Effect of preservatives and storage conditions on quality of Marula (*Sclerocarya birrea*) fruit juice

A thesis

by

Lekhuleni Isobel Lerato Gosh (67203507)

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Supervisor: Prof. MK Maluleke (University of South Africa)

Co-supervisors: Dr. F Malongane (University of South Africa)

Dr. AM Shabalala (University of Mpumalanga)

Dr. RR Mphahlele (DoA)

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DECLARATION

I, **Isobel Lerato Gosh Lekhuleni,** student number **67203507**, declare that this thesis entitled "**Investigating the effect of preservatives and storage conditions on quality of Marula (*Sclerocarya birrea subsp. caffra)* fruit juice"** which I hereby submit for the degree of Doctor of Philosophy in Life Sciences at the University of South Africa and is my own work and has not previously been submitted by me for a degree at this or any other institution.

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I declare that I have not copied and pasted any information from the Internet, without either specifically acknowledging the source or paraphrasing, and have included appropriate references to these sources in the in-text citation and reference section of the dissertation.

I declare that during my study, I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval **(2023/CAES_HREC/068)** for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions (Appendix **I and II**).

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ABSTRACT

The aim of the United Nations Sustainable Development Goals (SDGs) is, among other things, to eradicate hunger and malnutrition and guarantee that all people have access to sufficient nutrient-dense food by 2030. The introduction of underutilised indigenous fruit species such as marula (*Sclerocarya birrea* subsp. *caffra*), which is reported to be abundant in vitamin C and minerals needed by humans for the fulfilment of their recommended daily dietary requirements, seems to be a reliable strategy to achieve these goals. This multi-disciplinary study was conducted to investigate (i) the impact of geographical region on the physicochemical properties of marula fruits and juice, and (ii) the effect of a treatment combination of preservatives and storage conditions on the physicochemical properties, mineral retention and microbial activities of 100% marula fruit juice. The aim of the study was therefore to establish the most suitable preservatives and storage conditions for processed marula fruit juice, for the purpose of the future commercialisation of this product. Fruit and its juice utillised for for the analysis of physicochemical properties including calcium, iron, fruit mass, total soluble sugars, total flavonoids, and vitamin C were harvested from Bushbuckridge, Giyani and Tzaneen, all of which are classified as lowveld regions of South Africa. The effect of treatment combinations (preservatives and storage settings) on quality, including phytochemicals and minerals, was assessed by subjecting marula fruit juice to preservatives (sodium metabisulphite and sodium benzoate) and storage conditions (4, 6, and 10˚C) for a duration of 20 days. Concerning the assessment of microbial activity, the spread plate count approach was employed, encompassing incubation, enumeration of colony-forming units, and species identification. For the physicochemical properties investigation, study revealed that fruits harvested in the Bushbuckridge region exhibited a greater mass (44.6 g) compared to those from other places. In comparison to other regions, the Tzaneen region fruit had a higher total soluble sugar concentration of 12.9 ˚Brix. Regarding the impact of varying presevatives and storage conditions on quality retention, results demonstrated that the combination of sodium benzoate and a storage temperature of 10˚C resulted in the highest preservation of total flavonoid content (2.49 mg QE/ml) after 20 days, compared to other treatments. Retention of higher vitamin C content (83.26 mg/100 ml) was noted under the treatment combination (+ positive control - pasteurized at 10˚C). For minerals, the treatment combination of sodium benzoate and 4˚C yielded enhanced calcium retention (81.3 mg/L) relative to alternative treatments. The maximum retention of iron content (33.1 mg/L) was recorded with the treatment combination of sodium benzoate and 4˚C, in comparison to other treatments. Regarding microbiological quality, juice samples treated with sodium metabisulphite, irrespective of storage conditions, exhibited the lowest yeast count (3.2×10^2 cfu/ml) in comparison to other treatments. Therefore, regional variances, the use of preservatives, and low storage temperatures all have a direct impact on marula fruit and juice. As a result, marula fruit juice manufacturers should take these factors into account since they have a direct impact on the juice's marketability and potential for commercialisation.

