernel	Treatment for syphilis, leprosy, dysentery, hepatitis, rheumatism, gonorrhoea, diabetes, dysentery and malaria	Traditional healers, Households, community

(Shackleton *et. al.* 2002)

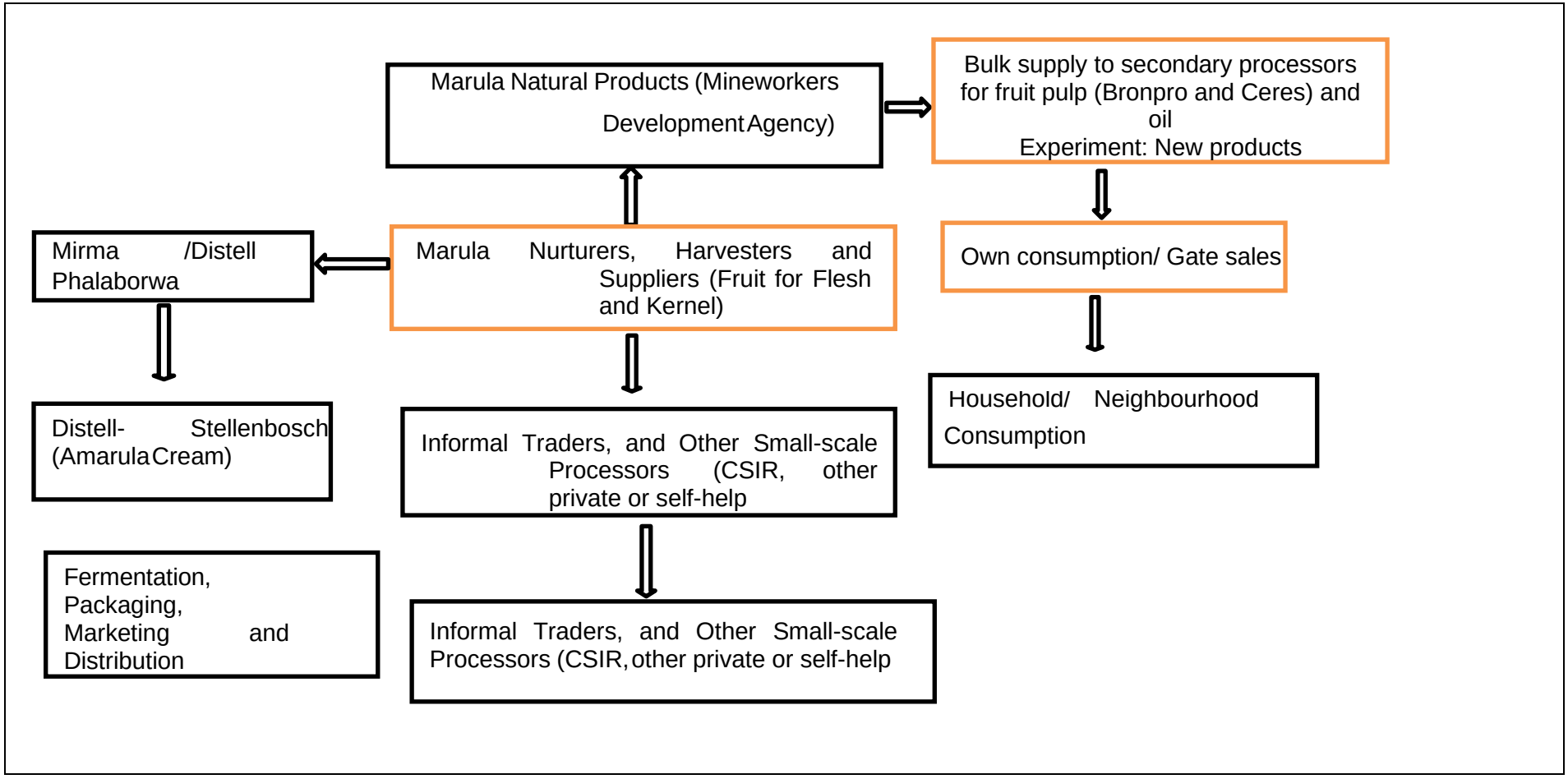


Fig 2.4. Marula commercial activity in South Africa.

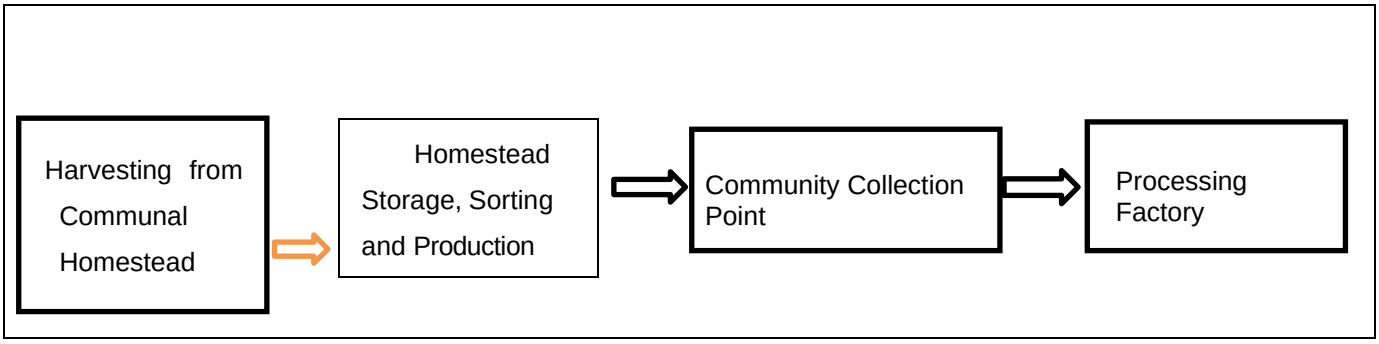


Fig 2.5. Marula supply chain.

2.9. Possible innovative marula products for commercialisation

Most unregistered informal traders such as producers, cooperatives, supplier associations, and household micro-processing of marula fruit products use laborious methods that yield little returns, have low productivity, and often produce poor-quality products. These entities purchase marula fruit from peers and supply it to local processors while the skin is processed into achar. Marula wood is commonly used for medicinal purposes, fencing and making utensils. Traditional healers use particularly the bark that is gathered before the first flush of the leaves to treat various illnesses including malaria, diabetes l, rheumatism, gonorrhoea, dysentery, hepatitis, syphilis, leprosy, and dysentery(van Wyk *et al.,* 2000; Mojeremane, 2004). There is a need to study the phytochemical properties of marula to develop innovative products that increase people's incomes and improve their quality of life.

Traditional processing of marula juice and beer is classified as inferior in terms of marula-like flavour. This could be because of heat processing of the pulp or due to the storage conditions of the pulp or juices before they are used by consumers or maybe it isbecause marula-like flavour compounds are left behind in the skin, which in many cases is discarded. Furthermore, very little is known about the flavour characteristics of marula products, and nothing has been published on the influence of storage and heat treatment on marula juice, pulp and its derived product flavour compounds. Therefore, there is a need to investigate marula processing to achieve the full potential for innovative commercialisation of marula products, such as carbonated marula drinks, vinegar and achaar production. The skin of marula in most cases is regarded as waste. Therefore, marula valorisation of wastes to pectin production may have a double advantage for the

rational use of bio-derived compounds that reduce land pollution and contribute to the sustainable development of the agro-food sector.

2.10 Possible innovative marula products for domesticated animals

Several authors have stated that Marula trees provide food for a variety of animals from game to livestock (www.krugerpark.co.za/africa_Marula.html; Mutshinyalo and Tshisevhe, 2003; Mlambo *et al.,* 2011). The tree is browsed, and the fruit is picked by animals. Marula fruit comprises the fruit skin, flesh and seed which hasa hard covering shell and the seed (Fig. 2.6).

Fig 2.6. Components of a ripe marula fruit.

Marula seed/nut is rich in crude protein and fat and has considerable amounts of minerals with low amounts of fibre (Mlambo *et al.,* 2011; Mdziniso *et al.,* 2016; Mthiyani and Mhlanga, 2017) which are adequate for different species of livestock. Mlambo *et al.* (2011) reported that marula seed cake (MSC) can be used in diets for feedlot cattle as a protein

source without negatively affecting the growth performance of cattle. Lactating dairy cows can be supplemented for protein with MSC in their diets without compromising milk production (Mdziniso *et al.,* 2016) whereas goats fed with hay can be given MSC as a protein supplement to improve their growth performance (Mlambo *et al.,* 2011). Broiler chickens can be given MSC as the protein source in limited amounts without negatively affecting their growth and carcass characteristics.

Many researchers have demonstrated that fruit skin wastes which are a result of fruit processing either for the juice market or alcoholic beverages can be used as an energy source for animals (Caparra *et al.,* 2007; Tayengwa and Mapiye, 2018). This gives an indication that fruit skins/pulp when included in the diets of animals, offer a source of supplemental energy to livestock. Marula fruit also, due to its high sugar content, has the potential to be used as an energy source or supplement to livestock. This, however, is an area that still needs a lot of research to ascertain its usefulness as an energy source due to the lack of information.

2.11 Challenges for commercialization of marula products

The South African legacy legislation restricts or prohibits livelihood trade-off in the commercialisation of multiple uses of marula which is an issue of concern and confusion surrounding the legality of harvesting marula. Again, marula compounds change significantly during the ripening stage and some compounds may be toxic if consumed in large quantities. There is a need for verification against the toxicity database for safe use. There is also a need for the establishment of quality control of marula products for new markets that require producers to satisfy international regulations concerned with consumer food safety, evidence of sustainable production, safe and hygienic manufacturing, local standards and testing infrastructure. The cost and complexity of securing access to new international food markets is expensive and complex while the traders are poor and lack financial support from the government. Although some marula products have a very strong history of safe use, they need formal documentation for international regulators and to be properly labelled to avoid misleading consumers. Figure 2.7 illustrates the challenges for innovative marula products for commercialisation purposes.

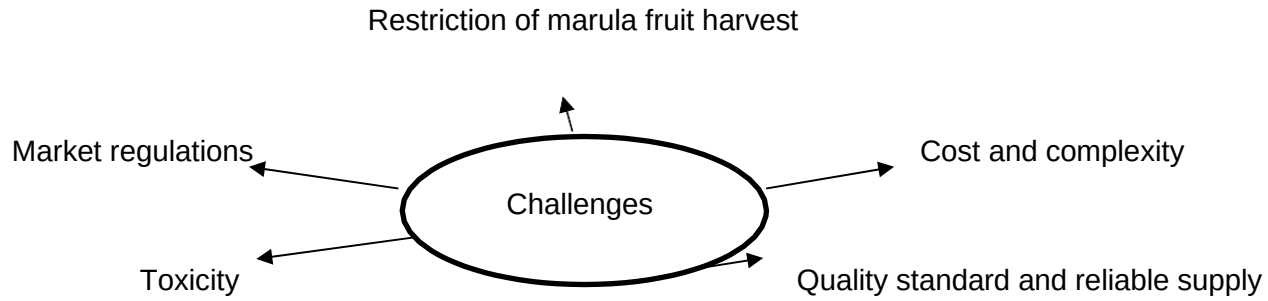


Fig 2.7. Challenges for innovative marula products for commercialisation.

2.12. Prospects for domestication and commercialization of marula

Several initiatives and programmes for the domestication of marula have been going on in southern Africa and started in the 1990s (Akinnifesi *et al.,* 2007; Kwesiga *et al.,* 2000). Research has been ongoing in the region on how to develop long-term domestication strategies such as collection of germplasm and improvement of tree genetic material, propagation systems and field management, harvesting and post-harvest technology, economic analysis and market research (Nyoka *et al.,* 2015; Mn'gomba *et al.,* 2012; Mokgolodi *et al.,* 2011). At the homestead level, marula trees are quite often preserved, and seedlings are often promoted (High and Shackleton, 2000). Erkkila and Siiskonen (1994) confirmed the purposeful cultivation of marula at household levels from seed or cuttings. However, national tree seed and/or seedling centres often do not stock indigenous tree species, such as marula, for sale in the way they stock exotic species, such as mangoes, citrus etc. since there is little demand for them. Farmers are, therefore, forced to plant only tree seedlings that are available in local government orprivate tree nurseries, rather than what they would have chosen (Jama *et al.,* 2008). Thisseems to limit the chances of local farmers to domesticate wild fruit trees, such as marula.Some farmers in rural communities for example in Namibia and Zimbabwe are also reported to be planting marula trees from cuttings (Du Plessis *et al.,* 2002; Ngorima, 2006).While there is limited extension material developed from research on indigenous fruit trees, the provision of improved germplasm may enhance the planting of indigenous fruittrees by expanding the markets for farmers, which can increase returns and encourage domestication (Cooper *et al.,* 1996; Akinnifesi *et al.,* 2007).

The commercialisation of marula products in southern Africa started when the liqueur Amarula was produced in South Africa (Strong, 2010; McCullum, 2000). Large-scale sustainable commercialisation of marula products would, however, mean planting marula trees in plantations for mass production. This could bring with it several threats to subsistence users, the resource base and the institutions regulating the marula resource use (Khan, 2002; Lombard *et al.,* 2000). It would mean that most of the forests must be converted into a monoculture of marula plantation, which would lead to a hostof other environmental challenges, such as pest control, loss of biodiversity, and reducedproduction of other food sources. Leakey and Simons (1998) reported the challenge of inconsistency in product quality and limited access to lucrative markets. Furthermore, farmers do not prefer to plant indigenous trees in their fields but would rather protect those that grow naturally for their individual use (Muok *et al.,* 2000; Shackleton *et al.,* 2001).

2.13. Climate and soils in marula producing regions

2.13.1. Climatic conditions

The goal of this study is to investigate the influence of environmental conditions (soils and climate) on the water use, growth, yield quality and quantity of marula trees and fruit. For this reason, the study was done in marula-growing regions with contrasting agro-ecological conditions. The regions include the Lowveld low rainfall (< 400 mm) Mopani District (blue dot to the north-east of Fig. 2.8), high rainfall (>700 mm) Bushbuck Ridge District (blue dot to the south of Fig. 2.8), and the medium rainfall Vhembe District (blue dot to the west of Fig. 2.8).

The low-veld region of the Limpopo Province has a wide variety of indigenous tree species which include marula (*Sclerocarya birrea* subspecies caffra). Because of the high demand for marula and its products, this species is classified as a protected endangered species in South Africa under the National Forest Act, 1998 (Act no 84 of 1998). Of the three Provinces in South Africa where marula trees grow, the highest concentration of these trees is found in Ba-Phalaborwa municipality in Limpopo (Mutshinyalo and Tshisevhe, 2003). Ba-Phalaborwa local municipality falls under the Mopani district municipality which alsoincludes Greater Giyani local municipality, Greater Letaba local municipality, Greater Tzaneen Local municipality and Maruleng local municipality. Ba-Phalaborwa might be

dominating in terms of marula tree density (number of trees per unit area), but these trees are also found in dense stands in all the local municipalities mentioned above.

According to the Koppen-Geiger classification system, modified for South Africa by Schulze *et al.,* 2007, the climate of the Mopani District is classified as *BSh* (Fig. 2.8). This represents a hot semi-arid climate. Temperatures in this region often soar to above 40°Cin summer with very low relative humidity which raises the atmospheric evaporative demand substantially.

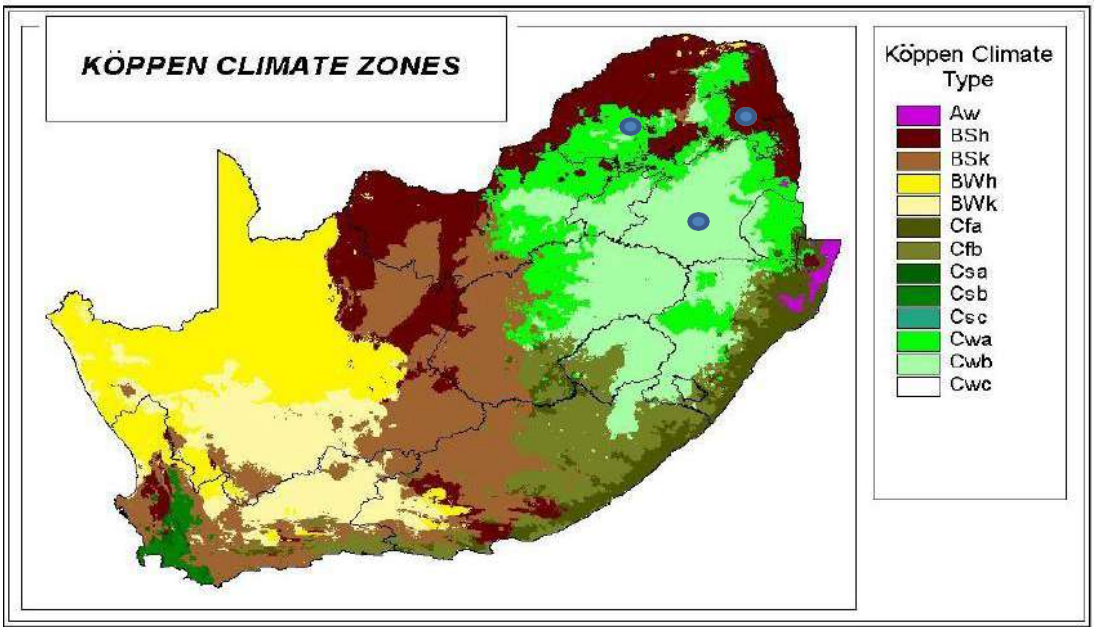


Fig 2.8. Koppen climate zones for South Africa (Schulze *et al.,* 2007).

The blue dots represent the location of the study sites.

Classification of the climate in the Vhembe region, whose long-term average annual rainfall is in the range of 400 to 600 mm can be classified as the *Cwa*, according to the Köppen-Geiger system. This classification represents a humid tropical to subtropical climatic region which is somewhat different from the Mopani region. The detailed long-term climatic conditions of this study area will be presented in future deliverables.

The third study region is the high-rainfall Bushbuck Ridge region, where significant standsof marula are also found. The climate of this region is classified as *Cwb* according to the Köppen system. This represents a subtropical highland climate with average temperatures for all months below 22°C. So, the temperatures are relatively milder than in the other two (2) regions, although this area has substantially higher rainfalls with the long-term average exceeding 800 mm per annum. Based on this information, we can hypothesise that the marula trees in the Bushbuckridge area do not experience as much water stress as at the other two (2) sites.

2.13.2. Soil characteristics

Studies conducted on marula trees growing at different locations show that this tree growsmostly in sandy, sandy loam soil and to a lesser extent in clayey soils (Mutshinyalo and Tshisevhe, 2003; Sinthumule and Mzamani, 2019). These soil types, especially sandy tosandy loam are generally the dominant soils in the Mopani district as well as the north- eastern region of the Bushbuckridge local municipality in the Mpumalanga Province (Fig.2.9). Lewis (1987) reported that marula trees are found in well-drained soils in large numbers, but they also grow in other different soil types in low rainfall areas (ranging from200 – 1 500 mm). A detailed description of the soil types on the map shown in Fig. 2.9 is given in the FAO, 1996 reference.

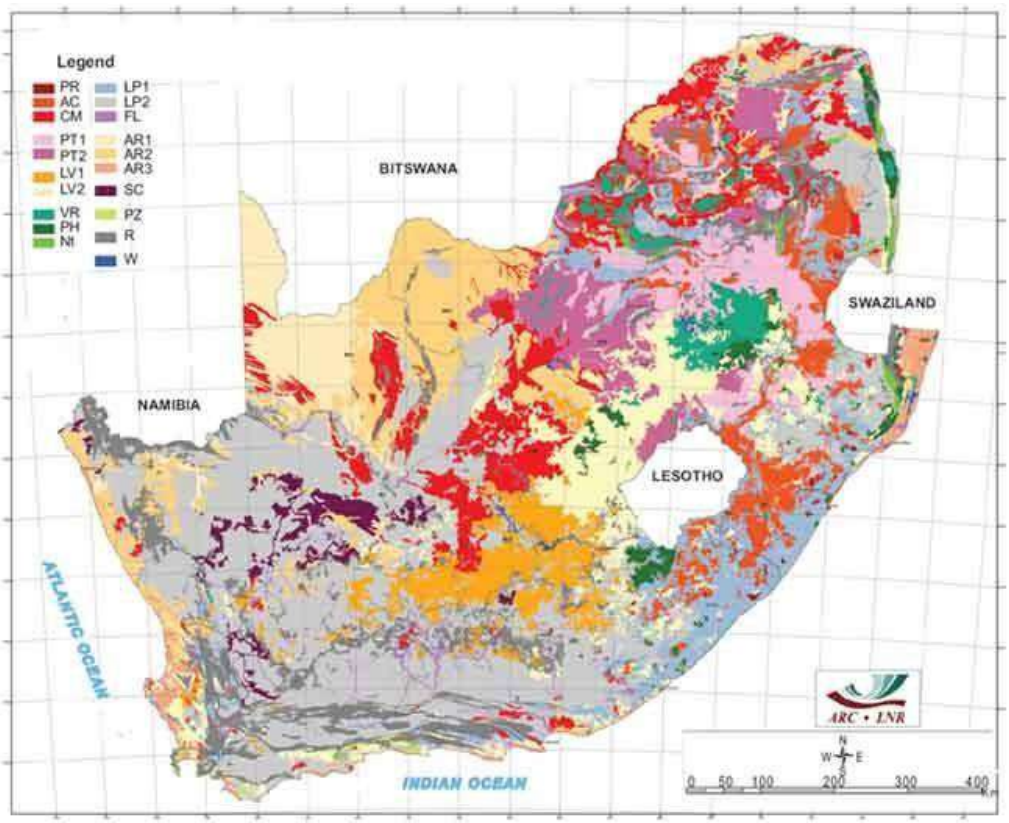


Fig 2.9. Soil map of South Africa (FAO, 1996).

2.14. Climate change in marula producing regions

Different marula-producing regions have different climate change projections. For example, in the Limpopo Province and the northern parts of Mpumalanga, drier conditions are predicted in future with fewer rainy days during the active marula growing season from December to May (DEA, 2019). The frequency of extremely hot days is also predicted to increase significantly. On the other hand, in most of the KwaZulu-Natal Province and the southern parts of Mpumalanga, no major changes are expected in the precipitation trends. But the maximum and minimum temperatures are expected to increase. A common feature of these production regions, however, is that the atmospheric evaporative demand will increase, leading to higher irrigation water requirements.

Given that water resources in some catchments in the marula-producing regions are already fully allocated, the production of some conventional crops, especially those that depend on irrigation will be very difficult, if not impossible in future. Therefore, there is a need to accelerate research on alternative crops that can withstand harsh growing conditions. Marula is a good example of such a crop as demonstrated by the studies in the Negev desert in Israel (Nerd and Mizrah, 2000). In that study, they planted marula trees in different environments within the desert. In some places, the treeswere exposed to extreme heat, low rainfall regimes, poor-quality brackish water etc. The performance of the marula trees in terms of growth and yield was satisfactory in nearly allenvironments. The best outcomes, however, were at the sites with poor-quality brackish water which was not surprising given that marula is generally salt tolerant. This raises the prospects of irrigation of marula with low-quality water e.g. reclaimed water, although further research is required locally to confirm this.

2.15. Quantifying water use, yield and water use efficiency of fruit trees

Three previous WRC-funded projects have quantified the water use of Indigenous trees (Dye *et al.,* 2008; Gush *et al.,* 2015; Ntshidi *et al.,* 2022). Dye *et al.* (2008) measured the sap flow of marula trees in the Kruger National Park using the heat ratio sap flow method though no yield or growth data were reported. Gush *et al.* (2015) on the other hand,measured both the transpiration and evapotranspiration components in severalIndigenous forests, but mostly for timber production. While stem growth measurements were taken, no yield data was reported. The study by Ntshidi and colleagues focused on fivedifferent indigenous fruit tree species. But it did not adequately capture the effect of the agro-ecological region on the performance of the trees given the large number of trees studied.

The present study used similar data collection methods as previous studies. However, the focus was on how the environmental conditions (climate and soils) affected the performance of marula trees in their natural habitats. Eco physiological variables quantified included:

- The full suite of weather variables using Automatic Weather Station (AWS)

sensors(solar radiation, temperature, relative humidity, wind speed and rainfall) at hourly and daily intervals.

- Transpiration / water-use (sap flow) monitoring using appropriate systems e.g. theHeat Ratio Method (HRM) or Thermal Dissipation Probe (TDP) systems also at hourly intervals
- Volumetric soil water content in the root zone of the trees at hourly intervals.
- Tree stem diameters and canopy dimensions.
- Tree phenology.
- Fruit growth rates and yield, and.
- Fruit internal quality for different study sites.

Further research was done under laboratory conditions e.g. to investigate the possibilityof developing new novel commercial products from marula fruit. In the sections that follow the team gives the details of the various data collection protocols used in different studies. Where appropriate we also provided information on key findings from the previous studies on indigenous trees. Water use efficiency in this study is defined as the ratio of yield produced per cubic meter of water transpired by the trees. Nyoka *et al.* (2015) reported for marula years when the trees had completely no fruit following a year of normal to high crop load.

2.16. Water use efficiency and fruit growth

Several different forms of water use efficiency exist depending on what is being measured, and clear definitions and as sociated units are important. These may be of a bio-physical or economic nature. Since many different definitions of WUE exist in the literature, it is important to clarify the specific definition of WUE to be adopted in the study, as well as the methods (and units) used to quantify this term. The plethora of definitions that existin the available literature is due to the many ways in which water use and yield can be measured/quantified and defined. For example, water use efficiency may be defined asthe ratio of yield (i.e. mass of utilisable fruit produced), against the accumulated water transpired by the tree over a calendar year, with resultant units being kg m^{-3}.

The preferred spatial scale is per unit hectare, but due to the non-uniform distribution of sample trees, it may be necessary to revert to results being on an individual tree basis. In an agricultural environment, production is commonly described as crop yield (kg or tons) per unit area (hectare). Agronomists therefore prefer the term water productivity, which is defined as crop production (as opposed to biomass production) per unit amount of water used over a specific area (e.g. ha). However, to avoid confusion, it is suggested that in this study the term WUE be used to describe bio-physical yield (fruit) produced per unit water used (e.g. kg m^{-3}), while the term WP be used to describe product value produced per unit water used (e.g. R m^{-3}) being an economic indicator of water productivity.

Besides the data collected on marula by Ntshidi *et al.* (2022), growth and fruit production data for marula is generally scant and frequently circumstantial (Nyoka *et al*., 2015). Fruit growth rate can be measured manually using Vernier calipers or continuously using electronic dendrometers. Fruit per tree will be determined according to the procedure described by Dzikiti *et al.* (2022).

2.17. Marula fruit quality

We hypothesize that differences in environmental conditions in various agro-ecological regions affect physiological processes such as photosynthesis and transpiration, and hence the marula fruit quality. This could be due to differences between the sites in the levels of water stress experienced by the trees due to variations in available water (rainfall), vapor pressure deficits, and the water holding capacity of the soils. To test this hypothesis different quality attributes for Marula fruit will be determined at harvested fromvarious sites using lab-based methods. The mineral profile of the harvested fruit will be conducted using ICP-AES and ICP-MS for the selected analytes as stated by Anyasi *et al.* (2018). The instrument will be calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. Dietary fibre was determined using the methods of Englyst *et al.* (1992) and Goni *et al.*(1996). Antioxidant activities were assessed on the marula fruit according to theapproach by Pfukwa *et al.* (2019). Total phenolics, flavonoids, tannins, and monomeric anthocyanins, were also determined according to Giusti

and Wrolstad (2001); Yang *et al.* (2009), among others. The phytochemical properties of the fruit were determined through a polyphenolic profile of the fruit and selected products and bi-products using Liquid Chromatography Coupled to Diode Array Detection and Electrospray Ionisation Mass Spectrometry (LC-DAD-ESI-MS). Food safety evaluations for products like marula fruit juices and jams were done to test for a range of pathogenic and non-pathogenic bacteria such as coliform bacteria and *Escherichia coli,* yeast and moulds, *Salmonella species, Bacillus cereus* and *Staphylococcus aureus.*

2.18. Review of water use and water use efficiency of indigenous trees

Over the last 10-15 years there have been many studies in South Africa that have quantified single-tree water use for indigenous tree species. Details of these studies are given in the reports to the Water Research Commission (Dye et al., 2008; Gush and Dye2015; Gush *et al.,* 2015). Examples of non-indigenous fruit tree species studied include *Trema orientalis*, *Celtis africana*, *Podocarpus falcatus*, *Ptaeroxylon obliquum*, *Olea europaea* subsp. *africana*, and *Berchemia zeyheri* in the KwaZulu-Natal midlands (Gush and Dye, 2009), *Vachellia* (formerly *Acacia*) *kosiensis* along the KwaZulu-Natal north coast (Gush, 2017), *Ilex mitis*, *Ocotea bullata* and *Podocarpus latifolius* in the Southern Cape region of South Africa (Mapeto *et al.,* 2017). In all these studies, the HRM sap flow method was used to collect data over one year. Patterns of water use throughout the year were different for the different species. Comparisons between the water-use efficiency of Indigenous trees with exotic species showed relatively lower water-use efficiencies in some indigenous species. These low water use efficiencies, calculated on the wood biomass basis, were a consequence of the slow growth rates of indigenous treesattributed to competition for resources, and not high water-use rates.

The water use rates of indigenous tree species were generally lower than that of co-occurring exotic species. According to Gush *et al.* (2015), the total sap flow volumes for the Indigenous trees cited above averaged 3 343 ± 1 242 L tree^{-1} year^{-1}. This was substantially lower than that for the introduced plantation species,

which averaged 10 300 ± 2 890 L tree^{-1} year^{-1} for *Eucalyptus* clonal trees, 7 994 ± 5 995 L tree^{-1} year^{-1} for *Eucalyptus grandis* trees and 7 488 ± 4 473 L tree^{-1} year^{-1} for *Pinus* species. The transpiration data reported by Gush *et al.* (2015) is of the same order of magnitude as thatreported by Dzikiti *et al.* (2022) on marula and *S. spinosa* trees, respectively in northern KwaZulu-Natal. The transpiring leaf surface areas of the two species were not vastly different, with the peak leaf area index (LAI) of *S. spinosa* being about 1.42 m^2/m^2 compared to approximately 1.20 m^2/m^2 for *S. birrea*. However, the maximum transpirationper unit leaf area was higher for *S. spinosa* at approximately 2.3 L/m^2/d compared to about 1.4 L/m^2/d for *S. birrea.* The corresponding daily peak transpiration of *S. spinosa* (~ 36 L/d) was almost double that of *S. birrea* (~19 L/d) reached during the summer months. The annual total water use of an individual *S. spinosa* tree was about 6 061 litres while marula used close to a third of this amount at only 2 160 litres per tree. The low annual water use of marula was attributed, firstly to the low daily transpiration rates per unit leaf area, which is presumably related to the physiology of the species. The second reason for the low annual water use of marula is the rather long dormant period for this species compared to other indigenous fruit trees. Even though the two (2) species grew in thesame locality, and therefore experienced the same resource constraints, their water use patterns responded differently to environmental factors. *S. spinosa* transpiration, for example, was somewhat correlated to the atmospheric evaporative demand (R^2 = 0.37). However, it was poorly correlated to the soil water deficit (R^2<0.10). The influence of these water-use drivers on marula transpiration, for example, was the opposite of those of *S. spinosa*. The atmospheric evaporative demand explained less than 20% of the variation in marula transpiration (R^2 = 0.19), but the soil water deficit in the root zone explained more than 65% of this variation (R^2 = 0.66). This example for the marula and Strychnos suggests that there exist significant physiological differences in the transpiration response of different indigenous fruit tree species even in the same growing region.

An additional study that quantified the water use of Indigenous fruit tree species was doneby Clulow *et al*. (2013). They measured the water use (transpiration) of *Syzygium cordatum*, *Eugenia natalitia* and *Drypetes natalensis* trees growing in peat swamp forest (PSF) and dune forest (DF) sites within the iSimangaliso

wetland park in northern KwaZulu-Natal. Over 12 months, transpiration was approximately 10,000 litres of water per tree for *S. cordatum* (PSF), 3 500 litres for *Eugenia natalitia* (DF) and just 600 litres for *D. natalensis* (DF). These results suggest that location in the landscape has huge effects on water availability and hence on the water use of indigenous trees. At the PSF site unconstrained by water availability, limits to transpiration were primarily driven by radiation interception levels and Vapour Pressure Deficit (VPD). At the drier DF site, however, soil water availability played a dominant role in limiting transpiration; with tree size and physiology playing a role at both sites.

CHAPTER 3

QUANTIFYING WATER USE AND YIELD OF MARULA TREES ACROSS A RAINFALL GRADIENT

3.1. Summary

Currently, no accurate quantitative information exists on how the water use–yield relationships of marula trees vary with environmental conditions. In this chapter, we attempt to close this important information gap by quantifying the transpiration, growth, and yield dynamics of these trees growing across a rainfall gradient. Data were collected in three study areas namely 1) the high rainfall Thulamahashe area in Mpumalanga, 2) the medium rainfall Moeketsi area in Limpopo, and 3) the low rainfall Phalaborwa area in Limpopo. Besides differences in rainfall, there were also variations in soil types leading to differences in the available soil water. Data were collected over periods ranging from two to three years per site. This included tree transpiration rates measured using the heat ratio method of monitoring sap flow, volumetric soil water content, and tree and fruit growth rates. Tree phenology was also documented across the three sites to compare the role of the local microclimates on tree and fruit development. Climate data were obtained from nearby weather stations operated by the Agricultural Research Council. Sap flow data showed that water use by individual trees was strongly dependent on tree size and the available soil water. Peak transpiration at the three sites exceeded 250 litres per tree per day on warm summer days when soil water was not limiting. Maximum yield per tree approached 50 kg, consistent with other published data. The measured transpiration and weather data were used to validate a water use model developed for marula by Dzikiti *et al.* (2022). This model was subsequently used to predict likely trends in water use patterns by the marula trees under future climate change scenarios for the period 1960 to 2099. Model simulations suggest that even if the atmospheric evaporative demand of the study regions was projected to increase over this period, the estimated increase in actual tree transpiration was insignificant. The reason for this is that as the atmosphere

becomes warmer and drier, the vapour pressure deficit of the air increases. In the case of marula trees, this seems to cause the stomata to close thus preventing excessive transpiration rates. Although we did not model the future yield trends, the reduction in the stomatal conductance of the marula trees would probably cause a greater reduction in transpiration than photosynthesis given that the water vapour gradient across the stomatal pore is much steeper than that of CO_2. This scenario will likely lead to enhanced water use efficiency for this species although more data is needed to confirm this. They begin this chapter by describing the long-term climate conditions in the three study sites. They then describe the experimental data collection and water use modelling in the later parts of the chapter.

3.2. Characterizing the long-term climatic conditions in the study areas

According to the project terms of reference, this study was to be done in three agro-climatic regions with contrasting microclimates, especially rainfall. Figure 3.1 shows a map of the Limpopo and Mpumalanga Provinces with the location of the three study sites indicated using green stars. The selected study sites include Thulamahashe, which is situated in a high rainfall area near Bushbuckridge in Mpumalanga. Data collection at this site started in November 2021. The second site is at ZZ2 farm located near Modjadjikloof in Tzaneen in Limpopo Province in a medium rainfall region. The equipment was installed at this site in August 2022. The third site is at the Wastewater Treatment Works in Phalaborwa, which is a low rainfall area. In addition to the terms of reference which required us to investigate and quantify the relationships between tree water use and yield along a climate gradient, several other factors were considered in the selection of the study sites used in this study. The highest priority was put into finding suitable sites in regions with contrasting microclimates. Other factors that influenced the choice of sites included the availability of trees that were suitable for quantifying water use using the heat ratio method of monitoring sap flow. This was important given that marula trees tend to be sparsely spaced in the wild and instrumenting several of them with the same equipment could be impossible. Security of the equipment from vandalism, theft and damage by wild animals were also considered. The availability of assistance to change batteries and to harvest the marula fruit that falls over a prolonged period were also important considerations.

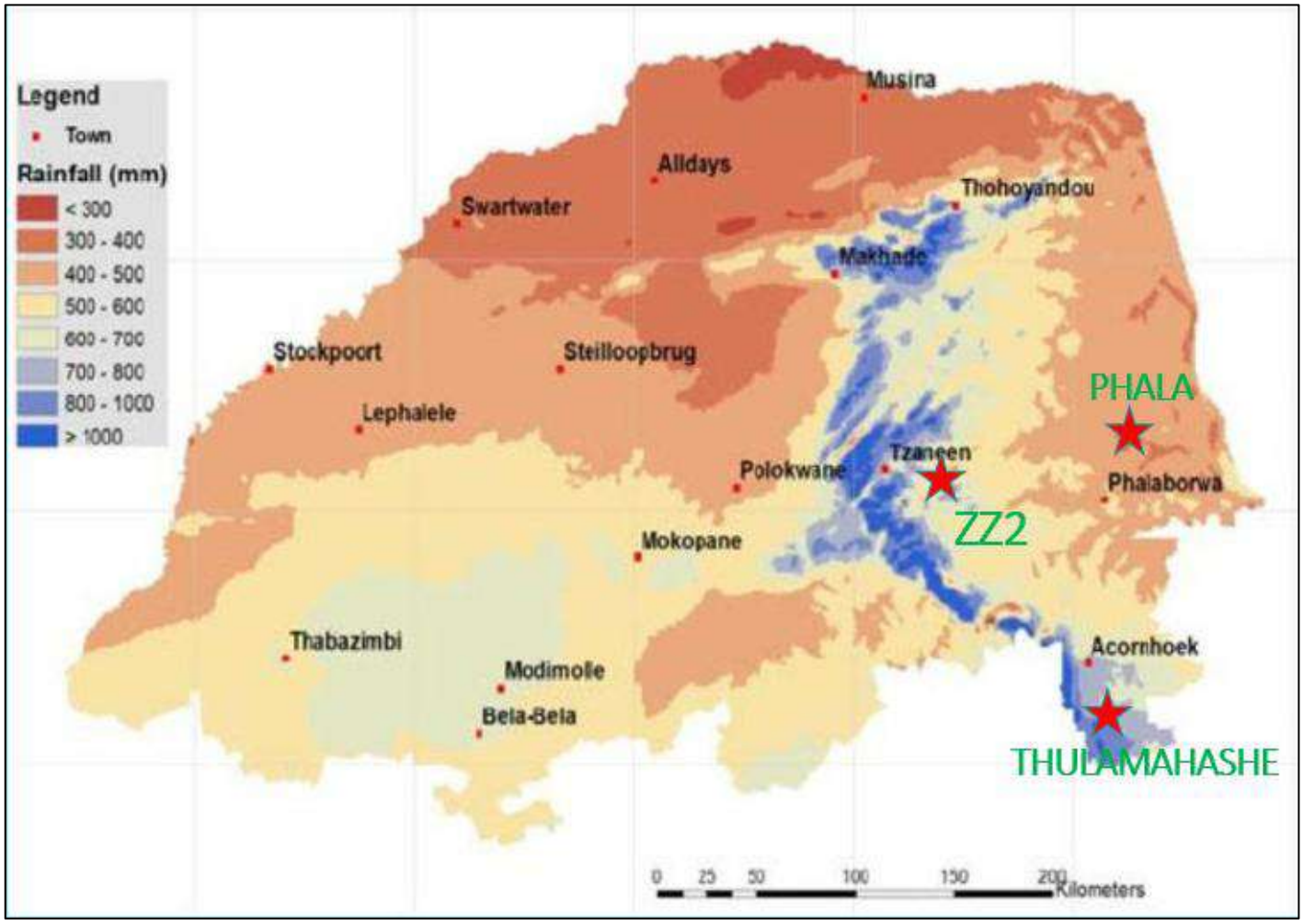


Fig 3.1. The three study areas namely Thulamahashe in the high rainfall area, ZZ2 near Modjadjikloof with medium rainfall, and Phalaborwa (Phala) in a low rainfall area.

To establish the differences in key climatic factors driving water use and yield, we obtained historic daily weather data from the Agricultural Research Council (ARC) stations located near each study site. This analysis was done to understand the long-term trends in the climatic variables and how these vary between seasons and between years. The length of the historical data varied from two years at Phalaborwa to five (5) years at Thulamahashe and ZZ2, respectively. The short duration of data from Phalaborwa was due to the weather station not working consistently due to technical problems until very recently.

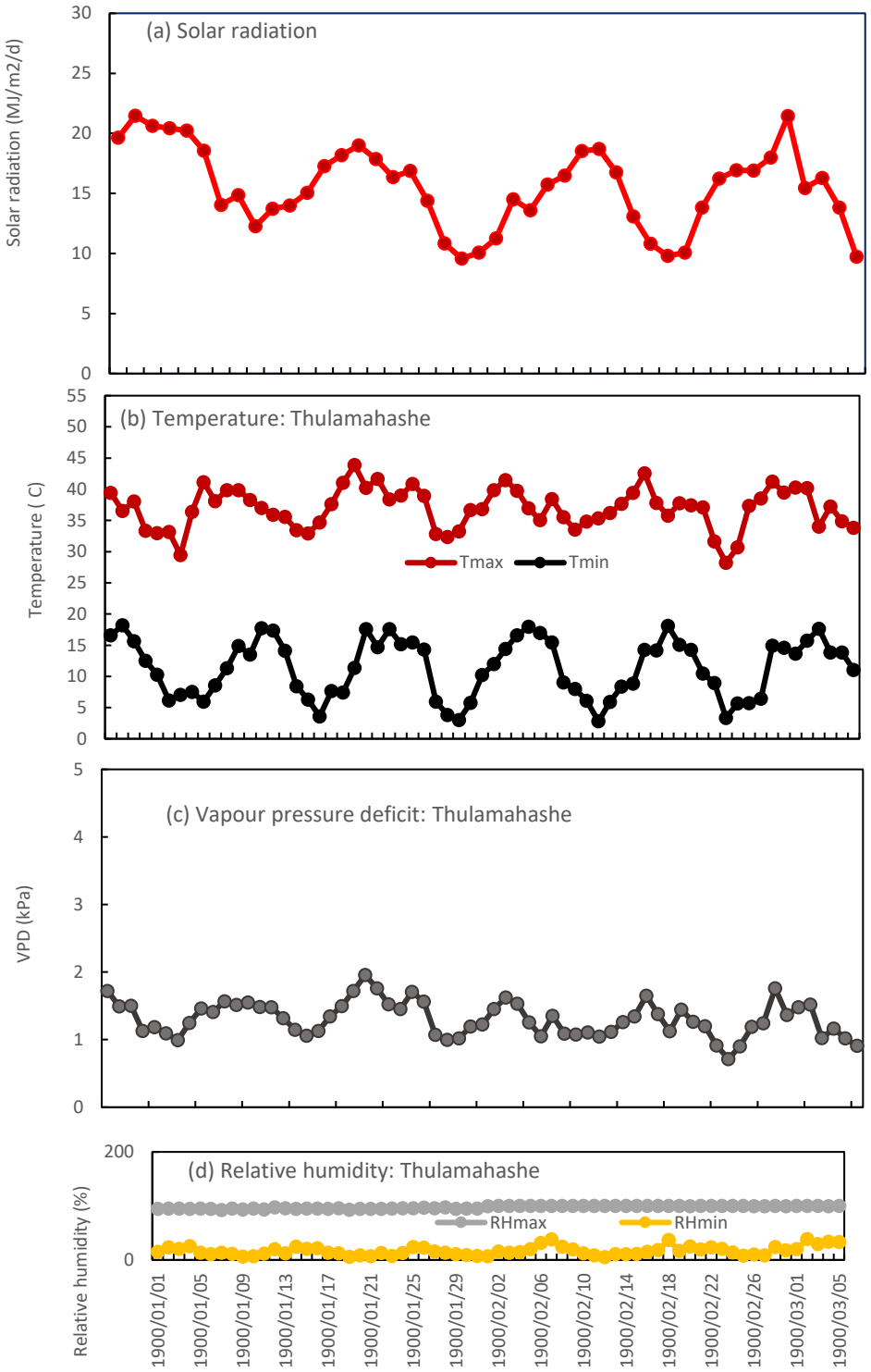


Fig 3.2. Monthly climate conditions for Thulamahashe from January 2018 to June 2023.

At Thulamahashe the daily total solar radiation (Fig. 3.2 a) peaked just above 21 MJ/m^2 in early summer (November) dropping somewhat in midsummer due to the high incidence of cloud cover. The maximum air temperature over the 5 years was around 44°C in early summer while the lowest temperature recorded was around 4.4°C.

Marula trees are known to be sensitive to frost, and frost incidences do not appear to be a regular occurrence in this region. The average vapour pressure deficit of the air (Fig. 3.2 d) was low being less than 2.0 kPa due to the high average relative humidity levels (Fig. 3.2 c). On hot and dry days, the humidity dropped to as low as 6%. As expected Thulamahashe received rainfall during the summer months which also had the highest reference evapotranspiration (Fig. 2.3). The five (5) year average of the total annual rainfall (2018 – 2022) for Thulamahashe was 430 mm. This is quite low compared to the long-term average rainfall which is close to 700 mm per year possibly because of the occurrence of droughts in the five years under review. The five (5) year average annual reference evapotranspiration was almost four times higher than the rainfall at 1 588 mm. These data gave an aridity index, calculated as the ratio of the rainfall to the reference evapotranspiration of 0.27.

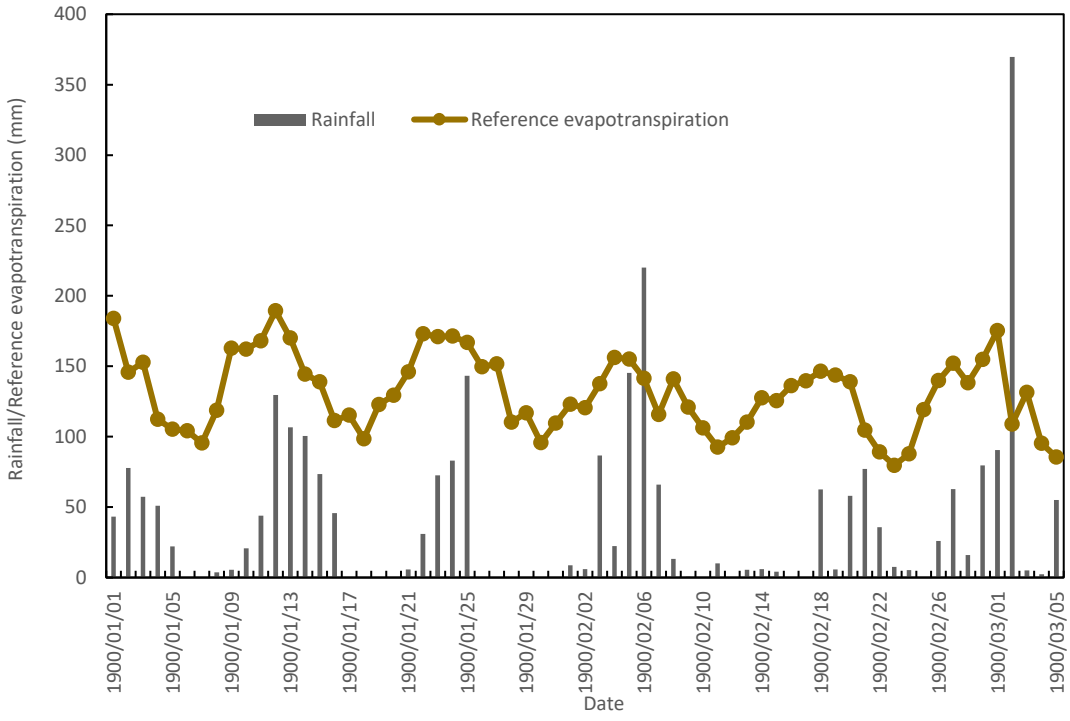
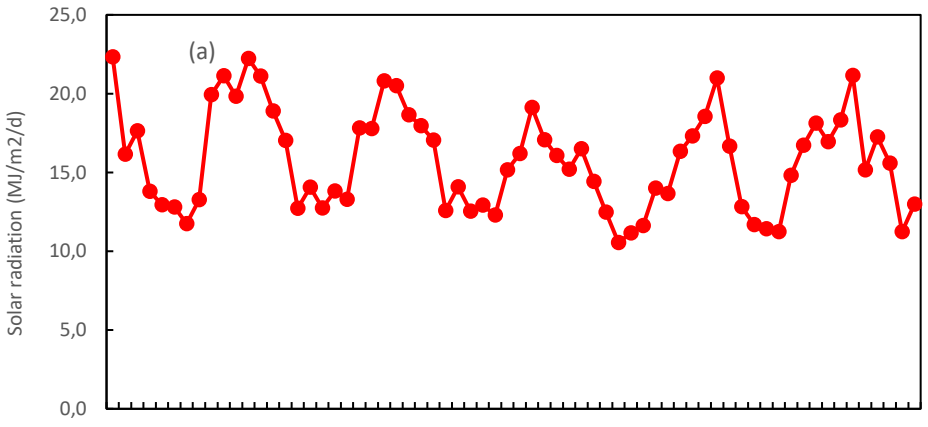


Fig 3.3. Monthly rainfall and reference evapotranspiration for Thulamahashe for the period January 2018 to June 2023.



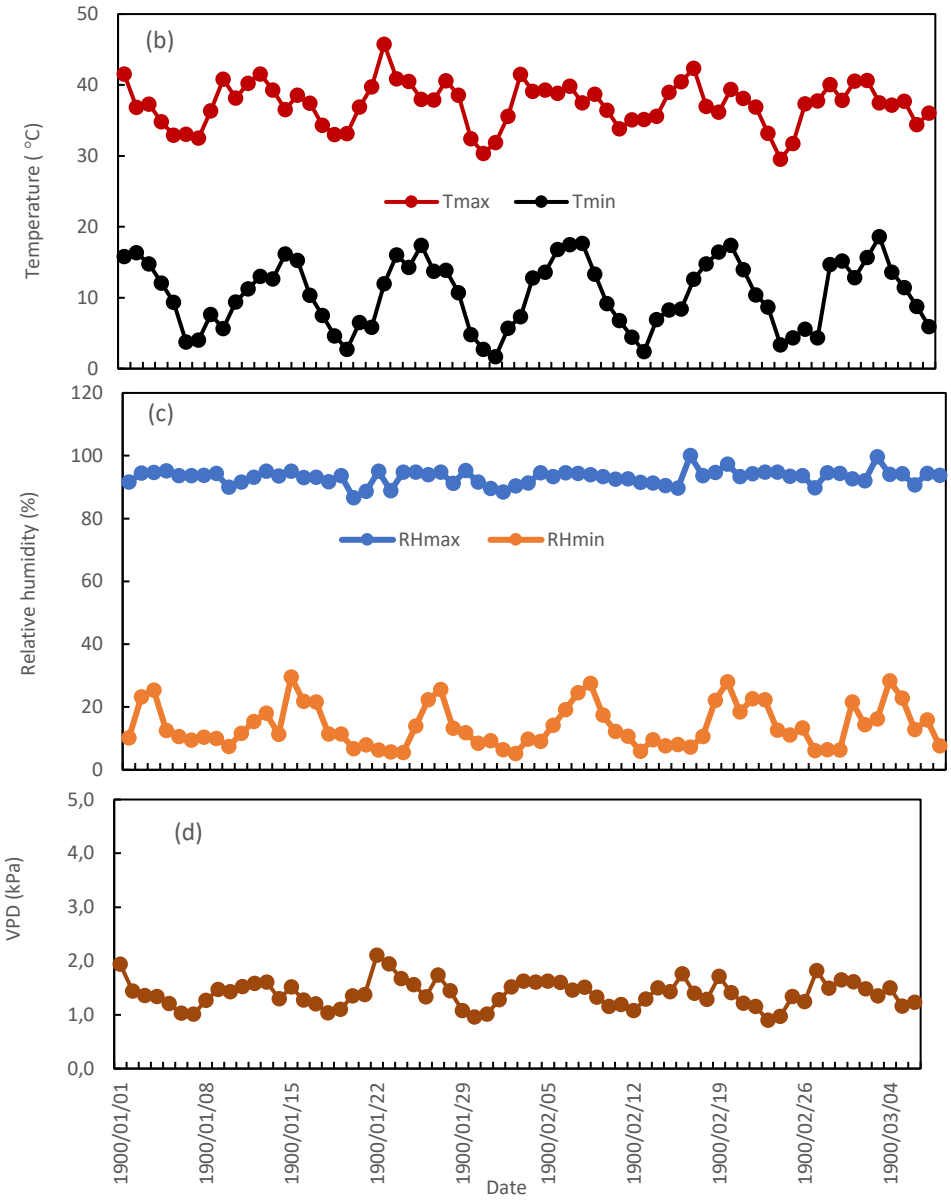


Fig 3.4. Monthly summary of climatic conditions at ZZ2 near Tzaneen.