Keywords: Marula fruit juice, physicochemical, minerals, microbial activities, SDG.

Isiswati

Inhloso yeMhlabuhlangene Tinjongo Tekutfutfukisa Letisimeme (SDGs) kutsi, emkhatsini waletinye tintfo, kucedza indlala kanye nekungadli kahle kanye nekucinisekisa kutsi bonkhe bantfu bayakhona kutfola kudla lokwenele lokunemsoco ngemnyaka wa 2030. Kwetfulwa kwetinhlobo tetitselo temdzabu letingakasetjentiswa kahle letifana nemaganu imarula (*Sclerocarya birrea* subsp. *caffra*), letisetjentiswa kancane. kuvithamin C kanye nemaminerali ladzingwa bantfu kwentela kugcwalisa tidzingo tabo tekudla letinconyiwe tetinsuku tonkhe, kubonakala ngatsi lisu leletsembekile lekufeza lemigomo. Lolucwaningo lolunetifundvo letiningi lwentelwe kuphenya (i) umtselela wesigodzi sendzawo etimphahleni te*physicochemical* tetitselo temarula kanye nejusi, kanye (ii) nemphumela wenhlanganisela yekwelapha yetintfo letilondvolotako kanye netimo tekugcina etimphahleni te*physicochemical,* kugcinwa kwemaminerali ejusi yemarula 100%. Inhloso yalolucwaningo bekukusungula tintfo letifanele kakhulu tekugcina kanye netimo tekugcina ijusi yetitselo temarula lecubunguliwe, ngenhloso yekutsengisa lomkhicito esikhatsini lesitako. Titselo kanye nejusi yato lesetjentiselwa kuhlatiya tintfo temtimba letifaka ekhatsi ikhalsiyamu, i-ayoni, bukhulu besitselo, shukela loncibilikako lophelele, ema *flavonoids* laphelele kanye nevithamin C kwavunwa eBushbuckridge, eGiyani nase Tzaneen, konkhe loku kuhlukaniswe njengetindzawo letiphansi eNingizimu Afrika Umphumela wekuhlanganiswa kwekwelapha (tintfo letilondvolotako kanye netindzawo tekugcina) ngelizinga, kufaka ekhatsi emakhemikhali etitjalo kanye nemamineral, kwahlolwa ngekubeka ijusi yetitselo temarula ngetintfo letilondvolotako (*isodium metabisulphite* kanye ne*sodium benzoate*) kanye netimo tekugcina (4, 6, kanye na 10℃) sikhatsi lseingemalanga langu-20. Mayelana nekuhlolwa kwemsebenti wetintfo letincane, indlela yekubala emapuleti lasakateke yasetjentiswa, lefaka ekhatsi kufukamela, kubalwa kwemayunithi lakha emakoloni kanye nekukhonjwa kwetinhlobo. Kuloluphenyo lwe*physicochemical*, lolucwaningo lwaveta kutsi titselo letivunwe esigodzini sase Bushbuckridge tikhombisa bukhulu lobukhulu (44.6 g) uma kucatsaniswa naleto letivela kuletinye tindzawo. Uma kucatsaniswa naletinye tifundza, sitselo sesigodzi sase Tzaneen besinesibalo lesiphakeme sashukela loncibilikako lesiphelele lesingu 12.9°Brix.