The daily peak radiation intensity at ZZ2 was similar to that at Thulamahashe at approximately 22 MJ/m^2 (Fig. 3.4a). A maximum temperature of 45.8°C was recorded in October 2019 while the long-term average maximum temperature was around 40.7 °C. The minimum air temperature dropped to around 1.7°C in July 2020 while the 5-year average minimum temperature was around 3.0°C. The lowest relative humidity

dropped to around 5.5% in early summer although the vapour pressure deficit of the air was quite low hovering around 2.0 kPa.

The five (5) year average annual total rainfall for ZZ2 from January 2018 to December 2022 was 443 mm (Table 3.1) compared to the reference evapotranspiration of 1472 mm (Fig. 3.5). This gave an aridity index of 0.30 which was slightly lower than that for Thulamahashe.

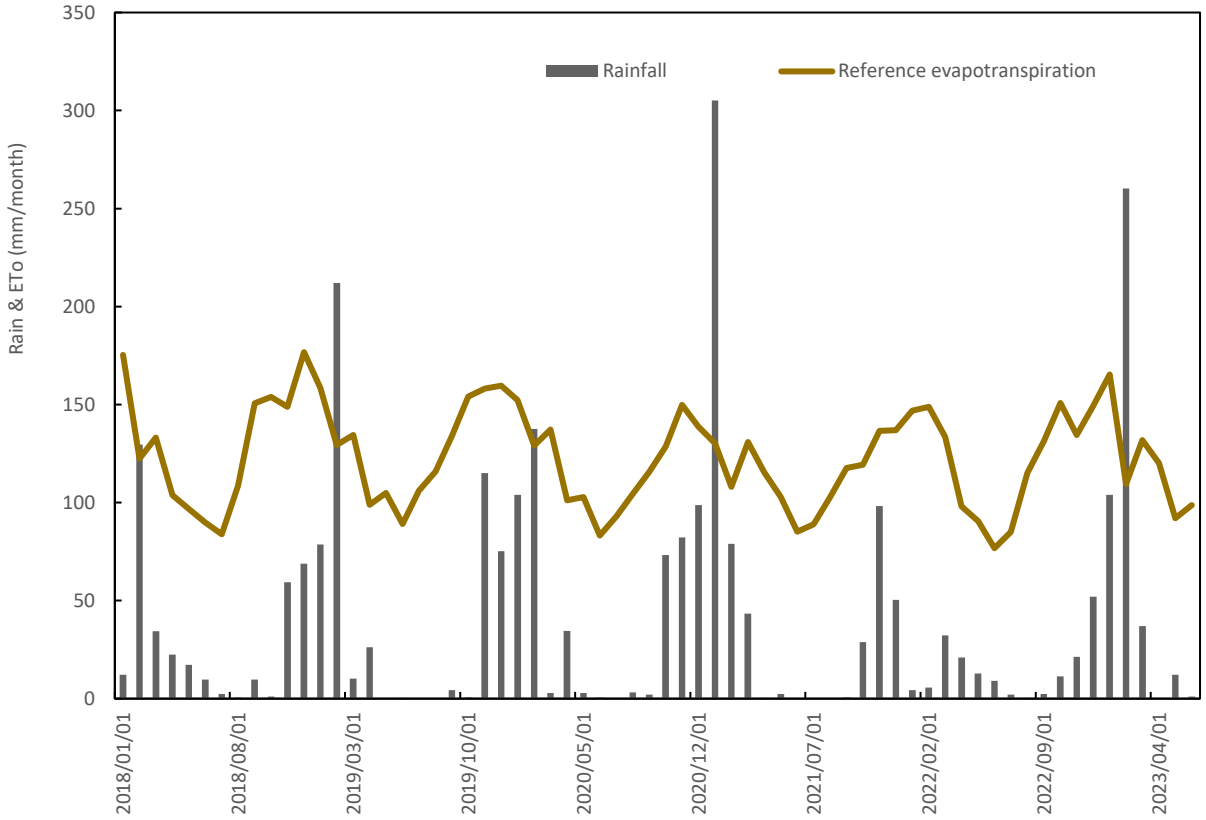
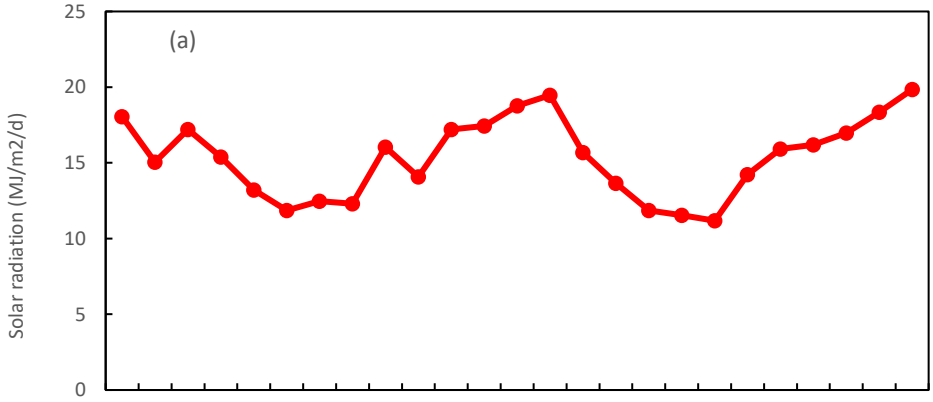


Fig 3.5. Monthly total rainfall and reference evapotranspiration for ZZ2 from January 2018 to April 2023.



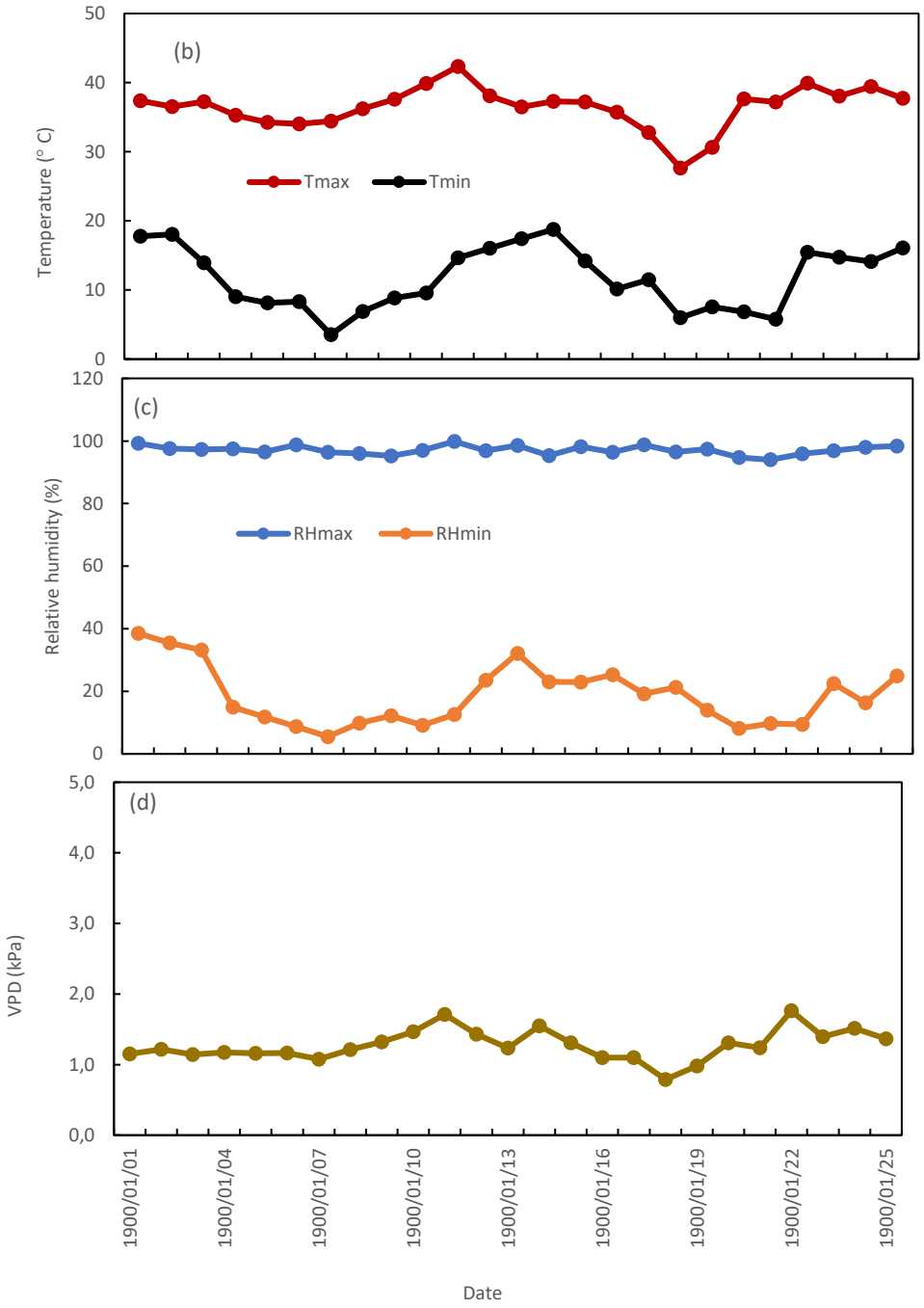


Fig 3.6. Monthly weather conditions in Phalaborwa for 2021 and 2022.

The two (2) year weather data from Phalaborwa shows somewhat milder conditions with the peak daily irradiance just lower than 20 MJ/m^2 (Fig. 3.6a). The maximum air temperature reached around 42.8°C while the lowest value was around 3.5°C. Over the two-year period, the maximum air temperature averaged 40.2°C while the monthly maximum averaged around 36.4°C.

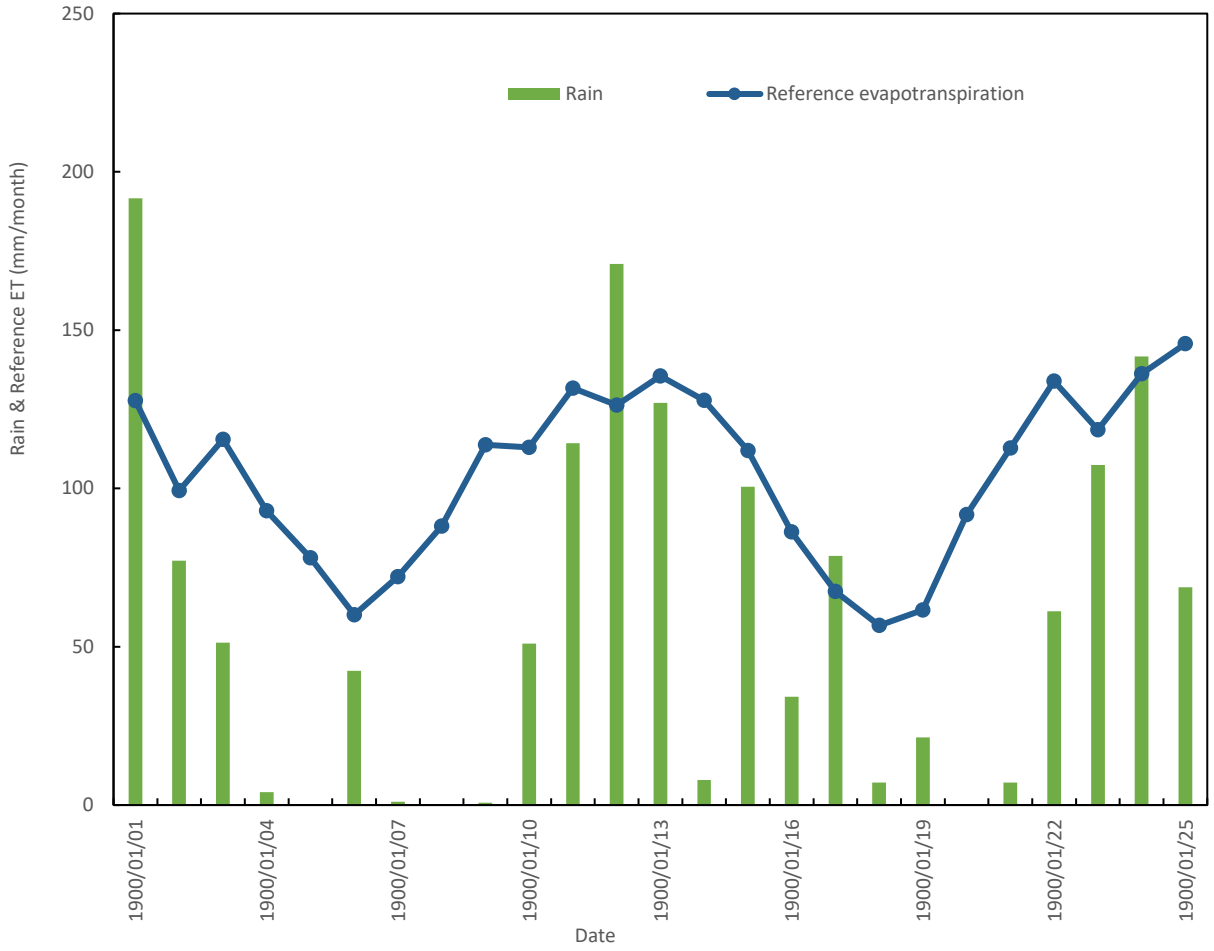


Fig 3.7. Monthly total rainfall and reference evapotranspiration in Phalaborwa from 2021 to 2023.

The average rainfall for the two (2) years at Phalaborwa was 699 mm while the reference evapotranspiration was 1230 mm. The aridity index was lower than at the other two sites at 0.57. The aridity index trends observed were unexpected and were possibly a result of the short climate data record used in this assessment. A comparison of the monthly indices is shown in Fig. 3.8 which shows very small differences between the sites. The significance of this analysis is that microclimatic differences between the sites could likely have small differences in the water use and yield quality and quantity. Variability in the soil's physical and chemical properties should therefore be carefully considered in studying the tree responses.

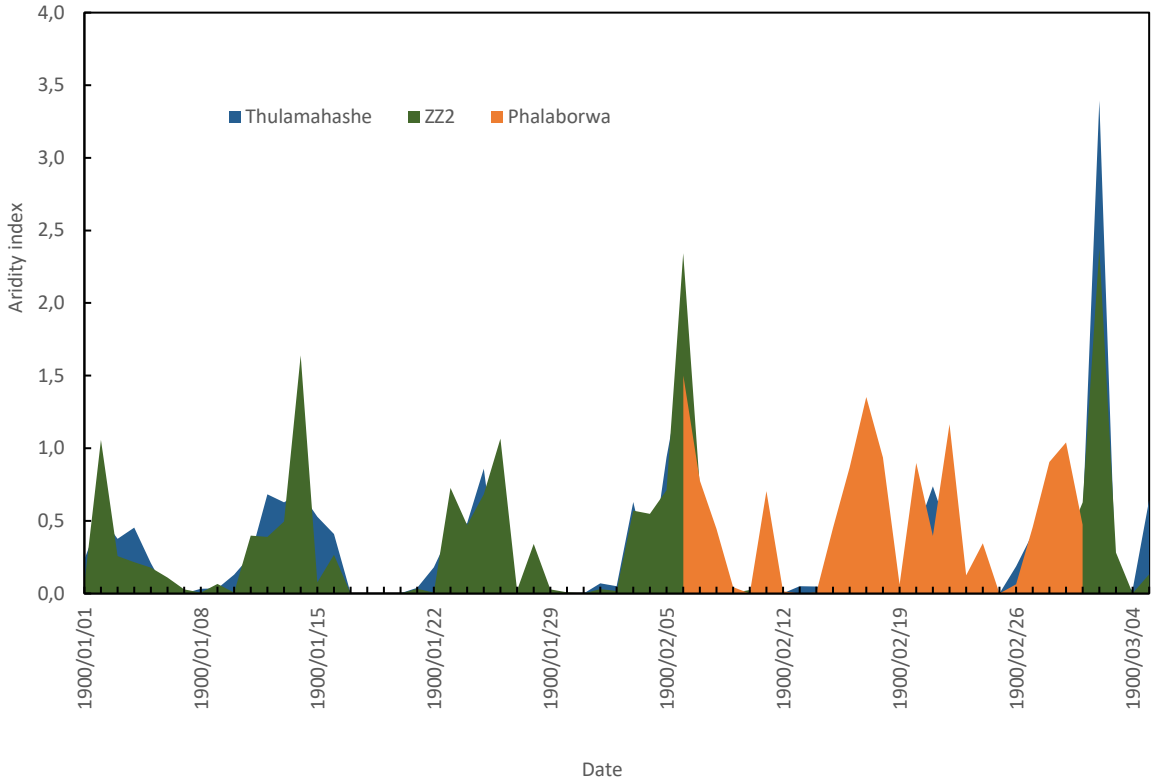


Fig 3.8. Monthly aridity index for the three study sites.

Table 3.1. shows the data for Thulamahashe (Thula) and ZZ2 are averages over five (5) years (2018 – 2022) while that from Phalaborwa Wastewater Treatment Works (Phala) is over two (2) years. Tmax is the maximum air temperature, Tmin is the minimum air temperature, RHmin is the minimum relative humidity, Rs is the daily total solar radiation, and ETo is the reference evapotranspiration over short grass. The aridity index is calculated as the ratio of the rainfall to the reference evapotranspiration.

Table 3.1. Summary of the 2 – 5 year average climatic conditions at the study sites.

	Tmax (°C)			Tmin (°C)			RHmin (%)			Rs (MJ/m2/d)			Rain (mm/mth)			ETo (mm/mth)			Aridity Index		
Date	**Thula**	**ZZ2**	**Phala**	**Thula**	**ZZ2**	**Phala**	**Thula**	**ZZ2**	**Phala**	**Thula**	**ZZ2**	**Phala**	**Thula**	**ZZ2**	**Phala**	**Thula**	**ZZ2**	**Phala**	**Thula**	**ZZ2**	**Phala**
January	37.8	39.0	36.9	16.8	16.0	17.6	23.8	19.3	35.3	20.4	19.3	18.4	115.2	100.9	159.3	161.8	152.7	131.7	0.7	0.7	1.2
February	37.1	37.6	36.9	16.6	16.3	18.4	24.9	24.8	29.3	19.1	17.8	17.3	50.0	112.8	42.5	139.9	127.6	113.6	0.4	0.9	0.4
March	38.1	38.7	37.2	15.6	14.2	14.1	21.0	20.0	28.1	17.7	17.0	16.5	40.4	24.6	75.9	144.7	133.9	113.7	0.3	0.2	0.7
April	36.1	36.8	35.5	12.1	10.5	9.6	19.6	16.1	20.1	14.2	13.3	14.5	35.0	20.9	19.1	111.9	103.5	89.6	0.3	0.2	0.2
May	32.9	33.3	33.5	8.3	7.4	9.8	14.6	10.7	15.5	12.7	13.0	12.5	11.7	7.0	39.4	106.5	99.6	72.8	0.1	0.1	0.5
June	32.3	32.2	30.9	5.1	3.8	7.2	12.0	9.4	15.0	11.1	12.0	11.7	3.6	4.0	24.8	94.1	84.8	58.4	0.0	0.0	0.4
July	32.7	32.9	32.5	4.4	3.0	5.5	9.0	9.3	9.7	11.7	12.2	11.8	1.1	0.9	11.2	103.0	91.4	66.9	0.0	0.0	0.2
August	36.9	36.4	36.9	6.5	6.5	6.9	8.8	7.3	9.0	13.0	13.1	13.3	3.7	0.8	0.0	120.1	109.4	89.9	0.0	0.0	0.0
September	39.0	39.8	37.4	7.7	6.3	7.3	9.8	7.5	11.0	16.4	16.7	16.0	9.9	3.9	3.9	139.4	129.8	113.3	0.1	0.0	0.0
October	40.5	40.7	39.9	11.1	11.5	12.5	10.5	7.9	9.3	17.8	17.4	15.1	41.1	23.0	56.1	150.1	141.3	123.5	0.3	0.2	0.5
November	40.7	40.1	40.2	14.4	13.7	14.7	14.3	13.4	17.5	18.7	18.6	17.1	31.0	75.2	110.8	154.0	145.6	125.1	0.2	0.5	0.9
December	39.9	39.7	38.8	14.8	14.3	15.1	17.6	17.5	19.9	19.5	19.1	17.9	87.5	69.0	156.3	162.1	152.2	131.2	0.5	0.5	1.2
Average	**37.0**	**37.3**	**36.4**	**11.1**	**10.3**	**11.6**													**0.2**	**0.3**	**0.5**
Total													**430.0**	**443.1**	**699.4**	**1587.5**	**1471.7**	**1229.7**			
Max	**40.7**	**40.7**	**40.2**	**16.8**	**16.3**	**18.4**															
Min	**32.3**	**32.2**	**30.9**	**4.4**	**3.0**	**5.5**	**8.8**	**7.3**	**9.0**	**11.1**											

3.3. Data collection: High rainfall Thulamahashe, Mpumalanga Province

3.3.1. Site description

According to the project Terms of Reference, this study was to be done at three sites with different agro-climatic conditions, especially rainfall. Figure 3.1 shows the location of the three study sites (marked with green stars) within the Limpopo and Mpumalanga Provinces, respectively. The study sites include Thulamahashe which is situated in a high rainfall area (> 700 mm/yr.) near Bushbuckridge in Mpumalanga. Data collection at this site started in November 2021. The second site is at ZZ2 farm located near Modjadjikloof in Limpopo with medium long-term rainfall (500-700 mm/yr.). The equipment was instrumented at this site in August 2022. The third site is in Phalaborwa which is a low rainfall area (300 – 400 mm/yr.). Data collection commenced at this site in September 2023.

Data collection in Thulamahashe stopped in May/June 2023 due to vandalism of the study site. Batteries were stolen from the sap flow tree boxes while the sap flow probes were routinely pulled out of the trees. The site was meant to be in a secure location within the boundaries of Mafemani Nxumalo Secondary School (031°12’E; 24°43’S; 489 m asl) shown in Fig. 3.1. We attempted to rectify this problem in two ways. Firstly, we looked for an alternative site with stricter security at Thulamahashe Municipal Court, however, installation of the equipment was delayed for a long time due to administrative bottlenecks at the University of Venda that resulted in long delays in the delivery of the replacement equipment that had been damaged. Secondly, to close gaps in the missing data at the Thulamahashe site, we used data on the water use of Marula trees done in another WRC project close by at the ARC Burgershal site in Hazyview (Ntshidi et al., 2022). These data were critical to complete the water use modelling section of this study for the Thulamahashe site.

Fig 3.9. Google Earth image of the study site at Thulamahashe, Bushbuck Ridge, Mpumalanga.

3.3.2. Soils and climate data at Thulamahashe

An accurate characterization of the soil type at the Thulamahashe study site was not done. However, Nell and Dreyer (2006), they described the geology of the the nearby Dingleydale irrigation scheme as comprising sediments of Swazian age that were classified as Makhutswi Gneiss consisting of light grey, medium-grained biotite gneiss with coarse-grained quartz-feldspar that had recrystallised in places. The soils at the specific study site comprised of shallow sandy soils in the top 20 to 30 cm which was followed by a hard layer of loamy-clay soils in the deeper horizons. Table 3.2

summarizes the main soil physical properties while Tables 3.3 a & b summarize the chemical properties of samples collected at 20, 40 and 60 cm depths. The soil samples were processed by Lab Serve in Cape Town. Soil texture varied from loamy sand in the shallow depths to course sandy loam at deeper depths with very low stone content (<3%). The field capacity of the soils varied between 12 and 15% at 100 kPa, and 23 to 25% at 10 kPa.

Climate data for this study was collected from an automatic weather station operated by the Agricultural Research Council at the Agricultural Research Council station at Hazyview, less than 50 km away. Weather variables measured at hourly and daily intervals included the solar irradiance, the maximum and minimum air temperature, the maximum and minimum relative humidity, wind speed and direction at 2.0 m height and rainfall. In this study the atmospheric evaporative demand, depicted by the reference evapotranspiration, was calculated for a short grass reference using the FAO modified Penman-Monteith equation as detailed by Allen *et al*. (1998).

Table 3.2. Soil physical properties at Thulamahashe.

Soil depth (mm)	Clay	Silt	Sand	Fine Sand	Medium Sand	Coarse Sand	Stone	Classification	Waterholding	Waterholding	Waterholding
	%	%	%	%	%	%	% (v/v)		10kPa %	100kPa %	mm/m
200	11.0	4.0	85.0	41.3	19.0	24.7	1.1	Loamy sand	23.00	12.42	105.85
400	15.0	10.0	75.0	34.0	18.6	22.4	1.3	Fine sandy loam	25.45	15.32	101.26
600	11.0	8.0	81.0	25.0	17.3	38.6	2.4	Course sandy loam	23.17	14.36	88.11

Table 3.3a. Soil chemical properties.

Depth(cm)	Type	pH	Resist.	H+	Stone	P (Bray II)	K	Ca	Mg	K	Na	Cu
		KCl	(ohm)	cmol/Kg	Vol %	mg/kg	mg/kg	Ca	Mg	K	Na	mg/kg
200	Sand	4.5	5890	0.52	1.93	3.1	55.2	1.4	0.44	0.14	0.16	0.48
400	Sand	5.0	4690	0.39	2.37	2.5	55.4	2.4	0.55	0.14	0.23	0.48
600	Sand	5.1	4580	0.34	4.19	3.4	56.9	2.0	0.54	0.14	0.15	0.59

Table 3.3b. Soil chemical properties continued.

Depth (mm)	Zn	Mn	B	Fe	C	S Am.acet	Na	K	Ca	Mg	T Value	Acid Sat.
	mg/kg	mg/kg	mg/kg	mg/kg	%	mg/kg	%	%	%	%	cmol/kg	%
200	<0.36	190	0.24	59.4	0.39	5.0	6.02	5.33	52.69	16.56	2.66	19.47
400	<0.36	168	0.24	87.6	0.57	4.0	6.20	3.83	64.68	14.82	3.71	10.52
600	<0.36	164	0.14	57.0	0.23	3.6	4.73	4.60	63.06	17.03	3.17	10.77

3.3.3. Tree statistics

Tree species at the Thulamahashe study site were predominantly marula trees with an average stand density of roughly less than 30 trees per hectare. The trees had mean diameter at breast height ranged from 10 to 15 cm as summarised in Table 3.3. There were a few Indigenous tree species that also grew in the neighbourhood with the kiaat (*Pterocarpus angolensis*) being the dominant species. However, these were much fewer in number compared to the marula with a density of less than 10 trees per hectare.

3.3.4. Transpiration measurements at Thulamahashe

Transpiration was measured on four sexually mature individual marula trees using the heat ratio method (HRM) of monitoring sap flow (Burgess *et al.,* 2001). More information on the sensors and instrumented trees is shown in Table 3.4.

Table 3.4. Details on the installation depths of the heat ratio method probes on marula trees at Thulamahashe, Mpumalanga.

Tree no.	Probe no.	Insertion depth (cm)	Circumference at probes (cm)	Tree height (m)	Canopy width (m)
1	1	1.5	181	100.8	8.85
	2	2.2			
	3	3.3			
	4	4.0			
2	1	1.5	159	220	13.60
	2	2.2			
	3	3.3			
	4	4.0			
3	1	1.5	178	200.3	12.91
	2	2.2			
	3	3.3			
	4	4.0			
4	1	1.5	157	13.0	9.15
	2	2.2			
	3	3.3			
	4	4.0			

The four-sap flow instrumented trees (Fig. 3.10) are hereafter referred to as Tree #1, Tree #2, Tree #3, and Tree #4. Details of each tree are summarised in Table 3.4. The trees grew in the same locality with the maximum distance between them being less than 20 m. The circumferences at breast height (~ 1.5 m) were approximately 181, 159, 178, and 157 cm, for Trees #1, 2, 3 and 4, respectively. The corresponding stem diameters at breast height are respectively, 57.6; 50.6; 56.7 and 50.0 cm. To install the sap flow sensors, a metal template with three precisely drilled holes aligned along the vertical axes of the trees and spaced 5 mm apart was used to minimise errors due to probe misalignment (Steppe *et al.,* 2010). Four HRM probe sets were installed per tree.

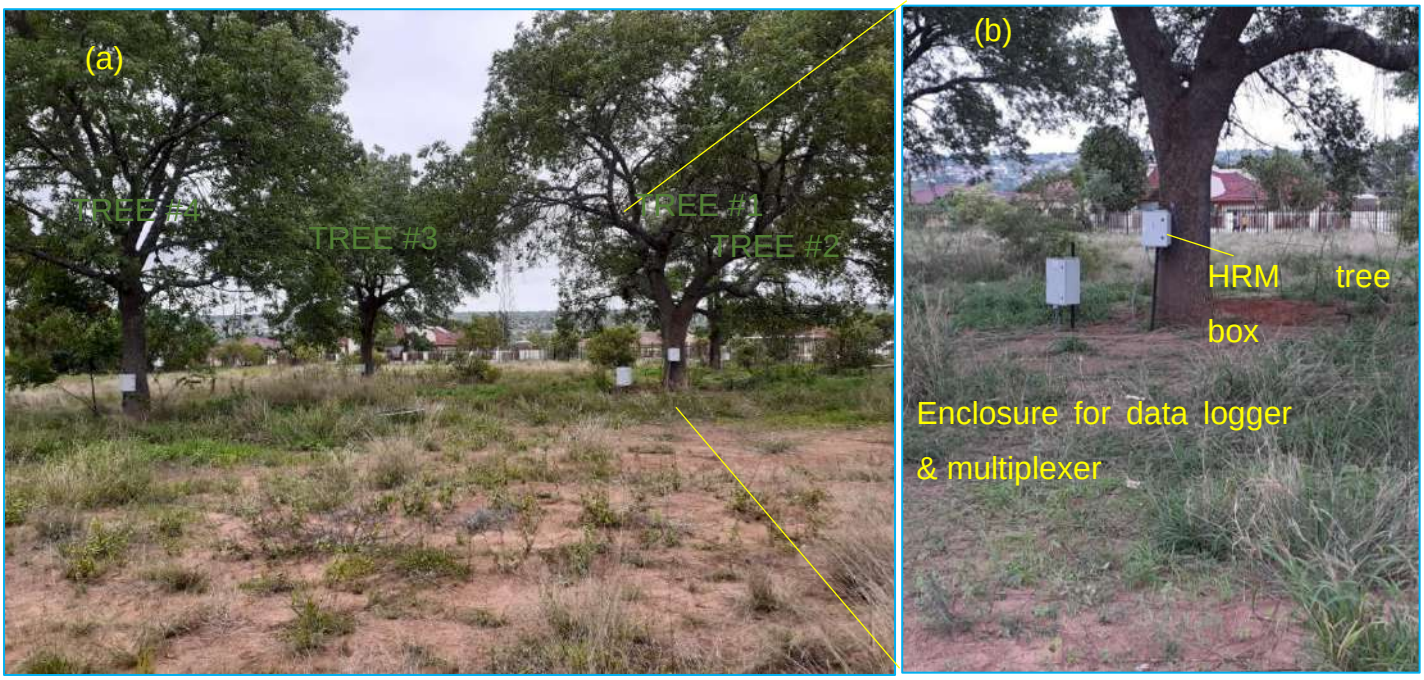


Fig 3.10(a). The four-sap flow instrumented marula trees at Mafemane High School, Thulamahashe. (b) An enlarged view of one instrumented tree showing the data logger and multiplexer enclosure and the HRM tree box.

Each probe set comprised a central heater that injected heat into the stems and two (2) T-type thermocouples that measured the sapwood temperature at equal distances up and downstream of the heater. The four (4) probes were installed in the four (4) cardinal directions around the trees and at different depths into the sapwood to account for the radial and circumferential variations in the sap velocity (Wulschleger and King, 2000; López-Bernal *et al.,* 2010). The probe installation depths were 1.5,

2.0, 2.8, and 3.5 cm, respectively from the outer bark of the trees. Average bark thickness was approximately 0.7 cm. The maximum installation depth of the probes was restricted by the length of the brass sleeves (4 cm) used to facilitate heat transfer between the heater probes and the sapwood. The length of the thermocouple probes also did not allow depths beyond 10 cm to be sampled.

Each tree had a single thermally insulated tree box measuring about 35 cm x 25 cm x 10 cm (Fig. 3.10) which contained the electronics for operating the HRM systems. Eight T-type thermocouple sensors and four heater probes, each about 1.5 m long, were connected to the tree box which also housed a precision thermistor which measured the reference temperature for the thermocouples. The four tree boxes were connected to one data logger (Model: CR1000, Campbell Scientific) and a multiplexer (Model AM16/32B, Campbell Scientific) via a 25-core plain wire cable.

Pulsing of the heat to the heaters, which lasted about 10 seconds, was done via control ports on the data logger (C3 to C6). The electronics in each tree box were powered by a 7 Ah battery and the sap flow data were collected at hourly intervals throughout the study period. Besides the data logger and multiplexer, all the other components of the sap flow system were custom-made. The zero-sap flow was determined manually by setting the base of the sap flow signal to zero for nighttime periods (i.e. 21h00 to 04h00). Ideally this should be done by cutting down the trees while measurements continued. But this was not an option given the protected status of the marula trees.

The sapwood area was determined by injecting methylene blue dye into the stems of the trees to determine the extent of the active xylem vessels. This was done by drilling thin (2.0 mm diameter) holes inclined at about 30°C from the horizontal to hold the dye. The dye was then injected using inverted 500 ml wash bottles tied to the stems on several non-sap flow instrumented trees. The drilled holes were flushed by running the liquid dye to remove air bubbles pbeforeattaching the containers. Wood samples were then extracted between 5.0 and 10 cm above the dye injection point after one to two days using a stem corer. The information on the sapwood depth was used to calculate the sap flow volumes by the individual trees. The heat pulse velocity data were corrected for wounding due to sensor implantation according to the procedure

by Burgess *et al.* (2001). The sap flow volume per tree (in litres per hour) was calculated as a weighted sum of the products of the sap velocity and the sapwood area represented by each probe set as detailed in Dzikiti *et al.* (2018b).

3.3.5. Tree leaf area index measurements

The leaf area index (m^2 of leaf area per m^2 of ground area – LAI) is an important measure of canopy size (Dzikiti *et al.,* 2018: Gush *et al.,* 2019). It gives an idea of the size of the transpiring leaf area that is projected on a unit ground area. In this study individual tree LAI data were collected on 18 November 2021 close to peak canopy cover (Fig. 3.11). This was measured on the sap flow instrumented trees using a leaf area meter (Model: LAI-2000, Li-Cor Inc., Nebraska, USA). The LAI data were collected under diffuse radiation conditions on an overcast day when the tree leaves approximated black bodies.

Fig 3.11. Measuring the LAI of a marula tree using a leaf area meter.

The leaf area meter was operated with a narrow 20 degrees slit/cap given the height of the canopy (see Fig. 3.11). The measurement procedure involved measuring the light incident at the top of the canopy at the beginning and end of each measurement cycle. This reading was taken in an open area away from the influence of the tree canopies or other obstructions. The assumption was that this measurement was equal to that incident at the top of the canopy. Then five readings were taken at various positions under the canopy with the operator's back to the main tree trunk as shown in Fig. 3.11. A key challenge as can be seen in Fig. 3.11 is the considerable influence of the woody plant material on the light measured by the LAI meter.

3.3.6. Soil moisture dynamics in the root zone of the marula trees

Indigenous tree species like marula can withstand extended drought periods because of their extensive root systems. In this study we quantified the available soil moisture by using three soil moisture reflectometer probes (Model CS616: Campbell Scientific Inc., Utah, USA). The probes were installed at depths of 15, 40 and 100 cm as shown in Fig. 3.12. Most of the fine root system appeared to be found in the 0 to 60 cm depth although the presence of roots from other surrounding plants made an accurate assessment of the extent of the root system difficult. A few thicker anchor roots were found beyond 1.0 m depth suggesting that the trees possibly have a deeper root system. The CS616 probes were not calibrated for the specific soils due to logistical challenges. Rather factory calibrations were used. This is not a problem given that these probes are most accurate in sandy type soils such as the ones found at the study site.

3.3.7. Phenology and yield monitoring

Quantifying the yield of marula at Thulamahashe during the first year of data collection proved challenging for two (2) reasons. Firstly, the study site was at least 300 km from the Univen. This made it difficult for us to know when the fruit drop at maturity had commenced. By the time we arrived, nearly all the fruit had dropped, so we missed the yield. The second problem related to the logistics of picking and weighing the fruit. At harvest, the fruit falls on its own from the trees. It is picked from the ground where it ripens rather than being plucked from the trees. This is a long process as the falling happens over time. We had no one on the ground to pick and weigh the fruit for us.

We made arrangements with a local person to ensure that yield was collected in the next harvest cycle. The individual not only picked and weighed the fruit, but they also change the batteries of the sap flow system monthly at a fee.

Fig 3.12. Installation of soil moisture monitoring probes at Thulamahashe.

3.4. Medium rainfall ZZ2 site in Moeketsi, Limpopo

3.4.1. Site description

This site was located within the ZZ2 premises in Moeketsi (23° 65’ 17’’ S, 30° 06’ 89’’ E, and 772 m above sea level) (Fig. 3.13). According to Nzanza (2012), the long-term temperature for the site was between 15 and 27°C while the rainfall varied from 800

to 1000 mm per annum. A suitable site was sought within the farm premises, but we were not successful after considering issues such as the security of the equipment and accessibility. The research team eventually settled for a cluster of mature marula trees located just outside the fence of the ZZ2 offices as can be seen in Fig. 3.13. This site was being used as a private vegetable garden by farm workers. These were kindly requested to cease growing the vegetables as watering the plants would bias the results. Besides the farm offices (to the south), the study site was bounded by an extensive grassland to the north, a mango orchard to the east and an irrigated lawn to the west. Water for irrigation did not reach the study site given the considerable distance between them

Fig.3.13. Study site at the medium rainfall ZZ2 study site in Moeketsi, Limpopo.

3.4.2. Soils and climate data

According to Ledwaba (2023), soils in the study area are dominated by the Glenrosa soil form of the 1.1.10 soil family and the dominant geology is granite. The dominating soil is shallow lithic, with predominantly sandy loam and loamy sand texture in the 0 – 60 cm depth range. The Ledwaba data is based on information derived from 132

samples that were collected at different locations with the ZZ2 farm in the 0 to 60 cm depth (Table 3.5). Results for soil samples that were collected within the marula stand studied here are shown in Tables 3.6 and 3.7. The soil textural data agrees with that of Ledwaba with predominantly sandy loam soils in the shallow layers (0 – 30 cm) and sandy clay in the deeper layers (30 – 60 cm). The pH of the soil was acidic (Table 3.7a) ranging from 5.7 to 7.1. The volumetric soil water content at 100 kPa was low ranging between 13 and 15% (Table 3.7).

Climate data for the ZZ2 study site were obtained from the weather station located within the farm. The weather station was situated less than 1.0 km from the instrumented marula trees, and it was operated by the Agricultural Research Council. Weather variables measured at hourly and daily intervals included the solar irradiance, the maximum and minimum air temperature, the maximum and minimum relative humidity, wind speed and direction at 2.0 m height and rainfall. As at Thulamahashe, the atmospheric evaporative demand, depicted by the reference evapotranspiration, was calculated for a short grass reference using the FAO-modified Penman-Monteith equation as detailed by Allen *et al.* (1998).

3.4.3. Phenological stages of marula

The period of phenological observations was August 2023–August 2024. Among the measured phenological stages were Bud Break, Leaf Emergence, Flowering, Fruit Set, Fruit Development, Fruit Drop, Leaf Senescence, and Dormancy. These phases were selected following well-established phenological research, which designates them as essential in deciduous trees' growth and reproductive cycles (Chuine, 2000). To track bud break, observations were made starting in the first week of August 2023. The day of the buds' initial swelling and breaking open was recorded as the "bud break." Every seven days, observations were conducted until the bud break on all the instrumented trees. The appearance of new leaves was observed after bud break. Every week, observations were made, and the date of leaf emergence was noted as the first completely developed leaves were seen. When the first flowers appeared, it was considered the start of flowering. From then onwards, observations were taken every seven days until the peak of flowering was reached. The fruit set began to be observed in November 2023. Every seven days, observations were made to determine

when the first little, green fruits were seen, marking the beginning of the fruit set (Cleland *et al.,* 2007).

3.4.4. Measuring tree water use and soil moisture

Four (4) marula trees were instrumented with the HRM sap flow sensors (Fig. 3.14). The full technical details of the setup are like those given earlier in this report for Thulamahashe. A summary of the probe installation depths and tree dimensions at this specific site is given in Table 3.8. The trees were much closer to each other at this site than at Thumalahashe. They were also relatively smaller in size than at Thulamahashe although indications from kernels on the ground were that yield was higher at ZZ2 than Thulamahashe. Soil moisture in the root zone was measured using CS616 probes at three depths i.e. 20, 40 and 60 cm (Fig. 3.14b).

Table 3.5. Soil physical properties at ZZ2.

Soil depth (mm)	Clay	Silt	Sand	Fine Sand	Medium Sand	Coarse Sand	Klip	Classification	Waterholding	Waterholding	Waterholding
	%	%	%	%	%	%	% (v/v)		10kPa %	100kPa %	mm/m
200	15.0	8.0	77.0	29.3	19.1	28.6	10.0	COARSE SANDY LOAM	22.07	13.59	84.81
400	15.0	10.0	75.0	27.5	16.4	31.1	9.8	COARSE SANDY LOAM	23.25	14.61	86.43
600	11.0	8.0	81.0	23.1	15.6	42.3	14.8	COARSE SANDY LOAM	20.45	12.86	75.97

Table 3.6a. Soil chemical properties.

Depth(cm)	Type	pH	Resist.	H+	Stone	P (Bray II)	K	Ca	Mg	K	Na	Cu
		KCl	(ohm)	cmol/Kg	Vol %	mg/kg	mg/kg	Ca	Mg	K	Na	mg/kg
200	Sand	6.1	1290		16.72	170	284	5.8	3.4	0.72	0.19	3.6
400	Sand	5.7	1870	0.30	16.34	62.4	149	3.4	4.3	0.38	0.17	1.1
600	Sand	5.9	1340	0.30	23.79	68.2	136	5.2	3.1	0.35	0.18	0.65

Table 3.6b. Soil chemical properties continued.

Depth (mm)	Zn	Mn	B	Fe	C	S Am.acet	Na	K	Ca	Mg	T Value	Acid Sat.
	mg/kg	mg/kg	mg/kg	mg/kg	%	mg/kg	%	%	%	%	cmol/kg	%
200	7.0	138	0.36	114	0.67	12.8	1.88	7.20	57.37	33.63	10.11	0.00
400	0.46	58.8	0.23	190	0.23	7.3	1.99	4.47	39.75	50.28	8.55	3.54
600	0.63	30.4	0.17	48.9	0.26	34.2	1.97	3.82	56.94	33.94	9.13	3.31

Table 3.7. Summary of the soil physical properties determined from 132 samples collected across the ZZ2 farm by Ledwaba (2023).

Profile	Horizons	Depth (cm)	TSD (m)	ERD (mm)	Soil colour		Slope (%)	Permeability (s)	Clay (%)	Silt (%)	Sand (%)	Textural class
1	Orthic A	0-30	0.6	200-300	2.5YR3/4	Dark reddish brown	0-3	1-3	16.16	21.28	62.56	Sandy loam
	Lithocutanic B	30-60										
2	Orthic A	0-30	0.6	200-300	2.5YR3/4	Dark reddish brown	0-3	1-3	10	18	72	Loamy sand
	Lithocutanic B	30-60										
3	Orthic A	0-30	0.6	200-300	2.5YR3/4	Dark reddish brown	0-3	1-3	14.16	17,28	68.56	Sandy loam
	Lithocutanic B	30-60										

Fig 3.14 (a). Installation of the heat ratio sap flow sensors in bearing marula trees and (b) CS616 soil moisture probes at the ZZ2 site in Moeketsi.

A detailed characterization of the soil's chemical and physical properties is summarized in Tables 2.6a & b. Data collection commenced in September 2022. The team arranged with personnel at ZZ2 to charge and change the batteries monthly, we are grateful for this help.

Table 3.8. Details on the installation depths of the heat ratio method probes on marula trees at ZZ2, Limpopo Province.

Tree no.	Probe no.	Insertion depth (cm)	Circumference at probes (cm)	Tree height (m)	Canopy width (m)
1	1	1.5	123.0	123	8.8
	2	2.2			
	3	3.3			
	4	4.0			
2	1	1.5	73.9	73.9	7.5
	2	2.2			
	3	3.3			
	4	4.0			
3	1	1.5	74.2	74.2	7.48
	2	2.2			
	3	3.3			
	4	4.0			
4	1	1.5	107.1	107.1	9.15
	2	2.2			
	3	3.3			
	4	4.0			

3.5. Low rainfall Phalaborwa site, Limpopo

3.5.1. Site description

The third study site was situated in Phalaborwa (31°17'E, 22°55'S and 350 to 450 m above sea level). The climate in Phalaborwa is classified as a hot semi-arid climate (Köppen climate classification BSh), with hot, dry summers, and mild, dry winters. Of the three (3) sites, the region receives the lowest rainfall with a long-term average annual rainfall of less than 500 mm (Munnik *et al.,* 1996). Average daily maximum temperatures for the hottest months (November - March) are around 30 – 32°C and the average daily minimum for the coldest months (June - August) are about 10-12°C (Weather Bureau, 1986). The study site was located within the Namakgale

Wastewater Treatment Works on the outskirts of Phalaborwa town (Fig. 3.7). The area was fenced, and it was deemed safe for equipment. Several large marula trees grew on the premises. The ones selected for instrumentation with sap flow sensors are shown in Fig. 3.15. The trees were sparsely populated with a density of less than 30 trees per hectare.

Fig 3.15. Google Earth image of the study site at the Namakgale wastewater treatment works in Phalaborwa.

3.5.2. Soils and climate data for Phalaborwa

Munnik *et al.* (1996) did a very detailed study of the soils around the Phalaborwa area. They state that the area is underlain by Goudplaats-Makhutswi granitic gneiss of the Swazian age (Geological Survey, 1987). Using 132 samples collected from 55 profiles pits dug in various parts of Phalaborwa, Munnik *et al.* (1996) concluded that the soils in the area were predominantly coarse, sandy, well-drained, reddish soils (Hutton

form) on the crests mostly followed by equally sandy and well-drained yellowish soils of the Clovelly form on the mid-slope.

Tables 3.9 and 3.10 a & b show the physical and chemical properties of the soil collected from open pits at the Namakgale Wastewater Treatment Works. The samples were collected only at the 40 and 60 cm depths because the shallower layers were too rocky, and it was difficult to extract the samples. The soils were predominantly sandy with more than 80% sand with the volumetric water content at field capacity (100 kPa) between 11 and 12% in the depth range 40 to 60 cm. The soils were generally acidic with a pH between 6 and 7.0 and nutrient-poor as summarised in Table 2.9b. Climate data were obtained from the nearest ARC-operated weather station in Phalaborwa.

3.5.3. Measuring tree water use and soil moisture at Phalaborwa

The marula trees at the Phalaborwa site were considerably much larger than those at the other two study sites as summarised in Table 3.10. Four (4) trees were instrumented with the HRM sap flow sensors (Fig. 3.16). The full technical details of the setup are like those given earlier in this report for Thulamahashe. A summary of the probe installation depths and tree dimensions at this specific site is given in Table 3.10. The trees were within 15 m of each other which was further than the ZZ2 stand but closer than the Thulamahashe stand. Indications from fruit kernels on the ground were that yield by most of the trees was high although many male trees did not bear fruit. Soil moisture in the root zone was measured using CS616 probes at three depths i.e. 20, 40 and 60 cm.

Table 3.9. Soil physical properties at Namagkale in Phalaborwa.

Soil depth (mm)	Clay	Silt	Sand	Fine Sand	Medium Sand	Coarse Sand	Klip	Classification	Waterholding	Waterholding	Waterholding
	%	%	%	%	%	%	% (v/v)		10kPa %	100kPa %	mm/m
200											
400	11.0	8.0	81.0	43.9	16.3	20.8	17.1	FINE SANDY LOAM	21.25	11.62	96.28
600	13.0	6.0	81.0	44.2	16.9	19.8	14.2	FINE SANDY LOAM	21.64	11.82	98.12

Table 3.10a. Soil chemical properties

Depth(cm)	Type	pH	Resist.	H+	Stone	P (Bray II)	K	Ca	Mg	K	Na	Cu
		KCl	(ohm)	cmol/Kg	Vol %	mg/kg	mg/kg	Ca	Mg	K	Na	mg/kg
200	Sand											
400	Sand	6.3	1210		27.08	114	110	4.5	3.1	0.28	0.19	11.5
600	Sand	6.8	1310		22.89	94.3	98.2	5.0	2.8	0.25	0.14	11.9

Table 3.10b. Soil chemical properties continued.

Depth (mm)	Zn	Mn	B	Fe	C	S Am.acet	Na	K	Ca	Mg	T Value	Acid Sat.
	mg/kg	mg/kg	mg/kg	mg/kg	%	mg/kg	%	%	%	%	cmol/kg	%
200												
400	11.0	142	0.27	107	0.40	8.0	2.35	3.50	55.76	38.41	8.07	0.00
600	12.1	170	0.28	113	0.38	8.6	1.71	3.07	61.05	34.19	8.19	0.00

Table 3.11. Details on the installation depths of the heat ratio method probes on marula trees at Phalaborwa, Limpopo Province.

Tree no.	Probe no.	Insertion depth (cm)	Circumference at probes (cm)	Tree height (m)	Canopy width (m)
1	1	1.5	167	167	11.68
	2	2.2			
	3	3.3			
	4	4.0			
2	1	1.5	86.1	86.1	5.35
	2	2.2			
	3	3.3			
	4	4.0			
3	1	1.5	182	182	12.25
	2	2.2			
	3	3.3			
	4	4.0			
4	1	1.5	165	165	11.61
	2	2.2			
	3	3.3			
	4	4.0			

3.4.4. Leaf area index

The tree Leaf area index (LAI) at Phalaborwa and Moeletsi was measured using a Ceptometer (AccuPAR LP-80). In each site, data were collected on instrumented trees; this was done to capture the correlation between water usage and the leaf area index. The alignment and sensitivity of the Ceptometer sensor were guaranteed by calibration following the manufacturer's instructions before data collection. To minimize the sensitivity of the Ceptometer to variations in light intensity, data collection was done at midday on clear sunny days, between 12h00 and 14h00. To determine reference light conditions, baseline readings were conducted in open spaces far from tree canopies (Pokovai and Fodor, 2019). On each instrument tree, five (5) random measurements were made below the canopy (four perpendicular measurements were made at each location) using a Ceptometer based on the procedures from the manufacturer to measure photosynthetically active radiation (PAR) waveband. In each tree, a total of twenty (20) radiation observations were averaged to get a single tree value. The Ceptometer was positioned horizontally at ground level beneath the canopy of each instrumented tree to ensure that the sensors were uniformly dispersed and were not blocked by big branches. The above data was collected three times at

each of the following stages: from 50% to 100% leaf development, flowering to fruit initiation, and at the end of the harvest period before the trees shed the leaves. This was to cover major developmental stages which are vegetative, reproduction, and maturity.

A simplified version of the Norman and Jarvis (1975) model was used to compute the leaf area index using the percentage of light intercepted by the canopy as determined by the LP-80 Ceptometer. This method takes into consideration the zenith angle (θ), canopy architecture, and leaf optical characteristics, as well as the influence of the sky circumstances during the measurement. It also presumes the leaves are dispersed erratically throughout the canopy (de Mattos, 2020).

The following equations were used to calculate LAI and intercepted radiation;

$$F_i = 1.0 - T_i \quad (3.1)$$

Where (F_i) is the fraction of incident light intercepted by the canopy and T_i is the fraction of the light transmitted through the canopy (De felipe *at al.,* 2020; Mubvuma *et al.,* 2021)

T_i is calculated using photosynthetically active radiation (PAR) data collected by (AccuPAR LP-80) Ceptometer;

$$T_i = 1\text{-} \left(\frac{PAR_{below\ canopy}}{PAR_{above\ canopy}}\right) \quad (3.2)$$

In order to determine the total intercepted radiation (IR), the following equation was used;

$$\text{IR} = F_i \times Q \quad (3.3)$$

Where Q is the daily incoming radiation.

Thus, LAI was calculated from the total incoming radiation and the radiation that has been intercepted using the following equation (De Mattos *et al.,* 2020; Mubvuma *et al.,* 2021)

$$\mathrm{LAI} = -\frac{in\left(1.0-\frac{IR}{Q}\right)}{k} \quad (3.4)$$

where k is the extinction coefficient.

Fig 3.16. Installing the heat ratio sap flow method for measuring transpiration of the marula trees at Phalaborwa.

3.6. Modelling marula tree water use efficiency

3.6.1. Description of the transpiration and yield models

Marula trees are widely seen as fruit tree crops for the future when growing conditions are expected to get harsher and unsuitable for the exotic tree crops. This assumption is based on the perceived ability of this species to grow and thrive in hostile environments. However, very little quantitative information exists on how marula trees respond to environmental factors. There is a need to quantify the ecophysiology of these trees under present-day growing conditions. This information can then form the basis for developing models to predict possible future responses to climate change, and other environmental conditions based on scenario modelling.

In this study, we further develop the species-specific transpiration model for marula initially proposed by Dzikiti *et al.* (2022a). Not only do we use the data collected in this project to further test and validate the water use model. But we also propose a yield prediction submodel. The two (2) models combined can provide simulations of water use efficiency (yield/transpiration) under present-day and future growing conditions. This information can be used by farmers and other decision-makers on the suitability of marula as an alternative tree crop for the future.

3.6.2. The transpiration model

According to Dzikiti *et al.* (2022a), the hourly transpiration of individual marula trees (T_c, in g/h) was modelled using the combination Penman-Monteith equation for hypostomatous leaves as initially proposed by Zhang *et al.* (1997):

$$\lambda T_c = L\frac{\Delta Rn_{tree}+0.93\rho C_p\frac{(e_s-e_a)}{r_b}}{\Delta+0.93\gamma(2+\frac{\bar{r}_s}{r_b})} \qquad (3.5)$$

where $Rn_{,tree}$ (W m^{-2}) is the net radiation absorbed by the tree canopy, L (m^2) is the total leaf area of the tree, Δ (Pa/K) is the slope of the saturation vapour pressure vs temperature curve, ρ (kg/m^3) is the density of the air, C_p (J/kg/K) is the specific heat capacity of air at constant pressure, e_s and e_a (in Pa) represent the saturation and actual vapour pressure of the air, γ (Pa/K) is the psychrometric constant, λ (J/kg) is

the latent heat of vaporisation, $\overline{r_s}$ (s/m) the average stomatal resistance, and r_b(s/m) is the aerodynamic resistance.

The total leaf area (L) of the selected trees can be estimated as the product of the LAI and the canopy cross-sectional area of each tree. This is a crude way to estimate the canopy leaf area, but it is also the most practical under the circumstances. The net radiation absorbed by the tree canopy ($Rn_{,tree}$) can be approximated as half of that absorbed by a horizontal short grass surface (Rn) according to the approach by Zhang *et al.* (1997). The Rn flux can be calculated according to Carasco and Ortega-Farias (2007) as:

$$Rn = (1 - \alpha)R_s + \sigma(\varepsilon_a - \varepsilon_s)T_a^4 \qquad (3.6)$$

where α is the surface albedo taken as 0.23, R_s (W/m^2) is the downward solar irradiance measured by the weather station, T_a (K) is the air temperature, σ is the Stefan-Boltzmann constant, ε_s is the emissivity of the surface, which can be taken as 0.98, and ε_a is the emissivity of the atmosphere for both clear and cloudy conditions which can be calculated as:

$$\varepsilon_a = 1.31(\frac{e_0}{T_a})^{\frac{1}{7}}(a - mk_t) \qquad (3.7)$$

The expression (a - mk_t) in equation 3.7 accounts for cloudiness as proposed by Alados *et al.* (2012). The rest of the equation is the emissivity under clear sky conditions according to Brutsaert (1975). In this expression, e_0 is the actual vapour pressure of the air in hPa, a and m are constants with values of 1.002 and 0.303, respectively. The parameter k_t represents the ratio of the measured solar irradiance to the extra-terrestrial radiation, calculated according to Allen *et al.* (1998).

The aerodynamic resistance can be derived according to the logarithmic wind profile as:

$$r_b = \frac{\ln\left(\frac{z-d}{z_0}\right)\ln\left(\frac{z-d}{h-d}\right)}{k^2 u_z} \qquad (3.8)$$

where h (m) is the average height of the trees, z (m) is the measurement height for wind speed (u_z), d (m) is the displacement height calculated as d = 0.67h, z_0 (m) is the roughness length which can be approximated as z_0 = 0.12h, k is the von Karman constant with a value of 0.4.

The stomatal conductance, g_s ($= {}^{1}/_{\overline{r_s}}$) can be modelled using the multiplicative Jarvis (1976) method. In this method, g_s is calculated from the maximum stomatal conductance (g_{max}, m/s) moderated by stress factors for radiation (R_s), vapour pressure deficit (VPD), air temperature (T, in °C), and soil moisture (ϑ) written as:

$$g_s = g_{max} f(R_s) f(VPD) f(\theta) f(T) \quad (3.9)$$

where $f(R_s)$, $f(VPD)$, $f(\vartheta)$ and $f(T)$ are the radiation, vapour pressure deficit, soil moisture and air temperature stress factors with values ranging from 0 to 1. Zero represents maximum stress and 1.0 represents no stress. The functional forms of the stress factors suggested by Jarvis (1976), Stewart (1988), Granier and Loustau (1994), Egea *et al.* (2011), and Dzikiti *et al.* (2022a) are:

$$f(R_s) = \frac{R_s(1+0.001k_r)}{k_r+R_s} \quad (3.10)$$

$$f(T) = \frac{(T-T_L)(T_h-T)^a}{(k_{Tp}-T_L)(T_h-k_{Tp})^a} \quad (3.11)$$

$$f(VPD) = e^{-k_{vpd}VPD} \quad (3.12)$$

$$f(\theta) = (\frac{\theta-\theta_{WP}}{\theta_{FC}-\theta_{WP}})^{\beta} \quad (3.13)$$

where k_r, k_{Tp}, k_{vpd} and β are parameters obtained by model optimisation, $a = (T_h\text{-}k_{Tp})/(k_{Tp}\text{-}T_L)$, T_h and T_L are the upper and lower temperatures at which g_s = 0, ϑ_{FC} and ϑ_{WP} are the volumetric water contents at field capacity and permanent wilting points of the soils, respectively. The Marquardt iterative method can berive model parameters that minimise the squared differences between the measured and modelled transpiration rates. Typical model parameters for marula trees derived from data from a previous WRC-funded project (Dzikiti *et al.* 2022) in KZN are shown in Table 3.12.

Table 3.12. Penman-Monteith model parameters

Parameter	Description	Value
β(-)	Describes the curvature of the f(θ) function	1.20
g_{max} (mm s^{-1})	Maximum stomatal conductance	6.50
kT_p (°C)	Describes the effect of temperature on stomatal opening	14.00
k_{d1} (kPa^{-1})	Describes the influence of the VPD stress factor	0.05
k_{d2} (kPa^{-1})	Describes the influence of the VPD stress factor	0.15
k_r (MJ m^{-2} d^{-1})	Describes the influence of solar radiation on stomatal opening	2.00
T_h (°C)	Maximum temperature for plant growth	45.00
T_L (°C)	Minimum temperature	5.00
θ_{FC} (cm^3 cm^{-3})	Volumetric soil water content at field capacity	0.21
Θ_{WP}(cm^3 cm^{-3})	Volumetric soil water content at the permanent wilting point	0.06

3.6.3. Description of the yield model

To develop this submodel, average tree yield data (in kg tree) were obtained for marula trees growing at Bonamanzi Game Reserve in KZN described by Dzikiti *et al.* (2022a) and Ntshidi *et al.* (2022). Estimates of the individual tree yield can be modelled using the approach by Stewart *et al.* (1977). According to this method, the relative yield reduction ($1 - Y_a/Y_m$) is related to the relative evapotranspiration deficit ($1 - ET_a/ET_m$) by

$$1 - \frac{Y_a}{Y_m} = K_y \left(1 - \frac{ET_a}{ET_m}\right) \quad (3.14)$$

where Y_a is the actual yield (kg ha^{-1}), Y_m is the maximum yield (kg ha^{-1}), ET_a is the actual annual total evapotranspiration (mm), ET_m is the maximum annual evapotranspiration (mm), and K_y is a water use – yield response factor. However, since only transpiration is being measured on individual trees in this study, we use a slightly different approach. Here we use the modified version of equation 10 following Gimenez *et al.* (2017) as

$$Y_a = Y_m - \frac{Y_m K_y T_d}{T_c} \quad (3.15)$$

Where T_c is the maximum possible annual transpiration by a marula tree (L/yr.), T_d is the observed annual transpiration by the tree under investigation (L/yr.).

The yield of indigenous fruit tree species has not been modelled before as far as we are aware. So, we attempted this for the first time, and we made some assumptions to achieve our goal. As a first approximation to the marula yield model, we propose that the water use – yield response factor (K_y) can be calculated based on the following assumptions and measured data from marula trees at Bonamanzi Game Reserve in KZN as reported in Dzikiti *et al.* (2022a). Maximum annual transpiration (T_c) ~ 20 000 L/tree. This figure is based on the maximum sap flow recorded for a huge marula tree in Hazyview that grew next to an irrigated banana orchard reported by Ntshidi et al (2022). Ntshidi *et al.* (2022) reported a peak transpiration of about 18600 L/yr, and 20 000 L/yr is a probable maximum for large trees of this species. The observed annual transpiration at Bonamanzi was much lower at ~ 2160 L/tree. A marula tree's maximum yield (Ym) was pegged at ~ 100 kg/tree although higher values are possible. The actual observed yield (Y_a) at Bonamanzi in KZN was ~ 4.3 kg/tree. Using these data yielded a water use–yield response factor (K_y) for marula of 8.9. This is the figure that will be used for yield modelling.

3.7. Results and Discussion

3.7.1. Thulamahashe

3.7.1.1. Weather conditions

Typical weather conditions in the Thulamahashe area for the period 01 August 2021 to 18 April 2024 are summarised in Fig. 3.17. As expected, the daily solar radiation peaked in the summer months with the maximum exceeding 25 MJ/m^2/d and approaching 30 MJ/m^2/d in some instances. On overcast days the daily total solar radiation was less than 5.0 MJ/m^2/d, especially in winter or during rainy days in summer. Data were lost between March and June 2022 due to a power failure at the weather station. However, the effect of the high frequency of cloud cover in summer is also evident in the slight decline in the peak daily irradiance between November 2021 and March 2022 which corresponds to the main rainfall season. The maximum air temperature peaked at 41°C just before the main rainy season in November 2021.

The lowest air temperature was about 7°C reached in winter in July 2022. The lowest relative humidity (~ 12%) was experienced just before the onset of the rainy season in September 2021. The vapour pressure deficit of the air reached a maximum of only 2.60 kPa in October 2021. This value is considerably lower than those observed elsewhere in the country. For example, Dzikiti *et al.* (2018a) recorded a VPD close to 9.0 kPa in the Koue Bokeveld region of the Western Cape Province which has a Mediterranean-type climate.

The diurnal course of the reference evapotranspiration is shown in Fig. 3.17. The reference evapotranspiration reached a peak of just over 7.2 mm/d in late spring with low average values reached during the winter months when the atmospheric evaporative demand was low. As with solar radiation, the effect of long periods of cloud cover is also evident on ETo in Fig. 3.17 and these correspond to periods of high rainfall. The significance of these results is that peak water use by the trees is unlikely to be reached during the peak summer season as the atmospheric evaporative demand will be relatively lower than the spring values. However, water use in spring is likely to be constrained by low soil water content (before the onset of the rainy season) and the low transpiring leaf area since marula trees are deciduous. Therefore, the correlation between peak transpiration and the atmospheric evaporative demand is unlikely to be straightforward. This assumption will be revisited in the next sections.

A summary of the monthly weather conditions at the Thulamahashe study site over the entire duration of the study is shown in Table 3.13. September and October 2021 were the warmest months with peak temperatures exceeding 40°C although the daily total solar irradiance was not necessarily highest during this period. Peak temperatures in the summer months varied from 35 to 39°C. January 2022 was the wettest month receiving over 200 mm of rainfall while August was the driest month receiving less than 1.0 mm of rainfall. The atmospheric evaporative demand was greatest in the January – February 2022 period when the reference evapotranspiration ranged between 135 to 138 mm/month.

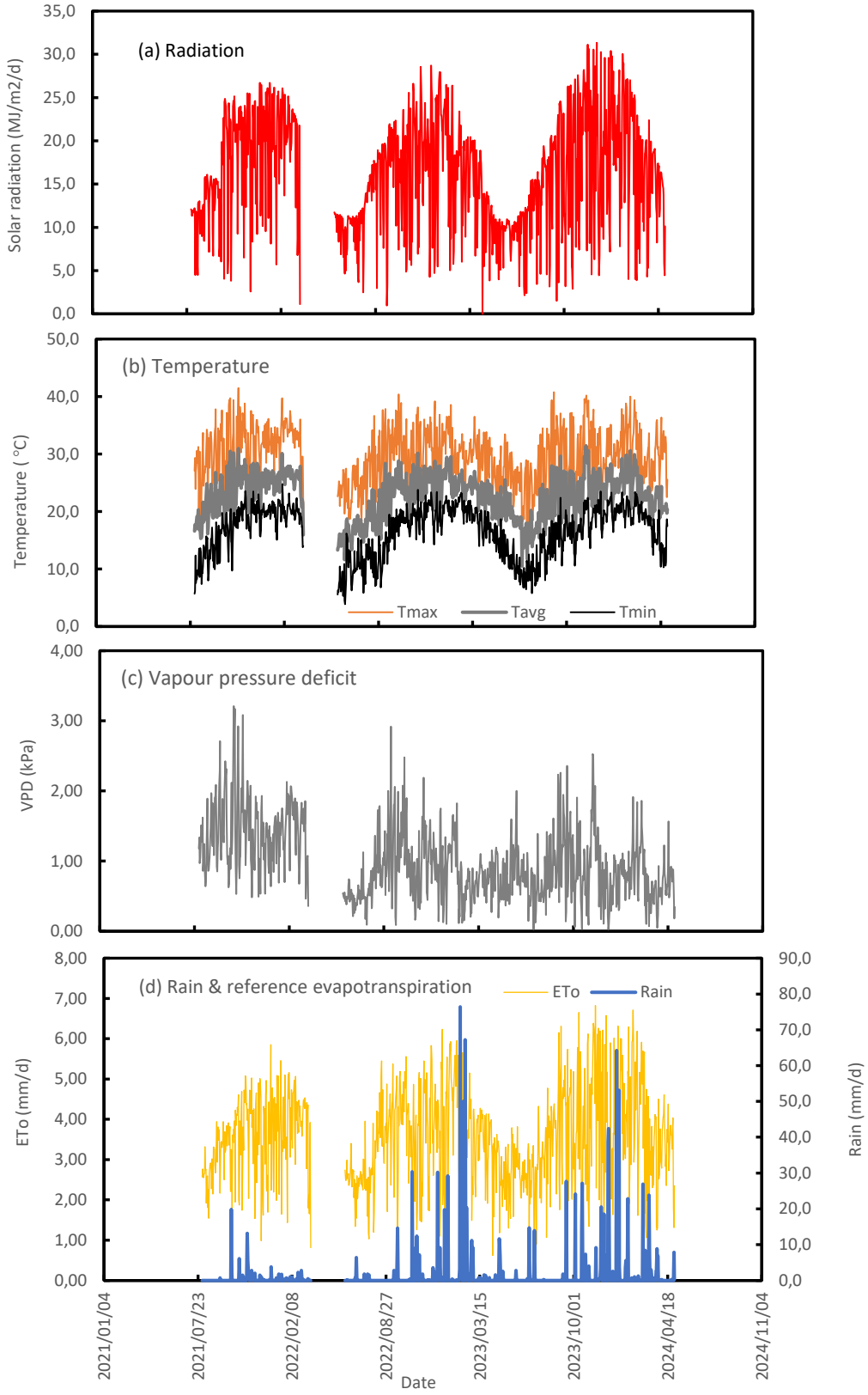


Fig 3.17. The seasonal course of the climate variables namely, (a) daily solar radiation, (b) maximum and minimum temperature, (c) vapour pressure deficit, and (d) rainfall and reference evapotranspiration at Thulamahashe, Mpumalanga.

Table 3.13. Summary of monthly weather conditions in the high rainfall Thulamahashe area, Mpumalanga.

Date	Tmax (°C)	Tavg (°C)	Tmin (°C)	Rs (MJ/m2/d)	Uavg (m/s)	Rain (mm/mth)	Rhmax (%)	Rhavg (%)	Rhmin (%)	ETo (mm/mth)	VPD (kPa)
2021/08/31	34.2	18.7	5.7	10.9	0.6	0.0	84.6	44.5	10.1	83.0	1.2
2021/09/30	37.8	22.1	9.2	13.8	0.8	0.8	84.6	40.1	7.8	105.0	1.6
2021/10/31	39.7	23.3	9.7	17.4	0.9	30.5	90.2	49.4	7.5	108.5	1.6
2021/11/30	41.5	25.3	15.3	19.8	0.9	33.7	91.9	55.1	11.3	119.7	1.5
2021/12/31	36.8	25.3	15.7	18.7	0.6	7.6	92.0	62.9	19.4	109.6	1.2
2022/01/31	36.1	25.6	15.1	20.3	0.7	9.0	90.4	59.6	26.3	118.2	1.3
2022/02/28	39.7	26.7	17.3	21.3	0.6	9.3	91.9	54.5	14.1	126.5	1.6
2022/03/20	37.5	25.2	13.8	19.3	0.5	7.6	92.2	59.5	25.3	115.8	1.3
2022/04/30	37.4	24.8	12.1	13.4	1.2	69.3	100	58.5	17	90.1	-
2022/05/31	33.0	21.9	10.8	12.3	1.1	113.7	100	62	24	76.8	-
2022/06/30	27.5	15.4	3.9	9.8	1.0	7.6	99.6	73.0	22.4	73.2	0.5
2022/07/31	30.1	17.0	5.9	10.1	0.8	5.2	100.0	72.5	15.2	78.4	0.5
						294.2				**1037.9**	
2022/08/31	30.1	17.0	5.9	10.1	0.8	5.2	100.0	72.5	15.2	78.4	0.5
2022/09/30	37.8	21.8	6.8	16.1	1.2	26.0	99.0	59.1	10.6	117.8	1.2
2022/10/31	40.4	24.6	15.4	16.9	1.3	49.6	98.8	64.6	9.7	118.7	1.1
2022/11/30	38.2	23.9	15.1	16.5	1.3	33.0	99.3	71.3	25.2	107.8	0.9
2022/12/31	39.2	25.1	14.3	18.0	1.3	74.0	99.4	71.6	20.5	117.8	1.0
2023/01/31	38.5	26.0	15.9	21.4	1.4	48.2	99.8	68.1	22.4	141.0	1.1
2023/02/28	34.6	24.5	17.9	15.3	1.3	400.6	100.0	83.2	38.4	100.0	0.5
2023/03/31	36.5	24.2	14.0	16.3	1.1	5.2	99.8	76.4	32.7	109.6	0.7
2023/04/30	33.8	22.9	14.1	12.2	0.9	32.6	100.0	73.4	33.7	93.0	0.8
2023/05/31	33.4	21.0	10.2	9.1	1.0	5.6	99.8	69.9	21.2	80.1	0.8
2023/06/30	30.3	18.4	7.3	9.5	1.1	18.0	98.7	61.3	15.6	83.4	0.9
2023/07/31	29.0	16.1	5.8	11.2	1.0	17.5	100.0	70.2	14.6	83.1	0.6
						715.5				**1230.6**	
2023/08/31	39.4	20.7	8.0	14.3	1.3	0.0	99.5	62.6	12.8	112.9	1.0
2023/09/30	40.8	22.7	10.6	16.0	1.4	29.5	98.6	62.0	6.2	121.8	1.1
2023/10/31	37.2	21.8	10.6	17.4	1.3	77.7	100.0	70.7	17.2	114.5	0.8
2023/11/30	40.2	25.4	13.6	20.6	1.2	49.0	99.5	67.4	17.3	138.6	1.2

2023/12/31	34.7	24.2	15.4	18.3	1.1	202.4	99.9	80.9	45.1	107.7	0.6
2024/01/31	37.9	24.9	16.4	19.9	1.1	114.2	100.0	76.3	36.7	122.0	0.8
2024/02/29	40.0	26.3	17.2	21.2	1.2	35.5	99.0	69.5	26.2	141.0	1.1
2024/03/31	35.4	22.5	13.8	13.8	1.1	75.4	100.0	83.1	35.4	91.4	0.5
2024/04/30	36.3	21.3	10.3	15.2	0.8	0.5	100.0	71.8	15.2	106.1	0.7
2024/06/30	-	-	-	-	-	-	-	-	-	-	-
2024/07/31	-	-	-	-	-	-	-	-	-	-	-
						584.2				**1055.9**	

The 2021/2022 growing season received the least rainfall (~ 294.2 mm) likely because of the drought that ravaged the study area.

The 2022/23 growing season had the highest rainfall peaked at 716 mm and was closer to the long-term average for the study area. The annual total reference evapotranspiration was 1038, 1231 and 1056 mm for the 2021/22, 2022/23 and 2023/24 growing seasons, respectively.

3.7.1.2. Soil moisture and tree growth

The soil moisture dynamics in the root zone of the marula trees at Thulamahashe are shown in Fig. 3.18 for the period November 2021 to February 2023. Part of the data collection was disrupted by vandalism as reported earlier. The dotted line in Fig. 3.18 indicates the approximate position of the volumetric soil water content at field capacity which we assume was similar at different depths. Over the measurement period, a few heavy storms were recorded that led to increases in the soil water content at all depths with the biggest rain event recorded in January 2023. The tree soil water extraction patterns suggest that the marula trees at the Thulamahashe site relied heavily on the rainwater stored in the shallow soil profile while to soil water content trend at the 100 cm depth remained relatively constant for much of the time.

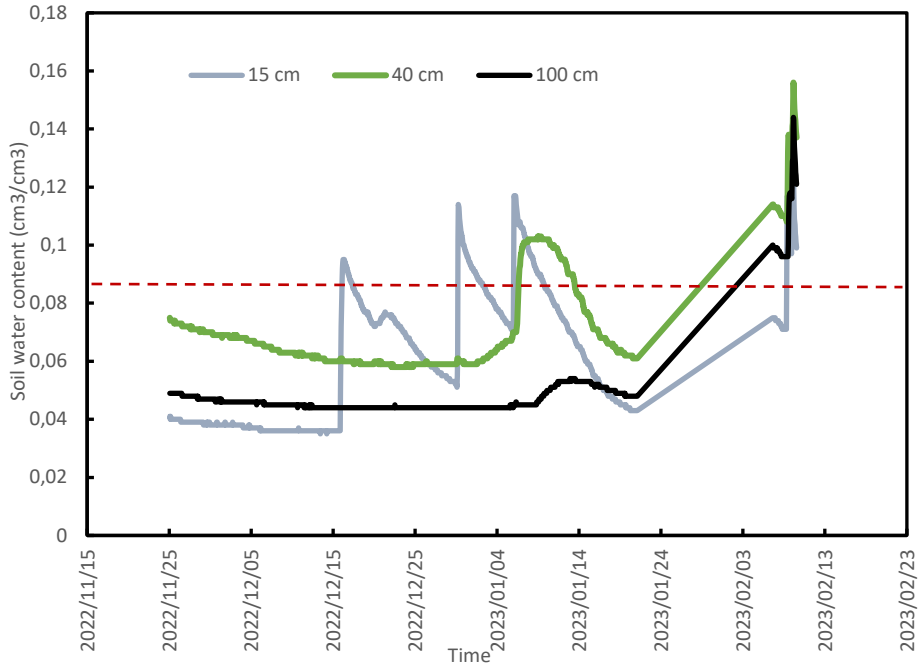


Fig 3.18. Seasonal changes in the volumetric soil water content in the root zone of the marula trees at Thulamahashe.

The leaf area index of the marula trees at Thulamahashe was measured at selected intervals as described earlier and Fig. 3.19 shows a typical four-stage LAI curve. More intense measurements of the LAI were not possible due to logistical reasons that include the distance between the study site and Univen campus and the shortage of appropriate equipment. The typical average LAI curve for the marula trees at Thulamahashe showed minimum (0) values during the winter months rising during spring flush around mid to end of September and reaching a peak averaging around 2.6 by mid to late October when maximum canopy cover was reached. The leaf fall phase commenced during mid to late June when the temperatures dropped, and the trees entered dormancy (Fig. 3.19). The spot measurements that were recorded at various stages in the growing season at the various sites are shown in Table 3.14.

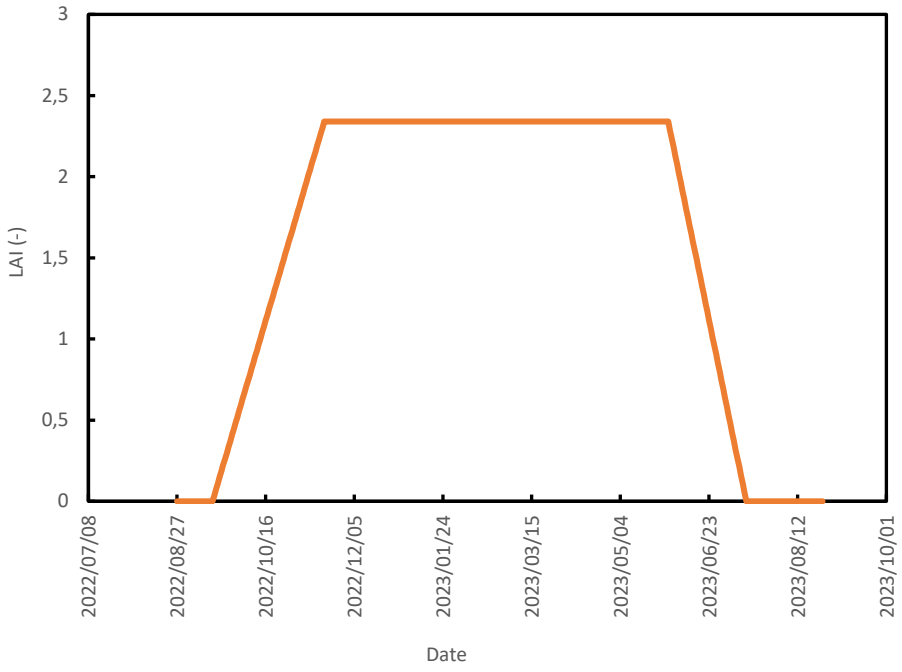


Fig 3.19. Seasonal changes in the mean leaf area index of the instrumented trees at Thulamahashe.

Table 3.14. Calculated LAIs for the three sites

Site	Tree	PAR Above (µmol m^{-2} s^{-1})	PAR Below (µmol m^{-2} s^{-1})	LAI
Phalaborwa	1	633	284.4	1.04
Phalaborwa	2	621	153.2	1.48
Phalaborwa	3	626	137.2	1.66
Phalaborwa	4	634	336.6	0.84
Mooketsi	1	1075	739	0.58
Mooketsi	2	1024	200	1.71
Mooketsi	3	1095	194.8	1.85
Mooketsi	4	1077	563.8	0.89
Thulamahashe	1	911	105.4	2.51
Thulamahashe	2	1016	72.4	3.13
Thulamahashe	3	1052	441.6	1.38

At Phalaborwa, because of the region's low rainfall, the LAI values ranged from 0.84 to 1.66, suggesting decreased canopy density and possibly lower leaf area. This result is consistent with other research showing that water stress can result in decreased LAI and decreased leaf output (Kallarackal *et al.,* 2004). This may also be because of growth stage at which the data was collected which is the physiological maturity stage.

A different pattern may be experienced when more data is being collected at other growth stages. Thulamahashe had the highest LAI values, which ranged from 1.38 to 3.13. Higher rainfall supports denser canopies and larger leaf areas, as seen by these numbers. This finding is in line with research by Ambrose *et al.* (2010), who found that more water availability promotes stronger tree growth and denser canopies. The LAI values in Mooketsi varied from 0.58 to 1.85. This site's medium canopy density, which is supported by a significant amount of leaf area but exhibits greater variability because of microenvironmental factors, is indicative of a moderate water supply (Bréda, 2003).

The LAI data show notable variations in leaf area and canopy density between the three sites, which are indicative of how rainfall affects marula tree growth. Phalaborwa's lower LAI values suggest that water stress causes less leaf growth and sparser canopies. This result aligns with the findings of Gower and Norman's (1991) research, which indicated that a key determinant of LAI in a variety of tree species is water availability. Although there is clear variety in canopy density, the intermediate LAI values in Mooketsi indicate that there is enough rainfall to support a significant leaf area. Archibald and Scholes (2007) have pointed out that fluctuations in soil moisture and other environmental conditions may be responsible for this. Thulamahashe’ s higher LAI values suggest that more rainfall is necessary to maintain denser canopies and larger leaf areas.

Phalaborwa, Moeketsi, and Thulamahashe marula trees were observed during their various phenological stages, which were noted and examined. The table and charts below offer a summary of the findings:

Table 3.15. Summary of phenological data as observed at Phalaborwa, Mooketsi and Thulamahashe from 2023 to 2024

Phenological Stage	Phalaborwa Start	durarion in days	Phalaborwa End	Mooketsi Start	duration in days	Mooketsi End	Thulamahashe Start	duration in days	Thulamahashe end
Bud Break	13-Aug-23	20	02-Sep-23	11-Sep	25	06-Oct	11-Aug	20	31-Aug
Leaf Emergence	02-Sep-23	30	02-Oct-23	15-Oct	30	14-Nov	29-Sep	20	19-Oct
Flowering	15-Oct-23	30	14-Nov-23	14-Nov	20	04-Dec	15-Sep	20	05-Oct
Fruit Set	20-Nov-23	20	10-Dec-23	21-Nov	20	11-Dec	17-Oct	30	16-Nov
Fruit Development	20-Nov-23	60	19-Jan-24	21-Nov	60	20-Jan	03-Nov	60	02-Jan
Fruit drop	15-Jan-24	45	29-Feb-24	15-Feb	30	16-Mar	10-Jan	30	09-Feb
Leaf Senescence	15-Mar	60	14-May-24	21-Apr	30	21-May	15-Feb	30	16-Mar
Dormancy	16-May	90	14-Aug-24	30-May	90	28-Aug	30-Apr	90	29-Jul

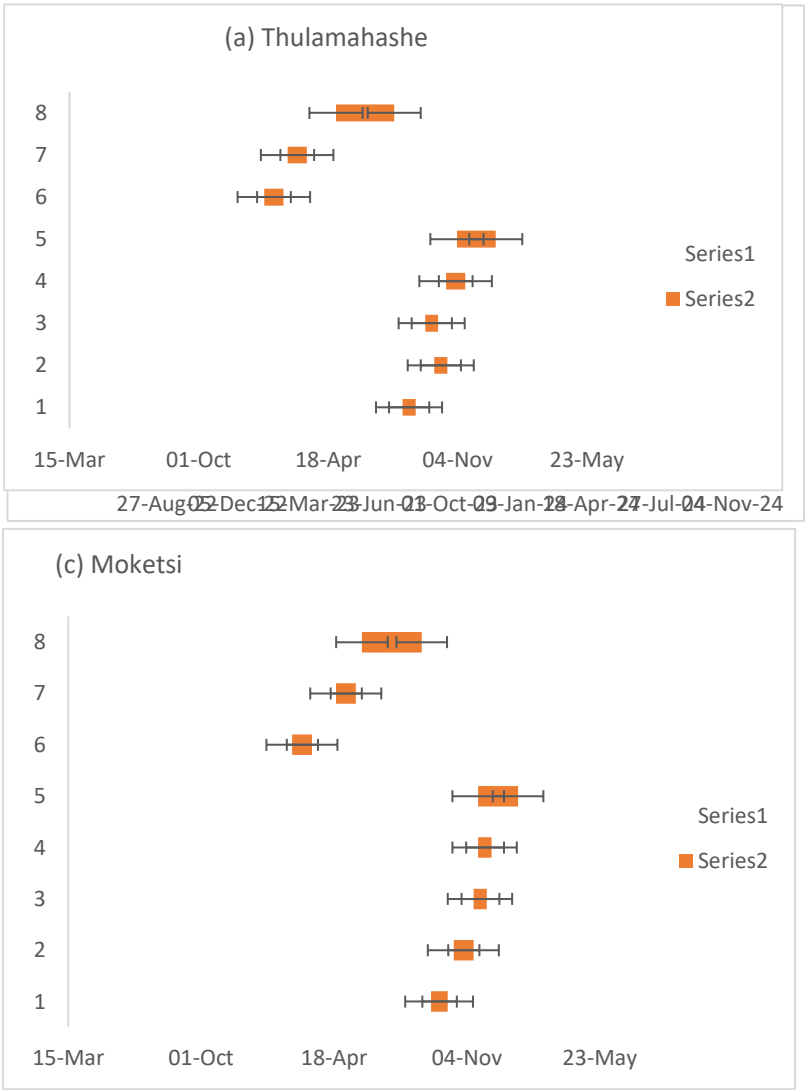


Fig 3.20. Phenological changes for the marula trees at Moeketsi, Phalaborwa and Thulamahashe.

Due to variations in the three (3) experimental sites, the phenological stages of marula trees also differed. These variations are significant as they provide insights into the

adaptability of marula trees to different environmental conditions. The timing and length of phenological episodes are influenced by climatic parameters like temperature and rainfall, as highlighted by earlier research on plant phenology (Cleland *et al.,* 2007; Parmesan and Yohe, 2003). The observations are consistent with these findings.

Higher rainfall may have contributed to the early bud break in Thulamahashe, leading to the earlier vegetative growth. According to Cleland *et al.* (2007), more precipitation can hasten bud break. There were significant variations in Thulamahashe's flowering and fruit set compared to Phalaborwa and Mooketsi. Perhaps because Thulamahashe received more rainfall than Phalaborwa, which receives less, the early onset occurs there. Early fruit set and flowering may result in extended times for fruit development, which will increase fruit output and size. Phalaborwa had longer periods of leaf senescence and dormancy than the other sites. This might be because of the area's higher temperatures and less rainfall, which forced trees to go into dormancy earlier and for longer periods to save energy and water.

3.7.1.3. Transpiration and its drivers at Thulamahashe

The average daily transpiration rate of a typical tree at the study site over two growing seasons is shown in Fig. 3.21. Sap flow data collection commenced in mid-August 2021 when the leaf area index of the trees was greater than 2.0. Peak daily transpiration reached around 200 litres of water per tree per day in the 2022 – 2023 season.

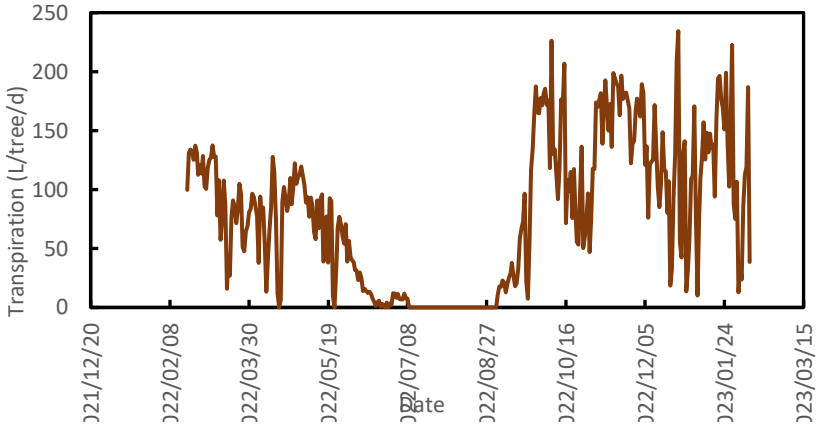


Fig 3.21. Daily total transpiration of a marula tree about 50 cm diameter at breast height at Thulamahashe.

The higher transpiration rates coincided with the higher rainfall received at the site as indicated in Table 3.13. There was a clear decline in transpiration rates during dry periods e.g. from early February to late March. This observation is consistent with data published by Dzikiti *et al.* (2022a) which showed that the transpiration of marula trees is strongly limited by the available soil water, which is correlated to rainfall. This is most probably because of the low density of fine feeder roots in marula trees that are responsible for absorbing water and nutrients.

A closer look at the relationship between individual climate drivers of transpiration i.e. the solar radiation and the vapour pressure deficit of the air is shown in Fig. 3.22. The solar radiation provides the stimulus for stomatal opening through the blue light responses. When blue light falls on the leaves, phototropins are stimulated which activate the ATPase proton pump. This in turn pumps protons from the inside to the outside of the guard cell apoplast leading to the acidification of the guard cell walls. This opens the K+ channels leading to a reduction in the osmotic potential inside the guard cells leading to stomatal opening.

Figure 3.22a shows a strong relationship between the radiation intensity and the whole tree sap flow for two days. Interestingly, the relationship between these variables is linear from morning to mid-afternoon as shown in Fig. 3.22b. However, in the declining phase from afternoon to early evening, there is a strong non-linear relationship which is not expected. This hysteresis effect is probably related to the capacitance of the trees wherein large volumes of water are stored in the stem cells.

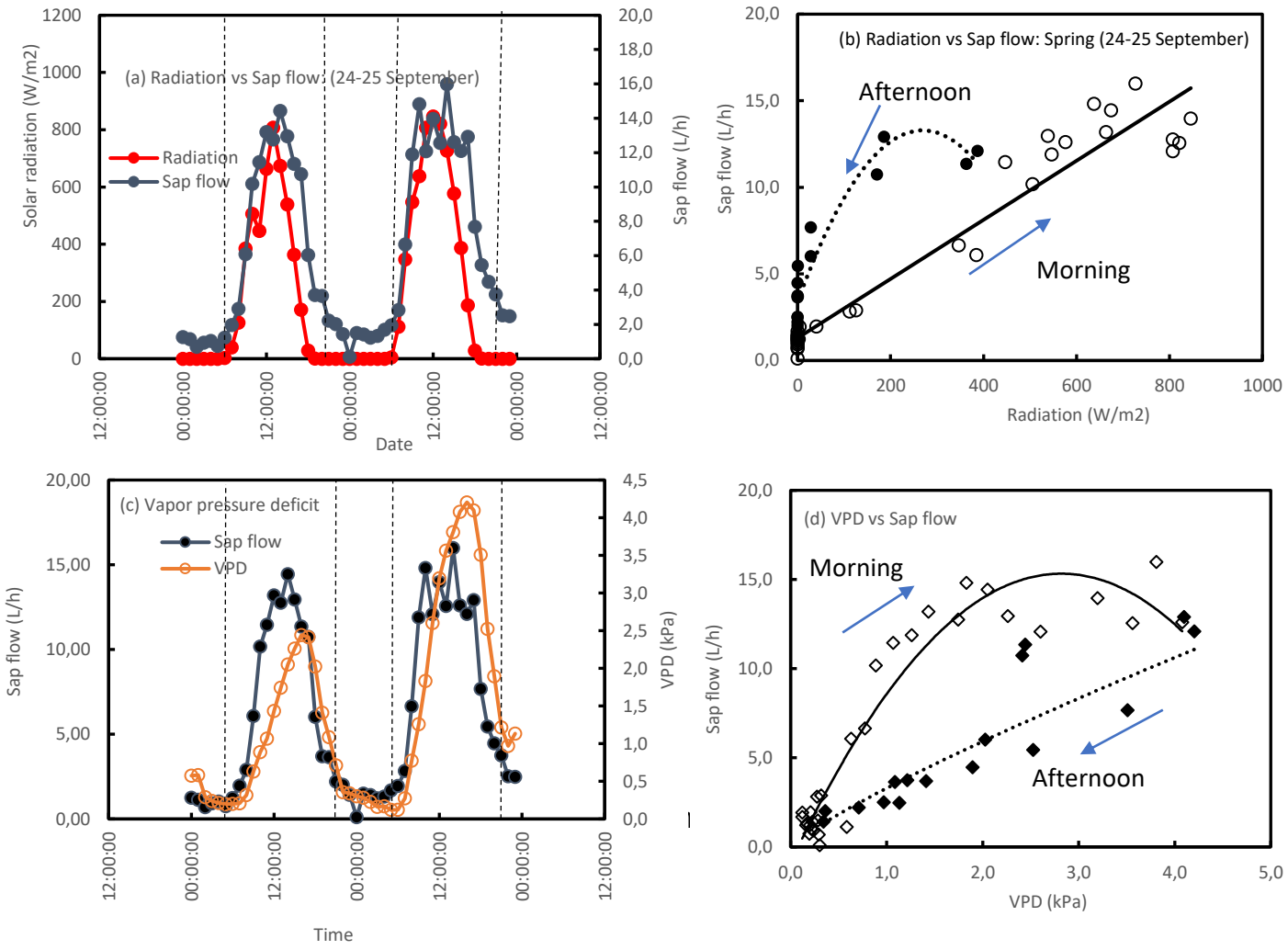
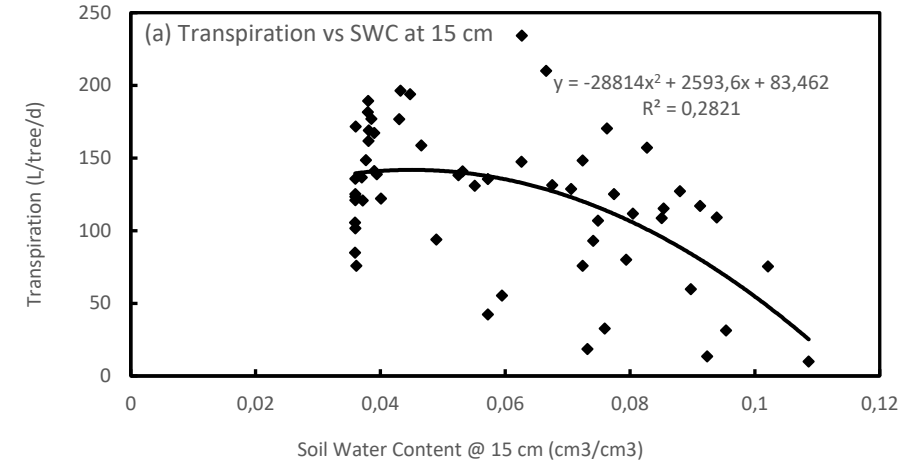
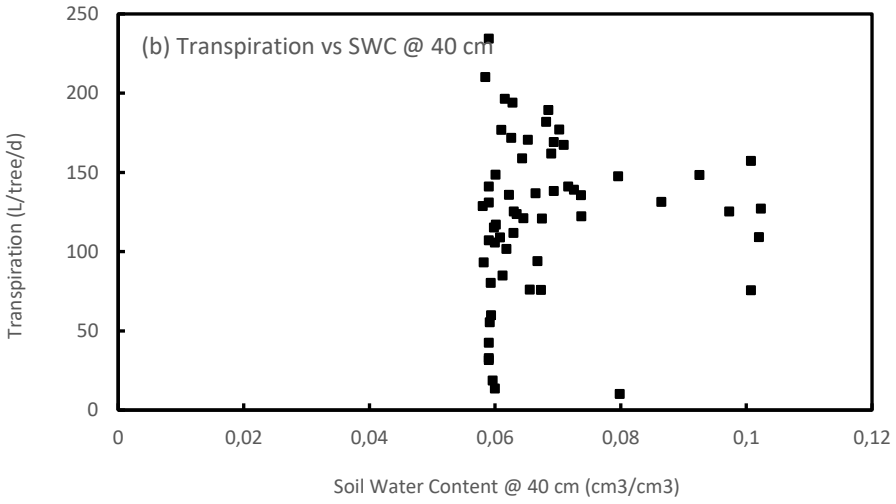


Fig 3.22(a). The relationship between the radiation intensity and the whole tree sap flow for two days. (b) The relationship between these variables is linear from morning to mid-afternoon.

The reverse trend is, however, evident with the vapour pressure deficit of the air (Fig. 3.23 c & d). A significant time lag is evident between the tree transpiration and the air's VPD with the VPD reaching a peak several hours after transpiration has peaked. Again, the reason for this behaviour is unclear.





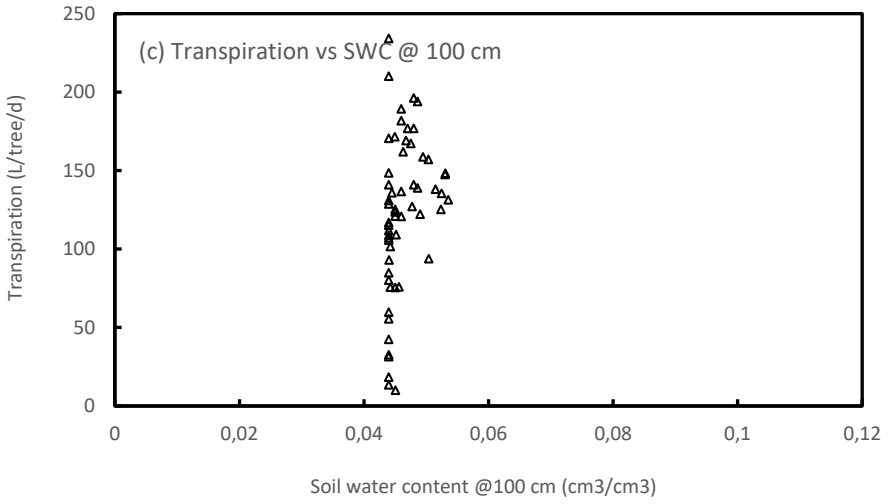


Fig 3.23. Relationship between tree transpiration and soil water depletion at 15, 40 and 100 cm depths, respectively.

The transpiration and VPD are strongly linearly related during the declining phase of transpiration from midday to sunset (Fig. 3.23d). From morning to midday, there was a non-linear relationship between the tree transpiration and the VPD. These data illustrate the complex relationships between water use by the marula trees and the environmental drivers. This also informs the complexity that is involved in modelling the water use of this species under a range of growing conditions.

Twenty-eight per cent (28%) of the variation in the soil water content in the 0 to 15 cm depth at Thulamahashe could be explained by the water uptake by the trees as shown in Fig. 3.23a. There was a weaker correlation between transpiration and soil water depletion at larger depths suggesting that the marula trees at Thulamahashe depend strongly on rainwater stored in the shallow soil layers.

3.7.2. Moeketsi - ZZ2 site

3.7.2.1. Weather conditions at ZZ2

Weather conditions in the ZZ2 area for the period August 2022 to April 2024 are summarised in Fig. 3.24. As expected, the daily solar radiation peaked in the summer months with the maximum exceeding 25 MJ/m^2/d and approaching 30 MJ/m^2/d in some instances. On overcast days the daily total solar radiation was less than 5.0 MJ/m^2/d, especially in winter or during rainy days in summer. As at Thulamahashe, the effect of the high frequency of cloud cover in summer is also evident by the slight decline in the peak daily irradiance between November and March which corresponds to the main rainfall season. The maximum air temperature peaked at a slightly lower value of about 40°C just before the main rainy season in November. The lowest air temperature was about 7°C reached in winter in July. The lowest relative humidity (~ 12%) was experienced just before the onset of the rainy season in September. The vapour pressure deficit of the air reached a maximum of only 3.2 kPa in October.

The diurnal course of the reference evapotranspiration is shown in Fig. 3.24. The reference evapotranspiration reached a peak just over 7.4 mm/d in late spring with low average values reached during the winter months when the atmospheric evaporative demand was low. As with solar radiation, the effect of long periods of

cloud cover is also evident on ETo in Fig. 3.24 and these correspond to periods of high rainfall. The significance of these results is that peak water use by the trees is unlikely to be reached during the peak summer season as the atmospheric evaporative demand will be relatively lower than the spring values. However, water use in spring is likely to be constrained by low soil water content (before the onset of the rainy season) and the low transpiring leaf area since marula trees are deciduous. Therefore, the correlation between peak transpiration and the atmospheric evaporative demand is unlikely to be straightforward. This assumption will be revisited in the next sections.

A summary of the monthly weather conditions at the ZZ2 study site over the entire duration of the study is shown in Table 3.16. September and October were the warmest months with peak temperatures exceeding 40 °C although the daily total solar irradiance was not necessarily highest during this period. Peak temperatures in the summer months varied from 35 to 39°C. December 2022 and February 2023 were the wettest months over the study period receiving over 200 mm of rainfall while August tended to be the driest month with less than 1.0 mm of rainfall. The atmospheric evaporative demand was greatest in the January – February 2022 period when the reference evapotranspiration ranged between 135 to 138 mm/month. The 2022/2023 growing season received a total of 530 mm which is somewhat lower than the long-term average for the region. The annual total reference evapotranspiration was about 1 240 mm.