Mayelana nemtselela wetintfo letehlukene tekugcina kanye netimo tekugcina ekugcineni lizinga, imiphumela yakhombisa kutsi kuhlanganiswa kwe*sodium benzoate* kanye nelizinga lekushisa lekugcina la 10℃ kwaholela ekugcineni lokusetulu kwalokucuketfwe kwe*flavonoid* lephelele (2.49 mg QE/ml) ngemuva kwemalanga lamashumi lamabili (20), uma kucatsaniswa naletinye tindlela tekwelapha. Kugcinwa kwe vithamin C lesetulu (83.26 mg/100 ml) kwaphawulwa ngaphansi kwekuhlanganiswa kwekwelashwa (+ kulawula lokuhle - *pasteurized* ku 10℃). Kumaminerali, inhlanganisela yekwelapha ye-*sodium benzoate* kanye na 4℃ kwakhicita kugcinwa kwe khalsiyamu lokukhulisiwe (81.3 mg/L) uma kucatsaniswa naletinye tindlela tekwelapha. Kugcinwa lokukhulu kwe-ayoni (33.1 mg/L) kwabhalwa ngekuhlanganiswa kwekwelapha kwe*sodium benzoate* kanye na 4℃, uma kucatsaniswa naletinye tindlela tekwelapha. Mayelana nelizinga letintfo letincane, emasampula ejusi laphatfwe nge*sodium metabisulphite*, kungakhatsaleki kutsi timo tekugcina, tikhombisa sibalo lesiphansi semvubelo (3.2 x 102 cfu/ml) uma kucatsaniswa naletinye tindlela tekwelapha. Ngako-ke, kwehluka kwesifundza, kusetjentiswa kwetintfo letilondvolotako kanye nelizinga lekushisa leliphansi kwekugcina konkhe kunemitselela lecondzile esitselweni semarula kanye nejusi. Ngenca yaloko, bakhiciti bejusi yetitselo temarula kumele batsatse letintfo njengoba tinemtselela locondzile ekutsengiseni kwejusi kanye nemandla ekutsengisa.

Emagama labalulekile: Ijusi yetitselo teMarula, ikhemikhali yemtimba, emaminerali, imisebenti yetintfo letincane, iSDG.

Xitsonga

Xikongomelo xa Swikongomelo swa Nhluvukiso lowu Yisekaka Emahlweni (tiSDG) swa United Nations, exikarhi xin'wana, i ku herisa ndlala na nsiko na ku tiyisisa leswaku vanhu hinkwavo va na mfikelelo wa swakudya leswi nga tala swiaki swa mirhi swo ringanela hi kwalomuya ka 2030. Ku sungula ku tirhisiwa kwa mixaka ya mihandzu ya xintu leyi tirhisiwaka switsongo yo tanihi makanyi (*Sclerocarya birrea subsp. caffra*), leyi yi vikiwaka ku va yi tele yi vhitamini ya C na timinerali leswi dingiwaka hi vanhu ku fikelela swilaveko wa vona swa madyelo ya siku na siku leswi bumabumeriwaka, swi tikomba swi ri qhinga ro tshembeka ku fikelela swikongomelo leswi. Ndzavisiso lowa marhavinyingi wu endleriwile ku lavisisa (i) nkhumbo wa xifundza xa ndhawu ya misava hi mayelana na swihlawulekisi swa fizikokhemikali swa mihandzu ya makanyi na juzi ya makanyi (ii) xitandzhaku xa nkatsaniso wa makhomelo wa swisivelakubola na swiyimo swa vuhlayiselo, na nhlayiso wa timinerali na migingiriko ya switsongwatsongwana ya 100% ta juzi ya mihandzu ya makanyi. Hikokwalaho, xikongomelo xa ndzavisiso lowu a ku ri ku tumbuluxa swisivelakubola leswi ringaneleke swinene na swiyimo swa vuhlayiselo swa juzi ya mihandzu ya makanyi leyi phurosesiweke, hi xikongomelo xa vubindzurisi bya nkarhi lowu taka wa xikumiwa lexi. Muhandzu na juzi ya wona leswi tirhisiweke eka nxopaxopo swihlawulekisi swa fizikokhemikali ku katsa na khalisiyamu, ayoni, ntiko wa mihandzu, machukela lama n'okaka hinkwawo, tifulavhonoyidi hinkwato na vhitamini ya C swi hlengeletiwile eBushbuckridge, Giyani na Tzaneen, hinkwaswo swa swona swi ntlawahatiwa tanihi swifundza swa le timbalankoveni ta Afrika-Dzonga. Xitandzhaku xa mikatsaniso ya makhomelo (swisivelakubola na tindhawu ta vuhlayiselo) eka risima, ku katsa na tifayitokhemikali na timinerali, xi kamberiwile hi ku endla juzi ya mihandzu ya makanyi yi va na swisivelakubola (sodium metabisulphite na sodium benzoate) na swiyimo swa vuhlayiselo (4, 6, na 10˚C) ku ringana nkarhivunavi wa 20 wa masiku. Hi mayelana na makambelelo ya nghingiriko wa switsongwatsongwana, nhlayelo wa puleti yo hangalasela wu tirhisiwile, leswi katsaka andziso wa switsongwatsongwana, nhlayelo wa tiyuniti leti vumbaka tikholoni na ntiveko wa mixaka ya swihanyi. Eka vulavisisi bya fizikokhemikali, ndzavisiso lowu wu paluxe leswaku mihandzu leyi hlengeletiweke eka xifundza xa Bushbuckridge yi kombe ntiko lowukulu wa (44.6 g) loko wu pimanisiwa na lowuya wa le tindhawini tin'wana. Hi ku

pimanisiwa na swifundza swin'wana, xifundza xa Tzaneen xi vile na nkhuvangano wa machukela lama n'okaka hinkwawo wa 12.9 ˚Brix. Hi mayelana na nkhumbo wa swisivelakubola leswi hambanaka na swiyimo swa vuhlayiselo eka nhlayiso wa risima, mivuyelo yi kombise leswaku nkatsaniso wa sodium benzoate na mahiselo ya vuhlayiselo ya 10˚C wu vange nhlayiso wa henhlahenhla wa vundzeni wa tifulavhonoyidi hinkwato (2.49 mg QE/ml) endzhaku ka 20 wa masiku, loko ku pimanisiwa na makhomelo man'wana. Nhlayiso wa vundzeni bya vhitamini ya C bya le henhla (83.26 mg/100 ml) byi lemukiwile ehansi ka nkatsaniso wa makhomelo (+ vulawurinene - ku hisisa kufika eka 10˚C). Eka timinerali, nkatsaniso wa makhomelo wa sodium benzoate na 4˚C swi nyike nhlayiso wa khalisiyamu lowu antswisiweke (81.3 mg/L) loko ku fananisiwa na makhomelo man'wana. Nhlayiso wa mpimohenhla wa vundzeni bya ayoni (33.1 mg/L) wu rhekodiwile eka nkatsaniso wa makhomelo wa sodium benzoate na 4˚C, hi ku pimanisiwa na makhomelo man'wana. Hi mayelana na risima ra ntivoswitsongwatsongwana, tisampulu ta juzi leti cheriweke sodium metabisulphite, swi nga ri na mhaka swiyimo swa vuhlayiselo, ti kombe nhlayelo wa xiviriso wa le hansihansi (3.2 x 102 cfu/ml) hi ku pimanisiwa na makahomelo man'wana. Hikokwalaho, mihambano ya swifundza, ntirhiso wa swisivelakubola na mahiselo ya vuhlayiselo ya le hansi hinkwaswo swi na nkhumbo wo kongoma eka mihandzu ya makanyi na juzi ya makanyi. Hikokwalaho, vamaki va juzi ya mihandzu ya makanyi va fanele ku tekela enhlokweni swihlohloteri leswi tanihileswi swi nga na nkhumbo wo kongoma eka vumaketeki bya juzi leyi na vuswikoti bya vubindzuriseki.

Maritokulu: Juzi ya mihandzu ya makanyi, fizikokhemikali, timerali, migingiriko ya switsongwatsongwana, SDG.