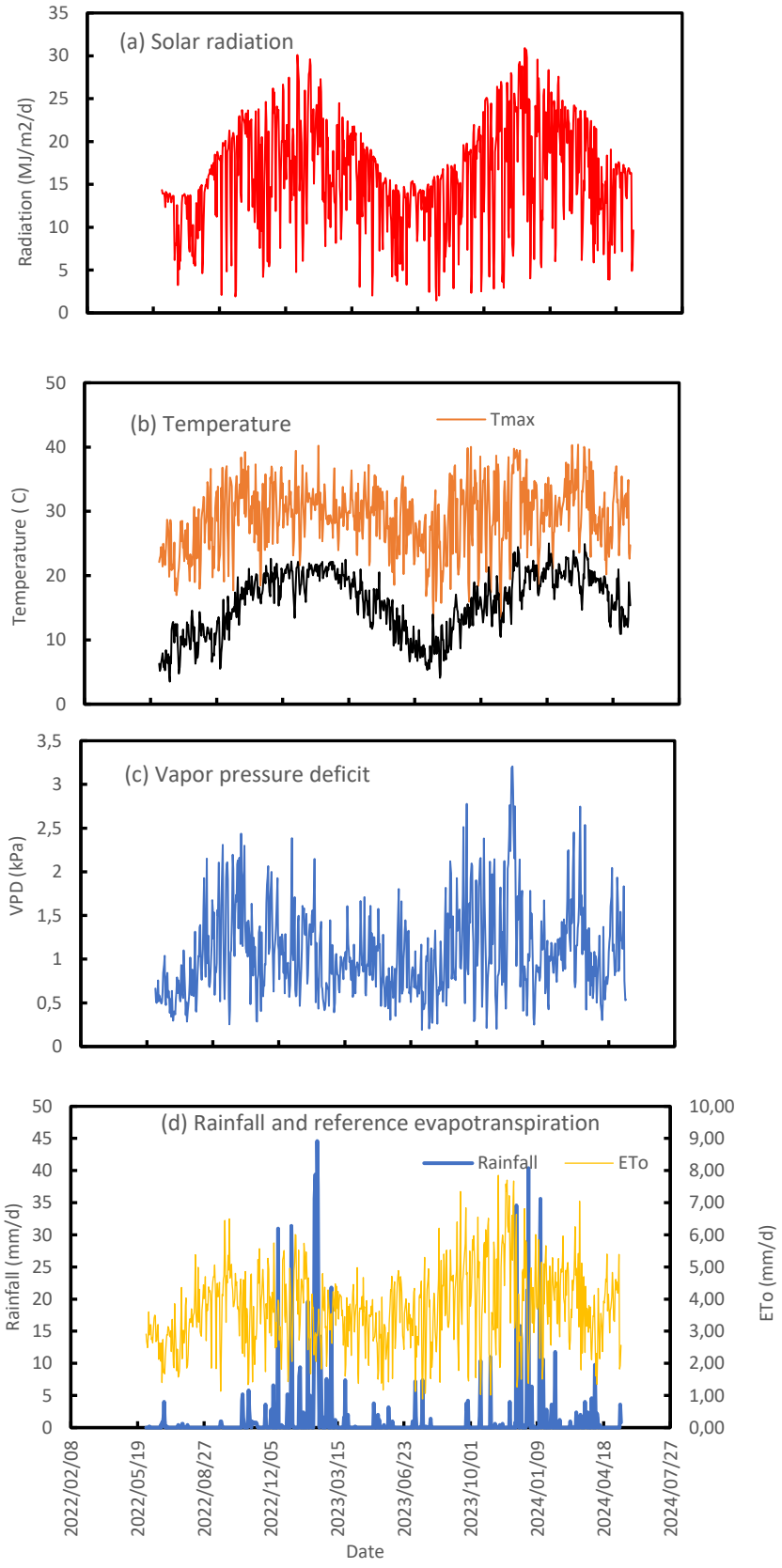


Fig 3.24. Daily climate variables for the period 2022 to 2024 at ZZ2 in Moeketsi representing (a) the solar radiation, (b) maximum and minimum temperatures, (c) vapour pressure deficit, and (d) rainfall and reference evapotranspiration.

Table 3.16. Summary of the monthly weather conditions at Moeketsi.

Date	Tmax (°C)	Tavg (°C)	Tmin (°C)	Rhmax (%)	Rhavg (%)	Rhmin (%)	Rs (MJ/m2/d)	U2 (m/s)	Rain (mm)	ETo (mm)
June 2022	28.7	14.8	3.5	92.8	66.0	13.2	11.4	1.0	9.0	80.6
July 2022	30.7	16.5	5.8	93.3	64.3	14.8	11.2	0.8	2.0	82.3
August 2022	36.6	18.7	6.6	89.4	50.9	7.1	15.2	1.0	0.4	106.9
September 2022	37.0	21.2	5.5	94.3	49.7	7.5	16.7	1.3	2.4	119.2
October 2022	39.2	24.4	15.4	94.1	54.8	7.6	18.0	1.3	11.2	122.8
November 2022	37.0	23.9	15.3	93.7	64.0	23.8	16.9	1.1	21.6	103.4
December 2022	39.4	24.8	13.4	94.5	65.6	15.7	18.0	1.1	52.0	107.8
January 2023	40.2	25.3	15.9	94.0	62.7	19.1	21.6	1.0	103.2	128.3
February 2023	36.7	24.3	18.8	93.8	75.2	34.7	15.3	0.8	279.8	92.7
March 2023	36.4	23.7	13.9	93.9	69.0	24.9	17.1	0.7	36.8	104.6
April 2023	37.2	22.5	11.7	90.3	62.7	15.0	15.3	0.7	0.2	104.2
May 2023	33.6	19.9	9.1	93.5	67.5	18.3	11.4	0.7	11.0	84.7
Total									**529.6**	**1237.7**
June 2023	35.5	18.3	6.6	93.5	53.4	8.2	13.0	1.2	2.2	111.4
July 2023	33.3	15.6	4.1	94.2	60.4	12.6	12.3	1.3	18.6	97.2
August 2023	37.2	18.5	6.7	91.0	56.2	6.9	12.7	1.5	1.4	109.0
September 2023	40.0	22.2	11.6	93.7	51.9	5.9	16.4	1.6	10.6	133.3
October 2023	38.7	22.1	10.5	93.1	56.5	12.7	17.8	1.8	23.4	128.8
November 2023	39.8	25.2	12.5	91.8	52.5	11.1	20.3	1.6	5.8	146.9
December 2023	38.1	24.3	14.8	94.7	68.3	21.2	18.5	1.6	175.0	122.8
January 2024	35.3	24.6	16.3	92.2	68.7	34.9	19.7	1.3	88.0	120.0
February 2024	40.4	26.1	17.3	91.7	62.4	22.0	18.7	1.3	20.4	125.7
March 2023	40.0	25.1	14.7	94.3	64.7	17.4	15.5	1.4	18.0	118.3
April 2023	37.0	21.7	10.9	92.3	63.9	13.9	13.3	1.1	15.2	101.8

3.7.2.2. Soil moisture dynamics and canopy growth at ZZ2

Soil water content in the rootzone of the marula trees at ZZ2 closely followed rainfall events as shown in Fig. 3.25. As expected, the soil water content spiked after rainfall events. There were many instances when data collection did not happen due to power failures as shown by the numerous gaps in the graph. The volumetric soil water content at field capacity was at about 0.14 cm^3/cm^3. The recorded moisture levels were lower than this value for most of the season suggesting that the trees did suffer water deficit stress on some occasions. The seasonal trends in canopy leaf area were similar to that at Thulamahashe as shown in Fig. 3.26. The only difference, however, is that there was a leaf-eating beetle that inflicted the trees at certain times and this contributed to a lower transpiring leaf area.

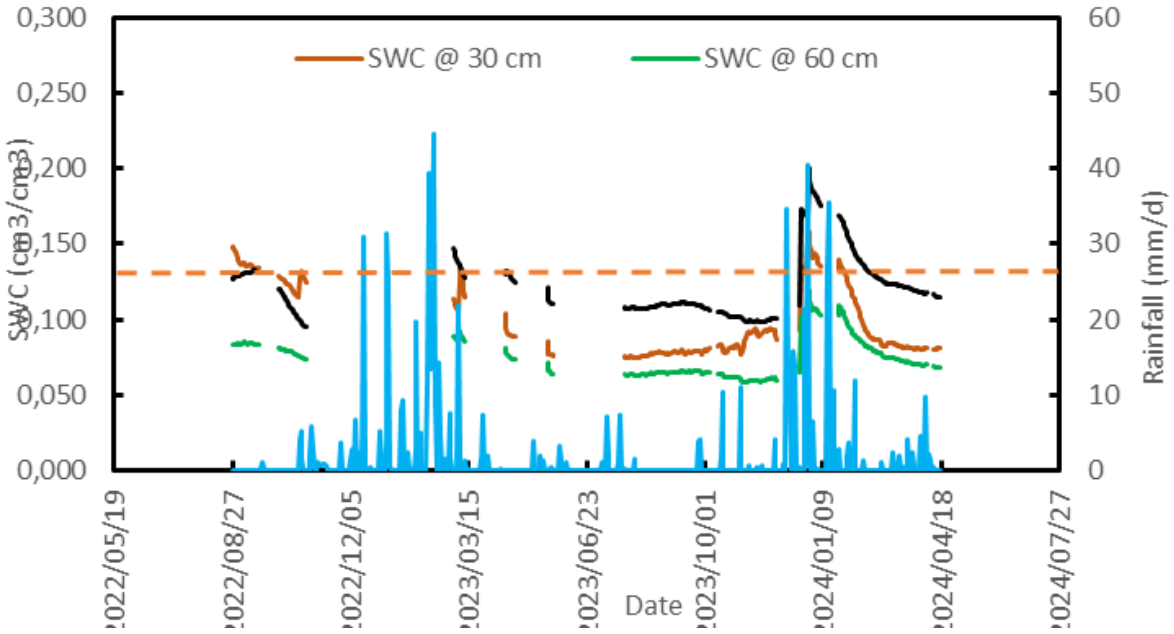


Fig 3.25. Rootzone soil water content at different depths is affected by rainfall at ZZ2 in Moeketsi. Missing data were due to the power failure of the datalogger.

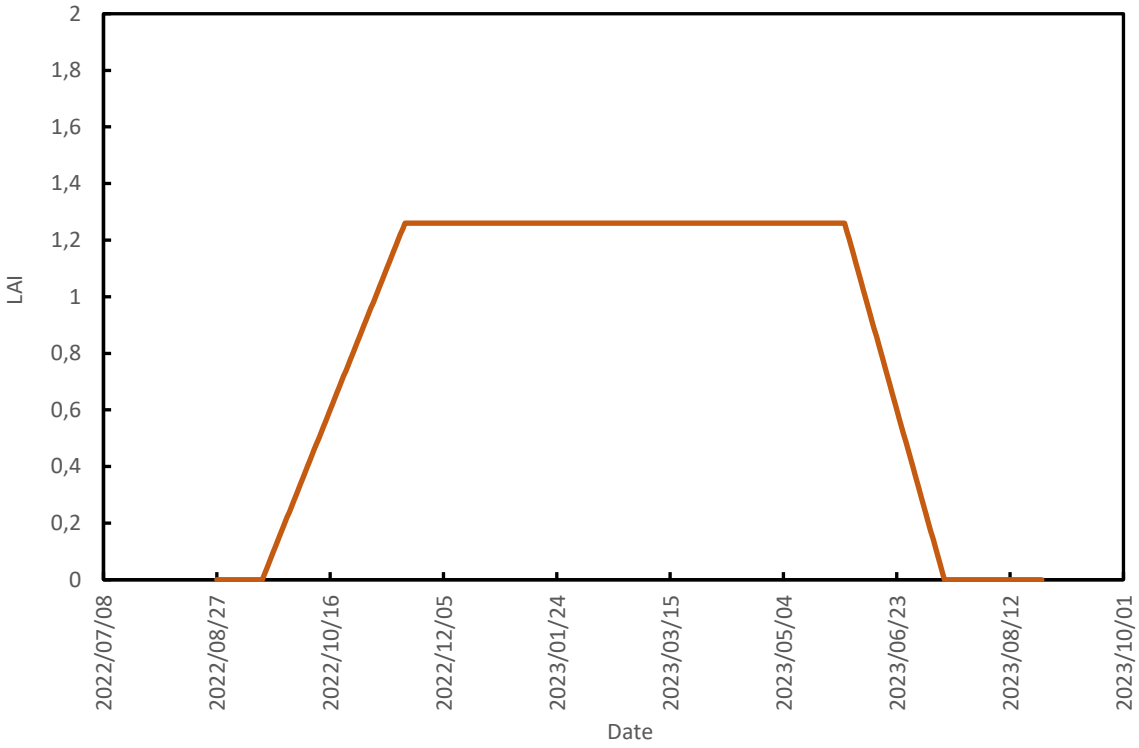


Fig 3.26. Average leaf area index of the four instrumented marula trees at ZZ2 in Moeketsi.

3.7.2.3. Transpiration dynamics and its drivers at ZZ2

Marula tree transpiration at the ZZ2 also showed clear seasonal variations (Fig. 3.27). However, the delayed leaf falls in winter, whose cause is unclear, likely resulted in non-zero sap flow measurements during much of the winter months. The trees were leafless for a much shorter period than at the other two sites for unclear reasons. The average maximum transpiration rate per tree was around 123 litres per day at this site.

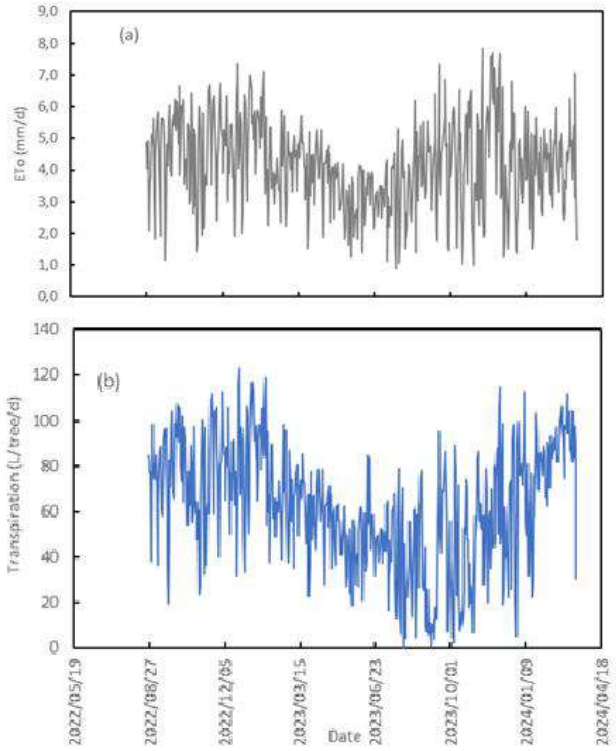


Fig 3.27. The daily reference evapotranspiration (a) and transpiration (b) of a marula tree at ZZ2 from August 2022 to March 2024.

Unlike the Thulamahashe site, there was a consistent curvilinear relationship between tree transpiration and the driving climatic variables namely the solar radiation, VPD and the reference evapotranspiration as shown in Fig. 3.28. There was no evidence of hysteresis possibly indicating a lower storage capacity of the relatively smaller trees than at Thulamahashe.

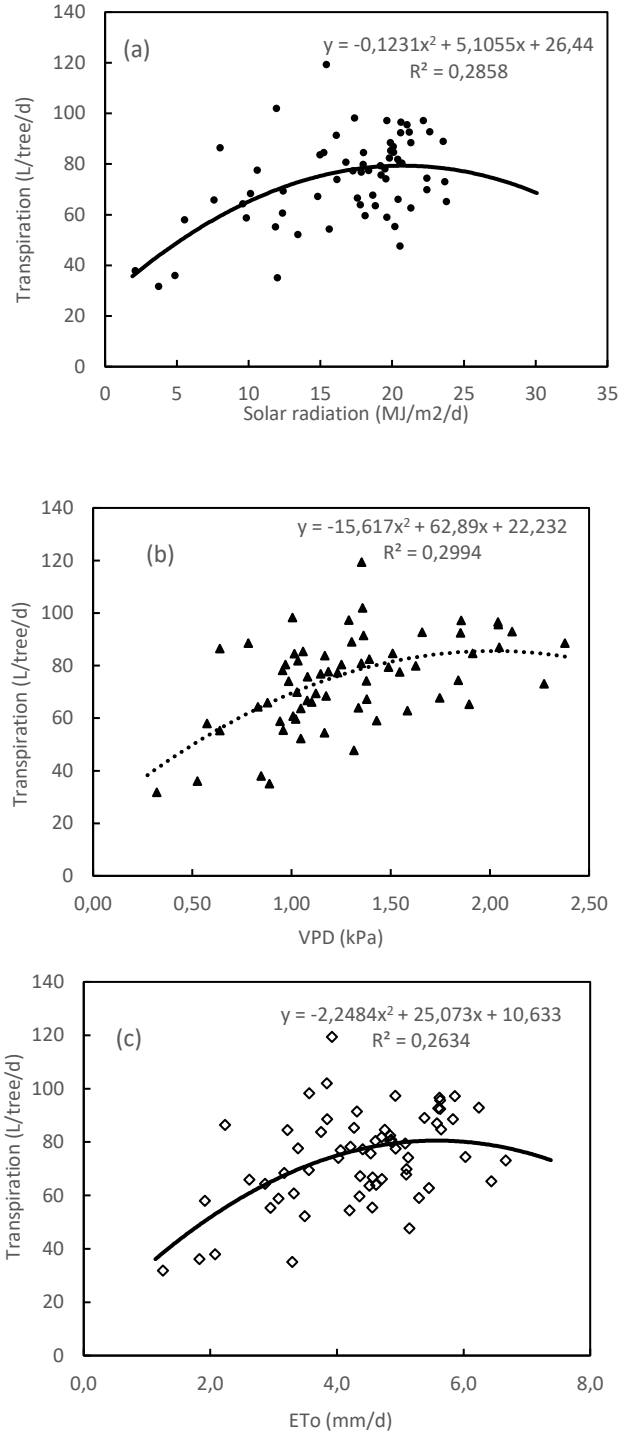


Fig 3.28. Relationship between whole tree transpiration and (a) the daily total solar radiation, (b) the vapour pressure deficit of the air, and (c) the reference evapotranspiration.

It appears that the root water extraction patterns at ZZ2 were uniform across all the measured depths as shown in Fig. 3.29. Much of the changes in daily total transpiration could be explained by the changes in the soil water content with coefficients of determination ranging from 0.27 to 0.41.

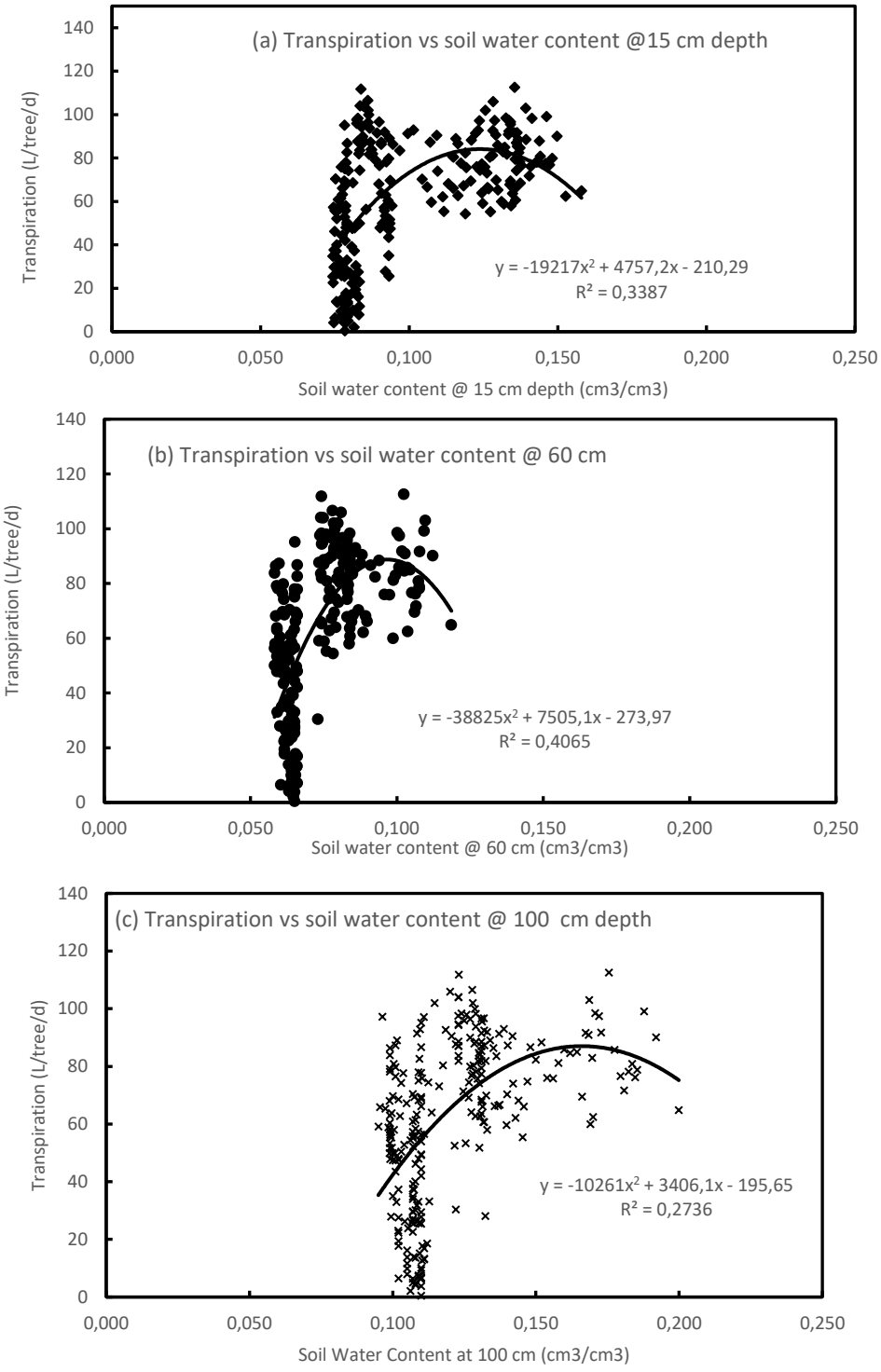


Fig 3.29. Water uptake patterns at different depths in the root zone of the marula trees at ZZ2.

3.7.3. Phalaborwa study site

3.7.3.1. Weather conditions and rootzone soil moisture in Phalaborwa

Weather data for the current growing season was available for a short period of time (December 2023 to April 2024) due to a system failure at the ARC-run weather station. Figure 3.30 summarises the available data although not many seasonal variations can be discerned due to the reduced data collection period.

The soil water content in the rootzone fluctuated between 0.05 and 0.1 cm^3/cm^3, which was quite low because of the high stone content of the soils which reduced the amount of available soil water (Fig. 3.31). Despite this, the trees at this site probably were the largest highlighting the ability of this species to adapt to harsh growing conditions. The larger size of the trees likely reflected their older ages rather than optimal growing conditions.

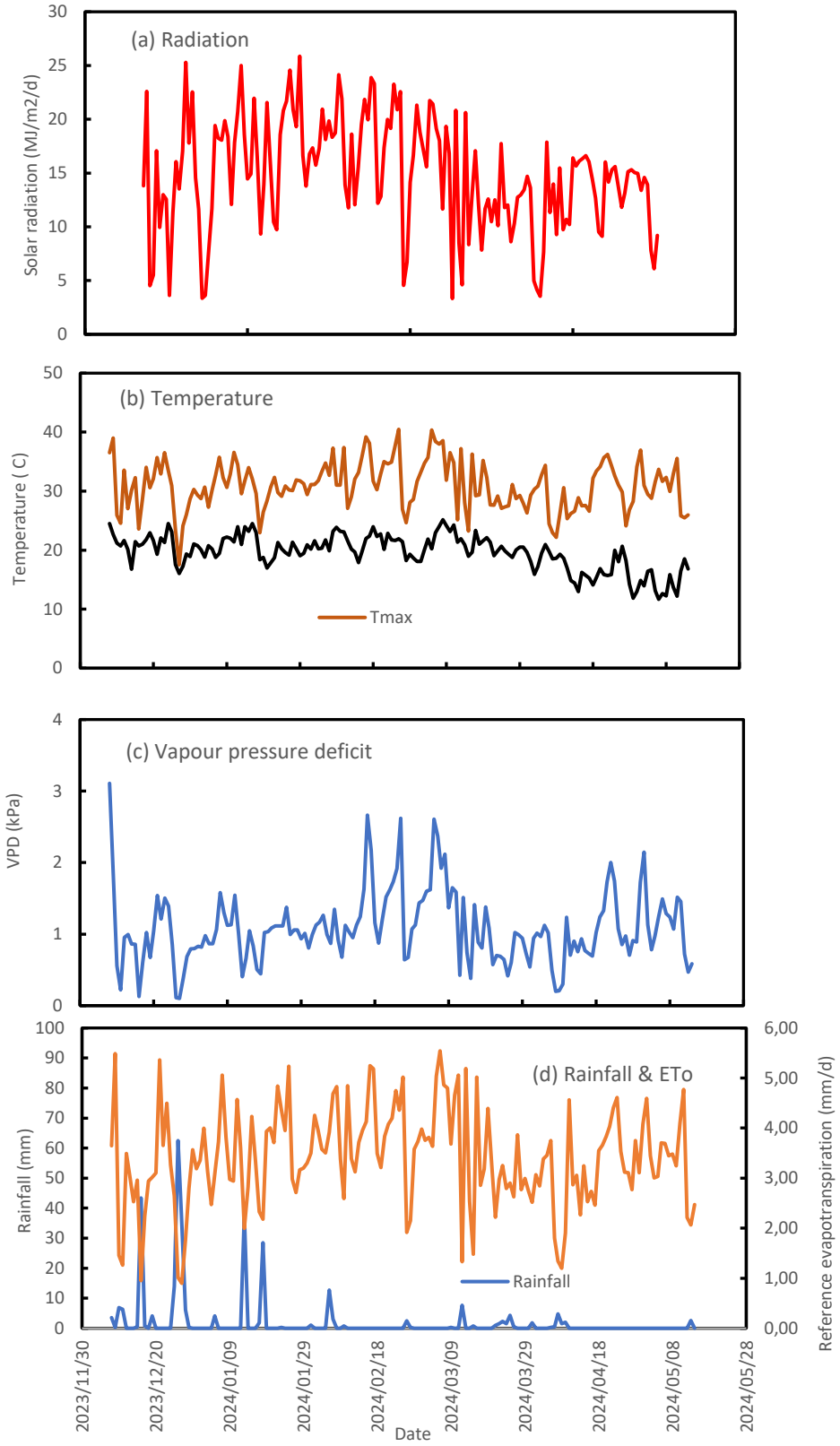


Fig 3.30. Summary of the daily weather conditions in Phalaborwa from December 2023 to May 2024.

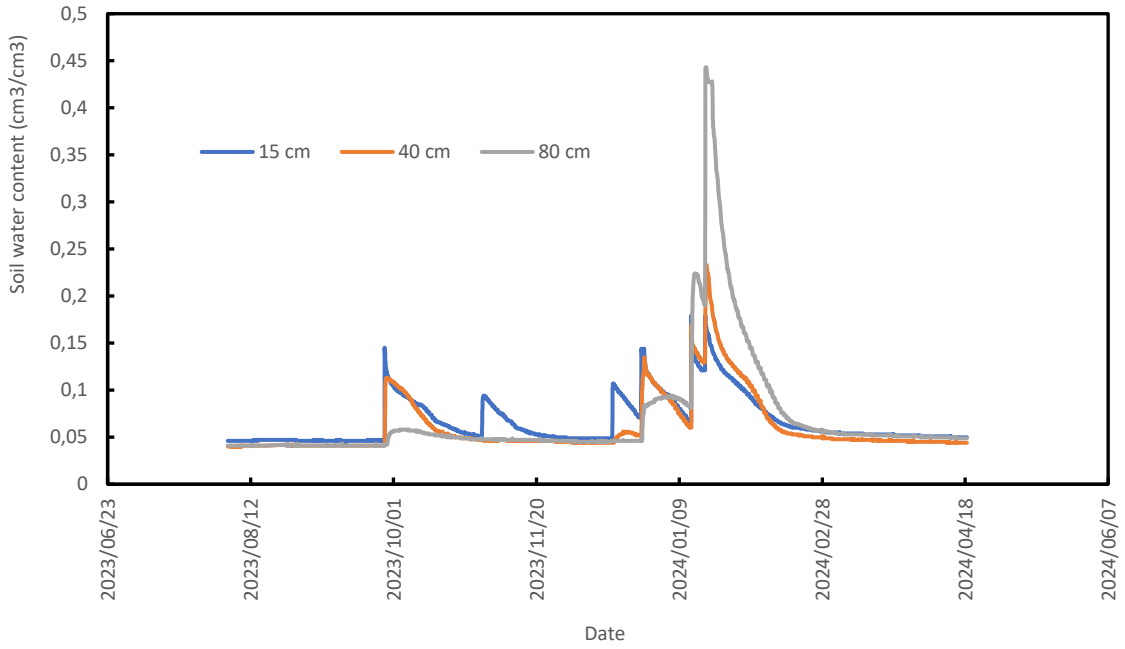


Fig 3.31. Soil water content in the root zone of the marula trees in Phalaborwa.

3.7.3.2. Transpiration and its drivers at Phalaborwa

Despite the large tree sizes at Phalaborwa, the average peak transpiration of these trees was somewhat lower than at the other two (2) sites as shown in Fig. 3.32. This likely reflects the impact of the rather harsh growing conditions in Phalaborwa i.e. low rainfall, higher temperatures, and poor soils. A comparison of the water use values normalised per unit leaf area will be presented in the next deliverable. This should show more clearly the effect of the growing region on the water use characteristics of the trees. As at the ZZ2 site, the driving climatic variables seem to be curvilinearly related to the tree transpiration at Phalaborwa as shown in Fig. 3.33.

However, a more complex trend seems to emerge relating to the relationship between tree water use and soil moisture depletion. It appears from Fig. 3.34 that transpiration of the marula trees at Phalaborwa was high after rainfall events when soil moisture levels were high. But as soon as the soil moisture depletion accelerated as the soil dried, the transpiration rates seemed to drop at a rapid rate and this needs further investigation.

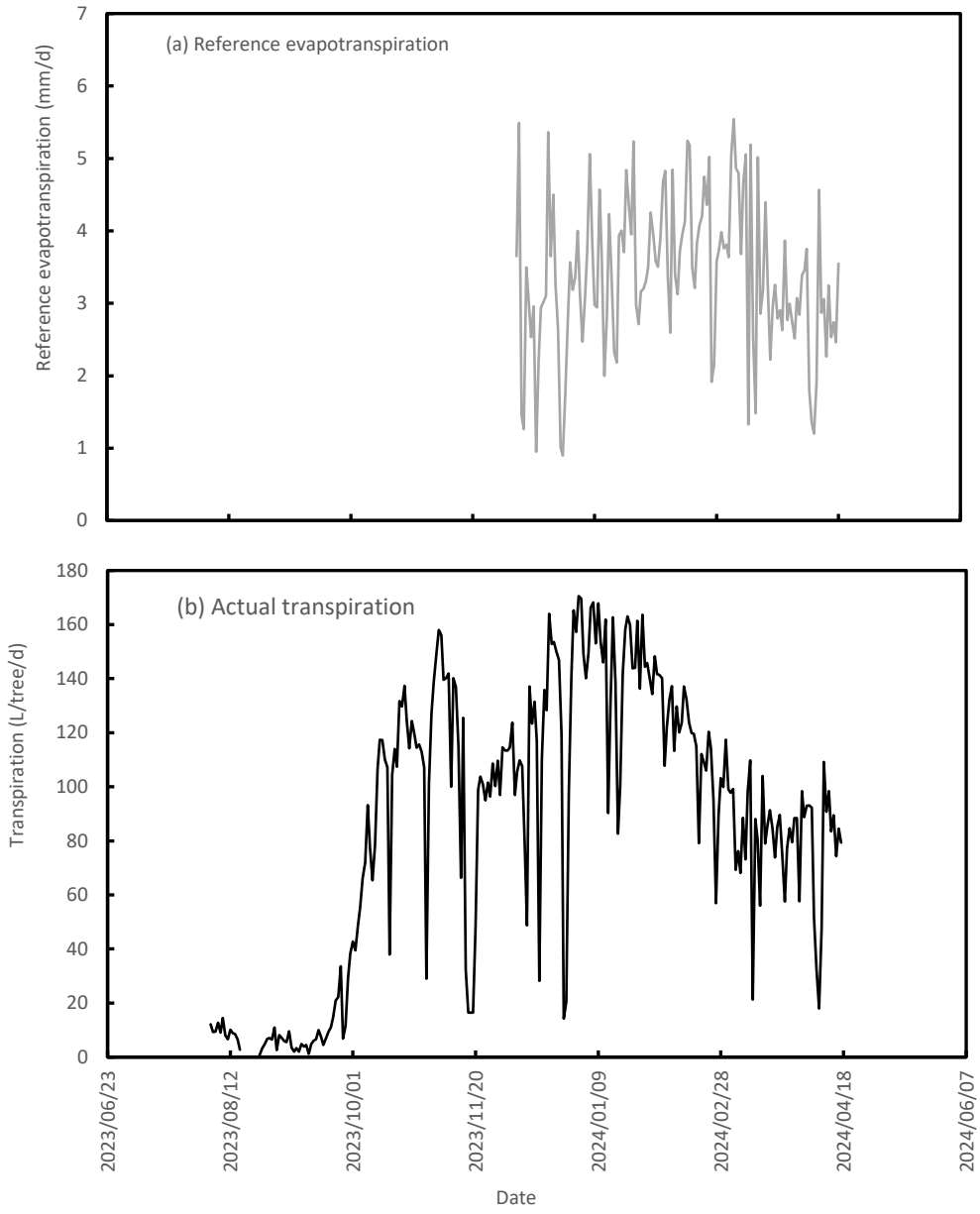


Fig 3.32. Seasonal changes in (a) the reference evapotranspiration (ETo) and (b) the actual tree transpiration at Phalaborwa.

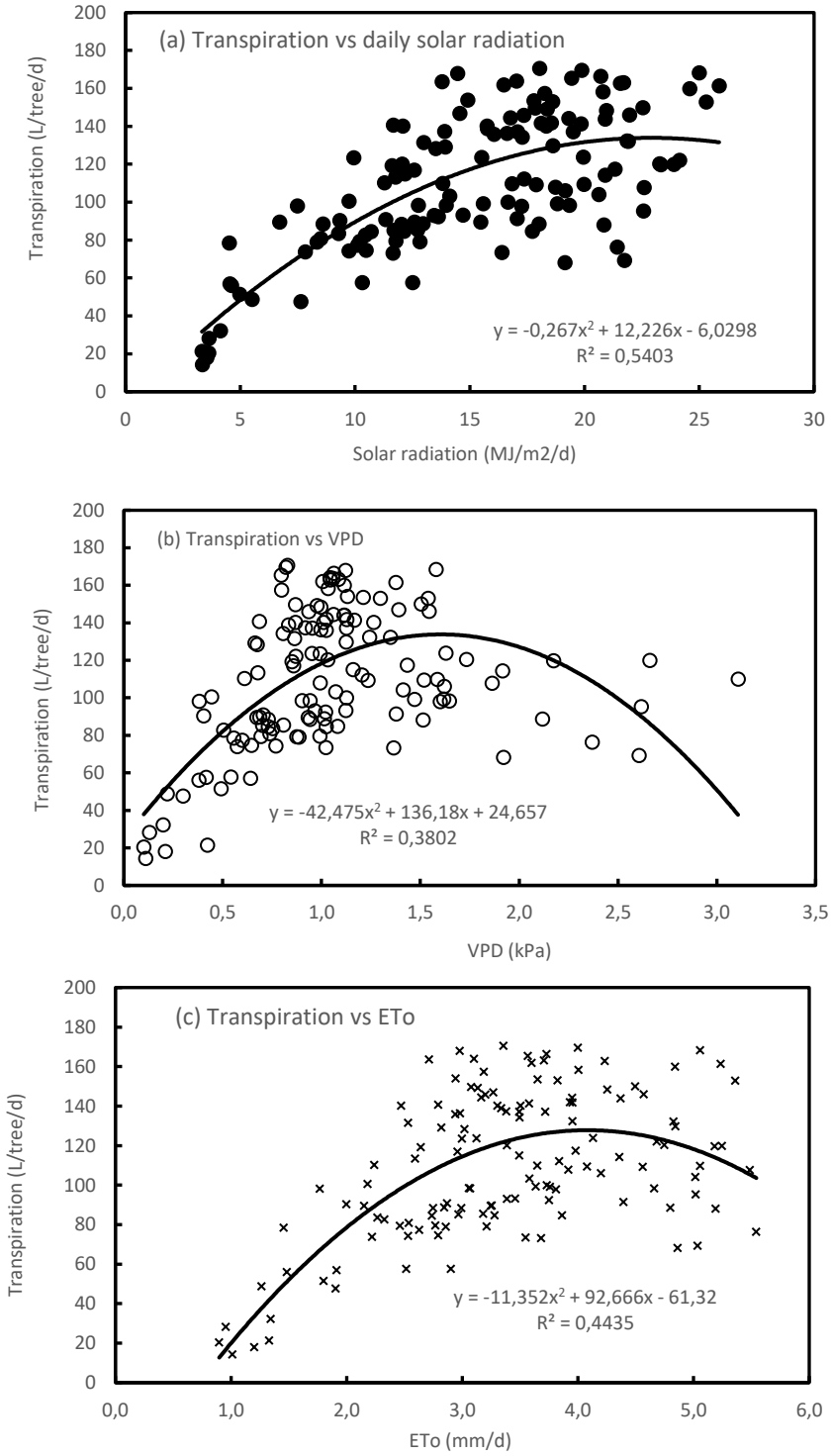


Fig 3.33. Effect of (a) the daily total solar radiation, (b) vapour pressure deficit of the air, and (c) the reference evapotranspiration on the average daily total transpiration of marula trees at Phalaborwa.

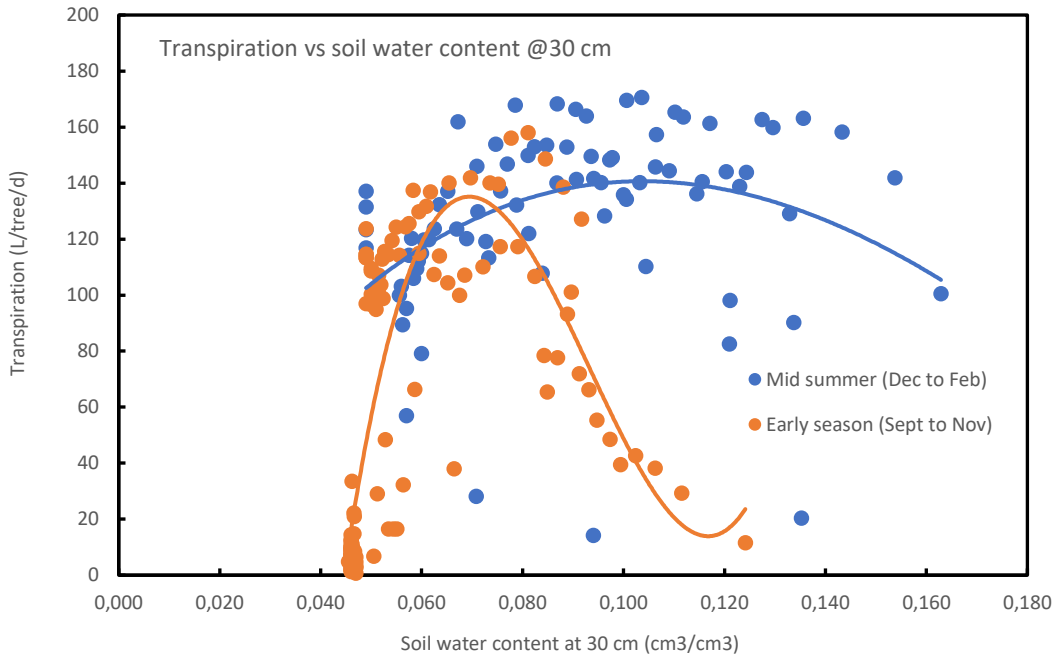


Fig 3.34. Effect of tree water uptake on changes in soil water content at various stages in the season in Phalaborwa.

3.8. Water use and yield modelling

3.8.1. Transpiration model sensitivity tests

The model sensitivity test results shown in Fig. 3.35 confirm the observed transpiration responses for marula trees based on measurements reported in the previous section. As expected, changing the maximum stomatal conductance caused significant changes in the modelled transpiration (Fig. 3.35a).

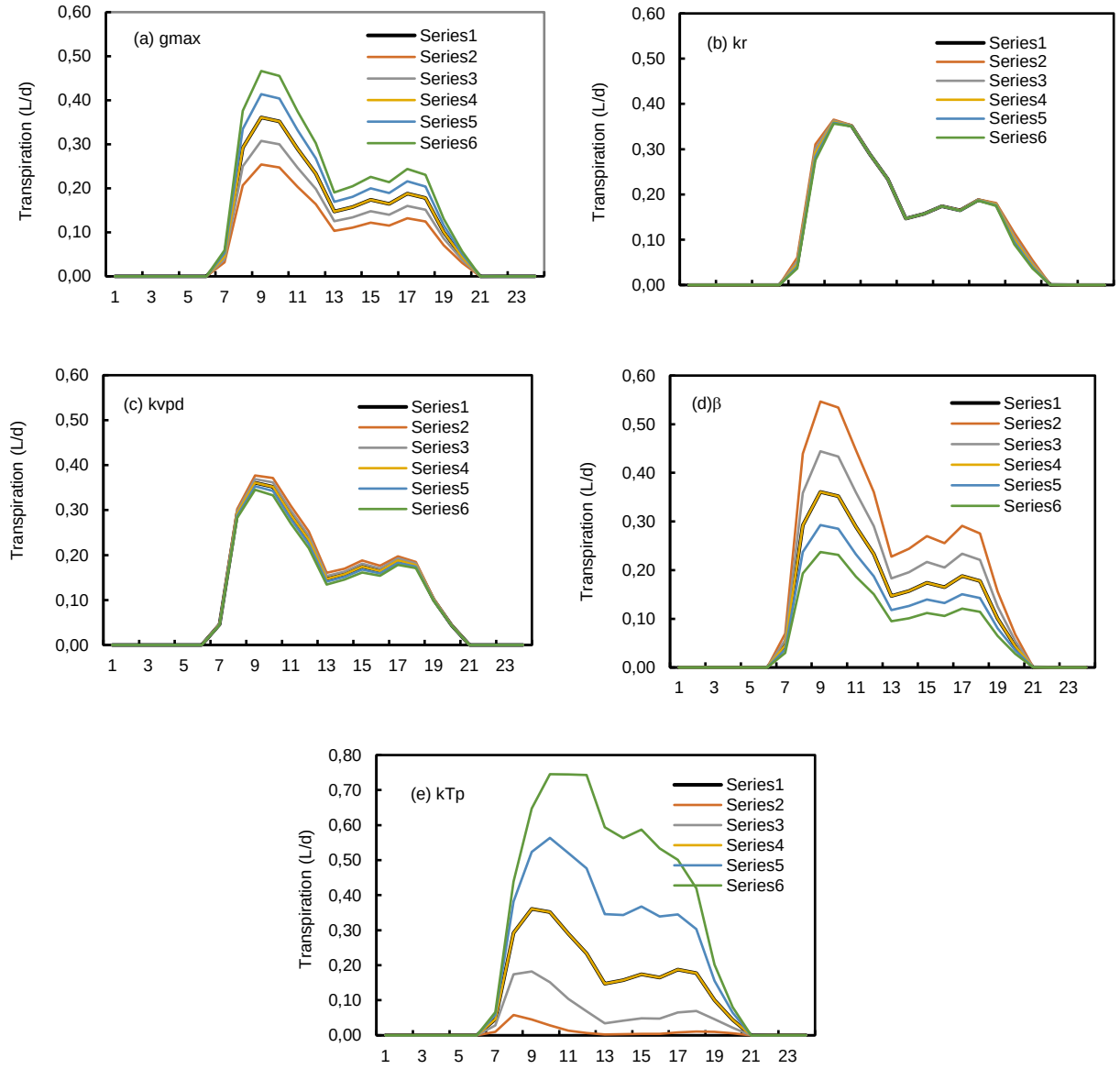


Fig 3.35. Sensitivity tests for the transpiration models representing (a) the maximum leaf stomatal conductance (g_{max}), (b) the radiation stress factor (k_r), (c) the vapour pressure deficit stress factor (k_{vpd}), (d) the soil water content stress factor (β), and (e) the air temperature stress factor (k_{Tp}).

The solar radiation and VPD stress factors had the weakest effect on transpiration (Fig. 3.35b & c). The soil water content (Fig. 3.35d) and air temperature (Fig. 3.35e) have the biggest effect on the transpiration response of marula trees. Detailed simulations of the seasonal transpiration dynamics and yield will be presented in the next deliverable.

3.8.2. Validation of the transpiration model

The water use model described above was validated using data collected in a related study close by on the water use of marula trees by Wilkens et al (under review). The model could simulate the tree transpiration reasonably well as summarised by the statistics in Table 3.17.

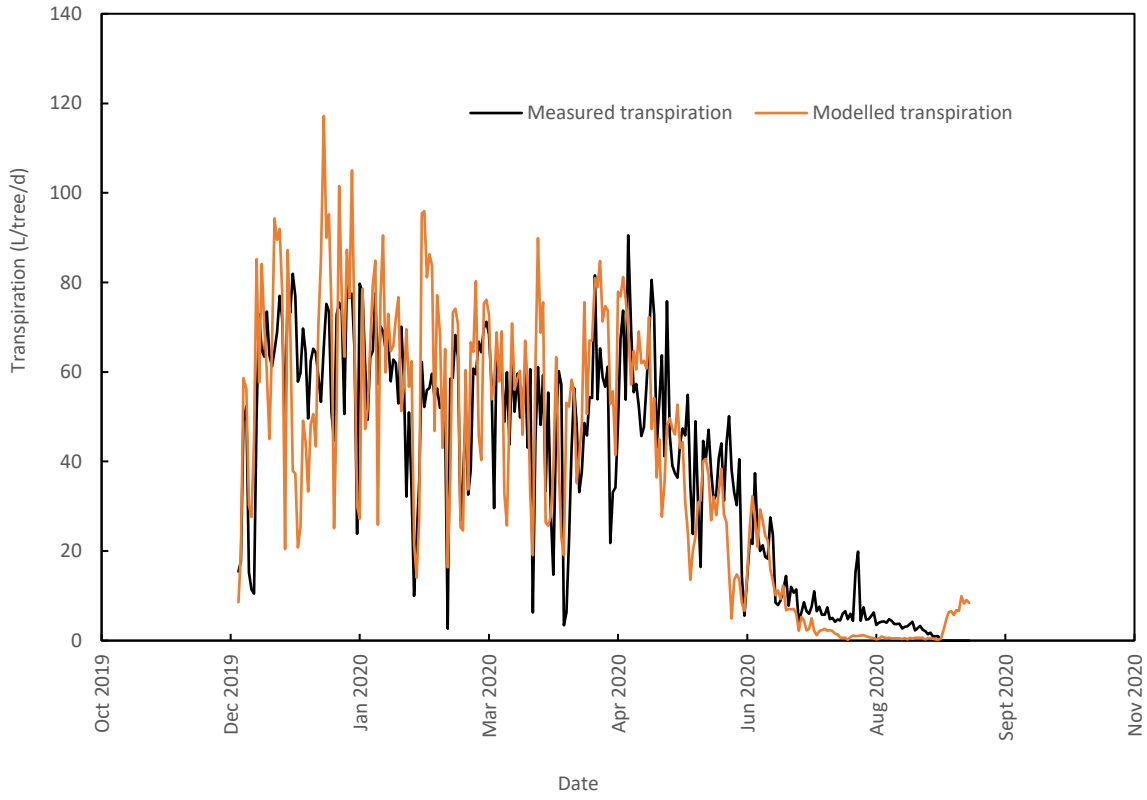


Fig 3.36. Validation of the marula tree transpiration model using data over an entire growing season using data from Hazyview near Thulamahashe.

The next step was to apply this model to predict the future changes in water use by the marula trees under climate change conditions. First, we describe the projected changes in climatic conditions in one of the growing regions (Thulamahashe). Next we then use these data to simulate the changes in the water requirements of typical marula trees.

Table 3.17. Water use characteristics of the marula trees and performance of the Penman-Monteith model against the measured data.

Species	Tree water use attributes				Model performance			
	Tree	E_{max} (L/d)	E_{avg} (L/d)	E_L (L/m^2/d)	R^2	MAE (L/m^2/d)	RMSE (L/m^2/d)	N
Marula	L	90.5	55.9	0.34	0.62	0.096	0.119	179
	S	19.8	10.0	0.43	0.48	0.154	0.187	179
	S	8.5	4.8	1.07	0.57	0.296	0.386	283

Emax represents the maximum transpiration per tree, Eavg, is the average tree transpiration during the summer months (November to March), EL is the average transpiration per unit leaf area, MAE is the mean absolute error, RMSE is the root mean square error and N is the Sample Size.

3.8.3. Climate change projections for Thulamahashe

3.8.3.1. Climatic conditions

In this report, the team present climate change data for the Thulamahashe area only as we could not get data for the other two sites. Given the uncertainties in the climate change projections of the available models, we evaluate the projections from six (6) different models summarised in Fig. 3.37 to 3.39. These models assume the business-as-usual pathway for which no mitigation actions are taken. The data presented is daily data for the period January 1961 to December 2099 for key water use driving variables.

All six models predict higher maximum temperatures for Thulamahashe for the period 1961 to 2099 as shown in Fig. 3.37 increasing by between 3 and 6 degrees Celsius over the period. The rising temperatures will likely lead to increased atmospheric evaporative demand as depicted by the rising VPD trends in Fig. 3.38 and the daily reference evapotranspiration in Fig. 3.39. These projections point towards higher future water demand by the marula trees.

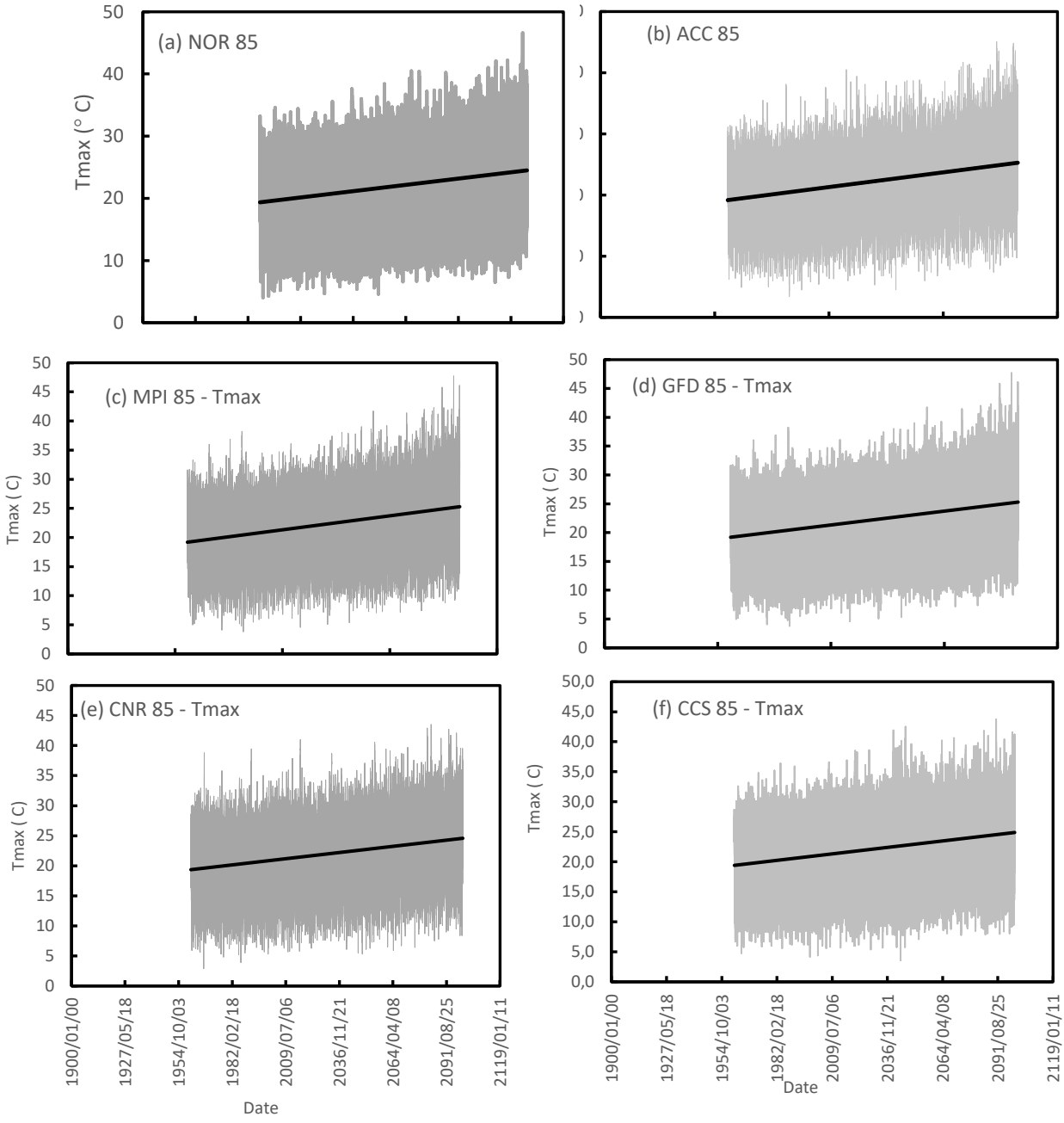


Fig 3.37. Projected changes in the daily maximum air temperature for the Thulamahashe area from January 1961 to December 2099 using six different models following the business-as-usual scenario.