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ABBREVIATION LIST

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
CE	Catechin equivalents
DPPH	1,1-diphenyl-2-picrylhydrazyl
GAE	Gallic acid equivalent
GC-MS	Gas chromatography–mass spectrometry
GSO	GCC standardisation organisation
RDI	Recommended daily intake
SDG	Sustainable development goals
TSC	Tropical and Sub-tropical Crops
TSS	Total soluble sugars
TFC	Total flavonoid content
TPC	Total phenolic content
UN `	United Nations
WHO	World Health Organisation

GLOSSARY

Shelf-life — shelf life is the maximum duration during which a product has acceptable safety and quality characteristics under designated storage settings before becoming unsatisfactory to customers (Islam et al. 2014). This duration is affected by factors such as the product's formulation, production method, packaging, storage temperature, and handling procedures (Mosqueda-Melgar et al., 2012).

Preservatives — preservatives are compounds, either natural or synthetic, that are incorporated into food products to prevent rotting, prolong shelf life, and avert degradation from microbial growth, oxidation, or other chemical alterations (Shahnawaz et al., 2023). They preserve the quality and safety of food by suppressing the proliferation of organisms such as bacteria and fungi (Kabir et al., 2019).

Malnutrition — malnutrition is a disorder resulting from a shortfall, surplus, or imbalance of calories, protein, vitamins, and minerals, which adversely affects body composition, function, and clinical results (Troesch et al., 2015).

Titratable acidity — Titratable acidity is determined by titrating a food or beverage product with a standard base until a certain pH endpoint is attained (Yu & Rupasinghe, 2012). This measurement is not the same as pH, which solely measures free hydrogen ions (Saeed & Al-Tinawi 2010).

Total soluble sugars	Total Soluble Sugars (TSS) in food science denotes the aggregate of all sugars in a food sample that are soluble in water, predominantly comprising glucose, fructose, and sucrose (Maluleke et al., 2021). This measurement serves as a crucial indicator of sweetness and quality in both fresh and processed fruits. Total soluble sugars are evaluated by methods such as Brix refractometry, which employs a refractometer to assess the refractive index of the sample's liquid, hence offering an estimation of soluble sugars (Tuckeldoe et al., 2023).
Total flavonoids	Flavonoids are phytochemicals found in fruits, and their dietary intake is linked to several health advantages, such as prolonged lifespan, reduced cardiovascular issues, and lower incidence of metabolic illnesses (Lekoba et al., 2024; Mogale & Maluleke, 2025).
Total phenols	Total phenols (or phenolics) are the total concentration of all compounds in a food sample that have one or more aromatic rings with hydroxyl groups (Maluleke & Thobejane, 2025). These chemicals are abundant as natural plant secondary metabolites that contribute to the color, flavor, and bitterness of food (Wern et al., 2016). They are also known for their strong antioxidant qualities, which can keep food components from oxidizing and provide health benefits by scavenging free radicals (Adilah et al., 2023).
Macro minerals	macrominerals, also known as major mineral, are necessary nutrients that the body needs in substantial amounts to facilitate critical biological

functions, metabolic processes, and growth (Lekhuleni et al., 2024). Typical examples of macrominerals include calcium, phosphorus, magnesium, sodium, potassium, and sulfur (Molnár & Pal 2024).

Micro minerals micro-minerals, also known as trace minerals, are vital elements that the human body needs in minute amounts (Sigdel & Janaswamy, 2020). These elements are essential for healthy physiological development and iron, zinc, iodine, selenium, copper, manganese, and chromium are a few examples of microminerals (Lekoba et al., 2024).

Microbial activity In food science, microbial activity denotes the metabolic processes, proliferation, and growth of microorganisms such as bacteria, fungi, and viruses within food, resulting in biochemical transformations that can either enhance or diminish food quality and safety (Lima et al., 2009). These processes are influenced by the food's characteristics and ambient conditions, such as temperature and humidity, and are essential for understanding food deterioration, shelf life, and the development of fermented products (Mandha et al., 2023).

Reccommended daily intake: The Recommended Daily Intake (RDI), frequently synonymous with Recommended Dietary Allowance (RDA), represents the average daily dietary intake level deemed adequate to fulfill nutrient requirements for specific life stages and gender groups. It aids individuals in achieving their nutritional requirements by the daily intake of a specific nutrient-dense diet to ensure sufficient nourishment and avert malnutrition (Dang et al. 2001).