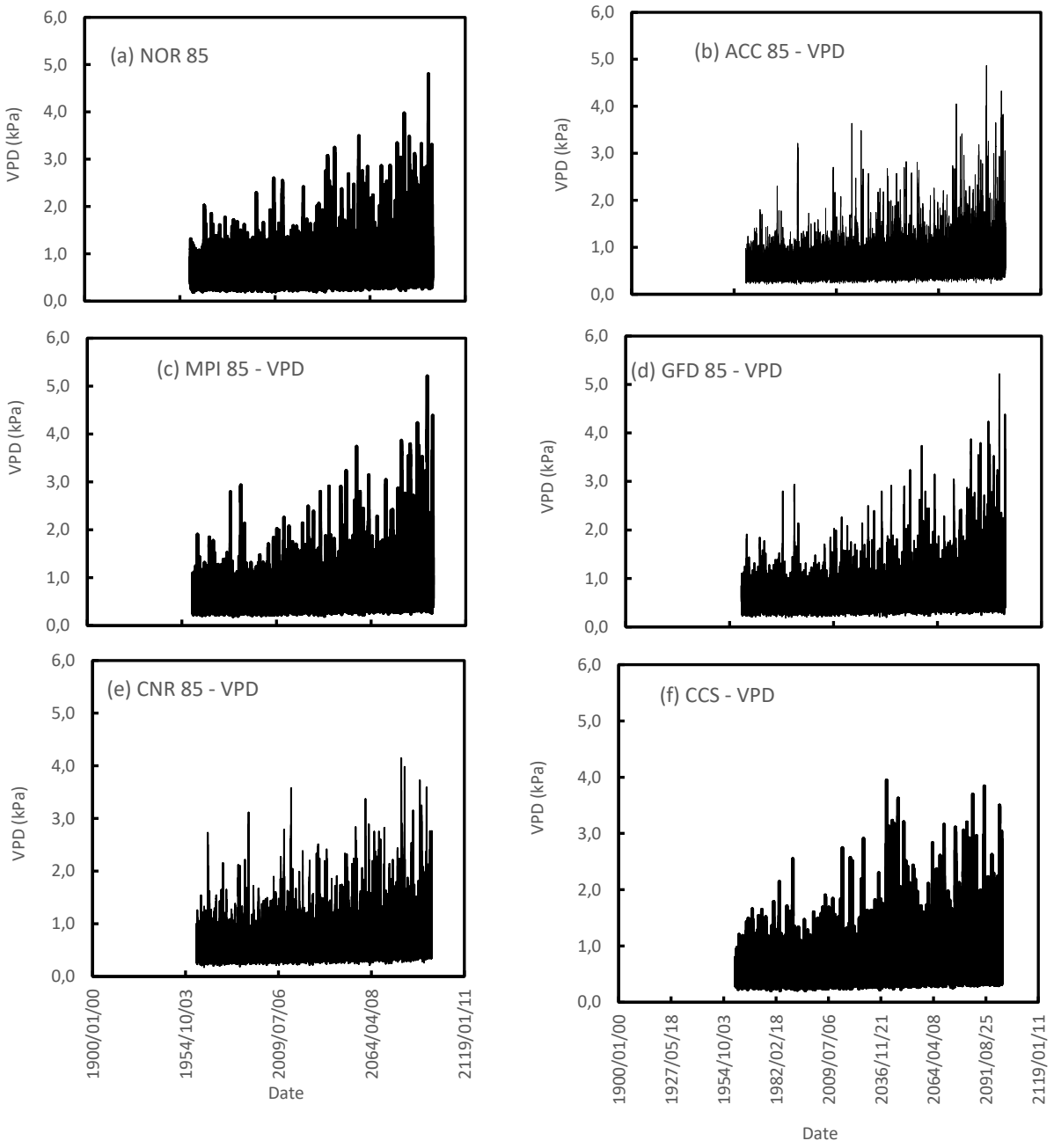


Fig 3.38. Changes in the vapour pressure deficit of the air projected with six (6) climate change models following the business-as-usual scenario (no mitigation) from January 1961 to December 2099 for the Thulamahashe area.

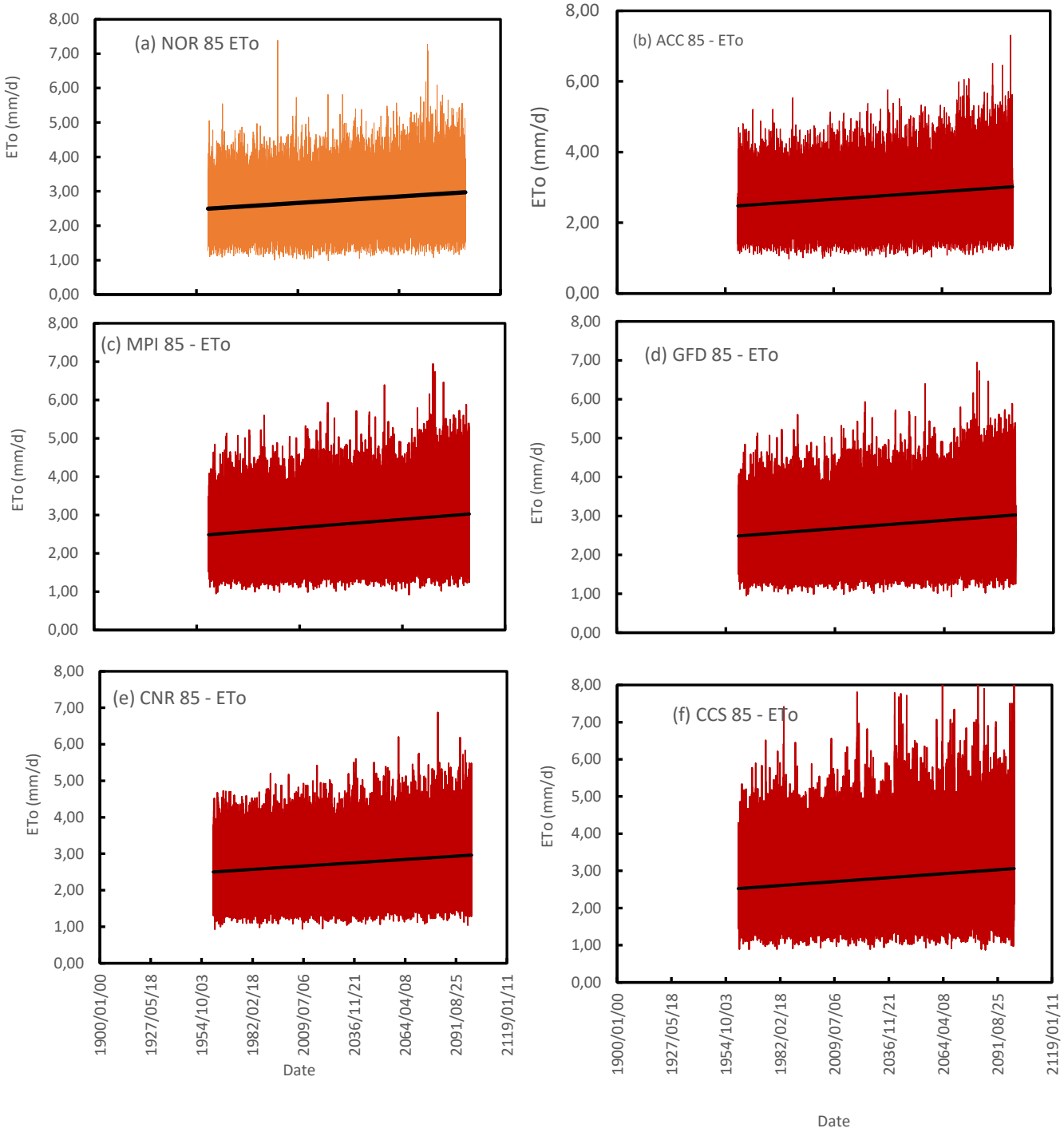


Fig 3.39. Changes in the reference evapotranspiration projected with six climate change models following the business-as-usual scenario (no mitigation) from January 1961 to December 2099 for the Thulamahashe area.

3.8.3.2. Water use of marula trees under climate change

However, applying the data described above to the water use model yields an unexpected outcome. Rather than the tree water use increasing under the increased atmospheric evaporative demand, it appears the change in water use rates will be negligible as summarised in Fig 3.40 for the Thulamahashe site. The reason for the projected insignificant increase in water use is that the increasing VPD levels will enhance the VPD stress factor which will induce further closure of the stomata leading to reduced transpiration rates as summarised in Fig. 3.40. If confirmed, this is a very significant outcome of this study wherein marula trees can indeed be possible alternative tree crops under increasingly drier conditions. Further simulations and refinement of the model are required to confirm this result.

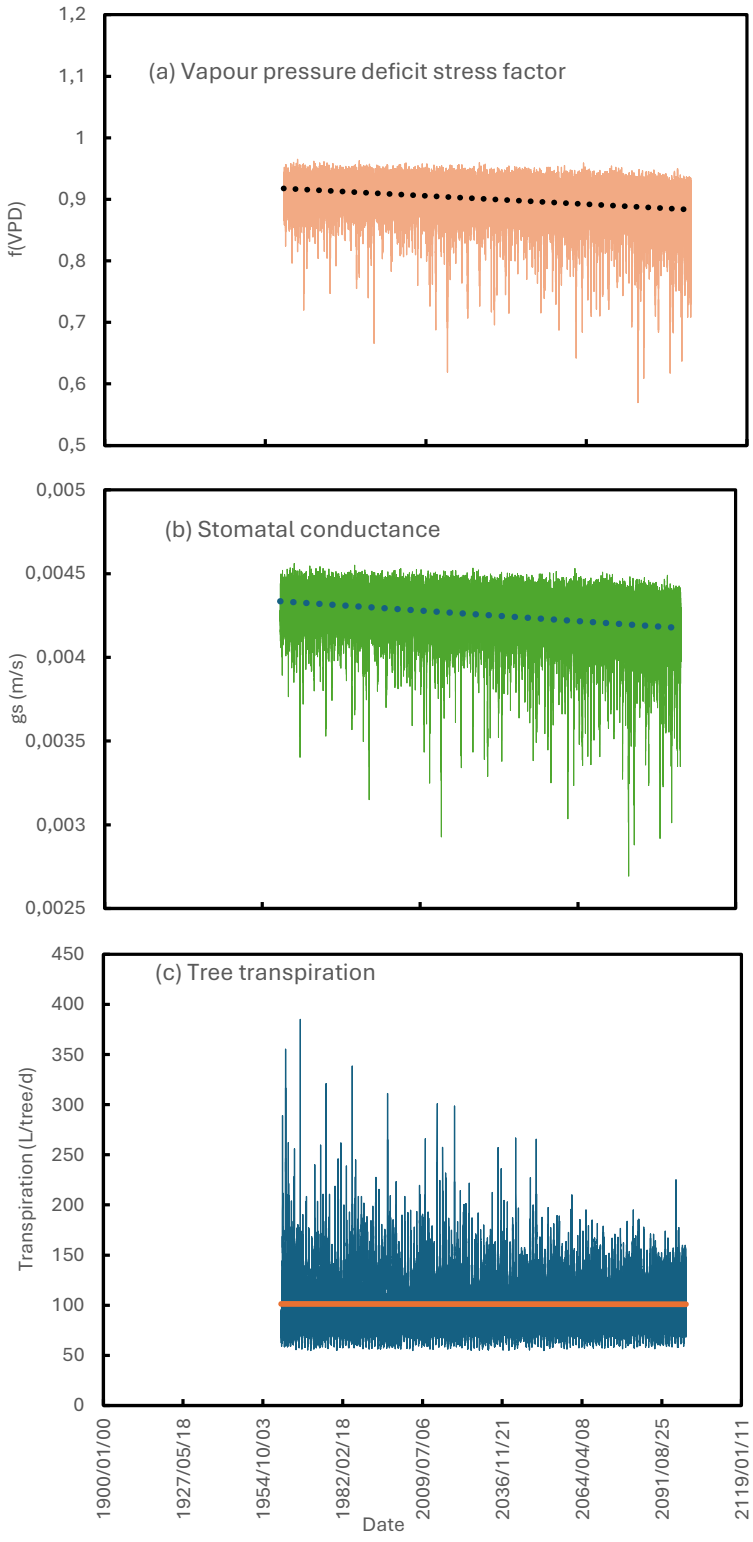


Fig 3.40. Simulated changes in (a) the vapour pressure deficit stress factor, (b) stomatal conductance and (c) whole tree transpiration for a mature Marula tree at Thulamahashe.

3.9. Conclusion

In this chapter, we have determined the water use of marula trees, their growth and yield in different growing regions. Our data suggests that these trees can use substantial amounts of water exceeding 200 litres per tree per day when water is available. This is likely because of their large sizes and lower transpiration rates are expected when smaller dwarfing trees are planted. We subsequently used the measured data to validate a marula-specific model, and good predictions of individual tree water use were obtained. Applying this model to predict future trends in tree water use under climate change suggests that the water use of marula trees is unlikely to increase under future climate change due to the response of the trees to increasing VPD levels which cause stomata to close. These trees are therefore likely to have higher water use efficiencies in future suggesting that they are potentially important alternative crops with lower demands. More data and analyses are required to confirm these observations.

CHAPTER 4

DRYING OF MARULA PEELS USING DIFFERENT METHODS

4.1. Summary

This chapter aimed to determine the effect of oven drying (OD), microwave drying (MD), solar drying (SD) and Shade drying (SHD) on the phytochemical and antioxidant properties of marula peel flour with two particle sizes (250 and 106 µm). The flour was evaluated for the effect of drying methods on ascorbic acid, phenolic compounds, antioxidants, and colour pigments (chlorophyll, carotene, and anthocyanins). The application of heat during 60°C oven drying resulted in a reduction of the chemical and bioactive properties, specifically total flavonoids (32.82 to 22.87 mg/g) and total phenolic compounds (8.35 to 6.79 mg/g), which declined gradually after drying and kept declining as particle size shortened. The smaller particle size (106 µm) resulted in a reduction in the examined parameters. Vitamin C decreased when heat was applied to the peels, with oven drying resulting in a 16% loss in vitamin C, 5% in carotene, and 12% in chlorophyll content, primarily at the lower (106 µm) particle size, which is less dense than the others. The study discovered that the final properties of phytochemicals and antioxidants are influenced by particle size. Furthermore, shade drying is recommended for fruit peels since it keeps most of the bioactive compounds.

4.2. Introduction

Marula (*Sclerocarya birrea* ssp. caffra) is a large deciduous savannah tree belonging to the Anacardiaceae that is indigenous to southern Africa (Caleja *et al.,* 2017). The fruit tree bears pale yellow plum-sized fruits 3–4 cm in diameter with a plain tough peel and a fibrous juicy sweet-sour mucilaginous flesh (Warinporn and Geoffrey, 2018). The flesh surrounds a hard nut consisting of 1–4 soft white edible kernels rich in oil and protein (Caleja *et al.,* 2017). The seeds are enclosed in locules embedded in the lignified tissue of the drupe (Chinma *et al.,* 2017). The seeds are about 10% of the drupe dry weight. The fruits abscise when green and firm, turning yellow as they ripen (Kugedera, 2019). The fruits are highly aromatic with a sweet-sour taste and can be eaten fresh or used for the preparation of juices, jams, conserves, dry fruit rolls, and alcoholic beverages (Weinert *et al.,* 2020). Fresh marula fruit can be consumed by

biting or cutting through the thick leathery skin and sucking the juice or chewing the mucilaginous flesh after removal of the peel (Caleja *et al.,* 2017).

The peel can be used for essential oil production for cosmetic purposes (Ngemakwe *et al.,* 2017). Several papers reported the medicinal effects of the marula bark and leaves (Eloff, 2001, Ojewole, 2013). Marula fruit is mostly used in the beverage production business, making it one of the most commercially significant indigenous fruits in South Africa (Kugedera, 2019). The physical properties and chemical properties of the fruit are broad, and such are described as physiochemical properties. The physicochemical properties of food are the physical and chemical components that are found in the food product (Macamo *et al.*, 2021). Physical properties such as moisture, density, and viscosity are important as they analyse the behaviour of the product during processes such as picking, storing, pH, drying and processing (Yenge *et al.,* 2018). The fruit is reported to contain reasonable amounts of antioxidants which is described based on its properties and capacity (Gokoglu, 2019).

Antioxidants are substances that control oxidation by delaying the oxidation chain reaction. Antioxidants are also food additives used in food processing that help extend food shelf life. Almost all edible plants have antioxidants naturally. Antioxidant compounds such as vitamin E, vitamin C and polyphenols are found in fruits and vegetables (Khoza, 2019). The earliest method of food preservation is drying. Fruits, meats, cereals, and herbs have historically been dried using the sun, wind, and a smoky fire (Murphy, 2013). Food dehydration is the removal of water from food using heated air, which prevents the development of bacteria and enzymes (Gauglitz, 2016). Dried foods are delicious, wholesome, portable, simple to make, simple to store, and simple to use. Compared to canning jars and freezer containers, there is less energy input, and less storage space required for freezing and canning (Joseph, 2020).

Food nutrition is not much impacted by drying. Fruit processing involves various steps that include discarding fruit peels after processing (Ceresa *et al.,* 2021). The peels contain reasonable nutritional values and henceforth reacquired, dried, and processed into flour. The aim of drying the waste (peels) was to reduce environmental waste, promote a new product of marula peels flour as well as preserve the nutritional benefits and compositions that are contained in the fruit peels.

4.3. Materials and Methods

4.3.1. Research sampling

The marula fruits were collected in three different locations that are ZZ2 in Modjadji Kloof Tzaneen region (-23°S 30°E), Phalaborwa (23.94°S, 31.14°E) and Thulamahashe (24.7242° S, 31.2097°E). The fruits were then transported to the University of Venda packaged in sacks while stored in a cool shaded area to reduce field heat. The fruits were stored in the Pilot plant chiller at a low temperature for better preservation, then sorted based on maturity and graded based on colour carefully selecting yellow fruits for processing. The fruits were peeled using a knife and the peels were dried using four different drying methods as shown in Table 4.1.

Table 4.1. Procedures for different drying methods.

Drying method	Procedure	References
Oven Drying	Samples are prepared onto a tray weighed at 500 g per tray and, thereafter, placed in a preheated oven at 60°C for 24 hrs.	Latha *et al.* (2017)
Shade Drying	Samples are prepared onto a tray weighed at 500 g per tray and, thereafter, placed on a table surface for air drying for 5 days.	Garau *et al.* (2013)
Microwave Drying	Samples are prepared onto a tray weighed at 500 g per tray and, thereafter, placed in a microwave and rotated for one hour at 10-minute intervals checking the integrity of the drying method.	Morais *et al.* (2015)
Solar Drying	Samples are prepared onto a tray weighed at 500 g per tray, thereafter, placed in a Solar drying tunnel for 4 – 5 days.	Moussaoui *et al.* (2021)

After drying, the peels were ground and sieved thereafter to get the desired particle sizes of (250 and 106 µm).

The flour was packaged in small plastics and stored in the pilot plant chiller for analysis. The flow processing of marula peel flour is shown in Figure 4.1. (Use of Clear Hi-abuse bags 30 mm x 30 mm stored at pilot plant chiller at 7°C for 14 days.)

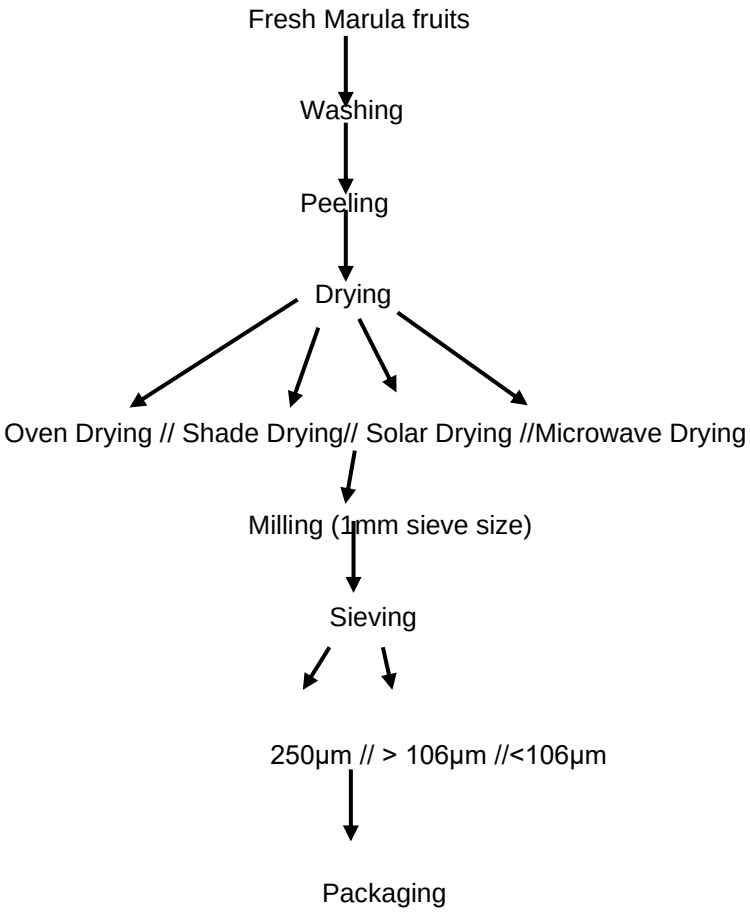


Fig 4.1. Process flow diagram for marula peel flour.

4.3.2. Sample extraction for bioactive compounds

Two (2) grams of marula peel flour were weighed using the AACC (2000) method and 20 ml of 1% methanolic acid was added (Comprising 1 mL of methanol and 99 mL of distilled water). The mixture was sonicated for 15 min in an ultrasonic bath. Thereafter the mixture was centrifugated at 3000 rpm for 10 min in a centrifuge model of T-8BL Lab TM (Laboratory instruments, Ambala Cantt, India) and filtered.

4.3.3. Antioxidant properties

4.3.3.1. DPPH assay

The free-radical scavenging capacity was measured by a DPPH assay according to a modification of the method of Souza *et al.* (2012). A 2 mL of the sample extract was added to 2 mL of 0.1 Mm DPPH (2.93 mg in 100 mL dissolved in methanol) in 10 mL test tubes. The mixture was then shaken vigorously, kept at room temperature for 30 min, and then its absorbance was read at 517 nm (A517) using a UV–visible spectrophotometer (model of T-28BL Lab TM Laboratory instruments, Blatant Laboratory, Denmark). The DPPH scavenging effect was calculated from the following equation 4.1:

$$\text{Scavenging effect } \% = \frac{Ac - As}{Ac} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.1)$$

Where Ac is the absorbance of the control (methanol with DPPH solution) and as is the absorbance of the polysaccharide sample.

4.3.3.2. Total phenolic content

A 0.15 mL of Folin Ciocalteu reagent (1:2 v/v diluted with distilled water) was added to 0.5 mL of test sample extract. The mixture was then allowed to stand at room temperature for 5 min. After 5 min, 2 mL of 7.5% w/v sodium carbonate (7.5 g of sodium carbonate and dissolved in 100 mL of distilled water) was added and the mixture was placed in the dark for 45 min with occasional shaking. The formation of a blue colour was observed after incubation. Because the colour was excessively dark, around 10 mL of distilled water was added to each test tube to dilute it. Finally, the absorbance of the blue colour in samples was determined using a spectrophotometer at 725 nm.

4.3.3.3. Total flavonoid content

In a 10 mL test tube, 0.3 mL of extract, 3.4 mL of 30% methanol, 0.15 mL of 0.5 M $NaNO_2$, and 0.15 mL of 0.3 M $AlCl_3.6H_2O$ were mixed. About 1 ml of 1 M NaOH was added after 55 min. A spectrophotometer (DR2800, Hach, USA) was used to test the absorbance against the reagent blank at 506 nm. The standard curve for total flavonoids was created using a catechin standard solution (1 mg/ml of catechin stock solution was formed by dissolving 100 mg in 100 mL of 1% methanolic acid and dilutions of 0, 1, 0.2, 0.4, 0.8 and 1 mg/mL were used as working standards). Total flavonoids were expressed in milligrams of catechin equivalents per gram of dried fraction (Gamage *et al.,* 2021).

4.3.3.4. Vitamin C/ascorbic acid

The content of vitamin C was collected and estimated according to Hiwilepo-van Hal *et al.* (2013). To create a standard, 250 g of ascorbic acid was added into a 250 mL volumetric flask. The 100 mL of distilled water was added, and the solution was allowed to dissolve. Distilled water was added into the conical flask up to the mark. In a 100 mL conical flask, 25 mL of the vitamin was added to it together with 10 drops of starch solution. A burette was rinsed with distilled water and filled with 0,025M iodine. The iodine solution was used to titrate the vitamin C solution until there was a colour change. The initial and final volume of the iodine solution on the burette was recorded. This procedure was done in triplicates, equation 4.2. This method was repeated,

$$\text{Vitamin C} = \frac{volume\ of\ iodine}{average\ of\ the\ standards} \times 0{,}250 \quad \text{.....(4.2)}$$

4.3.4. Statistical analysis

The experiment was repeated twice, and all analyses were conducted in triplicate and raw results were stored in Microsoft Excel. Data was measured by a two-way analysis of variance (ANOVA) and the difference in mean values was determined by Duncan's multiple-range test. Statistical analyses were done using SPSS version 23. Significance was accepted at $p<0.05$.

4.4. Results and Discussion

4.4.1. Effect of drying methods on the phenolic compounds of marula peel flour

The total phenolic and total flavonoid contents of marula peel flour obtained after drying using oven, microwave, solar, and shade drying techniques are presented in Table 4.2. The total phenolic content ranged from 6.79 to 8.64 mg/g with shade drying having the highest value. According to Mehmet *et al.* (2020), several factors might have contributed to the increase in phenolic content of marula peels powder after shade drying such as continued enzymatic activity in the peels leading to the synthesis or transformation of phenolic compounds. Another factor that might have contributed to the high values of phenolic compounds in shade-dried marula peel powder is the lower temperatures, which might have reduced the degradation of phenolic compounds compared to higher-temperature drying methods such as oven drying (Ahmed, 2021). High temperatures can cause thermal degradation of sensitive phenolic compounds, but shade drying helps preserve them. In a similar study conducted by Shih-Chuan Liu (2012), it was reported that drying with high heat (oven drying) or a long time demonstrated a decrease in phenolic compounds.

Table 4.2. Polyphenols of marula fruit peel flour dried with different drying methods and particle size.

Method of drying	Particle sizes (µm)	TPC (mg/g)	TFC (mg/g)
OD	<250	$6.79 \pm 0,54^{abc}$	$32.82 \pm 1,52^{f}$
	<106	$7.36 \pm 0,20^{bc}$	$30.34 \pm 1,11^{ef}$
	>106	$8.35 \pm 1,60^{bc}$	$22.87 \pm 0,68^{c}$
MD	<250	$6.15 \pm 2,39^{abc}$	$29.61 \pm 1,55^{d}$
	<106	$6.37 \pm 1,75^{abc}$	$26.05 \pm 0,49^{c}$
	>106	$8.20 \pm 1,97^{bc}$	$22.53 \pm 0,07^{d}$
SD	<250	$4.50 \pm 0,15^{a}$	$25.42 \pm 0,29^{b}$
	<106	$5.55 \pm 0,30^{ab}$	$22.00 \pm 3,00^{c}$
	>106	$7.79 \pm 1,47^{bc}$	$13.86 \pm 0,03^{cd}$
SHD	<250	$8.05 \pm 2,20^{bc}$	$24.95 \pm 3,49^{cd}$
	<106	$8.35 \pm 1,59^{bc}$	$19.40 \pm 2,25^{a}$
	>106	$8.64 \pm 0,91^{c}$	$10.61 \pm 0,47^{e}$

OD for Oven Dying, MD for Microwave Drying, SD for Solar Drying, SHD for Shade drying, TPC for Total phenolic content, and TFC for Total flavonoid content. Values followed by the same superscript(s)

within the same columns are not significantly different at ($p<0,05$). Values are the mean± standard deviation of triplicate determinations.

On the other hand, phenolic compounds are sensitive to oxidation, which can be accelerated by exposure to sunlight. UV radiation from the sun causes phenolic compounds to oxidise, leading to a decrease in their concentration and antioxidant activity hence solar drying had a low TPC value (4.50 mg/g) (Nesrine *et al.,* 2012). A study on grape skins found significant losses in phenolic content due to prolonged sun exposure, and this was attributed to both oxidation and enzymatic degradation (Ahmed, 2021). It can be concluded that shade drying typically involves lower temperatures compared to solar, microwave, and oven drying. Lower temperatures help preserve heat-sensitive phenolic compounds, preventing their degradation therefore retaining total phenolic compounds in fruit peels highlights the importance of gentle drying techniques in preserving the nutritional and functional quality of plant-based products.

The results show that the flavonoid content of dried marula peel flour values ranged from 10.61 to 32.82 mg/g in which shade drying had the lowest value and oven drying had a high value. It was also reported that high temperatures in oven drying can inactivate enzymes that degrade flavonoids, preserving these compounds. In contrast, shade drying, with its lower temperatures, may not inactivate these degradative enzymes, leading to a loss of flavonoids (Walker, 2019).

Kavita (2014) reported a similar study wherein the flavonoid content of banana peel flour decreased as heat increased mainly in oven drying and stated that it is temperature dependent. The higher the heat (oven drying at 60°C, the lower the flavonoid compound). It was further reported that samples with lower particle sizes yield a low flavonoid content as it is not dense enough to withstand heat application during drying (Whang, 2012). Microwave drying and solar showed a gradual but not excessive decrease in the flavonoid compounds, microwave drying can create localized hot spots due to uneven distribution of microwave energy. These hot spots can lead to the degradation of flavonoids in certain areas of the fruit. The high temperatures generated by microwave energy can cause thermal degradation of heat-sensitive flavonoids, resulting in lower overall flavonoid content (Whang, 2012). To

retain the flavonoids, a good method of drying needs to be carefully selected, oven drying retains flavonoids however it destroys phenolic compounds.

4.4.2. Effect of drying methods on the antioxidant activity of marula fruit peel flour

The ABTS values of dried marula peel flour ranged from 10.77 to 22.38% as shown in Table 4.3. High ABTS values were recorded on high particle size flour whilst low particle size had low ABTS values. All drying methods influenced the ABTS content of marula peel flour in which a higher value was noted on microwave drying and lower ABTS content was noted on solar drying.

Table 4.3. Effect of different drying methods on antioxidant properties.

Method of drying	Particle sizes (µm)	ABTS (%)	DDPH (%)
OD	<250	17.92 ± 2.47^{ab}	99.90 ± 0,00^{ab}
	<106	17.80 ± 2.05^{ab}	99.92 ± 0,01^{c}
	>106	15.50 ± 1.84^{ab}	99.93 ± 0,00^{d}
MD	<250	22.38 ± 1.54^{ab}	99.89 ± 0,00^{ab}
	<106	21.50 ± 1.61^{a}	99.89 ± 0,00^{a}
	>106	18.60 ± 1.77^{b}	99.91 ± 0,01^{ab}
SD	<250	14.57 ± 1.73^{ab}	99.90 ± 0,01^{ab}
	<106	12.96 ± 1.63^{a}	99.90 ± 0,00^{b}
	>106	11.84 ± 1.73^{ab}	99.93 ± 0,00^{d}
SHD	<250	18.15 ± 1.83^{ab}	99.93 ± 0,00^{d}
	<106	13.00 ± 1.57^{ab}	99.93 ± 0,01^{d}
	>106	10.77 ± 1.37^{ab}	99.96 ± 0,01^{ab}

OD for Oven Dying, MD for Microwave Drying, SD for Shade Drying, and SHD for Shade Drying. Values followed by the same superscript(s) within the same columns are not significantly different at ($p<0,05$). Values are the mean± standard deviation of triplicate determinations.

There was a significant decrease in ABTS values from a higher particle size to a lower, as particle size decreases, ABTS decreases. In oven drying, high heat degrades antioxidants (Yuan and Chen, 1998), this is due to the Maillard reaction that occurs due to high heat in which a non-enzymatic browning reaction between amino acids and reducing sugars occurs which degrades antioxidants and further reduces ABTS activity. Yuan and Chen (1998) further reported that microwave drying helps preserve antioxidants by minimising oxidation and enzymatic degradation thus potentially maintaining or increasing ABTS activity hence it was recorded as higher than on the other drying methods.

The highest particle sizes that were denser could withstand heat application to have at least a higher ABTS value than lower particle sizes. Shade/air drying had a massive decline in ABTS values when particle size decreased, this was due to size reduction, thermal degradation, oxidation, volatile compound loss, enzymatic degradation as well as moisture loss in the lowest particle size (Nesrine *et al.* 2012). In a similar study by Walker (2009), it was reported that the impact of drying methods on ABTS antioxidant activity depends on the specific antioxidant compounds present in marula peels, their stability and the drying conditions.

The antioxidant activity of marula peel flour dried with different methods is shown in Table 4.3. The DPPH values ranged from 99.89 to 99.96%. Lower DPPH values were identified in high particle size flour while high DPPH was recorded in low particle size. There was not much effect of these drying methods on the DPPH content of the fruit peel flour and different particle sizes also had minimal effect on the DPPH. Inshik (2013) reported that drying fruit peels may not significantly affect the DPPH due to several factors such as the stability of the antioxidants in the peels in which they are resistant to degradation and further reported that drying methods like solar, shade, and microwave use relatively low heat, which may not be enough to degrade antioxidants. Inshik (2013) further reported that smaller particle sizes have a larger surface area which leads to increased exposure to the DPPH radical, resulting in higher antioxidant activity on small particle sizes. The lesser the particle size, the more it increases. It can be concluded that heat treatment did not affect DPPH of marula fruit peels, the scavenging assay was retained after drying but varies on different drying methods. It is highly recommended to use such drying methods to preserve the quality of the antioxidants on the fruit peels thus making them easily accessible.

4.4.3. Effect of drying methods and particle size on the bioactive compounds of marula fruit peel flour

Table 4.4 shows the bioactive compounds of dried marula peel flour and vitamin C was noted to increase when particle size increases. The increase might be because of less density in particle size as well as more concentration of particles in 106 µm (Davis, 2016). Oven drying had a huge decrease as compared to the other drying methods. This was mainly because the oven uses higher heat than the other drying

methods and vitamin C is heat sensitive thus increasing the rate of vitamin C degradation (Hillman *et al.,* 2008). Vitamin C is a heat-sensitive nutrient that breaks down when exposed to high temperatures (above 60°C), oven drying had the lowest vitamin C content than the other drying methods due to hot air circulation when drying in the oven chamber and thus initiating the Maillard reaction that potentially formed new compounds that degraded vitamin C (Yuan and Chen, 1998).

Table 4.4. Bioactive compounds for marula peels with different drying methods and particle size.

Method of drying	Particle sizes (µm)	Chlorophyll (mg/g)	Vitamin C (mg/g)	Anthocyanin (µg/g)	Carotene (µg/g)
Oven dry	<250	22,28 ± 0,68^{a}	69.78 ± 1.46^{a}	52,27 ± 0,87^{a}	0,63 ± 0,03^{c}
	<106	21,94 ± 1,23^{ab}	71.77 ± 1.89^{ab}	103,09 ± 8,00^{bc}	0,57 ± 0,14^{bc}
	>106	18,41 ± 3,19^{b}	75.76 ± 1.97^{b}	121,07 ± 3,18^{ef}	0,49 ± 0,15^{a}
Microwave dry	<250	21,52 ± 3,19^{a}	103.67 ± 3.39^{a}	111,10 ± 10,22^{de}	0,43 ± 0,43^{a}
	<106	19,49 ± 2,11^{b}	105.67 ± 3.68^{bc}	119,45 ± 5,30^{ef}	0,54 ± 0,26^{bc}
	>106	18,49 ± 3,02^{bc}	120.23 ± 3.73^{c}	165,07 ± 15,01^{a}	0,62 ± 0,32^{c}
Solar dry	<250	22,32 ± 3,78^{c}	93.70 ± 3.12^{b}	95,85 ± 6,65^{bc}	0,46 ± 0,33^{a}
	<106	19,82 ± 2,80^{ab}	99.68 ± 3.28^{bc}	113,44 ± 0,77^{de}	0,65 ± 0,25^{c}
	>106	18,03 ± 0,99^{a}	103.67 ± 3.39^{c}	130,64 ± 7,13^{f}	0,63 ± 0,20^{b}
Air/Shade dry	<250	22,41 ± 1,04^{a}	77.75 ± 2.12^{a}	115,44 ± 3,08^{de}	0,78 ± 0,18^{c}
	<106	21,11 ± 2,58^{a}	81.74 ± 2.29^{ab}	124,44 ± 1,25^{de}	0,67 ± 0,34^{bc}
	>106	21,03 ± 2,45^{ab}	89.72 ± 2.33^{b}	145,44 ± 20,15^{b}	0,79 ± 0,21^{c}

OD= Oven Dying, MD= Microwave Drying, SD= Shade Drying, SHD= Shade Drying. Values followed by the same superscript(s) within the same columns are not significantly different at ($p<0,05$). Values are the mean± standard deviation of triplicate determinations.

Hillman *et al.* (2008) reported in a similar study of banana peels that vitamin C can turn into vapour (volatilise) during rapid drying however shade drying was the best method for preserving vitamin C because it uses low heat that prevents degradation and oxidation, it was further reported that the low temperature and gentle drying conditions in shade drying inhibits the activity of enzymes like ascorbic acid oxidase which can break down Vitamin C. Hillman *et al*. (2008) further reported that shade drying does not involve high heat thus Maillard reaction is unlikely to occur hence preserving vitamin C.

The chlorophyll content of marula peel flour ranged from 18.03 to 22.41 mg/g with shade drying retaining much of the chlorophyll as compared to the other drying methods. There was a trend of chlorophyll degradation when particle sizes decreased,

the lesser the particle size, the lesser the chlorophyll. Shade drying retained chlorophyll pigment more than the other drying methods due to several factors like low temperature, shade drying occurs at an ambient temperature usually below 30°C which according to Hiwilepo-van Hal *et al* (2013), slows down chlorophyll degradation and retaining colour and functionality. Since it is dried in a shade, there is a reduced light exposure which can cause chlorophyll to degrade as chlorophyll requires light to be productive. With reduced light exposure and low temperature, it is also minimized oxygen exposure which could potentially lead to oxidation and degradation of chlorophyll (Shackleton, 2022). Shade drying's slow rate of drying prevents rapid moisture loss hence chlorophyll is retained and thus the gentle conditions might have inhibited enzymes like chlorophyllase which could degrade chlorophyll when present (Shackleton 2022).

According to Hiwilepo-van Hal *et al.* (2013), minimizing degradation pathways and preserving cell structures of the fruit peels helps shade drying to maintain the vibrant greenish colour of chlorophyll in marula fruit peels. According to Marta Pasławska (2020) lemon peels, due to water loss and colour loss in high-heat drying methods, cause chlorophyll degradation due to oxidation, and the presence of enzymes that break down chlorophyll. Chlorophyll is a sensitive molecule that can degrade quickly when exposed to heat, light, and oxygen. The high heat and rapid drying of the oven and microwave drying caused significant degradation while solar drying's gentler heat and longer drying time helped preserve more chlorophyll. Shade drying's slow and low-temperature process makes it the most suitable method for preserving chlorophyll content.

The anthocyanin content of marula peel flour ranged from 52.27 to 165.07µg/g with microwave drying emitting the highest values and oven drying showing the lowest values. It was noted that a decrease in particle size resulted in a decrease in anthocyanin content. Spayd *et al.* (2011) reported that the increase in temperature accelerates the destruction of anthocyanins, these results indicated that both the temperature and the drying time affected the degradation of anthocyanins. Anthocyanins are sensitive to heat, and the oven uses heat above 60°C which leads to colour loss during drying and degradation (Spayd *et al.,* 2011). Oven drying can activate enzymes like polyphenol oxidase, which can break down anthocyanin. Oven

drying recorded a large decrease in anthocyanins from 121 to 52 µg/g. Oven drying uses more heat and in an isolated chamber thus promoting heat circulation leading to volatile compound loss, oxidation, Maillard reaction thus promoting anthocyanin degradation (Spayd *et al.,* 2011). The microwave method of drying is not effective as there are hot spots in which it does not get efficiently dried up, thus maintaining the colour and retaining the pigments. Shade drying and solar drying portray decent anthocyanin values in which they did not rapidly degrade, this is due to low heat, slow drying rate, minimal oxidation, and no direct sunlight. Khoo (2017) reported that both processes are gentle and slow processes that preserve anthocyanins.

Carotene content of marula peel flour ranged from 0.43 to 0.79 µg/g. The carotene level of arula peel flour decreased during the drying process. It further decreased with the reduction of particle size, the lesser the particle size, the lesser the carotene content. Oven drying with high heat had an impact on the carotene with a decrease from 0.63 to 0.49 µg/g and microwave dropping from 0.62 to 0.43 µg/g. This is because the two drying methods use high heat as compared to solar and shade drying, and the high heat leads to water loss that cause carotene degradation, colour loss, and oxidation (Witter, 2012). According to Chuyen *et al.* (2016), drying of carotenoid-rich materials such as carrots and mangoes has indicated that thermal-drying methods can cause substantial loss of carotenoids in the dried products. Carotene is a sensitive pigment that can degrade quickly when exposed to heat, light, and energy. According to White (2014), the slow drying and low-temperature process of shade and solar make them more suitable for preserving carotene content.

4.4.4. Conclusion

In conclusion, the results of this study demonstrated that, mostly through oven and shade drying, marula peel flour's phytochemical qualities and antioxidant capacity were degraded. The heat caused total phenolic compounds, flavonoids, vitamin C, and chlorophyll to diminish, but DPPH did not change because of drying. The research went on to demonstrate that the phytochemical and antioxidant properties are affected by changes in particle size. While oven drying yields reliable findings, heat-sensitive phytochemicals may be degraded. While light-sensitive molecules are effectively preserved by shade drying, certain phytochemicals may be susceptible to enzymatic

degradation. Rapid microwave drying is an excellent way to protect antioxidants, but it can also lead to uneven heating and possible thermal damage. Based on the results of this study, shade drying is recommended for drying fruits and their peels.

4.4.5. Recommendations

Further investigation of the thermal properties and microbial analysis of the marula fruit peels is required. Furthermore, a study on the effects of drying using a freeze dryer is required on antioxidant capacity and activity as compared to solar, shade, microwave, and oven drying used in the study.

CHAPTER 5

NUTRITIONAL COMPOSITION, FUNCTIONAL, ANTIOXIDANT PROPERTIES AND MICROBIAL QUALITY OF MARULA PEEL FLOUR

5.1. Summary

Flour refers to the end-product of grinding and milling cereals, beans or other seeds. It provides structure to baked products such as cakes, cookies, pastries and bread. Marula is one of the most used indigenous wild fruits in Africa, it serves several functions, and it is highly utilised by local people mainly for its fruits, cosmetic oil from the seeds and medicinal use from the leaves and bark. This study aimed to develop flour using marula peels and evaluate the nutritional composition, antioxidant properties, functional properties and microbial quality of the flour. The marula peel flour was subjected to nutritional composition analyses (fat, ash, protein and moisture), antioxidant, FTIR, physicochemical, functional and microbiological analysis. The results showed that marula peel flour is rich in vitamin C as the vitamin C content ranged from 126 to 162 mg/100 g showing that it aids in promoting good health. Mould, coliforms and *Escherichia coli* were not detected on the marula peel flour produced and the total plate count of the flour showed a significant difference ($p < 0.05$) in all regions. The colour attributes were significantly different ($p<0.05$) in redness (a^*), lightness (L^*) and yellowness (b^*) values for all three (3) regions. The FTIR spectra showed the O–H region of marula peel flour bands (peaks) ranging from 3474.30 to 3474.86 cm^{-1}. It was concluded that marula peel flour is a good source of vital nutrients such as proteins and compounds such as phytochemicals that aid in promoting good health concerning heart diseases, cancer and other chronic illnesses.

5.2. Introduction

Marula (*Sclerocarya birrea ssp. Caffra*) is a wild fruit that belongs to the family *Anacardiaceae* and is mostly found in the sub-Saharan parts of Africa (Khoza, 2019). It is one of the most used Indigenous wild fruits in Africa, it serves several functions, and it is highly utilised by local people mainly for its fruits, cosmetic oil from the seeds and medicinal use from the leaves and bark (Hiwilepo-van Hal *et al.,* 2014). The fruit

contains significant levels of dietary fibre, protein, vitamins (A, B_3, C and Carotene), minerals, amino acids and fatty acids (Mashau *et al*., 2022). The fruits are typically processed and preserved into a variety of products that are easily accessible on the market such as jams, flavour-enhanced water, candies, essential oils, traditional beer and internationally popular beverages such as Amarula Cream (Ndlovu, 2016).

The term flour refers to the end-product of grinding and milling cereals, beans or other seeds (Hughes, 2020). The degree of milling can vary, and each milled product is different and used differently (Chowdhury, 2018). There are several types of flour which include white flour, all-purpose flour, whole wheat flour, bread flour, cake flour etc. (Vali *et al.,* 2016). Flour provides structure to baked products such as cakes, cookies, pastries and bread (Gwirtz and Garcia-Casal, 2014).

Functional properties are the important physicochemical characteristics of foods that show the relationship between food composition, structure and molecular conformation (Awuchi *et.al*., 2019). The components of the food material, particularly the carbohydrates, proteins, fats and oils, moisture and other ingredients added to food or flour can influence the functional properties of foods and flour (Godswill, 2019; Awuchi *et al.,* 2019). Functional properties can also describe how food components behave during preparation and cooking as well as how they influence the final product's appearance, texture and flavour (Granato *et.al,* 2018). According to Godswill (2019) flour's functional properties are critical in the production of food goods. These features govern the manufacturing and use of flour as food ingredients in various foods, as well as the processing and storage of these commodities. Water absorption, oil absorption, and protein solubility, for example, have an impact on the texture and appearance of a product.

Antioxidants are chemicals that regulate oxidation by preventing or delaying the initiation of damaging oxidation chain reactions (Khoza, 2019). Marula fruit peels are higher in phytochemicals, namely antioxidants and phenolic compounds. According to Hiwilepo-van Hal (2013), the antioxidant activity of the marula fruit is greater than that of other fruits such as pomegranate and orange. Marula peels have a high content of ascorbic acid (150-250 mg/100 g). These phytochemical components are extremely beneficial to human health since they protect against heart disease, cancer, and

chronic disorders. This study aims to evaluate the nutritional composition, functional, antioxidant properties and microbial quality of marula fruit peel flour.

5.3. Materials and Methods

5.3.1. Sample collection

Marula fruits were collected from three (3) different locations namely, Thulamahashe, Phalaborwa and Mooketsi (ZZ2). The samples were stored in cold storage at a temperature of 4°C in the Pilot plant, Department of Food Science and Technology, University of Venda for further processing.

5.3.2. Preparation of marula fruit peel flour

Marula fruit peel flour was generated using the method of Reis *et al.* (2020). As indicated in Fig. 5.1. marula fruits from the three (3) locations were peeled using a knife and fork. The peels were well washed and dried at 50°C using an oven drier for 48 hrs. A laboratory miller was used to turn the peels into a fine powder using a 1 mm sieve.

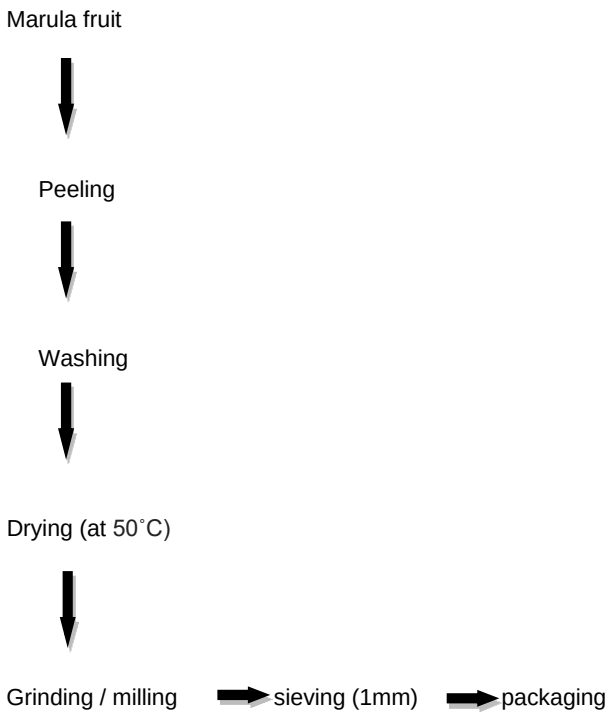


Fig. 5.1. Flow diagram of marula peels flour **(**Reis *et al.* (2020)

5.3.3. Some proximate composition of marula fruit peel flour

5.3.3.1. Ash content

Ash content of marula peel flour was determined using a muffle furnace (LASEC, laboratory & scientific equipment co (Pty) Ltd, model EMF035) at 550°C for 24hrs following AACC (2010) method number 08-01.01. Dry crucibles were weighed, and 2 g of flour sample was added into the crucibles. The ash content was determined using the difference in weight before and after burning using AACC (2010) method 08-01.01 using equation 5.1.

$$\text{Ash} = \frac{W2-W1}{W3} x\ 100\% \quad \text{.....5.1}$$

Where W1= crucible weight, W2= weight of crucible with ash, W3= mass of sample

5.3.3.2. Moisture content

The Moisture content was determined following AOAC (2005) method number 925.10. Before use, the crucibles were heated for 30 min at 105°C. The heated crucibles were then placed in a desiccator for storage. About 3 g of flour samples were weighed into the pre-heated crucibles and dried overnight at 60°C in a conditioned oven (Module 278, Labotech Ecotherm, South Africa). The samples were allowed to cool in a desiccator before being weighed, and the moisture content was calculated using the weight difference using equation 5.2.

$$\%\ \text{Moisture} = \frac{W2-W3}{W2-W1} x\ 100\ \% \quad \text{.....5.2}$$

Where: W1= crucible weight, W2 = weight of crucible with flour before drying, W3 = weight of crucible with flour after drying

5.3.3.3. Protein content

The crude protein content was determined using the AOAC (2002) method number 997.23 (2002) Kjeldahl method. About 2 g of the flour samples were placed in the digester tubes, along with two tables of the Kjeldahl catalyst and 25 mL of concentrated sulphuric acid (96%) and digested on a pre-heated BUCHI Digestion Unit (K-424) until the colour changed to a yellowish orange. After digestion, the

samples were allowed to cool to room temperature before being diluted with 60 mL of distilled water. A BUCHI Distillation Unit (K-355) was then used to distil the sample while collecting the distillate in a conical flask. Two (2) drops of the indicator were added to the distillate before it was titrated with a METTLER TOLEDO DL22 (Food and Beverage Analyser) using concentrated hydrochloric acid. The volume of hydrochloric acid was recorded during the first colour change of the distillate. The crude protein content was calculated using the conversion factor 6, 25, using equation 5.3.

$$\%\text{Nitrogen} = \frac{(Vs-Vb)X\ N(H2SO4)X14.007\ X\ 100}{W\ X\ 1000} \quad \text{.....5.3}$$

W

here Vs= volume of H_2SO_4 used to titrate the sample in ml, Vb= volume of H_2SO_4 used to titrate blank in ml, N (H_2SO_4) = Normality of acid titrant, W= weight of sample in grams.

5.3.3.4. Fat content

The fat content of marula peel flour was determined using Soxhlet extraction (BUCHI fat extractor, E-500) following AACC (1999) method number 30-25.01. It was accomplished by extracting fat from 2 g of marula peel flour which was weighed into a thimble with an organic solvent, namely petroleum ether with a boiling point of 40-60°C. The solvent was poured into a pre-weighed Soxhlet beaker before immersing the thimbles containing the sample in the solvent for 30 min, washing for 1 hr, and finally recovering the fat for 5 min. The distillate was then dried in an oven at 102°C for 30 min and chilled in a desiccator for 20 min. The weight difference between an empty container and one containing fat allowed the total fat content to be calculated using equation 5.4.

$$\%\text{Fat} = \frac{W3-W2}{W1}\ x\ 100 \quad \text{.....5.4}$$

Where W1=weight of sample, W2= Weight of beaker, W3= Weight of beaker with oil

5.3.4. Functional properties of marula peel flour

5.3.4.1. Bulk density

The bulk density (BD) of the flour was determined using the Mudau *et al.* (2021) method. Approximately 10 g of flour was weighed and placed in a 25 mL measuring cylinder, with the volume recorded as a loose volume. A bench was tapped on the bottom until a steady volume was detected. The dense volume was captured. The loose BD and packed BD were computed as the ratio of flour weight to flour volume before and after tapping. The bulk density (g/ ml^3) of flour was calculated using a ratio of the sample weight to the volume of the cylinder using equation 5.5.

Bulk density= $\frac{weight\ of\ sample}{volume\ of\ the\ cylinder}$.....5.5

5.3.4.2. Water holding capacity

The water-holding capacity of marula peel flour was determined by the method of Lu *et al*. (2020). One gram of flour was placed in 50 mL centrifuge tubes, and 10 mL of distilled water was added, homogeneously mixed with a glass rod, and placed in a 30°C water bath for 30 min. The centrifuge tubes were expressed at x g for 15 min in a 3000-rpm laboratory equipment type T-8BL Laby TM centrifuge (Ambala Cantt, India) using equation 5.6.

Water holding capacity = $\frac{V1-V2}{V2}$ 5.6

Where: V1= initial volume of water and V2= final volume of water

5.3.4.3. Oil absorption capacity

The OAC was evaluated by combining 1 g marula peel flour samples with sunflower vegetable oil (10 mL) in a centrifuge tube. Subsequently, the sample was allowed to remain at ambient temperature (26 ± 2°C) and then centrifuged at 1500 rpm for 30 min. The resulting supernatant was weighed, and OAC was calculated using the method of Mathobo *et al.* (2023) using 5.6.

Oil absorption capacity = $\frac{V1-V2}{V2}$……5.6.

Where V1= Initial volume of water and V2= Final volume of water

5.3.5. Physicochemical properties of marula fruit peel flour

5.3.5.1. Colour attributes of marula peel flour

The colour of the flour samples was determined using a Hunter Lab Colorimeter (MiniScan XE Plus, Model CM-3500d, Hunter Associate Laboratory, Reston, VA, USA) equipped with a D65 light source, 8° observer, diffuse/O mode, an 8 mm aperture for illumination, and an 8 mm aperture for measurement. Black and white tiles were used to calibrate the device. Hunter values for L* (lightness), a* (redness), and b* (yellowness) were used to indicate the colour reading. The hue angle (equation 5.7) and chroma (C) (equation 5.8) were calculated using the method (Mudau *et al.,* 2022).

$Hue\ (H°) = \{\frac{b}{a}\}$ ……5.7.

$Chroma\sqrt{(a)^2 + (b)^2}$……5.8.

5.3.5.2. pH measurement

The pH values of the flour samples were determined using a Crison digital pH meter (Crison instrument, SA) by the AOAC (2002) method number. 981.12. To check that the pH meter was operating properly, it was calibrated before use using buffers 4, 7, and 9. The pH was determined by dipping an electrode in a beaker filled with distilled water and a sample. Between changing samples, the electrode was washed with distilled water.

5.3.5.3. Viscosity of marula fruit peel flour

The viscosity of marula peel flours was measured with a Brookfield viscometer (Model RV, Brookfield Engineering, Inc., Middleboro, MA, USA) following a method described by (Ramashia *et al.,* 2019). In a beaker, 10 g of the flour samples were hydrated for 30 min with 90 mL of distilled water. The mixture was stirred on occasion until it created a slurry, the viscosity of which was measured, and the viscosity of the cold paste was recorded. The viscosity of the cooked paste was measured by heating the slurry in a

water bath until it boiled at 95°C. The cooked paste was chilled to 30°C and tested, and its viscosity was noted.

5.3.6. Antioxidant properties of marula fruit peel flour

For sample extraction of bioactive compounds, about 2 g of marula peel flour sample was weighed in a 100 mL beaker, and 20 mL of 1% methanolic acid was added. The mixture was then sonicated in an ultrasonic bath for 30 min before being centrifuged at 3000 rpm for 10 min in a laboratory equipment model T-8BL Laby TM centrifuge (Ambala Cantt, India).

5.3.6.1. 1-Diphenyl-2-picrylhydrazyl assay (DPPH assay)

About 2 mL of the flour sample extract was added to 2 mL of 0.1 Mm DPPH (2.93 g in 100 mL dissolved in 1% methanolic acid) in 10 mL test tubes. The mixture was agitated and kept at room temperature for 30 min shielded from light by foil, its absorbance was then measured at 517 nm with a UV-visible spectrophotometer (DR2800, Hach, USA) (Amarasinghe *et al.,* 2022), using equation 5.9.

Scavenging effect %= $\frac{Ac-As}{Ac} \times 100$ ……………………………………………………….5.9

Where Ac= the absorbance of the control (methanol with DPPH solution) and As = the absorbance of the polysaccharide sample.

5.3.6.2. Ferric reducing antioxidant power assay (FRAP assay)

Ferric reducing antioxidant power assay was analysed using a method by Tian *et al*. (2016). As with the extract, a standard calibration curve was created by combining different concentrations of Fe (II) (100-500 g) with FRAP reagent. 5.6 x 10-5 Fe^{2}+/ml is present in one (1) mM ferrous solution. The formula mole = mass/molar mass was used to determine μmol Fe^{2+}.

Preparation of reagents

About 300 mmol/L acetate buffer of pH 3.6 (3.1 g of $C_2H_3NaO_2.3H_2O$ and 16 mL of acetic acid per litre of buffer solution). To make 40 mmol/L HCl: Add 0.3 mL of HCl to

100 mL of distilled water. To make 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine): About 624.66 mg of TPTZ ($C_{18}H_{12}N_6$, Mw. 312.33) (Sigma-Aldrich) was dissolved in 100 mL of the 40 mmol/L of HCl and made up to 200 mL with same. To make 20 mmol/L $FeCl_3.6H_2O$: dissolve 1.0812 g of ferric chloride ($FeCl_3.6H_2O$, Mw. 270.3) in 20 mL of distilled water. Working FRAP reagent preparation: 25 mL acetate buffer was mixed with 2.5 mL TPTZ solution and 2.5 mL of $FeCl_3.6H_2O$ solution with a ratio of 10:1:1.

Standard preparation

About 1mM (1000 µM) ferrous sulphate solution (standard): prepared by dissolving 27.8 mg of $FeSO_4.7H_2O$ (Mw. 278.02) in distilled water and made up to 100 mL with the same. Ferrous Standard curve: Add 0, 1, 2.5, 5, and 7.5 of 1 mM ferrous standard into 10 mL volumetric flasks to generate 0, 100, 250, 500 and 750 µM of ferrous standard. The volume was adjusted to 10 mL with distilled water. Take 40 µL of each standard and add 2 mL of FRAP reagent, incubate for 15 min in the dark and measure the absorbance at 593 nm.

Sample preparation

About 40 µL of the sample extract and 2 mL of the FRAP reagent were mixed, incubated for 15 min in the dark and measured the absorbance at 593 nm Tian *et al*. (2016).

5.3.6.3. Total flavonoid content

In a 10 mL test tube, 0.3 mL of extract was combined with 3.4 mL of 30% methanol, 0.15 mL of 0.5 M $NaNO_2$, and 0.15 mL of 0.3 M $AlCl_3.6H_2O$. After 55 min, 1 cc of 1 M NaOH was added. At 506 nm, a spectrophotometer (DR2800, Hach, USA) was used to compare the absorbance to the reagent blank. A catechin standard solution (1 mg/ mL of catechin stock solution was generated by dissolving 100 mg in 100 mL of 1% methanolic acid, and dilutions of 0, 1, 0.2, 0.4, 0.8, and 1 mg/ mL were employed as working standards) was used to create the standard curve for total flavonoids. The total flavonoids were calculated as milligrams of catechin equivalents per gram of dried fraction (Pérez-Báez *et al.,* 2020).

5.3.6.4. Total phenolic content

About 0.15 mL of Folin Ciocalteu reagent (1:2 v/v diluted with distilled water) was added to 0.5 mL of test sample extract. The mixture was then allowed to stand at room temperature for 5 min. After 5 min, 2 mL of 7.5% w/v sodium carbonate was added, and the mixture was left in the dark for 45 min with intermittent shaking. After incubation, a blue hue was seen. Because the colour was too dark, each test tube received 10 mL of distilled water to dilute it. Finally, the absorbance of blue colour in samples was measured with a spectrophotometer at 725 nm. The total phenolic content was calculated as Gallic acid Equivalents (GAE/g) using a Gallic acid reference curve. (To make 10mg/mL of Gallic acid, weigh 1000 mg into a 100 mL volumetric flask and dissolve 10 mL ethanol before filling the mark with distilled water. Dilutions are required to achieve concentrations of 1, 2, 3, 4, and 5 mg/mL). Gall done in triplicates was used to express the results (Pérez-Báez *et al.,* 2020).

5.3.6.5. Vitamin C

The Indophenols technique was used to determine the vitamin C concentration (AOAC, 2005). method number. To make a standard, about 250 g of ascorbic acid was weighed into a 250 mL volumetric flask, distilled water was then added up to the mark and shaken until the liquid dissolved. Using a syringe, about 25 mL of vitamin C was transferred into a 100 mL conical flask, followed by the addition of 10 drops of starch. After rinsing with iodine, the burette was filled with 0.025 M iodine. The vitamin C was titrated until a change in colour from the initial volume of 0 was observed, and the final volume of iodine solution on the burette was recorded. This was repeated three times, with the average being taken. This was done three times, and the average was taken. This method was then repeated with 250 mg of samples weighed into a 250 mL volumetric flask and all the steps that followed to assess the vitamin C in the samples (Acham *et al*., 2020) using equation 5.10.

$$\text{Vit C} = \frac{volume\ of\ iodine}{average\ of\ the\ standards} \text{x } 0.250 \quad \text{.....5.10}$$

5.3.7. Microbial quality of marula fruit peel flour

The microbial quality of marula peel flour was determined using a modified method of Abdul-Nadi Al-Nasiry (2020). About 10 g of each flour sample was homogenised with 90 mL of sterile Buffered Peptone Water (BPW) to prepare the sample solution. The samples were diluted as 10^{-1} to 10^{-5} by adding 1 mL of sample solution to 9 mL of BPW. About 1 mL of the samples were transferred into culturing plates in triplicates and various media were added. The different media used for the isolation and culturing include Total plate count agar (TPC) which is a general-purpose media, Coliform chromogenic agar (CCA) for coliforms and *Escherichia coli* and Potato dextrose agar (PDA) used for yeasts and moulds following pour plate method. Incubation periods and temperature were 37°C for 24 hr (CCA), 37°C for 48 hr (TPC) and room temperature for 5 days (PDA). All media were prepared according to the manufacturer's instructions. BPW, TPC, CCA, and PDA were all sterilized in an autoclave for 15 min at 121°C. After that, the media was placed in a hot water bath to avoid solidifying.

5.3.8. Fourier-Transform Infrared Spectra of marula fruit peel flour

FTIR spectroscopy was done using a spectrometer 8700 (Thermo Scientific, Inc., Santa Clara, CA, USA. The PFPF spectrum was acquired in the 4000 to 400 cm^{-1} range (20 scans per experiment at a resolution of 4 cm^{-1}). About 0.5g of marula flour samples were mounted on the instrument and analysed, and the sample spectra for each sample were obtained (Mudau *et al.,* 2022).

5.3.9. Statistical analysis

All experiments were performed in triplicates. The data collected was processed using SPSS version 26 (IBM Chicago, USA) and means were separated using the Duncan multiple range test, 95% confidence interval ($p < 0.05$) was used to evaluate significance.

5.4. Results and Discussion

5.4.1. pH and viscosity of marula fruit peel flour

Table 5.1 shows the Viscosity and pH of marula peel flour from different locations in South Africa. The pH values of flour are used to determine the alkalinity and acidity of the flour (Ogunmodimu *et al*., 2015). The pH of the flours ranged from 4.05 to 4.35. The variation in pH could be due to the genetic makeup of the marula fruit, soil, weather, and geographic conditions. The lowest pH value was found in marula peel flour from the Phalaborwa region. According to Ramashia *et al.* (2018) lower pH in flour indicates good quality of the flour because it reduces the microbial load in the flour.

Table 5.1. Viscosity and pH of marula fruit peels flour

Sample name	Hot paste Viscosity (cP)	Cold paste Viscosity (cP)	pH
Thulamahashe	312.33±5.41^{b}	100± 1.30^{c}	4.35± 0.43^{c}
Phalaborwa	361.33±4.31^{c}	80±1.17^{b}	4.05±0.21^{a}
ZZ2	209.33±3.01^{a}	40± 1.09^{c}	4.14±0.27^{b}

Values are expressed as the mean ± standard deviation. A column with different letters indicates a significant difference ($p < 0.05$).

There was a significant difference ($p < 0.05$) among the three locations regarding hot and paste viscosities. Marula peel flour from ZZ2 had the lowest viscosity of 209.33 cP on hot paste and 40 cP on cold paste. Factors such as increased moisture, lower protein content and the presence of specific starch-breaking enzymes can contribute to low viscosity in flour (Ramashia *et al.,* 2021). Therefore, from the three (3) regions, marula peel flour from ZZ2 can be used for several cooking applications because using low-viscosity flour might result in softer and more tender finished products. A significant difference was observed in cold paste viscosity from the three regions. Marula peel flour from Thulamashe showed a higher value of 100 cP on cold paste viscosity. High cold paste viscosity in flour may be a sign of increased protein content and water adsorption capacity (Edun *et al.,* 2019).

5.4.2. Colour attributes of marula fruit peel flour

The colour attribute results of marula peel flour, as presented in Table 5.2 were significantly different ($p<0.05$) in redness (a^*), lightness (L^*) and yellowness (b^*) values for all three (3) regions. Thulamahashe flour had the highest value in lightness (L^*), the higher lightness value obtained might be due to the oven drying temperatures as higher temperatures have been found to inactivate phenolase activity (Ngoma *et al.,* 2019).

Table 5.2. Colour attributes of marula peels flour

Sample region	L*	a*	b*	Hue	Chroma
Thulamahashe	69.24 ± 0.01^c	6.95 ± 0.02^a	25.22 ± 0.04^a	74.60 ± 4.33^a	26.16 ± 0.04^a
Phalaborwa	62.13 ± 0.01^a	10.33 ± 0.03^c	28.46 ± 0.03^b	70.05 ± 0.06^a	30.28 ± 0.02^c
ZZ2	64.21 ± 0.01^b	9.54 ± 0.02^b	27.79 ± 0.04^c	71.05 ± 0.6^a	29.39 ± 0.03^b

Different letters in the same column are significantly different ($p < 0.05$), n = 3. L*, a*, and b* denote lightness, redness, and yellowness respectively.

In terms of a* values, a higher a* value was obtained in marula peel flour from Phalaborwa while a lower a* value was obtained in marula peel flour from ZZ2 and Thulamashe respectively. This means that marula peel flour from the Phalaborwa region contained higher red pigmentation than the one from ZZ2 and Phalaborwa. The hue angle of the flours ranged from 70.05 to 74.60. The hue angle shows the yellow, green and blue hues which are represented by angles of 90, 180 and 270°C and the red hue is represented by 360°C, as a result, the flour samples were closer to red (Mudau *et al.,* 2021). The chroma value of the flour samples ranged from 26.16 to 30.28.

5.4.3. Function properties of flour

Table 5.3 presents the functional properties of marula peel flour from three different regions. The loose bulk density of the flours ranged from 0.50 to 0.60 g/m^3 with no significant difference ($p<0.05$). For PDB marula peel flour from ZZ2 had the highest value of PBD while the flour from Phalaborwa and Thulamashe had the same value of PBD. Marula peel flour from ZZ2 had the highest value in terms of LBD and PBD. According to Oguntoyinbo *et al.* (2022) the bulk density of flour increases as it gets denser, suggesting that marula peel flour from ZZ2 is denser than marula peel flour

from the other two regions. High bulk density suggests that the flour can be used as a thickener in the food processing industry, as well as in food preparation since it can assist in lowering paste thickness, which is a major factor in convalescent and child feeding (Mahloko *et al.,* 2019).

Table 5.3. Functional properties of marula peel flour

Sample region	LBD (g/m^3)	PBD (g/m^3)	WAC (g/g)	OAC (g/g)
Thulamahashe	0.50 ± 0.11^a	0.77 ± 0.01^a	6,31± 0.04^b	2.41 ± 0.33^b
Phalaborwa	0.57± 0.11^a	0.77 ± 0.01^a	5.98± 0.03^a	2.47 ± 0.33^b
ZZ2	0.60± 0.11^a	0.85 ± 0.02^b	5.85 ± 0.03^a	2.22 ± 0.21^a

Different letters in the same column are significantly different ($p < 0.05$), n = 3. LBD = loose bulk density; PBD = packed bulk density; WAC = water absorption capacity; OAC = Oil

WAC is a measurement of the interaction between flour and water in a variety of foods. The capacity of flour to absorb water depends on the availability of hydrophilic groups that bind water (Mudau *et al.,* 2020). The WAC of marula peel flour from three different regions ranged from 5.85 to 6.31g/g. A higher value of WAC was obtained in flour from Thulamahashe. According to Barkar *et al.* (2018), a high WAC content in flour can change the texture and viscosity of food, enhancing the effects of bulking, thickening, and gelling. OAC is known for the ability of flour fat to bind to the non-polar side chains of proteins. It is an important functional property that contributes to improving mouth feel while retaining the flavour of food products (Bongjo *et al*., 2022). The OAC of the flours ranged from 2.22 to 2.47 g/g. These findings were like the ones reported by Khanitta *et al.* (2019) on the OAC of banana peel flour. A higher OAC value of 2.47g/g was recorded in marula peel flour from Phalaborwa among the three regions as shown in Table 5.3. Then 2.22 g/g of marula peel flour in ZZ2 was recorded to be the lowest. The relatively high OAC of marula peel flour from Phalaborwa indicates that it may be useful in food formulations requiring oil-holding capacity, such as sausage and bakery goods (Godswill, 2019).

5.4.4. Proximate composition of marula fruit peel flour

The approximate composition of marula peel flour including moisture content, protein, crude fat, and ash is presented in Table 5.4. the ash content indicates the amount of minerals present in food (Raj and Masih, 2014). Ash content of marula peel flour from

Thulamahashe region was higher compared to the other regions. Moisture content is a key factor in influencing food shelf life (Nilusha *et al.,* 2021). The moisture content of the flours ranged from 5.54 to 6.99%. The moisture level range (5.54 to 6.99%) is in line with the protein advisory committee of the United Nations, which suggested that the moisture content of flour should not exceed 10% to preserve a floury product for a reasonable amount of time (Zubair *et al.,* 2023). The protein content of marula peel flour ranged from 3.76 to 4.38 % among the three regions. The highest protein content was recorded in marula peel flour from ZZ2 and the lowest in Thulamahashe.

Table 5.4. Proximate composition of marula fruit peel flour

Sample region	Fat (%)	Ash (%)	Moisture (%)	Protein (%)
Thulamahashe	3.92±1.12^{b}	0.49±0.03^{c}	5.81± 0.57^{a}	3.76±1.00^{a}
Phalaborwa	3.56±1.12^{b}	0.34±0.01^{a}	6.99±1.15^{b}	3.89±1.00^{a}
ZZ2	3.20±0.49^{a}	0.21±0.01^{a}	5.54±0.57^{a}	4.38±1.12^{b}

Values are expressed as the mean ± standard deviation. A column with different letters indicates a significant difference ($p < 0.05$).

The differences in the protein content of the marula peel flour samples could be due to environmental factors such as soil fertility and environmental conditions (Chisenga *et al.,* 2019). The fat content of marula peel flour ranged from 3.20 to 3.92 %. Flour from Thulamahashe showed a higher value in fat content. These results indicate that marula peel flour is a good source of fat.

5.4.5. Polyphenols and antioxidant activity of marula peels flour

Table 5.5 summarises the polyphenols and antioxidant activity of marula peel flour. The DPPH ranged from 19,28 to 62,52%. The highest DPPH was obtained on marula peel flour from the Thulamashe region. The effect of the extraction solvent that dissolved the sample has contributed to the behaviour of the DPPH results acquired. According to Hasmadi et al. (2020) when the concentration of phenolic substances and their hydroxylation increases, so does DPPH. However, this was not the case in this study as the DPPH was increasing while TPC was decreasing. The total phenolic compounds of the marula flour ranged from 8.59 to 14.56 mg/100 g. Marula peel flour from Thulamashe showed the lowest value of TPC. A lower concentration of TPC in

flour can indicate lower levels of antioxidants and health-promoting compounds (Martin *et al.,* 2019).

Table 5.5. Polyphenols and antioxidant activity of marula peel flour**.**

Sample region	DPPH (%)	TPC (mg/100 g)	TFC (mg/100 g)	Vitamin C (mg/100 g)	FRAP (100mg GAE/g) P
Thulamahashe	62.52 ± 2.96^{c}	8.59 ± 0.05^{a}	35.82 ± 1.24^{a}	162.03±1.10^{C}	1479.17± 1.67^{b}
Phalaborwa	26.21 ± 0.18^{a}	14.56 ± 0.52^{b}	46.31 ± 0.97^{b}	154.99±0.94^{b}	1546.94 ± 4.19^{c}
ZZ2	19.28 ± 3.16^{b}	14.31 ± 0.11^{b}	40.96 ± 0.77^{c}	126.81±0.88^{a}	1437.50 ± 14.53^{a}

Different superscripts in the same column are significantly different ($p < 0.05$). TPC for total phenolic content, TFC for total flavonoid content, and FRAP for ferric-reducing antioxidant power.

The FRAP results for the flour ranged from 1437.50 to 1479.17 100 mg/GAE/g. Flour from ZZ2 had a higher value of FRAP and this may be due to the Millard reaction during oven drying of the peels. TFC concentration of marula peel flour ranged from 35.82 to 46.31 mg/100 g with a significant difference ($p<0.05$). The vitamin C of the flours ranged from 126 to 162 mg/100 g. Flour from Thulamahashe has the greatest value of Vitamin C meaning it can promote good health for heart diseases, cancer and other chronic illnesses.

5.4.6. Microbial quality of marula fruit peel flour

Table 5.6. summarises the microbial quality of marula peel flour. The microbial quality of flour is important for ensuring food safety. Coliforms are commonly used as an indication of the sanitary condition of food and water, while most *Escherichia coli* strains are not harmful, some can make individuals very ill (Akin *et al*., 2023). In this study mould, coliforms and *E. coli* were not detected on the marula peel flour produced. Therefore, the flour is fit for human consumption. The TPC of the flour showed a significant difference ($p<0.05$) in all regions. According to World Health Organization regulations (1999), the flour produced is safe for human consumption as the results obtained fall within the range where the maximum limit for total microbial plate count is 2.0 x 10^{5} cfu/ g^{-1}, *coliform* bacteria is 200 Most Probable Number (MPN/g^{1}, yeast and mould is 1.0 x 10^{4} cfu/g^{-1}, and *E. coli* is absent.

Table 5.6. Microbial quality (log cfu/g) of marula fruit peel flour

Sample region	Thulamashe	Phalaborwa	ZZ2
E. coli	ND	ND	ND
Coliform	ND	ND	ND
TPC	1.45±0.02^{C}	1.19±0.08^{b}	1.29±0.01^{a}
Yeast	1.79±0.06^{C}	1.66±0.11^{c}	1.49±0.17^{a}
Mould	ND	ND	ND

Values are mean ± standard deviation. Different letters indicate a significant difference ($p < 0.05$). ND for not detected, TPC for total plate count and *E. coli* for *Escherichia coli.*

5.4.7. Fourier-Transform Infrared Spectra of marula fruit peel flour

Figure 5.2. below displays the FTIR spectral regions (O–H stretch region, C–H stretch region of marula peel flour from different locations. The FTIR spectra of flour can reveal details about its chemical structure and content as various peaks in the spectrum correspond to different functional groups in the molecule (Lin *et al*., 2021). The O–H region of marula fruit peel flours showed bands (peaks) within the 3474.30 to 3474.86 cm^{-1} range. The absorption peaks of

Marula peel flour produced in Phalaborwa ranged from 3180.16 to 3180.74 cm^{-1}. These peaks can be caused by the stretching vibrations of the O-H bond. The moisture content of samples can contribute to the behaviour of peaks that occur in the O-H region (Mudau *et al*., 2022). The peaks at around 2480.40 in marula peel flour from ZZ2 and Thulamahashe showed that the flours contained O–C–H, C–C–H, and C–O–H.

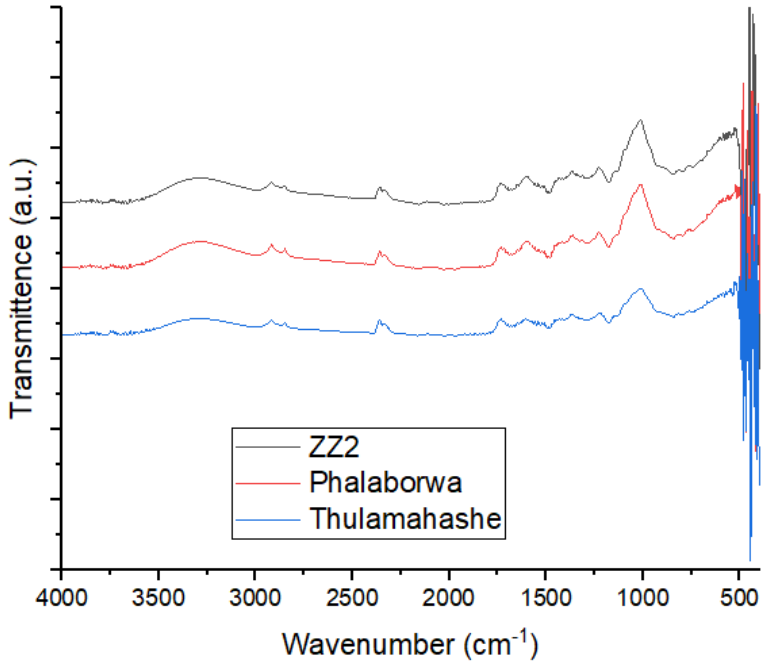


Fig. 5.2. FTIR spectra of marula fruit peels flour

5.4.8. Conclusion and Recommendation

5.4.8.1. Conclusion

From the study conducted an overview of the nutritional composition, functional, antioxidant properties and microbial quality of marula fruit peel flour was thoroughly examined. Marula peel is regarded as a waste of easy availability and low cost. It can be concluded that marula peel flour is a good source of vital nutrients such as proteins and compounds such as phytochemicals that aid in promoting good health concerning heart diseases, cancer and other chronic illnesses.

5.4.8.2. Recommendations

From the study conducted marula peel flour may be useful in food formulation such as sausages and bakery goods. It is recommended to conduct more studies on marula peel flour particularly it is cooking and baking properties to increase several gluten-free food products in the market.

CHAPTER 6

USE OF MARULA FRUIT PEEL TO MAKE BISCUITS

6.1. Summary

Biscuits made from composite flour are becoming more popular because of the economic and nutritional advantages of composite flour. This chapter aimed to develop wheat biscuits fortified with marula peel flour and to evaluate their proximate composition, physical, antioxidant and structural properties. The formulations used in the ratios were 5, 10, 15, and 20% marula peel flour and 100% wheat flour biscuits were used as a positive control. The wheat-marula (WM) peel composite biscuits were analysed for proximate composition, physical properties, antioxidant properties, and FTIR (Fourier-Transform Infrared Spectra). The ash content of the biscuits significantly increased with the incorporation levels of marula peel flour while moisture, fat, and protein contents decreased significantly ($p< 0.05$). Increased marula flour inclusion decreased the L*, a* and b* values. The thickness, mass, spread ratio, and diameter of the biscuits decreased significantly ($p< 0.05$) while hardness increased with the inclusion of marula peel flour. The wheat-marula peel composite biscuits had high antioxidant activities, and the vitamin C increased with the increase in levels of marula peel flour incorporation. The O-H region of composite biscuits had peaks ranging from 3267.78 to 3249,01 cm-1 which revealed the vibrations of O-H bonds. In conclusion, marula peel flour can be utilised as a functional ingredient in bakery products such as biscuits.

6.2. Introduction

One of the most popular processed food items in the world is confectionery products (Caleja *et al*. 2017). Biscuits are one of the largest categories of ready-to-eat foods as they are made from simple and easily accessible ingredients. They are consumed worldwide as they have a very acceptable taste and long shelf life due to low water activity (Warinporn and Geoffrey, 2018). If they are consumed regularly, they can impact negatively one's health as they contain high-energy and easily digestible foods such as wheat flour and fats (Caleja *et al*. 2017). The overall nutritional composition of biscuits can be improved by reducing the content of wheat flour. In developing

countries, researchers have begun to find solutions to substitute wheat flour with soybean or plantain (Chinma *et al*., 2017).

Marula (*Sclerocarya birrea*) grows well in the sub-Saharan regions of Africa and forms part of the most important trees in dry areas of Zimbabwe as it provides resources such as the marula fruits and the barks that can be used as medicine (Kugedera, 2019). The marula tree is also found in South African parks and rural areas of Mpumalanga, Limpopo, KwaZulu Natal, and Eastern Cape (Sinthumule and Mashau, 2019). It is part of the most commercially important indigenous fruits in South Africa, mainly in the beverage manufacturing industry (Kugedera, 2019). Marula also forms part of the major income-generating project for farmers as several products can be produced such as juice, soda, and oil (Tapiwa, 2019). To allow regeneration and continual growth of the tree, proper harvesting techniques must be done (Mguni *et al*., 2023).

The tree (3) produces flowers from September to November and bears fruit from January to March (Ngemakwe *et al*., 2017). The marula fruits are round or oval and 3-5 cm in diameter when mature enough to be harvested. They taste acidic when they are not yet mature but sweet when fully ripe. The edible parts of the marula fruits are smaller compared to the fruit size just like the Baobab fruit (Chaibva *et al*., 2023). The female trees are the ones that bear plum-sized fruits and have a thick yellow peel and white sweet-sour fruit that can be consumed raw (Kugedera *et al.* 2020). These fruits develop in clusters of 4 to 5 at the ends of twigs when a new growth develops. The products of marula products such as jam, juice and beverages, the peels are thrown away making them the underutilised part of the marula (Tapiwa 2019).

The physicochemical properties of food are the physical and chemical components that are found in the food product (Macamo *et al*. 2021). Physical properties such as moisture, density, and viscosity are important as they analyse the behaviour of the product during processes such as harvesting, storing, pH, drying, and processing (Yenge *et al*. 2018). Compounds that control oxidation by delaying the oxidation chain reaction are called antioxidants. In food production, antioxidants are also food additives that are effective in prolonging the shelf life of food (Gokoglu 2019). Almost all edible plants have antioxidants naturally. Antioxidant compounds such as vitamin

E, vitamin C, and polyphenols are found in fruits and vegetables including fruits such as lemons, oranges, and bananas (Khoza, 2019).

The oil in the marula seed was classified as containing a high content of oleic acid (Macamo *et al*. 2021). The marula fruit also has anti-inflammatory and antibacterial properties. The chewing of marula leaves can help with indigestion (Ngemakwe *et al*. 2017). Marula contains Vitamin C approximately 70% of the total antioxidant capacity of most common fruits (Fyfe *et al*., 2020). There are no studies yet regarding the effect of thermal treatment on antioxidants. From a study conducted by Mashau *et al*. (2022), Marula juice has a high concentration of polyphenols and tannic acid. The study aims to evaluate the physicochemical properties, antioxidant activities, and structural properties of the wheat-marula composite biscuits

6.3. Materials and Methods

6.3.1. Sample collection

The marula fruits were collected in three (3) different locations, namely, Phalaborwa, Thulamahashe and ZZ2. They were stored in the pilot plant located in the Department of Food Science and Technology at the University of Venda until further processing. The wheat flour was purchased at the local retail store in Thohoyandou together with ingredients that were used such as sugar, milk, salt, and butter.

6.3.2. Preparation of marula peel flour

The production of marula peel flour was done following the method used by (Santos *et al.* 2022). The marula fruits were washed peeled and dried using an oven dryer. Once dry, a miller from the Department of Animal Science at the University of Venda was used to mill the peels (Fig. 6.1). The miller was a 1 mm sieve size.

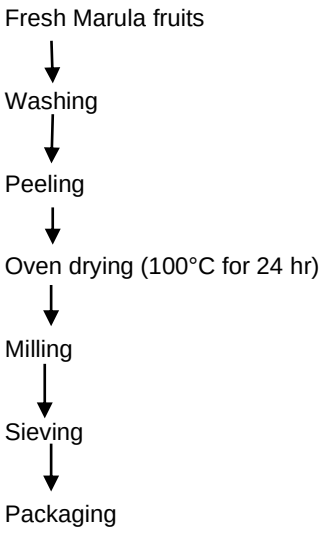


Fig. 6.1. Flow diagram to produce marula flour (Santos *et al.,* 2022)

6.3.3. Formulation of flour blends

The composite flour produced is indicated in Table 6.1 below. The control was wheat flour, which is sample A. Samples B, C, D, and E incorporated wheat flour and Marula peel flour (MPF) with different ratios, respectively. The formulations below were used on the Marula peel flour for all three locations (Phalaborwa, Thulamahashe, and ZZ2). The flour blends were mixed thoroughly and stored in polyethylene bags and stored in a cool dry place at room temperature until further use.

Table 6.1. Composite flour formulations

Samples	Wheat flour (%)	Marula peel flour (%)
A	100	0
B	95	5
C	90	10
D	85	15
E	80	20

(Laganà *et al.,* 2022)

6.3.4. Preparation of wheat-marula peel composite biscuits

The baking of the biscuits was conducted following the method of Setyaningsih *et al.* (2019). The biscuits were baked using an electric flour place stove (Defy) at the pilot plant, Faculty of Science, Engineering and Agriculture. The quantity and ingredients used are listed below in Table 6.2.

Table 6.2. Baking of marula peel biscuits.

Samples	Wheat flour (%)	Marula peel flour (%)	Sugar(g)	Fat(g)	Salt(g)	Milk(ml)
A	100	0	50	80	1	100
B	95	5	50	80	1	100
C	90	10	50	80	1	100
D	85	15	50	80	1	100
E	80	20	50	80	1	100

(Saadoudi *et al.,* 2017)

Firstly, the oven was preheated at 180°C. In a bowl, 80 g of butter was mixed with 50 g of sugar, and 200 g of Wheat flour, salt, and milk were added bit by bit using a wooden spoon. The dough was formed, and a rolling pin was used to roll it out into a sheet. A 5 cm dough cutter was used to cut the dough and place it into a butter baking tray. They were then baked for 15 minutes until golden brown. They were then allowed to cool down, packaged into zip-lock bags and stored in the fridge till further use. Fig. 6.2 shows the baking process of the biscuits and Fig. 6.3 indicates the baked biscuits.

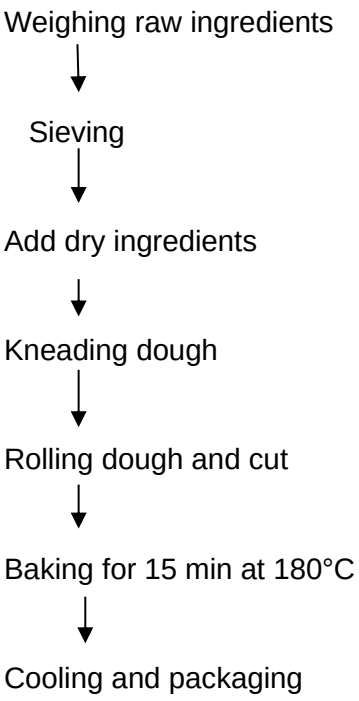


Fig. 6.2. Flow diagram of the baking process (Thongoram *et al.,* 2016)

Fig 6.3. Indicate the biscuits produced from different wheat-marula formulations.

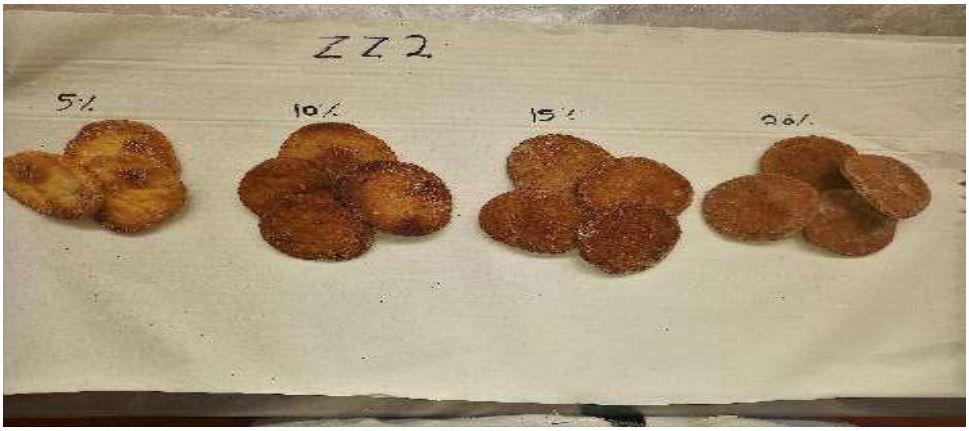


Fig 6.3. Biscuit samples from three regions (ZZ2 (Moeketsi), Thulamahashe and Phalaborwa) Control= 100% Wheat flour, 95:5% wheat- marula peel composite biscuits, 90:10% wheat-marula peel composite biscuits, 85:15% wheat-marula peel composite biscuits and 80:20% wheat-marula peel composite biscuits.

6.3.5. Proximate composition of wheat-marula peel composite biscuits

6.3.5.1. Moisture content

The moisture content was using AOAC (2005) method 925.10. The oven dryer was preheated for 30 mi at 105°C before use together with the crucibles. The heated crucibles were then stored in desiccators. Two (2) g of crushed biscuits were weighed into the pre-heated crucibles and then dried in a conditioned oven (Module 278, Labotech Ecotherm, South Africa) at 105°C overnight. The samples were then allowed to cool in a desiccator, and the moisture content was calculated by the difference in weight using equation 6.1 (Belokurova *et al*., 2021)

% Moisture = (W2-W3)/(W2-W1) X 100%.....6.1.

Where: W1= crucible weight, W2 = weight of crucible with biscuits before drying and W3 = weight of crucible with biscuits after drying.

6.3.5.2. Fat content

The total fat content was carried out by Soxhlet extraction (BUCHI fat extractor, E-500) following AACC (1999) method no. 30-25.01. It was conducted by extraction of fat in 2 g of dry biscuits into a thimble with an organic solvent of petroleum ether with

a 40-60°C boiling point. The solvent was poured into a pre-weighed Soxhlet beaker then immersing the thimbles with the sample in solvent for 30 min, washed for 1 hour and finally recovered the fat into the beaker for 5 min. The distillate was dried in an oven at 102°C for 30 min and chilled in a desiccator for 20 min. The difference in weight between an empty container and one containing fat allowed the total fat content to be calculated using equation 6.2

%Fat= (W3-W2)/W1 X100…….6.2.

Where W1=weight of sample, W2=weight of beaker, W3=weight of beaker with oil.

6.3.5.3. Ash content

Ash content of the composite biscuits was accomplished by sample incineration in a muffle furnace (LASEC, laboratory and scientific equipment co (Pty) Ltd, model EMF035) at 550°C for 24 hrs following AACC (2010) method 08-01.01. Dry crucibles were weighed, and 2 g of biscuit samples were added to the crucibles. The ash content of the sample to be determined by the difference in weight before and after burning allowed the sample using equation 6.3.

Ash= (W2-W1)/W3 x 100%.....6.3.

Where W1= crucible weight, W2= weight of crucible with ash, W3= mass of sample.

6.3.5.4. Protein content

The crude protein content was determined following the Kjeldahl method of AOAC (2002) method 997.23 (2002). About 2 g of the biscuits were added into the digester tubes, two (2) tables of the Kjeldahl catalyst and 25 mL of concentrated sulphuric acid (96%) were added into the digester tubes and digested on a pre-heated using a BUCHI Digestion Unit (K-424) until the colour changes to a yellowish orange. After digestion, the samples first cooled down to room temperature then 60 mL of distilled water was added to dilute the sulphuric acid. The sample was then distilled while collecting the distillate in a conical flask by a BUCHI Distillation Unit (K-355). Two (2) drops of the indicator were added to the distillate which was then titrated with a METTLER TOLEDO DL22 (Food and Beverage Analyser) using concentrated

hydrochloric acid. The volume of hydrochloric acid was recorded during the first colour change of the distillate. The crude protein content was calculated using the conversion factor 6, 25 using equation 6.4.

$$\%\text{Nitrogen} = \frac{(Vs - Vb)\ X\ N(H2SO4)\ X\ 14.007\ X\ 100}{W\ X\ 1000} \quad \ldots\ldots 6.4.$$

Where Vs= volume of H_2SO_4 used to titrate the sample in mL, Vb= volume of H_2SO_4 used to titrate blank in ml, N (H_2SO_4) = Normality of acid titrant, W= weight of sample in grams.

6.3.5.5. Fat content

The total fat content was carried out by Soxhlet extraction (BUCHI fat extractor, E-500) following AACC (1999) method no. 30-25.01. It was done by extraction of fat in 2 g of dry biscuits and flours weighed into a thimble with an organic solvent namely petroleum ether with a 40-60°C boiling point. The solvent was poured into a pre-weighed Soxhlet beaker then immersing the thimbles with the sample in solvent for 30 min, washing for 1 hr and finally the fat into the beaker for 5 min. After that, the distillate was dried in an oven at 102°C for 30 min and chilled in a desiccator for 20 min. The difference in weight between an empty container and one containing fat allowed the total fat content to be calculated, equation 6.5.

$$\%\text{Fat} = \frac{W3 - W2}{W1}\ x\ 100 \quad \ldots\ldots 6.5.$$

Where W1=weight of sample, W2= Weight of beaker, W3= Weight of beaker with oil

6.4. Physicochemical properties of wheat-marula peel composite biscuits

6.4.1. Colour properties

The colour properties (L*, a*, and b*) of the biscuits was analyzed using a Hunter lab colour flex colour analyser (Lovibond Colourimeter, RM200, USA) where positive or negative results were recorded. The L* indicates the whiteness or brightness, a* indicates red or green coordinates and b* indicates yellow or blue coordinates, and H°

angle (equation 6.6) and Chroma (C*) equation 6.7 were calculated using the method of Aboshora *et al*. (2016).

$$Hue\ (H°) = \{\frac{b}{a}\} \quad \text{.....6.6}$$

$$Chroma\sqrt{(a)^2 + (b)^2} \quad \text{.....6.7.}$$

6.4.2. Texture characteristics

The texture of the biscuits was done following the method described by Armi and Fkri-Ershad (2019). The hardness analysis of the biscuit samples was determined using the TA-XT Plus texture analyser (Stable Micro Systems Serial No. 5014 England) located in the chemistry lab in the food science and technology department, University of Venda. The texture analyser was set to operate on single-cycle measurements which had a measurement speed of 2 mm/s and 5 mm was applied. The samples were analyzed for fractubility (mm) and hardness (mm). This procedure was done in triplicates on each biscuit sample.

6.4.3. Physical properties of wheat-marula peel composite biscuits

The diameter and thickness of the wheat-marula peel composite biscuits were measured with a Verner caliper as described by Zheng *et al*. (2020). The weight of the biscuits was measured by weighing on a weighing balance and measured in grams (g). The thickness was divided by diameter to get a spread ratio of biscuits.

6.4.4. Antioxidant properties of wheat-marula biscuits

6.4.4.1. DPPH assay

In a 100 mL beaker, 2 g of the crushed biscuit sample was weighed and 20 mL of 1% methanolic acid was added. The mixture was then sonicated in an ultrasonic bath for 30 minutes and centrifuged at 3000 rpm for 10 in a model T-8BL Laby TM centrifuge (laboratory instruments, Ambala Cantt, India).

1, 1-Diphenyl-2-picrylhydrazyl assay (DPPH assay)

In test tubes, 2 mL of each sample solution was added, and the procedure was conducted in triplicates. Into the test tubes, 2 mL of 0.1 Mm DPPH was added, the

solution was mixed, and a foil was used to cover the test stubs to prevent light from entering for 30 min. The absorbance was then measured at 517 nm with a UV-visible spectrophotometer (DR2800, Hach, USA) (Thongram *et al*., 2016) using equation 6.8.

Scavenging effect %=$\frac{Ac-As}{Ac} \times 100$……..6.8

Ac is the absorbance of the control (methanol with DPPH solution) and so is the absorbance of the solution extracted.

6.4.4.2. Ferric reducing antioxidant power assay (FRAP assay)

A method by Tian *et al*. (2016) was used to determine the ferric-reducing ability. A standard calibration curve was prepared by mixing various concentrations of Fe (II) (100-500 µg) with FRAP reagent as carried out with the extract. One (1) mM ferrous solution contains 5.6 x 10-5 Fe2+/mL. The µmol Fe2+ was calculated using the formula: mole = mass/molar mass.

Reagent preparation

Approximately 300 mmol/L acetate buffer of pH 3.6, 3.1 g of C2H3NaO2.3H2O) and 16 ml of acetic acid per Liter of buffer solution were added in a conical flask.

To make 40 mmol/L HCl: Add 0.3 ml of HCl to 100 ml of distilled water. To make 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine): About 624.66 mg of TPTZ (C18H12N6, Mw. 312.33) (Sigma-Aldrich) was dissolved in 100 mL of the 40 mmol/L of HCl and made up to 200 mL with same.

To make 20 mmol/ll $FeCl_3.6H_2O$: dissolve 1.0812 g of ferric chloride ($FeCl_3.6H_2O$, Mw. 270.3) in 20 mL of distilled water. Working FRAP reagent preparation: 25 mL acetate buffer was mixed with 2.5 mL TPTZ solution and 2.5 mL of FeCl*3*.6H*2*Osolution with ratio of 10:1:1.

Standard preparation

About 1 mM (1000uM) ferrous sulphate solution (standard): prepared by dissolving 27.8 mg of $FeSO_4.7H_2O$ (Mw. 278.02) in distilled water and made up to 100 ml with the same. Ferrous Standard curve: Add 0, 1, 2.5, 5, and 7.5 of 1 mM ferrous standard into 10 mL volumetric flasks to generate 0, 100, 250, 500, and 750 UM of ferrous standard. The volume was adjusted to 10 mL with distilled water. Take 40 µL of each

standard and add 2 mL of FRAP reagent, incubate for 15 m in the dark, and measure the absorbance at 593 nm.

Sample preparation

About 40 uL of the sample extract and 2 mL of the FRAP reagent were mixed, incubated for 15 minutes in the dark, and measured the absorbance at 593 nm.

6.4.4.3. Total flavonoid content

In a 10 mL test tube, 0.3 mL of extract, 3.4 mL of 30% methanol, 0.15 mL of 0.5 M $NaNO_2$, and 0.15 mL of 0.3 M $AlCl_3.6H_2O$ were mixed. About 1 mL of 1 M NaOH was added after 55 min. A spectrophotometer (DR2800, Hach, USA) was used to test the absorbance against the reagent blank at 506 nm. The standard curve for total flavonoids was created using a catechin standard solution (1 mg/mL of catechin stock solution was formed by dissolving 100 mg in 100 mL of 1% methanolic acid and dilutions of 0, 1, 0.2, 0.4, 0.8 and 1 mg/mL were used as working standards). Total flavonoids were expressed in milligrams of catechin equivalents per gram of dried fraction (Gamage *et al*., 2021).

6.4.4.4. Total phenolic content

Approximately 0.15 mL of Folin Ciocalteu reagent (1:2 v/v diluted with distilled water) was added to 0.5 mL of test sample extract. The mixture was then allowed to stand at room temperature for 5 min. After 5 min, 2 mL of 7.5% w/v sodium carbonate was added and the mixture was placed in the dark for 45 min with occasional shaking. The formation of a blue colour was observed after incubation. Because the colour was excessively dark, around 10 mL of distilled water was added to each test tube to dilute them. Finally, the absorbance of the blue colour in samples was determined using a spectrophotometer at 725 nm. The total phenolic content was estimated to be Gallic Acid Equivalents (GAE/g) using a Gallic acid reference curve.

(To make 10 mg/mL of Gallic acid, 1000 mg was weighed into a 100 mL volumetric flask and 10 ml ethanol must be dissolved and then filled to the mark using distilled water. Dilutions must be made to make concentrations of 1, 2, 3, 4, and 5 mg/mL). The results were expressed as Gall done in triplicates (n=3), (Gamage *et al*., 2021).

6.4.4.5. Vitamin C

The content of Vitamin C was collected and estimated according to (Satpathy *et al.* 2021*).* To create a standard, 250 g of ascorbic acid was added into a 250 mL volumetric flask. 100 mL of distilled water was added, and the solution could dissolve. Distilled water was added into the conical flask up to the mark. In a 100 ml conical flask, 25 mL of vitamin C was added to it together with 10 drops of starch solution. A burette was rinsed with distilled water and filled with 0,025 M iodine. The iodine solution was used to titrate the vitamin C solution until there was a colour change. The initial and final volume of the iodine solution on the burette was recorded. This procedure was done in triplicates. This method was repeated 10 g of the samples weighed into a 250 mL volumetric flask and all the procedures were done to evaluate the vitamin C content in the biscuit samples, equation 6.9.

Vitamin C = $\frac{volume\ of\ iodine}{average\ of\ the\ standards}$ x 0.250……6.9.

6.5. Fourier-Transform Infrared Spectra of wheat-marula peel composite biscuits

An FTIR spectrometer Nicolet 8700 (Thermo Scientific, Inc., Santa Clara, CA, USA) was used to analyse WMPCB, with wavelengths ranging from 400 to 4000 cm $^{-1}$. Approximately 0.5 g of milled wheat-marula peel composite biscuits were mounted on the instrument and analysed, and the sample spectra were obtained (Mudau *et al*., 2022).

6.6. Statistical analysis

The experiment was repeated twice. All data obtained from all analyses of each sample were carried out in triplicates. The collected data were processed using SPSS version 26 (IBM Chicago, USA) and means were separated using the Duncan multiple range test. To evaluate significance, the 95% confidence interval ($p<0.05$) was used.

6.7. Results and Discussion

6.7.1. Some proximate composition of wheat-marula composite biscuits

Table 6.3 shows the proximate composition of the wheat-marula peel composite biscuits. The ash content of the biscuits increased significantly ($p<0.05$) with the increasing levels of marula peel flour from all three (3) locations. The increment in ash content of biscuit samples as the levels of marula peel flour increase could be due to the high ash content of marula peel flour. marula peel is potentially a good source of some essential mineral elements especially calcium, potassium, sodium and magnesium which are found in high amounts (Muhammad *et al.,* 2015). Gondim *et al.* (2005) indicated that fruit peels have more minerals than edible parts, which explains the high ash content of composite biscuits in this study. Ramashia *et al.* (2021) reported similar results whereby the incorporation of *Parinari curatellifolia* peel flour enhanced the ash content of the biscuits.

The protein content of the biscuits decreased significantly ($p<0.05$) with the incorporation of marula peel flour, irrespective of the location. The decrease in protein content of the composite biscuits might be due to lower protein content in marula peel flour. Furthermore, the reduction in protein content might be due to the decrease in the quantity of wheat flour used, leading to a decrease in the protein constituent, especially, the gluten content. Protein content in food products is said to oversee processes like in vitro digestion and texture (Klunklin and Savage, 2018). The protein content of wheat flour biscuits was 10.25%, according to Dauda et al. (2019), which is comparable to the protein content found in this study.

Table 6.3. Some proximate composition of wheat-marula composite biscuits.

Sample region	Ash (%)	Protein (%)	Fat (%)	Moisture (%)
ZZ2 (Moeketsi)				
Control	0.43 ± 1.18^{a}	10.4 ± 1.60^{a}	24.81 ± 0.79^{a}	9.99 ± 0.31^{a}
95:5	0.97 ± 5.46^{b}	9.94 ± 1.60^{b}	22.33 ± 0.54^{b}	7.54 ± 0.35^{b}
90:10	1.24 ± 2.89^{c}	9.55 ± 0.67^{c}	21.92 ± 0.22^{c}	6.91 ± 0.01^{a}
85:15	1.34 ± 6.08^{d}	8.89 ± 1.22^{d}	21.24 ± 0.79^{d}	6.74 ± 0.09^{a}
80:20	1.47 ± 0.69^{e}	8.61 ± 1.43^{d}	20.09 ± 1.20^{e}	6.30 ± 0.48^{a}
Thulamahashe				

Control	0.43 ± 1.18[a]	10.4 ± 1.60[a]	24.81 ± 0.79[a]	9.99 ± 0.31[a]
95:5	0.92 ± 1.42[b]	8.58 ± 1.96[b]	23.99 ± 1.92[b]	7.33 ± 0.58[b]
90:10	1.24 ± 6.64[c]	8.44 ± 1.43[c]	22.61 ± 0.84[c]	7.09 ± 1.50[b]
85:15	1.35 ± 1.51[d]	8.21 ± 0.66[d]	20.39 ± 1.06[d]	6.46 ± 0.67[c]
80:20	1.54 ± 0.33[e]	8.09 ± 0.61[e]	19.22 ± 0.85[e]	6.37 ± 0.29[d]
Phalaborwa				
Control	0.43 ± 1.18[a]	10.4 ± 1.60[a]	24.81 ± 0.79[a]	9.99 ± 0.31[a]
95:5	0.80 ± 1.28[b]	8.68 ± 1.96[b]	23.75 ± 1.92[b]	7.88 ± 1.00[b]
90:10	0.91 ± 1.82[c]	8.31 ± 1.43[c]	22.45 ± 0.84[c]	7.67 ± 1.05[c]
85:15	1.02 ± 1.65[d]	8.22 ± 0.66[d]	21.27 ± 1.06[d]	7.21 ± 0.25[d]
80:20	1.43 ± 2.69[e]	8.08 ± 0.61[e]	20.01 ± 0.85[e]	7.04 ± 0.09[e]

Values are mean ± standard deviation. Control= 100% wheat flour, 95:5= 95:5% wheat-marula peel composite biscuits, 90:10= 90:10% wheat-marula peel composite biscuits, 85:15= 85:15% wheat-marula peel composite biscuits and 80:20= 80:20% wheat-marula peel composite biscuits.

The fat content of the biscuits decreased significantly ($p<0.05$) with the inclusion of marula peel flour, irrespective of the location. The decreasing trend observed as the levels of marula flour incorporation might be due to lower fat content in marula peel flour as compared to wheat flour. Marula peel has low crude lipid contents (2.42%), the value is lower than the 8.33% reported in the peels of Hasta la pasta fruit (Hassan *et al.,* 2009). Thongram *et al*. (2016) stated that adding fats like butter to the dough when making biscuits enhances the product's overall texture and quality. Egwujeh *et al*. (2018) reported that wheat biscuits had a 22.1% fat content in their study. The results of this study's analysis of the fat content of wheat-marula peel biscuits are comparable to those of biscuits made with 5% defatted mustard flour (Zbikowska *et al.,* 2022)

As compared to the control sample, the moisture content of the composite biscuits was significantly lower ($p<0.05$) and decreased significantly with the increasing levels of marula peel flour. The moisture content of all biscuits was within the recommended range of 0 to 10% for the storage of biscuits (Ranjana *et al.,* 2000). The amount of moisture in any food product impacts its general suitability for storage, packaging, and transportation (Man *et al*., 2021). The water added to baked goods acts as a channel for the physicochemical reactions that transform raw ingredients into baked goods (Klunklin and Savage, 2018).

6.7.2. Colour attributes of wheat-marula composite biscuits

Colour attributes are a major component that affects the quality of the final product (Starowicz and Zieliński, 2019). Colour attributes (L*, a*, b*) of the biscuit samples are shown in Table 6.4. The L*, a* and b* values of the biscuits decreased significantly ($p < 0.05$) as the levels of marula peel flour inclusion increased, irrespective of the location. The L* value of the control biscuit sample was significantly higher than the composite biscuits. The decrease in the L* and a* values of the biscuits might be attributed to the formation of Maillard reactions, as marula peel contains glucose, fructose, sucrose, and protein (Banerjee *et al.,* 2018; Mahloko *et al.,* 2019). Furthermore, the decrease in the L* and a* values of composite biscuits might also be influenced by heat exposure during baking, which led to processes such as caramelisation and dextrinisation (Mahloko *et al.,* 2019). Previous studies have shown that biscuits supplemented with pineapple core flour had lower L* and hue angle values than the control (de Toledo *et al.,* 2017). On the other hand, the observed colour change in the b* value could be attributed to the browning effect of the marula peel. Different studies have shown that the darker colour might be attributed to high total polyphenols in the peels of fruits such as marula (Aboshora *et al.,* 2019; Ajila *et al.,* 2008).

Table 6.4. Colour attributes of wheat-marula composite biscuits.

Sample region	L*	a*	b*	Hue	Chroma	ΔE
ZZ2 (Moeketsi)						
Control	58.82 ± 0.06^{a}	13.53 ± 0.05^{a}	35.35 ± 0.49^{a}	69.17 ± 39.34^{a}	37.82 ± 0.45^{a}	0
95:5	51.16 ± 4.94^{b}	09.72 ± 3.94^{b}	20.54 ± 5.57^{b}	64.68 ± 3.18^{b}	22.72 ± 6.72^{b}	17.09 ± 0.94^{a}
90:10	50.04 ± 7.61^{b}	10.29 ± 2.89^{c}	22.03 ± 1.77^{c}	64.96 ± 4.77^{c}	24.31 ± 2.86^{c}	17.26 ± 1.61^{a}
85:15	47.19 ± 2.77^{c}	10.92 ± 2.66^{c}	22.73 ± 3.42^{c}	64.34 ± 2.35^{c}	25.22 ± 4.22^{d}	17.35 ± 1.77^{a}
80:20	43.64 ± 2.56^{d}	10.01 ± 0.36^{d}	26.88 ± 2.09^{d}	62.47 ± 1.32^{d}	30.31 ± 2.00^{e}	17.39 ± 1.56^{a}
Thulamahashe						
Control	58.82 ± 0.06^{a}	13.53 ± 0.05^{a}	35.35 ± 0.49^{a}	69.17 ± 39.34^{a}	37.82 ± 0.45^{a}	0
95:5	50.57 ± 5.75^{b}	12.31 ± 1.32^{b}	29.64 ± 1.54^{b}	65.19 ± 03.27^{b}	32.09 ± 0.85^{b}	10.09 ± 1.75^{a}
90:10	47.99 ± 2.67^{c}	12.79 ± 0.92^{b}	28.29 ± 0.98^{c}	65.67 ± 1.28^{d}	31.05 ± 1.17^{c}	12.94 ± 1.67^{b}
85:15	45.78 ± 2.29^{d}	13.03 ± 1.06^{c}	27.34 ± 1.46^{d}	64.52 ± 1.03^{c}	30.29 ± 1.71^{d}	15.31 ± 1.29^{c}

80:20	42.73 ± 3.46^{e}	13.23 ± 1.05^{c}	24.85 ± 1.72^{e}	61.43 ± 3.17^{d}	28.29 ± 1.27^{e}	19.21 ± 1.46^{d}
Phalaborwa						
Control	58.82 ± 0.06^{a}	13.53 ± 0.05^{a}	35.35 ± 0.49^{a}	69.17 ± 39.34^{a}	37.82 ± 0.45^{a}	0
95:5	45.72 ± 2.83^{b}	10.44 ± 1.29^{b}	27.03 ± 2.50^{b}	64.57 ± 1.83^{b}	30.23 ± 2.63^{b}	15.50 ± 1.83^{a}
90:10	42.80 ± 2.33^{c}	11.62 ± 1.25^{c}	24.87 ± 1.09^{c}	63.31 ± 1.09^{e}	27.45 ± 1.91^{c}	19.22 ± 1.33^{b}
85:15	41.77 ± 2.07^{d}	13.24 ± 0.64^{d}	21.86 ± 1.49^{d}	60.97 ± 1.56^{d}	25.56 ± 1.42^{d}	21.73 ± 1.07^{c}
80:20	39.63 ± 0.32^{e}	13.45 ± 0.13^{e}	18.81 ± 0.12^{e}	58.79 ± 0.42^{e}	21.51 ± 0.08^{e}	25.51 ± 0.92^{d}

Mean ± standard deviation. Different letters in the columns show significant differences ($p < 0.05$) in mean values. Control= 100% wheat flour, 95:5=95:5 % wheat-marula peel composite biscuits, 90:10% wheat-marula peel composite biscuits, 85:15% wheat-marula peel composite biscuits and 80:20% wheat-marula peel composite biscuits. L* =lightness; a* = redness; and b* = yellowness

All biscuits containing marula peel flour had significantly lower hue angles than the control sample. The hue angle values decreased as the marula peel flour content of the formulation increased, causing the biscuits trend to appear less yellow. The variation in hue angle might be due to the association between gelatinised flours and other compounds present in the marula peel formulation, potentially influencing the final colour of the biscuits (Mala *et al.,* 2024). The chroma* value decreased in composite biscuits with the incorporation of marula peel flour; this trend could be explained by both chroma*, and H° being dependent on a* and b* values.

The ΔE values, representing the total colour difference of composite biscuits compared to the control, significantly increased with the increase in the levels of marula peel flour incorporation. A high ΔE value indicates a significant perceptual difference between the colour of composite and control biscuits. This variation might be due to factors of hue, chroma, and lightness as well as ingredient compositions and the development of red pigmentation caused by the Maillard reaction (Pereira *et al.,* 2013). On the other hand, values greater than 3 suggest an obvious colour difference for the human eye (Silva *et al.,* 2018). The inclusion of marula peel flour showed a slight difference in colour in the control biscuit sample. In terms of a*values, all the wheat-marula peel composite biscuits from all three (3) sample regions were significantly lower than that of the control sample. The a* values of the marula biscuit were significantly higher in the region ZZ2 as it was ranging from 13.53 to 14.01. The marula biscuit's a* values ranged from 13.23 to 13.53 in the Thulamahashe and Phalaborwa ranged from 13.45 to 13.53, which was significantly higher. The chroma

values of ZZ2, Phalaborwa, and Thulamahashe varied significantly ($p < 0.05$) from 22.72 to 30.31, 28.29 to 32.09, and 21.51 to 30.23 with the addition of marula peel flour, respectively. The yellowness b* of ZZ2, Phalaborwa, and Thulamahashe biscuits ranged from 20.54 to 26.88,24.85 to 29.64 and 18.81 to 27.03, respectively. In all the samples, the lowest b* was obtained at Phalaborwa.

6.7.3. Physical properties of wheat-marula composite biscuits

Table 6.5 shows the physical properties such as texture, thickness, diameter, and spread ratio of biscuits. The hardness of the biscuit samples ranged from 11.85 to 18.63 N and increased significantly ($p<0.05$) when marula peel flour was added, irrespective of location. According to Zhang *et al.* (2021), the increase in hardness of composite biscuits could be related to starch composition and starch-protein interactions. Moreover, the inclusion of marula peel flour might also have reduced the gluten protein in the dough, further affecting the generation of the gluten network structure (Jia *et al.,* 2020). Gluten stimulates the development of the network by attracting water molecules. Mahloko *et al.* (2019) reported similar results where the inclusion of prickly pear and banana peel flour increased the hardness of biscuits.

The diameters of the biscuits decreased with the inclusion of marula peel flour, irrespective of the location. The findings revealed significant differences between composite and control biscuits.

Table 6.5. Texture and physical properties of wheat-marula composite biscuits.

Sample region	Hardness (N)	Diameter (cm)	Thickness (cm)	Mass (g)	Spread ratio
ZZ2 (Moeketsi)					
Control	11.86 ± 1.01^{a}	5.50 ± 1.23^{a}	0.83 ± 0.02^{a}	12.71 ± 1.06^{a}	7.93 ± 1.52^{a}
95:5	13.93 ± 1.54^{b}	5.01 ± 1.19^{b}	0.72 ± 1.02^{b}	12.26 ± 1.22^{b}	7.56 ± 0.12^{a}
90:10	15.63 ± 1.23^{c}	5.03 ± 1.01^{b}	0.66 ± 0.28^{c}	11.92 ± 2.92^{c}	7.48 ± 0.02^{a}
85:15	17.45 ± 1.76^{d}	4.90 ± 1.14^{c}	0.61 ± 0.43^{c}	11.71 ± 3.20^{c}	7.33 ± 0.22^{a}
80:20	18.63 ± 1.00^{e}	4.60 ± 1.01^{c}	0.55 ± 1.22^{d}	10.87 ± 1.01^{d}	7.17 ± 0.02^{b}
Thulamahashe					
Control	11.86 ± 1.01^{a}	5.50 ± 1.23^{a}	0.83 ± 0.02^{a}	12.71 ± 1.06^{a}	7.93 ± 1.52^{a}
95:5	12.16 ± 1.02^{b}	4.87 ± 1.07^{b}	0.63 ± 0.18^{b}	11.91 ± 2.26^{b}	7.63 ± 0.34^{a}
90:10	13.89 ± 1.51^{c}	4.67 ± 1.41^{c}	0.56 ± 0.12^{c}	11.44 ± 3.72^{b}	7.34 ± 0.21^{a}
85:15	16.26 ± 1.36^{d}	4.70 ± 1.11^{c}	0.53 ± 0.18^{c}	11.09 ± 4.02^{b}	7.27 ± 0.52^{a}
80:20	18.19 ± 1.24^{e}	4.53 ± 1.39^{d}	0.49 ± 0.02^{d}	11.18 ± 4.32^{b}	7.14 ± 0.11^{b}
Phalaborwa					
Control	11.86 ± 1.01^{a}	5.50 ± 1.23^{a}	0.83 ± 0.02^{a}	12.71 ± 1.06^{a}	7.93 ± 1.52^{a}

95:5	12.69 ± 1.04[b]	4.93 ± 1.03[b]	0.63 ± 1.02[b]	11.67 ± 2.11[b]	7.83 ± 0.23[a]
90:10	14.64 ± 1.49[c]	4.64 ± 1.21[c]	0.59 ± 0.28[c]	11.42 ± 2.49[b]	7.66 ± 0.32[a]
85:15	16.02 ± 1.31[d]	4.67 ± 1.09[c]	0.52 ± 0.43[c]	11.23 ± 2.71[b]	7.56 ± 0.16[a]
80:20	18.22 ± 1.02[e]	4.46 ± 1.11[d]	0.50 ± 1.22[c]	10.81 ± 3.54[c]	7.32 ± 0.09[b]

Values are mean ± standard deviation. Control= 100% wheat flour, 95:5= 95:5% wheat-marula peel composite biscuits, 90:10= 90:10% wheat-marula peel composite biscuits, 85:15= 85:15% wheat-marula peel composite biscuits and 80:20= 80:20% wheat-marula peel composite biscuits.

The addition of marula peel flour resulted in a decrease in gluten content due to the presence of fibre raw material, which disrupted the formation of gluten due to the fibre content inherent in the marula peel (Bakar *et al.,* 2022). According to Thongram *et al.* (2016), protein content and diameter have an inverse relationship; when the dough is exposed to heat, the gluten manages to gain mobility and can prevent the flow of biscuit dough, and starch polymer molecules are more tightly bound with granules.

The thickness and mass of the biscuit samples varied significantly across all locations. With the addition of marula peel flour, the thickness of the biscuit samples and mass decreased from 0.83 to 0.49 cm and 12.71 to 10.81 g, respectively. This weight reduction could be due to the inclusion of marula peel flour, which is lighter in weight than wheat flour (Oyet and Chibor 2020). Arepally *et al.* (2020) indicated that weight reduction could be due to an increase in the number of available water-soluble sites competing for the limited free water in biscuit dough. These findings were like those reported by Kurchatov *et al.* (2018), who found that adding grape seed flour to biscuits reduced their weight.

When adding marula peel flour to the biscuit samples, the spread ratio decreased. The observed decrease in the spread ratio has been thought to be due to gluten dilution and a decrease in the amount of water available for gluten hydration (Peng *et al.* 2022). The decrease in the biscuit spread ratio with the addition of marula peel flour suggests that the starches in these biscuit samples are very soluble in water (Thongram *et al.,* 2016). Dough viscosity limits the spread ratio because the lower viscosity of dough causes biscuits to spread faster (Peter-Ikechukwu *et al*. 2018). A study conducted by Manaf *et al*. (2019) obtained similar findings, which were 7.74.

6.7.4. Antioxidant properties of wheat-marula peel composite biscuits

The antioxidant properties of the control and composite biscuits are presented in Table 6.6. Results show that composite biscuits exhibited significantly higher ($p \leq 0.05$)

DPPH and FRAP values compared to the control biscuits. The generation of melanoidins during baking might be responsible for the increase in DPPH and FRAP values since they have antioxidant capacity (Sharma and Gujral, 2014). Moreover, high DPPH values might also be due to high TPC and TFC because of the inclusion of peel flour since it is rich in flavonoids and polyphenols. Higher DPPH and FRAP values are associated with stronger antioxidant activity, while lower values are related to a weaker antioxidant activity (Fatemeh *et al.,* 2012). The FRAP of the control sample measured in this study was 294.38 mg GAE/g, which was significantly lower than that reported in the study by Saeed *et al*. (2022), which was 340 mg/ml for a biscuit sample with 100% rice flour. The FRAP assay (831.33100 mg GAE/g) indicates that the composite biscuits contain high levels of protein and amino acids such as leucine, lysine, and methionine (Adegoke *et al*., 2017). Similar results of an increase in DPPH and FRAP were reported by Ramashia *et al.* (2021) for biscuits added with Parinari *curatellifolia* peel flour.

Table 6.6. Antioxidant properties of wheat-marula peel composite biscuit.

Sample region	DPPH (%)	TPC (mg/100g)	TFC (mg/100g)	Vitamin C (mg/100g)	FRAP (100mg GAE/g)
ZZ2 (Moeketsi)					
Control	67.36 ± 5.98^{a}	3.60 ± 1.23^{a}	25.55 ±1.18^{a}	77.49 ± 0.01^{a}	294.38 ± 29.89^{a}
95:5	79.64 ± 1.85^{b}	6.62 ± 0.54^{b}	28.94 ± 5.46^{b}	84.54 ± 0.00^{b}	564.70 ± 21.26^{b}
90:10	80.08 ± 5.63^{c}	6.75 ± 2.72^{b}	30.61 ± 2.89^{c}	91.53 ± 0.01^{c}	571.74 ± 41.34^{b}
85:15	84.81 ± 4.79^{d}	7.03 ± 0.64^{c}	31.88 ± 6.08^{d}	105.67 ±0.05^{d}	689.15 ± 112.66^{c}
80:20	87.94 ± 1.45^{e}	7.08 ± 1.92^{c}	32.58 ± 0.69^{e}	112.72± 0.01^{e}	799.19 ± 25.00^{d}
Thulamahashe					
Control	67.36 ± 5.98^{a}	3.60 ± 1.23^{a}	25.55 ± 1.18^{a}	77.49 ± 0.01^{a}	294.38 ± 29.89^{a}
95:5	71.05 ± 2.40^{b}	5.36 ± 1.22^{b}	27.45 ± 1.42^{b}	80.45 ± 0.21^{b}	551.22 ± 171.81^{b}
90:10	81.02 ± 0.32^{c}	6.09 ± 0.43^{c}	29.15 ± 6.64^{c}	90.86 ± 0.01^{c}	590.85 ± 121.99^{c}
85:15	85.64 ±10.89^{d}	7.54 ± 0.28^{d}	31.51 ± 1.51^{d}	101.79 ± 0.02^{d}	691.04 ± 128.57^{d}
80:20	89.52 ±11.47^{e}	7.91 ± 1.02^{e}	33.45 ± 0.33^{e}	109.43 ± 0.01^{e}	759.80 ± 135.23^{e}
Phalaborwa					
Control	67.36 ± 5.98^{a}	3.60 ± 1.23^{a}	25.55 ±1.18^{a}	77.49 ± 0.01^{a}	294.38 ± 29.89^{a}
95:5	72.16 ± 0.70^{b}	5.66 ± 0.02^{b}	28.45 ± 1.28^{b}	82.97 ± 0.01^{b}	506.19 ± 118.74^{b}
90:10	82.12 ± 4.05^{c}	6.77 ± 0.13^{c}	31.89 ± 1.82^{c}	95.43 ± 0.01^{c}	751.48 ± 150.17^{d}

85:15	84.47 ± 6.19^{d}	7.03 ± 0.12^{d}	32.58 ± 1.65^{d}	104.52 ± 0.02^{d}	790.78 ± 159.84^{d}
80:20	88.60 ± 7.62^{e}	7.76 ± 0.18^{e}	34.22 ± 2.69^{ae}	111.94 ± 0.01^{e}	831.33 ± 165.03^{d}

Different superscripts in the same column are significantly different (p>0.05). Control= 100% wheat flour, 5:5% wheat-marula peel composite biscuits, 90:10% wheat-marula peel composite biscuits, 85:15% wheat-marula peel composite biscuits, and 80:20% wheat-marula peel composite biscuits. TPC= total phenolic content, TFC= total flavonoids content, FRAP= ferric reducing antioxidant power

The incorporation of marula peel flour at different levels resulted in a significant improvement ($p<0.05$) in the TPC of the biscuits. The increase in the TPC of the composite biscuits might be associated with more bound phenolic acid from the release of cellular components during the baking process. It has been shown that phenolic compounds play a major role in the food's sensory qualities, including colour, flavor, and taste, as well as the total antioxidant value of foods derived from plants (Massini *et al.* 2018). Similarly, Nakov *et al*. (2020) found that the addition of white grape pomace increased TPC values of the biscuits. Moreover, this finding agrees with Toledo *et al.* (2019), wherein the inclusion of fruit by-product powder improved the TPC of the biscuits. Significantly higher TFC values were observed in the biscuits with marula peel flour incorporation compared to the control biscuits ($p\leq0.05$). The observed variations in TFC values may be due to the formation of brown-coloured pigments known as melanoidin's, which are byproducts of the Maillard reaction that takes place during the baking of biscuits (Saeed *et al*., 2022). Ahmed *et al*. (2022) found results with comparable trends for biscuits produced with wheat flour with 2% plant powder, which had a 28.18 mg/100 g concentration of TFC.

It was found that the marula peel flour substituted biscuits exhibited significantly higher ($p \leq 0.05$) vitamin C compared to the control biscuits. This was anticipated since marula fruit is rich in vitamin C and it is high in marula pulp ranging from 62 mg/100 g to above 400 mg/100 g in the fresh fruit (Mashau *et al.*, 2022). Due to the high vitamin C content, composite biscuits could act as an alternative source of vitamin C for individuals seeking to variegate from market-driven bakery products. Vitamin C is a less toxic antioxidant compound with a recommended daily intake of about 60 mg per day (Mapunda and Mligo, 2019).

6.7.5. Fourier-Transform Infrared Spectra of wheat-marula composite biscuits

The FTIR (Fourier-Transform Infrared Spectra) regions (O-H stretch region, C-H stretch region, and fingerprint region) of wheat-marula peel composite biscuits are

shown in Fig. 6.4. The O-H region of wheat-marula peel composite biscuits illustrates bands (peaks) ranging from 3267.78 to 3249,01 and this could be due to stretching vibrations of the O-H bond. Differences in peaks observed in this region could be due to the different moisture content observed in this study. According to a study conducted by Mudau *et al.* (2022), the moisture content of the sample has an impact on the peaks that occur in the O-H region. The absorption peaks in the C-H stretch region of wheat-marula peel composite biscuits ranged from 2894.22 to 2925.42 cm^{-1} and this was probably due to the stretching vibrations of aromatic and aliphatic C–H bonds (Adebiyi*et al*., 2017).

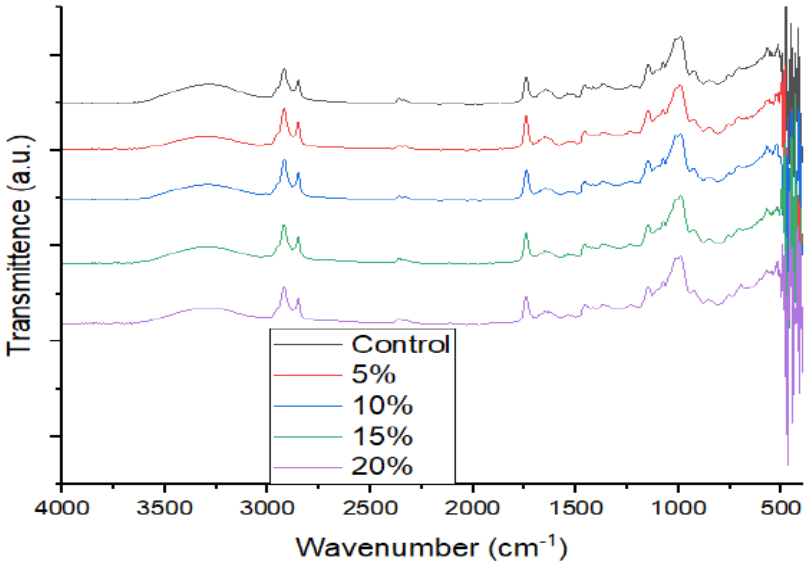


Fig 6.4. FTIR spectra of marula flour from ZZ2. Control= 100% wheat flour, 5%= 95:5% wheat-marula peel composite biscuits, 10%= 90:10% wheat-marula peel composite biscuits, 15%= 85:15% wheat-marula peel composite biscuits, and 20%= 80:20% wheat-marula peel composite biscuits.

The FTIR spectra are primarily influenced by the properties of bioactive compound profiles (Arslan *et al*. 2019), Fig. 6.5. The C-H bond peaks were detected in biscuits at 9541.26 to 1096.58 cm^{-1}. The FTIR spectra shows that peaks in the 1500 to 200 cm^{-1}range are almost similar. The spectral peaks obtained at different wavelengths of the biscuit samples are nearly identical, indicating that the fat did not change during the baking process (Pattnaik and Mishra, 2022).

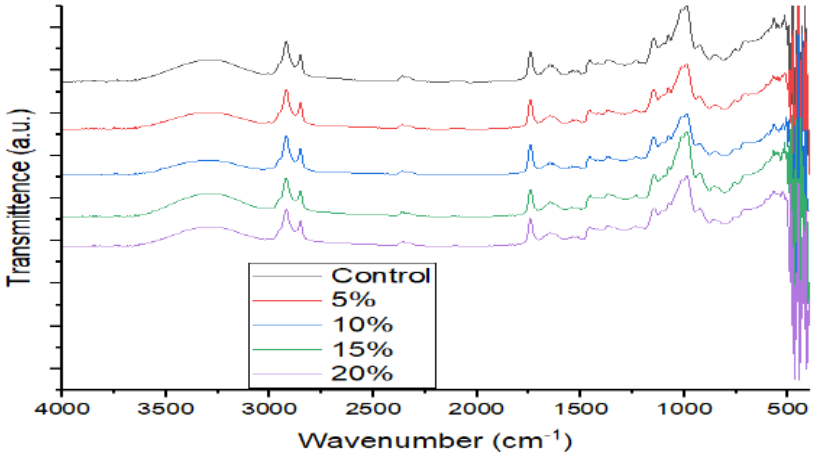


Fig 6.5. FTIR spectra of marula flour from Thulamahashe. Control= 100% wheat flour, 5%= 95:5% wheat- marula peel composite biscuits, 10%= 90:10% wheat-marula peel composite biscuits, 15%= 85:15% wheat-marula peel composite biscuits and 20%= 80:20% wheat-marula peel composite biscuits.

The C-H bond peaks were detected in biscuits at 9541.26 to 1096.58 cm^{-1}. The FTIR spectra in Figure 6.6 shows that peaks in the 1500 to 200 cm^{-1} range are almost similar. The spectral peaks obtained at different wavelengths of the biscuit samples are nearly identical, indicating that the fat did not change during the baking process (Pattnaik and Mishra, 2022).

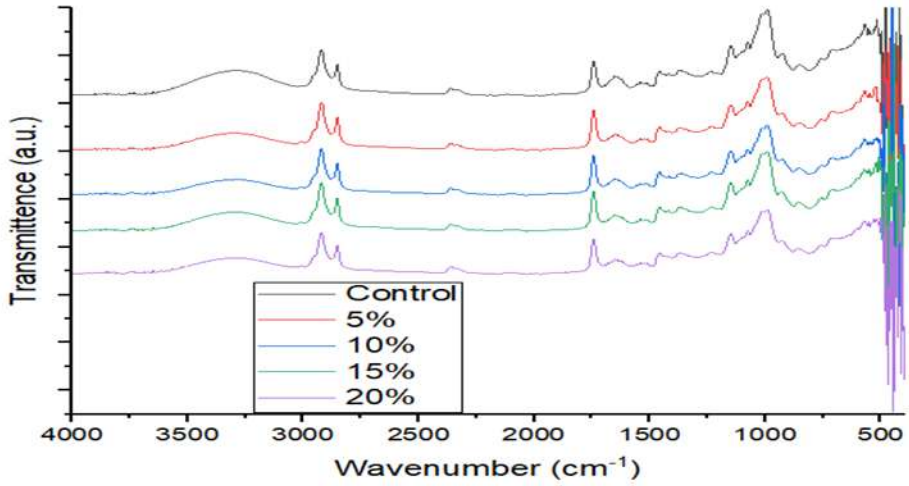


Fig 6.6. FTIR spectra of marula flour from Phalaborwa. Control= 100% wheat flour, 5%= 95:5% wheat-marula peel composite biscuits, 10%= 90:10% wheat-marula peel composite biscuits, 15%= 85:15% wheat-marula peel composite biscuits and 20%= 80:20% wheat-marula peel composite biscuits.

6.7.6. Conclusion and Recommendation

The study highlights the potential of marula peel flour as a valuable ingredient in producing nutritional and antioxidant-enhanced biscuits. The incorporation of marula peel flour in biscuits enriches ash content, TPC, TFC, vitamin C and antioxidant activity. The inclusion of marula peel flour shows significantly increased hardness while decreasing protein, fat, thickness, mass, spread ratio, and colour compared to the control. Further research on the effect of marula peel flour on the minerals, fatty acid profile, microbial quality, and morphology of the biscuits must be encouraged.

CHAPTER 7

BY-PRODUCTS AS FEED FOR PEN-FED LAMBS (EFFECT OF REPLACING MAIZE MEAL WITH DRIED MARULA FRUIT SKIN ON APPARENT DIGESTIBILITY, GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF SOUTH AFRICAN MUTTON MERINO LAMBS)

7.1. Summary

The purpose of this study was to determine the effect of location and drying methods on the nutrient composition of dried marula fruit peels and apparent digestibility, and growth performance as well as carcass characteristics of South African mutton merino lambs fed different levels of dried marula fruit peels. Marula (*Sclerocarya birrea*) is a medium-sized tree belonging to the *Brachystegia* genus. Several experiments were conducted to evaluate the chemical composition of dried marula fruit peels where the dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), and gross energy (GE) were determined. All the data in this experiment were subjected to analysis of variance for a 2 x 2 factorial in a completely randomised design using a GLM procedure of Minitab 19 (Minitab, 2019). Tukey's studentised multiple range test determined statistically significant differences among the means. The dry matter of fresh marula fruit peels has shown a significant difference ($p<0.01$).

The dry matter of fresh marula fruit peels from Tzaneen using freeze and oven-drying methods was higher than those from Phalaborwa. The dry matter, crude protein, ash, ether extract, acid detergent lignin, and gross energy contents of dried marula fruit peels for both locations and drying methods were not significantly different ($p>0.05$). The crude fibre, acid detergent fibre, and nitrogen detergent fibre results for both location and drying methods were significantly different ($p<0.01$). The crude fibre and nitrogen detergent fibre from Tzaneen were found to be higher than those from Phalaborwa when a freeze-drying method was used. However, when using an oven-

drying method, the crude fibre, nitrogen detergent fibre, and acid detergent lignin contents were higher in Phalaborwa.

The apparent digestibility of DM, CP, and OM of the diet was determined in a completely randomised design and the means were compared using a Tuckey method at a 95% confidence level. The experiment was conducted in the last ten days of growth trial. A total of 9 male South African mutton merino sheep of approximately 35kg of weight were used in this experiment. The results revealed that the inclusion levels of dried marula fruit peels in the animal diet did not affect ($p>0.05$) the CP intake. However, a significant difference ($p<0.01$) was observed in the OM intake by the lambs. Including dried marula fruit peels in the diet did not affect ($p>0.05$) the faecal excretion of nutrients by lambs. A non-significance difference ($p>0.05$) was observed in the digestibility of nutrients.

The determination of growth performance and carcass characteristics of the lambs fed different levels of dried marula fruit peels was conducted, where the average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and hot carcass weight, cold carcass weight, dressing percentage were measured respectively. A completely randomised design was used in this experiment with seven animals per treatment. Before the trial, the lambs were vaccinated against brucellosis, pulpy kidneys and treated for internal parasites. The inclusion levels of dried marula fruit peels had no significant impact ($p>0.05$) on the growth performance and carcass characteristics. This research has revealed that dried marula fruit peels can be used as a potential energy source for lambs without negatively affecting the growth and carcass characteristics of lambs at up to 10% inclusion level.

Keywords: By-products, Growth performance, Apparent digestibility, Carcass characteristics, Chemical composition, South African merino lambs, Dried marula fruit peels.

7.2. Introduction

The most used energy source for animals in South Africa and some other parts of Africa is maize and some of the alternative energy sources that have been used in animal nutrition are citrus pulp and popcorn waste (Nkosi et al, 2010; Oladejo et al,

2012; Steyn *et al.,* 2017). According to Nkosi *et al.* (2010) approximately 66% of maize produced around the world is used in animal nutrition while 25% is for human consumption. Due to the increase in population and rise in competition for maize between livestock and humans, it is important to look for an alternative energy source for animals. The amount of nutrients that an animal receives determines how well it will grow and develop, which is balanced energy and protein in their feed (Sayed, 2009; White and Hegarty, 2014). Energy is the most limiting factor in sheep, energy deficiency may result in reduced conception rate, lambing rate, milk production, and rate of gain and in severe situations it can result in loss of weight and death (Johnson, 1997). Energy and protein are the major dietary elements that affect growth performance, quantity of carcasses, and sheep productivity (Sayed, 2009). A significant amount of the marula fruit waste produced during the production of marula beer is found in the Lowveld of the province of Limpopo. Some of these wastes are consumed by ruminants at the disposal locations, of most of the waste remains unutilized, which poses a risk of land pollution. The marula fruit contains a significant amount of carbohydrates roughly 14% (Dube *et al.,* 2012). Fruit waste has a high energy content, making it a viable alternative to maize meals for ruminants, particularly in feedlots. Thus, this study's objective was to evaluate the use of dried marula fruit peels for feedlot lambs' consumption as an energy source.

7.3. Materials and Methods

7.3.1. Description of the study area

The agriculture experimental farm served as the study's location (22° 58' 32" S, 30° 26' 45" E; Altitude of 596 above sea level), and the samples were analysed in the animal science nutrition laboratory at the University of Venda, Thohoyandou. The daily temperatures at Thohoyandou range from a minimum of 18°C to a maximum of about 31°C (Rambau *et al.,* 2016). The area receives an average annual rainfall of ±500 mm that falls predominantly in summer.

7.3.2. Sample collection and preparation

Marula fruit peels were collected from Phalaborwa and Tzaneen from people who brew marula beer. A subsample from the samples collected from the abovementioned areas was dried using two (2) different drying methods, freeze-drying, and oven-

drying, before chemical composition analysis in the animal science and biochemistry lab at the University of Venda, Thohoyandou.

7.4. Chemical composition determination

7.4.1. Nutrient analysis

7.4.1.1. Determination of dry matter

The Association of Official Analytical Chemists' method was used in determining the DM (AOAC, 2000 method 934.01). Then, 5 g of fresh marula fruit peels were dried using an oven at 60°C until constant weight was achieved. The peels were ground using a Retsch ZM 200 grinder to pass through a 1 mm sieve for chemical analysis. The DM was calculated by dividing the mass of the crucible containing the dried samples by the mass of the crucible containing fresh samples multiplied by 100, equation 7.1

$$\% \text{ DM} = \frac{mass\ of\ dried\ sample}{mass\ of\ fresh\ samples}\ x \quad \text{.....7.1}$$

7.4.1.2. Determination of ash content and calculation of OM

According to the Association of Official Analytical Chemists (AOAC, 2000 official method 942.05), The sample was ignited at 500°C for six hours in a muffle furnace to determine the ash content. Two (2) hrs were given for the samples from the furnace to cool. The crucibles were then placed in the desiccator for 30 min. Ash content was calculated as follows, equation 7.2.

$$\text{Ash } \% = \frac{weight\ of\ test\ portion(g) - weight\ loss\ on\ ashing(g)}{weight\ of\ the\ test\ portion(g)} \text{ x } 100 \quad \text{.....7.2}$$

Then OM was calculated from the ash content of the sample as follows:
% Organic material on DM basis = 100 - % Ash on DM basis

7.4.1.3. Determination of ether extract (EE)

The EE was determined according to the Association of Official Analytical Chemists (AOAC, 2002, method 920.39) using the VELP® SCIENTIFICA SER 148 Solvent Extractor. An extraction vessel was placed in an oven overnight at 100°C. it was then cooled for 30 min in a desiccator, and its weight was recorded. Then, 2 g of sample was weighed and transferred to the extraction thimble. A small, defatted cotton wool was inserted in a thimble to keep the sample in place during the extraction. After the extraction unit has completed extracting fat, and ether has been evaporated, the extraction vessels were placed in an oven for 2 hr at 100°C. After 2 hr, the extraction vessels were placed in a desiccator for 30 min, and weight was taken. Then, 10 mL diethyl ether was added to the used cup to remove fat. The % EE was calculated as follows, using equation 7.3.

$$\%EE = \frac{Weight\ of\ the\ extraction\ vessel\ with\ fat + Weight\ of\ empty\ vessel}{Wt\ of\ the\ sample} x\ 100 \quad \text{.....7.3.}$$

7.4.1.4. Determination of crude protein

The nitrogen content of marula fruit peels was determined using the Dumas method with a LECO FP 528 based on AOAC, 2002, method 984.13). The sample was combusted at a high temperature of about 900°C in the presence of oxygen. The instrument was first calibrated by analysing a pure material with a known nitrogen concentration. The sample of 0.1000 g dried marula fruit peels was weighed and placed in a foil.

The foil was folded with a sample, and the weight was taken. The foil containing the sample was then placed in a carousel for a Leco instrument to analyse the sample automatically. Once the analysis was complete, the system was switched off and all three gas bottles were closed.
%percentage crude protein was calculated as follows using equation 7.4.

%Crude Protein = %Nitrogen x Protein conversion factor (6.25). ……7.4

7.4.1.5. Determination of crude fibre (CF)

The CF was determined according to AOAC (2000, method 978.10). Samples were boiled first with 1.25% sulphuric acid (H_2SO_4) and subsequently with 1.25% sodium hydroxide (NaOH) for 30 min. The residues containing crude fibre were dried to a constant weight, and the dried residues were then ignited in the muffle furnace. The crude fibre content was estimated as follows using equation 7.5.

$$\%\text{Crude Fibre} = \frac{pre-ash\ weight - post-ash}{weight\ of\ the\ sample}\ x\ 100 \quad \text{.....7.5}$$

7.4.1.6. Determination of neutral detergent fibre (NDF)

The Determination of NDF was done according to the method of Robertson and van Soest (1981) (Method 968.06). Then, 1 g of dried sample was dried and ground to pass through a 1mm sieve. The samples were transferred into a 500 ml beaker containing an NDF solution and 0.5 g sodium sulfide and heated on a hot plate. After 1 hour of heating, the sample mixture was allowed to cool for 10 minutes, and samples were filtered using hot water to remove residues. Following collection of the samples into a crucible, they were put in the oven for six hours at 105°C.

7.4.1.7. Determination of acid detergent fibre (ADF)

The ADF was determined according to the method of (AOAC, 2000 Method 968.06). Samples of marula fruit peels were digested in a pepsin-acid solution and acid detergent solution.

Determination of Acid Detergent lignin (ADL)

The ADL was determined according to the method of (Goering and Van Soest, 1970). Samples of marula fruit peels were subjected to the ADF procedure, and the residues were further digested in sulphuric acid.

7.4.1.8. Gross energy (GE)

The GE was determined by ignition of the sample using a Bomb MC-1000 modular Calorimeter.

7.4.2. Experimental design

A 2 x 2 factorial design was used, where 2 represents the sample sites (Phalaborwa and Tzaneen), and the other 2 represents the drying methods (Freeze and oven drying).

7.4.3. Data analysis

All the data in this experiment were subjected to analysis of variance for a 2x2 factorial in a completely randomised design using a GLM procedure of Minitab 19 (Minitab, 2019). Tukey's studentised multiple range test determined statistically significant differences among the means at a 95% significance level.

The statistical model used was as follows, equation 7.6.

$$Yij = \mu + \alpha i + Gj + (\alpha G) ij + eij \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 7.6$$

Where yij = is the observations: OM, DM, CP, NDF, ADF, ADL, Fat, Ash,

μ= overall mean
αi = the effect of sample sites
Gj = effect of the drying methods
(α G) ij = sample sites x drying methods interaction
eij= random error

7.4.4. Results

Table 7.1 shows the results of the chemical composition of marula fruit peels of the interactions between locations (Phalaborwa and Tzaneen) and drying methods (oven and freeze-drying method). The dry matter of fresh marula fruit peels has shown a significant difference ($p<0.01$). The dry matter of fresh marula fruit peels from Tzaneen using freeze and oven-drying methods was higher than those from Phalaborwa. The dry matter, crude protein, ash, ether extract, acid detergent lignin, and gross energy contents of dried marula fruit peels for both locations and drying methods were not significantly different ($p>0.05$). The crude fibre, Acid detergent fibre, and nitrogen detergent fibre results for both location and drying methods were significantly different ($p<0.01$). The crude fibre and nitrogen detergent fibre from Tzaneen was found to be higher than those from Phalaborwa when a freeze-drying method was used. However, when using an oven-drying method, the crude fibre, nitrogen detergent fibre, and acid detergent lignin contents were higher in Phalaborwa.

Table 7.1. Chemical composition analysis (%) of marula fruit peels from two locations (Phalaborwa and Tzaneen) using two drying methods (Oven and Freeze-drying method).

Location	Drying Method	DM (fresh peels)	DM (dried peels)	ASH (dried peels)	CP (dried peels)	EE (dried peels)	CF (dried peels)	ADF (dried peels)	NDF (dried peels)	ADL (dried peels)	ENEG (Mj/100g)
TZN	FM	38.37^{b}	89.95^{a}	8.43^{a}	4.28^{a}	2.44^{a}	18.13^{b}	22.30^{d}	25.64^{b}	5.63^{b}	1.431^{a}
	OM	49.81^{a}	88.89^{a}	7.24^{a}	4.06^{a}	1.91^{a}	18.88^{b}	28.61^{b}	26.29^{b}	7.10^{ab}	1.442^{a}
PLB	FM	35.99^{b}	88.91^{a}	8.21^{a}	4.30^{a}	1.93^{a}	17.57^{b}	25.43^{c}	24.98^{b}	7.41^{ab}	1.442^{a}
	OM	35.14^{b}	85.54^{a}	9.12^{a}	4.09^{a}	2.35^{a}	20.01^{a}	30.65^{a}	28.03^{a}	9.11^{a}	1.421^{a}
SEM		0.640	2.00	0.368	0.794	0.243	0.440	0.421	0.709	1.090	8.440
Location Means											
TZN		44.09^{a}	89.42^{a}	7.83^{a}	4.17^{a}	2.17^{a}	18.00^{b}	25.45^{b}	25.96^{b}	5.37^{b}	1.437^{a}
PLB		35.56^{b}	87.22^{a}	8.66^{a}	4.44^{a}	2.14^{b}	19.59^{a}	33.04^{a}	31.98^{a}	9.22^{a}	1.421^{a}
SEM		0.452	1.550	0.260	0.562	0.172	0.311	0.298	0.501	0.773	5.970
Drying method means											
FM		37.18^{b}	89.42^{a}	8.32^{a}	4.29^{a}	2.18^{a}	17.45^{b}	23.87^{b}	25.31^{b}	5.52^{b}	1.426^{a}
OM		42.48^{a}	87.22^{a}	8.18^{a}	4.07^{a}	2.13^{a}	19.75^{a}	34.63^{a}	32.64^{a}	9.06^{a}	1.432^{a}
SEM		0.452	1.550	0.260	0.562	0.172	0.311	0.298	0.501	0.773	5.970
Significance											
Location(L)		*	Ns	Ns	Ns	Ns	*	**	**	*	Ns
Drying methods(D)		*	Ns	Ns	Ns	Ns	*	**	**	*	Ns
LXD		*	Ns	Ns	Ns	Ns	**	**	**	Ns	Ns

*: significantly different (P<0.05); **: significantly different (p<0.01) ; Ns: non-significant (P>0.05) ; FM: Freeze-drying method; OM: Oven-drying method; TZN: Tzaneen ; PLB: Phalaborwa ; SEM: standard error means; DXL: Location X Drying methods; DM: Dry Matter; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber; ADL: Acid Detergent Lignin

7.4.4. Discussion

The DM content of fresh marula fruit peels from this study ranged between 35 and 45g. However, Makharamedzha (2017) reported the DM of marula peels to be 16 g/kg, which was lower than the DM found in this study. The difference in these DM results could be the locations where the peels were found. There was similar CP and EE content of dried marula fruit peels for both the locations and drying methods. Alnaimy *et al.* (2017) reported a CP of 6.9% and EE of 3.8% of dried citrus pulp, which was higher than the CP and EE content of dried marula fruit peels. The difference in the CP and EE content could be the difference in plant species, weather, and location where the plants are found. ARC (2020) results show that different locations may

receive different annual rainfall; hence, the annual rainfall received in 2020 in Phalaborwa was reported to be lower than that received in Phalaborwa the same year.

The non-significance difference in the gross energy of dried marula fruit peels in this study may be attributed to the similar EE and crude protein content. The fat content contributes to the energy content of the feed (Mikasi *et al.,* 2018). The chemical composition of dried marula fruit peels from Phalaborwa and Tzaneen in this study contradicts the chemical composition of dried citrus pulp obtained by Rahmahn (2023), who observed 90.63% DM, 7%CP, 21.6% NDF, and 15.9% ADF.

The DM and CP content contained in DMFP are lower than those obtained from DCP; however, the ADF and NDF content of dried citrus pulp were greater than those in dried marula fruit peels. The difference in these results could be that the peels came from different fruits and how the samples were processed. Muhammad *et al,* (2016) reported that fresh marula fruit's average crude protein content was 30.96%, much higher than the crude protein of dried marula fruit peels in this study. The crude protein content of dried citrus pulp obtained by Fegeros *et al.* (1995), Ibrahim *et al.* (2011), and Alsaied *et al*. (2019) was found to be higher than the CP found in this study. The CF and ash content reported by Alnaimy *et al.* (2017) was 12.68 and 3.71%, lower than the CF and ash content found in this study.

The NDF, ADL, and ADF content from both locations using the freeze-drying method were lower. These results concur with the results found by Jancik *et al.* (2017), who reported that freeze-dried samples had lower NDF, ADF, and ADL content than those that were dried using a forced air oven. This shows that; indeed, drying methods influence the nutritive value of samples.

7.4.4. Conclusion

The dry matter of fresh marula fruit peels in this study was found to be low, which suggests that its moisture content is high. Therefore, it can be concluded that there is a need to preserve marula fruit peels to prevent spoiling.

7.5. Digestibility trial

7.5.1. Description of the study area

The study was conducted at Suikerboschfontein at a farm in Bronkhorstspruit 50km east of Pretoria, Gauteng, South Africa-(25°48’18” S, 28°44’47” E). Bronkhorstspruit falls under Kungwini municipality in Tshwane Metro. The area has an average annual temperature of 16.8°C.

7.5.2. Management of animals

The apparent digestibility trial was conducted towards the end of the growth trial, on the last ten days, when animals were approximately 35 kg. A total of 9 male South African mutton merino sheep were used in this experiment. A diet for the sheep was formulated to be isocaloric and isonitrogenous. There were three (3) diets: one with maize serving as control, one with 5% dried marula fruit peels, and the third one was having 10% dried marula fruit peels. Three (3) animals were randomly allocated to each diet. The animals were fed ad *libitum daily* at 9 a.m. Clean water was always available. Animals were placed in metabolism cages individually designed to collect faeces, which were 117.5L x 57.8b x 83.9h in size. These animals were given a 3-day adaptation period before data collection. The metabolic crates were fitted with feeders and drinking bowls. Sheep were fitted with harnesses and faeces collection bags.

7.5.3. Data collection

The collection of faecal samples took seven days with the first three days serving as the adaptation period of lambs to the faecal bags. Faeces and orts were weighed daily in the morning immediately after collection, and 20% of representative samples were taken and frozen at -20°C and pooled over the collection period for each animal to be later analysed at the University of Venda laboratory.

7.5.4. Laboratory analysis

Before chemical analysis, the faeces and feed samples were thawed and dried in the oven at 60°C for 48 hr and ground to pass through a 1 mm sieve. The DM of the feed and faeces samples was determined according to the association method of the official analytical chemist (AOAC, 2000 Method 934.01). They were dried using an

oven at 60°C for 48 hr. The ash content of the feed and faecal samples was determined by ashing the samples in a furnace at 500°C overnight (until all carbon was removed from the ash). The organic matter was calculated by subtracting the ash from the dry matter content of the respective samples. The feed and faecal samples were analysed for OM and nitrogen to estimate nutrient intake and digestibility. The apparent digestibility of nutrients was calculated as indicated below using equation 7.7.

$$\%Nutrient\ digestibility = \frac{\text{nutrients consumed} - \text{nutrients in faeces}}{\text{nurient consumed}} x100 \ldots\ldots\ldots\ldots\ldots\ldots 7.7.$$

7.5.5. Experimental design

A completely randomised design was used with three animals per treatment.

7.5.6. The statistical analysis

Data were subjected to analysis of variance (ANOVA) for a complete randomised design using Minitab 19 (2019) (GLM). The treatment means were compared using a Tukey method at a 95% significance level.

The following model was used in this experiment: $Yij = \mu + Di + \varepsilon ij$

Where: Yij = is the experimental observation (Nutrient intake, faecal output, and nutrient digestibility)

μ = is the mean

Di = is the effect of the inclusion level in the diet (i=control diet, i=10% marula fruit skin, 20%marura fruit skin)

εij = random error

7.5.7. Description of results

7.5.7.1. Nutrient Intake and faecal output

Data on crude protein and organic matter intake for the three (3) groups of lambs are presented in Table 7.2. The results show that the inclusion levels of dried marula fruit peels in the animal diet did not affect ($p>0.05$) the CP intake. However, a significant difference ($p<0.01$) was observed in the OM intake by the lambs. The data on faecal

output (g/day) of Nitrogen (N), Organic matter (OM), and Dry matter (DM) for the three groups are presented in Table 7.3. The inclusion levels of dried marula fruit peels in the diet did not affect (p>0.05) the faecal excretion of nutrients by lambs.

7.5.7.2. Nutrient digestibility

Data on DM, OM, and CP digestibility of the diets for the three groups of lambs are presented in Table 6.4. There was no significant difference (P>0.05) observed in the digestibility of nutrients.

7.5.8. Discussion of results

7.5.8.1. Nutrient Intake

The nutrient intake of crude protein and by the lambs given the three diets was not significantly different, however, the lambs that were assigned a diet with a 5% inclusion level had a numerically high intake, followed by a 0% inclusion level, and lastly 10% inclusion level. This indicates that a 5% inclusion level of dried marula fruit peels resulted in higher CP intake than the other inclusion levels. For the nutrient intake of OM (organic matter), the lambs with a 5% inclusion level had the lowest intake, followed by the lambs with a 0% inclusion level and then the lambs with a 10% inclusion level. This suggests that OM intake decreased with the increased inclusion level of dried marula fruit peels in the diet of lambs.

Regarding DM (dry matter) intake, the lambs with a 5% inclusion level had the lowest intake, followed by the lambs with a 10% inclusion level, and then the lambs with a 0% inclusion level. This shows that the DM intake was the lowest, with a 5% inclusion level of dried marula peels. A study by Guimaraes *et al.* (2014), who evaluated the intake, digestibility, and performance of lambs fed with a diet containing different levels of cassava, reported that the dry matter and organic matter intake was not affected (p>0.05) by the inclusion level of cassava peels in the diet. This study concurs with the current study; both studies show that at a 0% inclusion level, the DM and OM intake was numerically higher than those at a 10% inclusion level. This indicates consistency in the impact of including dried peels in animal diets on DM and OM intake. Ramírez-Rivera *et al.* (2010) reported that there was a significant difference among the treatments; the total Nitrogen intake increased with the increase in the

inclusion levels of Tithonia in the diet of sheep which is contradictory to the findings of the current study. This suggests that different types of dried peels may have different effects on nutrient intake in animal diets.

7.5.8.2. Faecal output

The nutrient output (faecal output) of dry matter, organic matter, and nitrogen for the inclusion levels of 0%, 5%, and 10% dried marula peels were found to be similar. This concurs with the study by Mikasi *et al. (*2018), who evaluated the digestibility of Macadamia oil cake and marula seed cake and found that the nutrient in the faecal output was not significantly different for all the diets. Based on these results, it can be observed that the nitrogen output increased slightly with the inclusion of dried marula peels in the diet, reaching its highest value at a 5% inclusion level (1.86 g/kg). However, at a 10% inclusion level, the nitrogen output decreased slightly to 1.82 g/kg. This could be the result of the non-significant difference observed in the CP intake. There was a gradual increase in organic matter output with the inclusion levels of dried marula peels. The 0% inclusion level resulted in the lowest OM output, while the 10% inclusion level had the highest OM output.

7.5.8.3. Digestibility of nutrients

The digestibility values for dry matter, organic matter, and crude protein were significantly the same for all the treatments. These findings suggest that the inclusion of dried marula fruit peels did not negatively affect the digestibility of DM, OM, and CP in the lamb diet, which could have an impact on their overall nutritional efficiency. Supporting this observation, Mikasi *et al.* (2018) reported a non-significant effect on the digestibility of nutrients by lambs fed different levels of MOC and MSC. However, Ajagbe *et al.* (2020) contradicted these results; they reported that the digestibility values for DM, OM, and CP differed significantly among the treatments. This contradiction could be the result of different feeds given to the animals and the type of animals used in the experiments. Factors such as animal species (sheep and goats), breed, and feed composition affect nutrient digestibility (Khashkheli *et al.,* 2020).

7.5.9. Conclusion

This study revealed that incorporating dried marula fruit peels into the sheep's diet does not affect nutrient intake and digestibility; therefore, dried marula fruit peels can be a potential energy source for sheep.

7.6. Growth trial

7.6.1. Description of the study area

A farm mentioned in 7.1.1 served as the study's location.

7.6.2. Management of animals

A total of 21 male lambs were used in the experiment with an average weight of 20 kg at the beginning of the experiment, and they were fed until they were about 42 kg on average. The duration of the experiment was 3 months. The lambs were put individually in separate pens of 117.5L x 57.8b x 83.9h by size. Animals were randomly assigned to three treatment diets with seven animals in each diet. They were fed *ad libitum* daily at 9 in the morning and there was always access to clean water. The adaptation period for this experiment was 14 days. All lambs were dewormed with Prodose Orange, vaccinated against pulpy kidney with Pulpy vax, and treated for internal and external parasites with Ivemax before entering the feedlot.

7.6.3. Animal diets and treatments

A diet for the lambs was formulated to be isocaloric and isonitrogenous. The experiment had 3 diets thus one with 0% marula fruit peels serving as control, one with 5% dried marula fruit peels, and the third one was having 10% dried marula fruit peels.

7.6.4. Chemical analysis of feed material

The feed samples (0%,5%, and 10% dried marula fruit peel-based diets) were determined.

Table 7.2. Three lamb diets with 0%, 5%, and 10% dried marula fruit peels as an energy source.

Feed	Treatment 1 0%	Treatment 2 5%	Treatment 3 10%
Soya	10	10	10
Crashed maize	10	0	0
Wheat bran	10	10	10
Hominy chop	43.5	50.5	47.5
Hay	10	10	10
Molasses	10	8	6
Feed lime	5	5	5
Salt	1.5	1.5	1.5
Dried marula peels	0	5	10
Nutrient density of diets			
Dry matter	94.37%	89.06%	94.29%
Crude protein	10.11%	11.77%	11.39%
Ash	91.77%	91.98%	92.10%

7.7. Data collection

The lambs were weighed at the beginning of the trial, during the adaptation period, and at the beginning of data collection. Feed offered and refusals were weighed and recorded once daily before offering fresh feed, to determine the average feed intake. Lambs were weighed once a week before feeding to calculate the average daily gain and feed conversion ratio was calculated using the daily feed intake and daily live weight gained.

The following equations were using equations 7.8, 7.9, 7.10

$$ADFI = \frac{total\ feed\ consumed}{number\ of\ days\ on\ feed} \quad \text{.....7.8.}$$

$$ADG = \frac{weight\ gain}{number\ of\ days\ on\ feed} \quad \text{.....7.9.}$$

$$FCR = \frac{daily\ feed\ intake}{daily\ live\ weight\ gained} \quad \text{.....7.10}$$

All the experiment's lambs were slaughtered at a commercial abattoir when it was concluded. Captive bolt stunning was used to make lambs unconscious and insensitive to pain and dressed as per the industry-accepted procedure. Immediately after dressing, the carcass was weighed to obtain hot carcass weight. The carcasses were then chilled for 24 hr at ± 3°C and reweighed to determine cold carcass weight. Dressing percentage was calculated using hot carcass weight and live weight as follows:

$$Dressing\ percentage = \frac{hot\ carcass\ weight}{live\ weight} \quad \text{.....7.11}$$

The chilling loss was calculated using hot carcass weight and cold carcass weight using the following equation 7.12

$$Chilling\ loss\ \% = \frac{Hot\ carcass\ weight - Cold\ carcass\ weight}{Hot\ carcass\ weight}\ x\ 100 \quad \text{.....7.12.}$$

7.8. Experimental design

A total of 21 lambs were used in a completely randomised design with 7 lambs in each diet. Animals were randomly allocated to three diets (0% dried marula fruit peels, 5% dried marula fruit peels, and 10% dried marula fruit peels).

7.9. Statistical analysis

Data was subjected to analysis of variance (ANOVA) on a complete randomized design using Minitab 19 (Minitab, 2019). Means were compared using the Tuckey pairwise method at a 95% confidence level.

The following model was used.

Yij =μ + αi + eij

Where yij= is the observations: IBW, FBW, ADFI, AWG, FCR and Carcass measurements

μ= overall mean

αi = the effect of inclusion level

eij= random error

7.10. Results

Table 7.3. Effect of marula fruit peels-based diet on growth performance of South African mutton merino lambs.

Treatments	IBW(kg)	FBW(kg)	ADFI(kg)	ADG(kg)	FCR
0%	21.429a	41.857a	1.199a	0.244a	5.249a
5%	21.286a	42.714a	1.285a	0.258a	4.979a
10%	21.286a	39.143a	1.192a	0.250a	5.438a
SEM	0.84	2.20	0.08	0.03	0.46
Significance	NS	NS	NS	NS	NS

IBW: Initial body weight, FBW: Final body weight, ADFI: Average daily feed intake, ADG: Average daily gain, FCR: Feed conversion ratio, SEM: Standard error mean, NS: Non-significant (p>0.05),

Table 7.4. Effect of dried marula fruit peels on the carcass characteristics of South African mutton merino lambs

Treatments	SLT MASS	HOT C MASS	COLD C MASS	DRESSING %	CHILLING LOSS
0%	41.857[a]	16.871[a]	16.500[a]	39.414[a]	2.201[a]
5%	42.714[a]	17.743[a]	17.343[a]	40.557[a]	2.220[a]
10%	39.143[a]	15.929[a]	15.614[a]	40.000[a]	1.999[a]
SEM	2.20	1.01	0.991	0.698	0.149
Significance	NS	NS	NS	NS	NS

SLT MASS: Slaughter mass, HOT C mass: Hot Carcass Mass, COLD C MASS: Cold carcass mass, DRESSING %: Dressing percentage, NS: Non-significant (p>0.05)

7.11. Data description

7.11.1. Growth performance

The growth performance of lambs' results is shown in Table 6.1 above. The inclusion levels of dried marula fruit peels had no significant impact (p>0.05) on the growth performance.

7.11.2. Carcass characteristics

Carcass characteristics results are shown in Table 6.2 above. There was no significant difference in all the parameters (slaughter mass, hot carcass mass, cold carcass mass, dressing percentage, and chilling loss) among treatments ($p>0.05$).

7.12. Discussion of the results

7.12.1. Growth performance

The non-significant difference observed in this study may be due to the non-significant effect observed for Crude protein intake obtained in the apparent digestibility trial. The results obtained in this study mirror the results found in the study which has shown that the inclusion levels of dried citrus pulp in the diet of sheep did not influence the dry matter intake and body weight gain (Bakr, 2019). Yang *et al.* (2020) and Barreto *et al.* (2010) indicated that the level of nutrients contained in the diet affects the growth performance of livestock. However, the result of the current study shows that maize meal can be replaced by marula fruit peels at 5%, and in this study replacement of maize with 5 and 10% dried %levels of the diet. Lambs-fed high-energy diets containing more than 14% protein have not demonstrated improved growth performance (Rios-Rincon *et al.,* 2014). This study contradicts the current study because the diet of the lambs contained low protein content, and the growth performance was not negatively affected. Menezes *et al.* (2018) reported an average daily gain for SantaIne lambs of 0.0717 kg per day which was found to be lower than the average daily gain reported in this study.

Feed intake is positively correlated with body weight gain as it is generally acknowledged that lambs fed more feed picked up more weight each day (Mikasi *et al.,* 2018). Sayed (2009) reported that feeding lambs with diets that are high in energy significantly affects the average daily feed intake. However, lambs allocated on low-energy diets consume less feed and have a low conversion ratio compared to other treatment groups. This study contradicts the current study since a non-significant effect on feed intake and feed conversion ratio among all the treatment groups was observed.

Hu lambs were fed a diet containing metabolisable energy ranging from 9.17 to 10.82 MJ/kg and their average daily gain ranged from 0.108 to 0.1527 kg/d (Wang, 2020). These results were found to be lower than the results obtained in this study, where the metabolisable energy ranged from 14.26 to 14.42 MJ/kg and the average daily gain ranged from 0.244 to 0.258 kg. The difference in the two studies may be due to the level of metabolisable energy in the diets and the difference in the sheep breed used in both experiments. However, the study by Wang (2020) shows that increasing metabolisable energy results in an increased average daily gain of lambs.

7.12.2. Carcass characteristics

The non-significant difference amongst the parameters under investigation in this study suggests that the inclusion of dried marula fruit peels did not affect the lambs' overall carcass characteristics. The report published by Takele *et al.,* (2011) shows that the Horro lambs had a dressing percentage of between 36.7 and 42.5%. The results published by Mulugeta *et al.,* (2021) show that the dressing percentages of sheep were found to be (33.5 - 35.7%). The results from both the above-mentioned studies were found to be lower than the results observed in the current study. The difference in these results could be the type of feed fed to these lambs and the type of sheep breed used. Increasing energy levels in the diet results in increased dressing percentage (Hosseini *et al.,* 2008; Sayed, 2009, Chikwanha *et al.,* 2019). These three studies contradict the current study.

7.13. Conclusion

Based on the findings in this study, dried marula fruit peels can be added to lamb diets as a feed ingredient to provide an alternate energy source for the animals without compromising the animals' growth and carcass characteristics. However, additional studies with longer higher levels of marula fruit peels in the diets would contribute to a more robust evaluation of the effects of dried marula fruit peels on the growth performance of lambs.

CHAPTER 8

DEVELOPMENT OF MARULA CARBONATED JUICE ITS CHARATERISATION

8.1. Summary

In this study, the production and characterisation of carbonated marula juice using spontaneous fermentation was aimed. The physicochemical properties and bioactive compounds were investigated to determine the impact of fermentation. Results showed fermentation maintained a higher ascorbic acid (vitamin C), carotene, enhanced total acidity, and volatile acids in carbonate marula juice. On the other hand, fermentation decreased the residual sugar, total soluble sugar, pH and viscosity of carbonated marula juice. The colour of the carbonated marula juice was affected by fermentation with the L* value increasing from 55.17 to 57.76, while the a* and b* values decreased from 3.74 to -1.03 and from 13.55 to 7.25, respectively. The cloudiness and browning index of fermented carbonated marula juice decreased from 2.48 to 0.89 and from 1.86 to 1.41. Furthermore, the volatile acidity in beverages is within the regulatory limitations for alcoholic beverages. According to the findings, carbonated marula juice can be fermented spontaneously to produce probiotic beverages.

8.2. Introduction

Soft drinks are defined as carbonated or non-carbonated beverages containing sugars or artificial sweeteners, acidulants, flavourings, colourings and preservatives (Tahmassebi and BaniHani). Soft drinks contain large amounts of sucrose or high-fructose corn syrup that have cariogenic potential (González-Aragón Pineda *et al.,* 2019; BaniHani *et al.,* 2019). Frequent consumption of fruit drinks, carbonated beverage and fruit juice as well as bedtime consumption increase the severity of dental erosion. Soft drinks are often high in sugar content and acidity and a gram of sugar contains 4 calories. In addition, they supply energy only and are of little nutritional benefit (Bucher and Siegrist 2015; Chi and Scott 2019). Soft drinks can

contribute to detrimental general and oral health effects on children and adolescents including an increasing risk of overweight, obesity, type 2 diabetes, dental caries and dental erosion (Scientific Advisory Committee on Nutrition 2015; Chi and Scott 2019). Overweight and obesity can have major costs for individuals and their families as well as for the health care systems. They increase the risk of developing type 2 diabetes and heart disease as well as double the risk of dying prematurely (Pischon *et al.,* 2008). Along with the increased consumption of soft drinks, there has been a rapid and large increase in the reported incidence of type 2 diabetes (Hu and Malik 2010; Greenwood *et al.,* 2014).

Soft drinks have markedly acidic pH (2.5–4.0), 1.5 to 4 volumes of carbon dioxide (CO_2), with a typical carbonation level of about 3 volumes. Cola-type soft drinks commonly present a pH in the range of 2.5 to 2.8 and are acidified using a diluted phosphoric acid solution (Barnabé and Venturini Filho, 2010). The low pH caused by the addition of acidulants, the addition of preservatives and CO_2 comprise major barriers to microbial growth in soft drinks. Innovations in the field of soft drinks comprise the development of new tastes, the addition of not only higher amounts of juices, pulps and fruit cells, but also the addition of health-appealing beverages, the use of natural colourants, low-calorie formulations, the addition of proteins, and removal of chemical preservatives from soft drink formulations, among others (Data Monitor, 2013). In the present study, a functional carbonated marula drink was developed utilising some easily available and well-known indigenous marula fruit having nutritional potential.

The developed carbonated marula drink can provide an economical and feasible option for consumers with very good taste combined with potential health benefits. The present drink can replace the synthetic soft drinks available in the market for health-conscious consumers. The major problems which are generally encountered in traditional marula fruit juice beverages are the viscosity of solids the effectiveness of thermal treatments, and low shelf life (Hlangwani *et al.,* 2023). This fruit also has some phytochemicals such as polyphenols, flavonoids, tannins and pectin, which are good for the prevention of chronic and degenerative diseases. Marula is consumed or served fresh, juiced and can be preserved into jam and marula alcoholic beverage (Mashau *et al.,* 2022). Addition of carbon dioxide can improve the ‘mouthfeel’ of the

drink, and shelf-life span and filtering can reduce fruit solids material by increasing the viscosity of the marula juice. In the present study, carbonation was done to improve the sensory properties of marula juice. The objective of the study is focused on the development of carbonated marula juice to evaluate its physiochemical parameters and determine the sensory profile and degree of acceptability.

8.3. Materials and Methods

8.3.1. Processing of marula carbonated drink

Marula fruits were harvested at Phalaborwa and transported to a pilot plant in the Department of Food Science and Technology at the University of Venda. Marula fruits were stored in a cold room until further processing. Marula fruits were cleaned with running tap water and sorted by colour and free of bruises. Selected fresh marula fruits were boiled for 5 min to eliminate microorganisms, and the release of juice from fruit and other useful compounds was followed by filtration. Afterwards, the drink was carbonated by injection of CO_2 gas (non-fermented). The fermented carbonated marula juice was fermented for 72 hr. The fermentation process took place at a temperature of 35^{o}C, since fermentation of 30-40^{o}C ends up retaining high antioxidant activity which is positively correlated to its vitamin C and phenolic content. The carbonation process was carried out according to Fig 8.1.

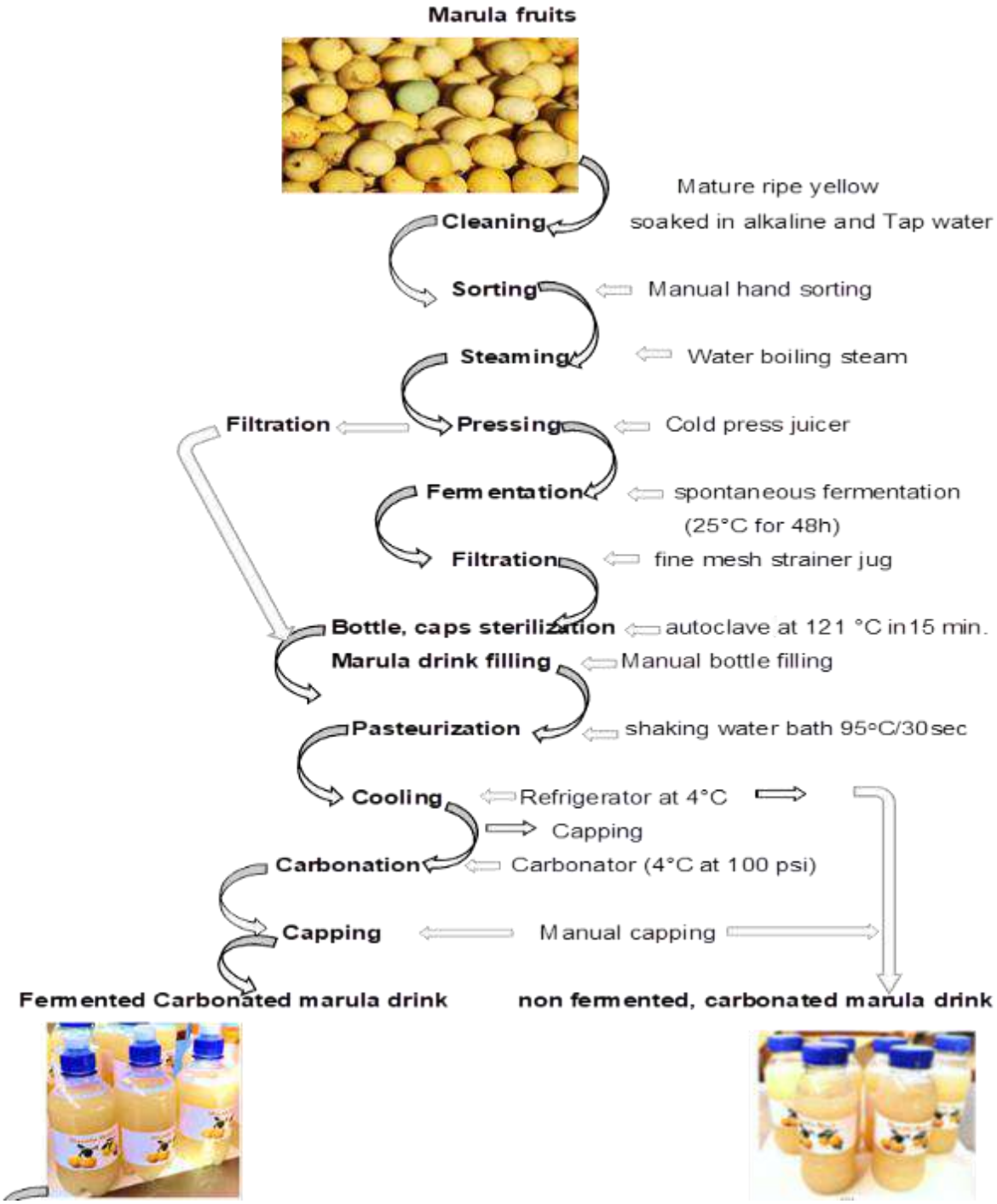


Fig 8.1. Processing flow diagram of carbonated marula drink.

The carbonate marula beverage was first pre-chilled in a refrigerator to decrease the temperature to about 2-4°C to facilitate the absorption of CO_2 gas which was injected into the extract with the aid of the carbonation machine (Carbonator). Transparent plastic bottles of 150 mL capacity equipped with screw caps were used in filling and

bottling the drinks prepared. The bottles were obtained from the local market. The products of carbonated and non-carbonated marula drinks were then bottled and capped immediately to maintain their gas contents. The drinks were analysed for their chemical characteristics and sensory evaluation.

8.3.2. Physical and chemical characteristics determination

The pH of the samples was determined using a digital pH meter (Hanna, HI 9321). The soluble solid content was measured using a digital portable refractometer and reported as °Brix. The titratable acidity of the juices was determined according to the method reported by the AOAC (2000) and is expressed using the citric acid conversion coefficient. The volatile acidity was determined by titration until reaching pH 8.1 using a 0.1 N NaOH solution according to the 11.047, AOAC (2000) method, the volatile content was measured as a percentage (w/v) of acetic acid. The ascorbic acid content in the sample was determined by the visual titration method using the dye 2,6-dichlorophenolindophenol. The electrical conductivity of all the samples was determined using “Electrical Conductivity Meter Model 1056. The density of soft drinks was calculated by using a glass hydrometer. The colour parameters of the fruit juices, where L* is the lightness, a* and b* are colour-opponent dimensions, redness, and yellowness, were determined using a hand-held Minolta Chroma Meter Model CR-400 (Minolta Co Ltd., Osaka, Japan). The determination method of browning degree was changed according to the method of Xing *et al.* (2015).

8.3.3. Data analysis

Experimental data were analysed using Minitab v.17 Statistical Software (Minitab Inc., State College, PA, USA). Differences within means of treatments were considered significant for $p \leq 0.05$ using ANOVA and Turkey’s tests.

8.3.4. Results and Discussion

8.3.4.1. Physicochemical properties of carbonated marula juice

The physicochemical characteristics of marula juice are shown in Table 8.1. These characteristics are important in marula juice for the fermentation process. There was a significant reduction of residual sugar in fermented marula juice compared to the

non-fermented juice. The reduction in the sugar content of fermented marula juice is undesirable in terms of taste. The non-fermented carbonated marula juice had more sugar, which seem to have the potential to maintain fruit juice sweetness. Contrarily, Wang *et al.* (2022) reported a decrease in sugar content in fermented kiwifruit juices. There was an inversely proportional relationship between fermentation and total soluble sugar (˚Brix) in marula juice. With longer fermentation times, fermentable sugars were metabolised more quickly, which reduced the number of soluble solids (˚Brix) present (Paredes *et al.,* 2022). There was a greater production of volatile acidity in fermented carbonated marula drinks than in non-fermented carbonated marula beverages. According to the International Code of Oenological Practices (2015), the maximum permitted limit of volatile acidity in wine is 0.12 g/100 mL (expressed as acetic acid). The acidity found in this research was within the limits permitted by the International Code of Oenological Practices (2015).

Table 8.2. Physio-chemical analysis of marula drink fermentation and non-fermented.

Chemical composition	**Fermented**	**non-fermented**
Residual sugar (g/L)	3.44±0.00[a]	4.03±0.01[b]
Brix	8.87±0.11[a]	13.97±0.18[b]
Volatile acidity(mg/L)	0.12±0.01[b]	0.10±0.01[a]
Total acidity(mg/L)	0.58±0.01[b]	0.38±0.[01a]
pH	3.05±0.14[a]	4.07±0.21[b]
Conductivity (µS/cm)	1256±0.01[a]	1256±0.01[a]
Density ((g/mL)	1.04±0.00[a]	1.05±0.00[a]
Water activity (aw)	0,90±0,00[a]	0,93±0,00[a]
Ascorbic acidity (mg/L)	198.30±0.03[a]	348.30±0.05[b]
Carotene(mg/L)	156.86±0.07[b]	198.44±0.04[a]
Viscosity (cP)	5.79 ±0.02[a]	7.89 ±0.03[b]
Colour Quality		
L*	57.76±0.035[b]	55.17±0.025[a]
a*	-1.03±0.005[a]	3.74 ±0.003[b]
b*	7.25±0.001[a]	13.55.012[b]
Cloudiness	0.89±0.07[a]	2.48±0.09[b]
Brown index	1.41±0.05[a]	1.86±0.08[b]

By the decrease in pH, the acidity of fermented carbonated marula juice increased directly with fermentation. The increase in total acidity may be due to the production of α-ketoglutaric and succinic acids in the glyceropyruvic fermentation pathway and pyruvic acid in the glycolytic pathway (Rios-Corripio *et al.,* 2019). Organic acid production in fermented foods lowers pH, increasing total titratable acidity (Puerari et al., 2015). Acidity has a decisive impact on the aroma and flavour of fermented drinks. Bueno *et al.* (2021) also reported a pH reduction in fruit juices fermented with 1% kefir grains from 3.80 to 3.48. Like apple juices, Esatbeyoglu *et al.* (2023) reported that the pH of Aronia melanocarpa juice fermented with kefir decreased. Bueno *et al.* (2021) stated that the pH values of red pitaya juices fermented with water kefir grains were reduced from 7.73 to 4.83. Similar patterns in total acidity values have been observed by Randazzo *et al.* (2016); wherein it increased after fermentation for apple, grape, pomegranate, and prickly pear. It is recommended that the pH in the fermentation wort be in the range 2.8–4.0.

The conductivity, density and water activity of fermented carbonated and non-fermented carbonated marula beverages did not show any significant differences. The fermented marula beverage had a higher ascorbic acid than the non-fermented juice. But all fermented juice consistently had higher levels of vitamin C than the control group, suggesting that although vitamin C is abundant in fresh marula juice, it was easy to oxidise and decomposed with the extension of shelf life, the fermentation slowed down the loss of vitamin C (Xu *et al.,* 2023). The carotene content of the fermented marula juice was higher than the non-fermented sample. A feasible explanation for the increase in carotene during fermentation may be due to the occurrence of some changes in the internal structure of the suspended solids, in which the pigments are integrated, enhancing their extractability (Cerrillo et al., 2014). This aspect is of particular interest because it would indirectly indicate an increased bioaccessibility of the carotenoids in the fermented juice, so further research should be carried out to confirm this hypothesis.

Carotenoids have been implicated in several biological processes including cardiovascular protection, immune responses, antioxidant activity, and bone metabolism (Tavani *et al.,* 1997; Fernández-García *et al.,* 2012). Humans need to acquire carotenoids through their diet because they are not able to synthesize them.

According to our results, the fermentation of marula juice induces an apparent increase of carotene content, so the fermented marula juice could be considered a good source of these bioactive compounds and may have potentially beneficial effects already tested in marula juice. Fermented carbonated marula juice had lower viscosity compared to non-fermented juice. This could be due to fermentation which breaks down complex sugars and pectin molecules, resulting in a thinner, more fluid consistency; this is primarily due to the production of organic acids like lactic acid during fermentation, which alters the pH level and impacts the structure of the drink components.

It was found that the colour of the fermented marula juice showed some differences from non-fermented juice (Table 8.1). For example, the L* value of fermented marula juice was higher, meaning that it had a lighter colour compared with that of fermented marula juice. Similarly, research observed that the colour of fermented smoothies obtained from different fruits was preserved and the rate of browning reactions slowed down when lactic acid bacteria (LAB) were dominant in the fermentation environment (Di Cagno *et al.,* 2011). Regarding a* values of marula juice samples, the control group (non-fermented) was found to be significantly different to the fermented juice. The fermented juice had significantly lower a* value representing that it had a close appearance in terms of redness ($P < 0.01$). On the other hand, for the b* parameter, the value for the fermented marula juice was found to be significantly lower than the non-fermented marula juice and this suggested there was some difference in color between the two juices. The colour of a fermented beverage is an important parameter for the consumer's acceptance. The cloudiness and browning decrease after fermentation. The browning degree is associated with the amounts of browning products, as well as the degree of diffusion of browning products and their binding to proteins.

8.3.5. Conclusion

The development of fermented carbonated marula juice by natural microorganisms is a complex and dynamic biochemical process. This study explored the changes in the physicochemical properties of marula juice during the fermentation process. The fermented carbonated marula juice maintained a higher ascorbic acid and carotene

content. In terms of physicochemical properties, fermentation decreased the pH, viscosity, and brix of the marula juice, while the titratable acidity and volatile acidity increased. At the end of fermentation, the colour parameters of the juice were significantly changed. These results provide valuable information about the development of innovative functional plant-based beverages for individuals with cow milk seeking nutritious meals with several health benefits. However, the research of the bioconversion in the marula juice fermentation system could provide a theoretical basis for the fermented fruit industry and help to optimise the fermentation time and screen a more suitable strain to obtain fermented marula juice with higher nutritional value.

CHAPTER 9

MICROBIAL QUALITY OF MARULA JUICE COLLECTED FROM PHALABORWA

9.1. Summary

Marula (*Sclerocarya birrea*, A. Rich) is an underutilised wild edible fruit that can be consumed fresh or prepared. Research has shown that marula fruit can be used in juice processing, alcohol-based products, jams and jellies, fruit leather, vinegar, and animal feed. This study aimed to isolate and detect the microbial quality of raw, fermented and carbonated marula juice. The marula fruit samples were collected from Phalaborwa because the consumers tested the juice and preferred the Phalaborwa as compared to Mooketsi ZZ2 and Thulamahashe. The marula fruit juice was prepared, fermented and later carbonated. Microbial populations including *Enterobacteriaceae* (Coliforms and *Escherichia coli)*, aerobic count, lactic acid bacteria, yeast and moulds were isolated. Coliforms, yeast, *E. coli*, lactic acid bacteria, and aerobic bacteria were all found in abundance in fermented marula juice. The fermented marula juice is not safe for consumption, however, make sure all equipment and surfaces are thoroughly cleaned and sanitised before handling marula juice. This helps prevent contamination from external sources. In addition, further processing is required.

9.2. Introduction

The marula tree (*Sclerocarya birrea*), which is native to southern Africa, produces small, yellowish fruits. It is a widespread species that lives in sub-Saharan Africa's semi-arid savannas. Its natural habitat ranges from Senegal to Ethiopia in the north, south to KwaZulu-Natal in South Africa, and east to Namibia, Angola, and the southern Democratic Republic of Congo. Caffra is the most important and dominant sub-species in southern Africa. It is found in Zimbabwe, Botswana, Namibia, South Africa, and Swaziland. The tree is widespread in South Africa's KwaZulu-Natal, Mpumalanga, Limpopo, and North-West provinces (Mashau *et al.,* 2022; Maluleke *et al.,* 2023).

Marula fruit juice is a delightful beverage derived from the pulp of the marula fruit (*Sclerocarya birrea*). It has a sweet, tangy, and slightly tropical flavour, like citrus and mango fruits. The juice is high in vitamin C, antioxidants and vital minerals such as potassium and magnesium, making it a healthy beverage. Marula juice can be freshly drunk or made into commercial goods, which are sometimes combined with other fruit juices. It is also used to make traditional African fermented drinks and as a foundation for alcoholic beverages like Amarula liqueur. Due to its high sugar content, marula juice spontaneously ferments if left too long, producing a moderately alcoholic beverage. They're also popular with wildlife, particularly elephants, who enjoy eating them as they fall to the ground (Okoye *et al.,* 2012; Lekhuleni *et al.,* 2024).

Fermentation has long been used to preserve food and create natural goods with practical benefits at a low cost. It was originally used for food preservation, but it now improves food quality by increasing shelf life, minimising the risk of foodborne illness, and enriching flavour profiles. Several investigations have indicated significant fermentation reactions that result in increased efficacy, assurance, costs, and overall quality (Brown and Jones, 2022; Smith, 2023).

Fermented marula juice is a traditional alcoholic beverage prepared from the marula fruit (*Sclerocarya birrea*), which is indigenous to southern Africa. The fermentation process converts the fruit's inherent sugars into alcohol, creating a beverage with a distinct flavour profile (Rugheimer *et al.,* 2023). The traditional preparation of marula fruit juice begins with the collection of ripe marula fruits, which are then washed and peeled. The pulp is obtained by crushing or squeezing the fruits, and the juice is collected. This juice is allowed to ferment ambient microorganisms, usually made of indigenous yeasts found on the fruit skin, utensils and in the environment. Fermentation factors, such as temperature and time are not controlled, resulting in inconsistent flavour and alcohol levels of the beverage from batch to batch. For example, fermenting at 30 to 40°C for 4 to 6 days has been demonstrated to retain strong antioxidant activity in the beverage (Mbhenyane and McKay, 2023; Nkosi and Steenkamp, 2023).

The precise microbial composition can have a substantial impact on the fermentation process and the sensory properties of the finished product (Maluleke *et al.,* 2023).

Carbonated marula fruit juice is a beverage derived from marula fruit, which is native to southern Africa. The juice is taken from the marula fruit and then carbonated to give it a fizzy or sparkling appearance. Marula fruit juice is well-known for its refreshing taste, which is generally described as fruity with a tinge of citrus. Carbonating creates a bubbly texture, akin to carbonated soft drinks or sparkling water. Carbonated marula fruit juice is a distinctive and pleasurable beverage choice due to its flavour combination (Rodney, 2002; Mabapa *et al.,* 2020).

Maluleke *et al.* (2023) found lactic acid bacteria, acetic acid bacteria, and fewer enteric species in marula juice during the spontaneous fermentation process. Lactobacillus species were isolated throughout the fermentation process but dominated the earlier stages of fermentation, together with non-*Saccharomyces* yeasts, whereas *Saccharomyces cerevisiae* and acetic acid bacteria dominated the later phases of wine production (Phiri *et al.,* 2022). *Escherichia coli, Bacillus cereus, Salmonella typhimurium, Listeria monocytogenes,* and *Staphylococcus aureus* were the bacteria detected and isolated from these samples (Tonk *et al.,* 2018). The most common bacterial isolates were *Glucanobacter* species. and *Lactobacillus* species. The most frequent bacterium was *Leuconostoc* sp. Some fungi were also found in the fruit juice sample, including *Penicillium expansum, Penicillium roquefori*, and *Penicillium glabrum* (Jadhav and Waghmare, 2021). Therefore, unwanted microorganisms can contaminate marula juice if not managed effectively. If pathogenic bacteria or moulds invade the juice, this can result in unpleasant smells, odours, and even safety problems. Therefore, this study aimed to isolate and detect the microbial quality of three (3) different types of marula juice (raw, fermented and fermented carbonated) produced from marula fruit.

9.3. Materials and Methods

9.3.1. Sample collection

Marula fruit was collected from Phalaborwa, Limpopo province (South Africa) and transported to the Department of Food Science and Technology, Food Micro Lab, University of Venda, for further analysis. They were packaged in polystyrene plastics, stored in a cooler box and transported to the laboratory. The fermented and carbonated marula juices were produced and tested for microbial analysis, and this

was done in duplicate (n=2). These samples were stored at 4-8°C until used further. Medium and other chemicals were purchased from Lasec, Midrand, South Africa and all were Oxoid grades.

9.3.2. Sample preparation

The raw marula fermented and carbonated marula juice samples were determined for Enterobacteriaceae (Coliforms and *Escherichia coli*), aerobic count, yeast and mould using the Peel Plate method. For each sample, 1 mL of juice was weighed and homogenised with 9 mL buffered peptone water (BPW), serial dilutions were prepared up to 10^{-7}, and the dilution was used for plating. The plates were labelled properly based on the different media used; Peel Plate EB for Enterobacteriaceae which detects coliforms and *E. coli*, Peel Plate AC for aerobic count incubated at 30°C for 48 hrs, Peel Plate YM for yeast and moulds incubated at 25°C for 72 hr (Fig 9.1). For each juice sample, two (2) measurements were taken. The plates were counted using a colony counter for each microorganism. The counts were expressed as colony-forming units per gram CFU/g (Ahmed *et al.,* 2020; Ramashia *et al.,* 2020). For Lactic Acid Bacteria MRS agar pour plate method was modified and employed. One ml of each sample was homogenized with 9 ml of 0.85% sterile saline solution and serially streaked to 10^{-7} onto de Man Rogosa and Sharpe (MRS) agar on MRS agar media, then, the plates were incubated at 37°C for 24 hr (Maluleke *et al.*, 2023).

Fig 9.1. Preparatory stage of microbial isolation and detections (a) Marula fruit, ((b) Fermented marula fruit juice, (c) Carbonated marula juice, (d) Peel Plates, and (e) Isolation and identification.

9.4. Results and Discussion

Table 9.1 indicates the microorganisms isolated and detected from raw, fermented and carbonated marula juices. The coliform counts ranged between 19.5 x 10^{-1} to 81.5 x 10^{-4} cfu/mL with the fermented marula juice having the highest counts of 81.5 x 10^{-4} cfu/ml. Lee *et al.* (2021) isolated the coliforms which ranged from ranged from 0.00 to 5.56 $\log_{10}$ cfu/ml, in addition to the specifications of coliforms is10^{-5}cfu/ml and the

results obtained were with the specification. The colour of the coliforms was shown as blue colonies. As a result, there is a need to implement food safety measures, because fermented marula juice can promote the growth of coliform bacteria under normal conditions. Coliforms are bacteria found in the environment and the intestines of warm-blooded animals, including humans. They are commonly used as indicators of sanitation and potential faecal contamination. The presence of coliforms in fermented marula juice may indicate inadequate processing cleanliness or contamination from handling. The initial microbial load of the raw materials, fermentation conditions (temperature, pH, oxygen availability), and post-fermentation handling processes all influence whether coliform bacteria proliferate in the juice (Garcia and Martinez, 2016; Smith and Jones, 2023).

Table 9.1. Microbial analyses of raw, fermented and fermented carbonated marula juices (cfu/ml) (n=2).

Microorganisms	**Raw marula fruit juice**	**Fermented marula juice**	**Fermented carbonated marula juice**
Coliforms	19.5×10^{-1}	81.5×10^{-4}	59.5×10^{-2}
Escherichia coli	Not detected	46×10^{-4}	Not detected
Aerobic count	92.5×10^{-3}	37.5×10^{-6}	41.5×10^{-5}
Yeast	28×10^{-4}	49.5×10^{-6}	22.5×10^{-5}
Moulds	None	None	None
Lactic acid bacteria	None	59×10^{-5}	92×10^{-4}

Escherichia coli was only detected in fermented marula juice at 46×10^{-4} cfu/mL, and it was not detected in the raw and carbonated marula juices. The colonies identified for *E. coli* were reddish pink, Fig. 9.2. Marula juice fermentation is frequently done in traditional, unregulated circumstances, which increases the danger of cross-contamination with ambient germs. The presence of *E. coli* in fermented marula juice raises food safety concerns because it suggests faecal contamination. Preventing measures for *E. coli* contamination in fermented marula juice requires several important measures to ensure safety and quality: Before using the marula juice, make sure to properly clean and sterilise all equipment, utensils, and surfaces that will come into touch with it. This prevents the initial infection. Maintain good personal hygiene

and ensure that everyone handling the juice adheres to stringent handwashing practices. This decreases the likelihood of introducing pollutants. *Escherichia coli* was not detected in the fresh fruit and vegetable juice and the detectable level is 0 cfu/ml (Lee et al., 2021).

Fig 9.2. Peel plates *Escherichia coli* colonies

The aerobic count ranged from 92.5 $x10^{-3}$ to 37.5 x 10^{-6} cfu/mL in which the highest count was found in the fermented marula juice. The aerobic bacteria count indicates the quantity of bacteria that can live and multiply in the presence of oxygen. This count is significant in a variety of disciplines, including food safety and environmental monitoring. Aerobic bacteria counts are important markers of food cleanliness and deterioration in the food manufacturing and processing industries. High numbers may suggest inadequate handling procedures or contamination during manufacture. Monitoring these counts ensures that food safety regulations are met (Smith, 2020).

Total aerobic bacteria were detected at the level of 4.21 ± 1.10 log cfu/mL and ranged from 2.00 to 6.70 log cfu/mL (Lee *et al.* (2021). According to MFDS (2020) fruit and vegetable juice microbial standards, the number of samples is 5, the number of detectable samples is 1, the minimum allowable standard is 100,000 (5.0 log cfu/g), and the maximum allowable standard is 500,000 (5.7 log cfu/g) or less in the case of total bacterial count (Lee et al., 2021).

The highest yeast count was isolated from fermented marula juice at 49.5 x 10^{-6} cfu/mL and the lowest was isolated from raw marula fruit juice at 28 x 10^{-4} cfu/mL. Yeasts are a group of unicellular eukaryotic organisms, which are widely used in the production of fruit wines, fruit enzymes, jams, and more. Throughout the fermentation

process, yeasts play a pivotal role in converting the glucose and fructose present in fruits into alcohol and carbon dioxide. Simultaneously, vitamins, amino acids, and other beneficial metabolites were produced. Saccharomyces cerevisiae is the most used strain in fruit wine fermentation (Yuan *et al.,* 2024). Lee *et al.* (2021) isolated yeast from fresh fruit and vegetable juice which ranged from 0.00 to 6.72 $\log_{10}$cfu/mL. The moulds were not isolated from the juice samples.

The fermented marula juice had the highest lactic acid bacteria counts of 59 x 10^{-5} and the lowest counts were isolated carbonated marula juice at 92 x 10^{-4} cfu/mL, Fig 9.3.

Fig 9.3. Lactic Acid Bacteria plate with colonies

Lactic acid bacteria are a group of gram-positive bacteria that can ferment sugars to produce lactic acid, which is widely distributed in nature, mainly in fermented foods, intestinal tracts of animals, soil, water, etc. Lactic acid bacteria play a crucial role in fruit fermentation by utilising carbon sources and free amino acids to produce abundant metabolites including organic acids, bioactive peptides, fatty acids, extracellular polysaccharides, vitamins, etc. (Narvhus and Gadaga, 2017; Zannini *et al.*, 2021).

Fruits, being high in water and nutrients, are prone to microbial attack, especially considering the diverse microorganisms present on their surfaces. In addition, the thin and easily broken skin of some fruits exacerbates the risk of spoilage. Microbial fermentation serves as an effective means to extend the shelf life of fruits. It can inhibit

the growth of harmful microorganisms by lowering the pH through the production of organic acids; producing alcohol and some antimicrobial substances such as organic acid, antimicrobial enzymes, and peptides; and reducing the supply of oxygen through the production of gases such as carbon dioxide. Hence, Coliforms, *E. coli,* Yeast, and Lactic acid bacteria are common strains in fruit fermentation (Liu and Tsao, 2022; Yuan *et al.,* 2024).

9.5. Conclusion

High counts of Coliforms, yeast, *E. coli*, lactic acid bacteria, and aerobic bacteria were isolated and detected in the fermented marula juice, however, coliforms were within the specified limits of 10^{-4}. Overall, the presence of yeast, *E. coli*, lactic acid bacteria, and aerobic bacteria in fermented marula juice demonstrates the complex microbial ecology involved in fermentation processes, as well as the importance of hygiene, environmental control, and understanding microbial dynamics for quality assurance and safety in fermented beverages. Thoroughly clean any equipment, containers, or surfaces that meet the marula juice. This may lower the initial microbial load.

CHAPTER 10

CONCLUSIONS AND RECOMMENDATIONS

The conclusion regarding the effect of drying methods on marula peels generally focuses on how different methods influence the nutritional and physicochemical properties of the peels. Specific points include:

Some drying methods, such as shade drying, are better at preserving sensitive nutrients like vitamins and antioxidants, while others, like sun-drying, may lead to greater nutrient degradation due to exposure to heat and UV light.

Drying methods can affect moisture content, colour, texture, and the concentration of bioactive compounds. For example, oven drying at controlled temperatures may maintain consistent quality, while air or shade drying might lead to variability. The choice of drying method impacts how marula peels can be used in value-added products. Methods that preserve functional properties, like antioxidant activity, make the peels more suitable for incorporation into health-focused products like biscuits or supplements. While methods like freeze-drying yield higher-quality products, they are typically more energy-intensive and costly, whereas sun-drying or air-drying may be more economical but less effective in preserving quality.

The conclusion on utilising marula peels in biscuit-making highlights that incorporating marula peels can enhance the nutritional value of biscuits. This includes adding dietary fibre, antioxidants, and other bioactive compounds derived from the peels. Additionally, such incorporation promotes sustainability by utilising a by-product that might otherwise go to waste. However, the extent of incorporation should be carefully optimised to ensure the sensory qualities of the biscuits, such as taste, texture, and appearance, remain acceptable to consumers. Further research and consumer testing may be necessary to refine formulations and maximise benefits.

It can be concluded that both the marula fruit and marula fruit peel silage are high moisture feed materials with dry matter contents of less than 50% and that the area where marula trees are located has little influence on the nutrient composition of the fruits. This study revealed that incorporating dried marula fruit peels and or marula fruit peel silage in the diets of growing lambs does not affect nutrient intake and

digestibility of the diet. Based on the findings of the two studies, dried marula fruit peels and or marula fruit peels silage can be added to lamb diets as an alternate energy source for up to 10% of the total diet without negatively affecting growth performance and carcass characteristics of the lambs. Marula fruit peels should be either dried or ensiled to prolong their shelf-life due to their high moisture content. Further studies should be conducted to determine the optimal inclusion levels of dried marula fruit peels and or marula fruit peel silage in the diets of growing lambs.

The microbial analyses were also conducted on the raw, fermented and fermented carbonated juices in which the fermented juice had the highest number of microorganisms. Thus, the high microbial growth in fermented juice could be attributed to the favourable conditions created during the fermentation process, such as increased nutrient availability, optimal temperature, and pH levels, all of which promote the proliferation of fermentative and naturally occurring microorganisms. Furthermore, the absence of carbonation, which can impede microbial activity by releasing carbon dioxide, may have contributed to the greater microbial count in the non-carbonated fermented juice.

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ANNEXURE 1. COMMUNITY ENGAGEMENT


Community Engagement Program


Deliverable 12 Revised marula Com


WRC-UNIVEN stakeholders 21 Marc


Community Engagement Program


WRC-UNIVEN stakeholders 21 Marc


WRC stakeholder 22 March 2023.pdf


Marula juice processing guideline.p

ANNEXURE 2. CONFERENCES

Conference 22nd World Congress of Food Science and Technology Rimini, Italy / September, 8-12, 2024

Nutritional composition, functional, antioxidant properties, and microbial quality of Marula fruit peel flour

Shonisani E. Ramashia; Christah Masinamela

Department of Food Science and Technology, Faculty of Science, Engineering, and Agriculture, University of Venda, Private Bag x5050, Thohoyandou, Limpopo Province, South Africa, 0950.

*Corresponding author shonisani.ramashia@univen.ac.za

Abstract

The final byproduct of milling and grinding seeds, legumes, or other grains is called flour. It gives baked goods like bread, cakes, cookies, and pastries structure. One of the most widely used native wild fruits in Africa, marula has multiple uses and is highly valued by the locals for its fruits, cosmetic oil derived from the seeds, and therapeutic properties found in the leaves and bark. The purpose of this study was to create flour from marula peels and assess the flour's microbiological purity, antioxidant capacity, functional qualities, and nutritional makeup. Analyses of the Marula peel flour's nutritional composition (fat, ash, protein, and moisture) as well as FTIR, antioxidant, physicochemical, functional, and microbiological tests were performed. The findings indicated that marula peel flour had a high vitamin C content. Mould, coliforms, and *Escherichia coli* were not detected on the Marula peel flour produced and the total plate count of the flour showed a significant difference ($p < 0.05$) in all regions. The colour attributes were significantly different ($p < 0.05$) in redness (a^*), lightness (L^*), and yellowness (b^*) values for all three regions. The FTIR spectra showed the O–H region of Marula peel flours bands (peaks) within the range of 3474.30 to 3474.86 cm^{-1}. It was concluded that Marula peel flour is a good source of vital nutrients such as proteins and compounds such as phytochemicals that promote good health to heart diseases, cancer, and other chronic illnesses.

Keywords: Flour, Marula, Marula peels, antioxidants properties, physicochemical, physical properties.

ANNEXURE 3: PUBLICATIONS

International Journal of Food Properties

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ljfp20

Nutritional composition, polyphenolic compounds and biological activities of marula fruit (*Sclerocarya birrea*) with its potential food applications: a review

Mpho Edward Mashau, Tsietsie Ephraim Kgatla, Mashudu Viginia Makhado, Masiza Samuel Mikasi & Shonisani Eugenia Ramashia

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ANNEXURE 4: CAPACITY DEVELOPMENT

1. Nutritional composition, functional, antioxidant properties and microbial quality of Marula fruit peel flour

By

Masinamela Christah

(18014380)

A mini dissertation presented as part of the academic requirement for the Bachelor of Science degree in Food Science and Technology.

Research project (FST 4081)

Department of Food Science and Technology

Faculty of Science, Engineering and Agriculture

Thohoyandou

South Africa

Supervisor: Dr. S.E. Ramashia

2024

2. Physicochemical, antioxidant properties and structural properties of wheat-Marula peel composite biscuits

By

Mafadza Elelwani Ullenda

(17004813)

A mini dissertation submitted in partial fulfillment of the academic requirement for Bachelor of Science degree in Food Science and Technology

Research project (FST 4081)

Department of Food Science and Technology
Faculty of Science, Engineering and Agriculture
Private Bag X5050
Thohoyandou
South Africa

Supervisor: Dr. S.E. Ramashia

2024

3. The effects of different drying methods on phytochemical, and antioxidant properties of Marula peels flour with different particle sizes

By

Tharollo Fine Makhubela
(Student no: 18000537)
A research project (FST 4801)
Department of Food Science and Technology
Faculty of Science, Engineering and Agriculture
Supervisor: Mr. T.E. Kgatla
2024

4. EFFECT OF LOCATION AND DRYING METHODS ON NUTRIENT COMPOSITION, APPARENT DIGESTIBILITY, GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF SOUTH AFRICAN MUTTON MERINO LAMBS FED DIFFERENT LEVELS OF DRIED MARULA FRUIT PEELS.

MUROVHI RONEWA
Student no: 15000963

A dissertation submitted to the Department of Animal Science, Faculty of Science, Engineering, and Agriculture, University of Venda in fulfilment of the requirement for a degree of Master of Science in Agriculture (AGMAAS).

Student: Mrs R Murovhi _____ 23/02/2023

Signature Date

23/02/2024

Supervisor: Dr M.S Mikasi _____ _____

2024