Vitamins In food science, vitamins are essential organic nutrients necessary for optimal growth, development, and physiological processes, and they must typically be acquired through the diet, as the body does not synthesise them in adequate amounts (Maqbool et al. 2017). Vitamins are essential for numerous metabolic processes and maintaining health, and are classified as either fat-soluble, such as vitamin E, or water-soluble, such as vitamin C (Awuchi et al., 2020; Barker, 2023; Maluleke et al., 2024).

CHAPTER 1: INTRODUCTION

1. Background and research justification

The United Nations set Sustainable Development Goals (SDGs) to be achieved by 2030, which include the eradication of severe poverty (SDG 1) and hunger (SDG2) for all people (Rao et al., 2018). Worldwide challenges such as poverty and food security are compounded by the rising number of cases, including the resurgence of hunger, as well as the ongoing rise in food costs (Obersteiner et al., 2016). According to Dorward (2013), extreme hunger and malnutrition continue to be an obstacle to achieving the Sustainable Development Goals as they create a trap from which societies are unable to escape. Societies that are experiencing hunger and malnutrition are less productive because they are more likely to be prone to lethargy and various illnesses, subsequently creating a situation whereby they are unable to generate enough income that may help them improve their standard of living (Renzaho et al., 2017; Lekhuleni et al., 2024). Thus, a collaborative and comprehensive strategy is necessary to provide equitable access to food for all people according to the Sustainable Development Goals agenda (Maluleke et al., 2024).

One such promising strategy with numerous benefits involves the introduction of indigenous fruit crops and their value-added products as a supplement to current mainstream crops and food products. These indigenous fruits are nutrient-dense, thrive in their natural habitat without the need for constant agricultural inputs such as fertilizers and irrigation, and can be easily consumed either as raw fruits or processed into value-added products (Omotayo & Aremu, 2020; Lekoba et al., 2024; Maluleke et al., 2024).

One of the well-known indigenous fruit trees that is considered underutilised is the marula tree (*Sclerocarya birrea*). According to Nwonwu (2006), archaeological evidence indicates that this tree species has been a source of nutrition for approximately 10,000 years, making it one of Africa's oldest botanical treasures.

The Marula tree is said to be widely dispersed throughout Africa's tropical and subtropical regions where it has been a highly nutritious indigenous fruit for centuries in South Africa, Botswana, Namibia, Angola and Zimbabwe (Omotayo & Aremu, 2020). Although there is minimal historical literature illustrating the economic activities involving the marula fruit and its value-added products within the regions where it is prevalent, the tree continues to play an important cultural and economic function in the region today. Thus, indigenous people of the lowveld regions of southern Africa hold the Marula tree in such high respect because of its many beneficial uses in their diets and customs (Omotayo and Aremu, 2020).

In terms of its botanical characteristics, the marula tree (*Sclerocarya birrea*) belongs to the Anacardiaceae family, and is considered as a medium to large tree with a rounded crown that grows up to 10 to 15 meters in height and has compound leaves with 10 or more pairs of sharply pointed leaflets (van Wyk, 2011). Male and female flowers are normally found on different trees, and the fruits are large, spherical, and somewhat flattened when ripe, with a rich covering of flesh surrounding a single stony seed (Mokgolodi et al., 2011). The seed or nuts are covered by a little, circular shell or lid that gets dislodged when the stone is cracked (Mariod & Abdelwahab, 2012). In South Africa, it is generally found in the rural areas of Limpopo, Mpumalanga, Eastern Cape and KwaZulu-Natal, but is more dominant in the Lowveld region of South Africa, including Giyani and Phalaborwa in Limpopo, and Bushbuckridge in Mpumalanga (DAFF, 2010; Ngemakwe et al., 2017). The tree is drought-resistant and naturally grows under warm, frost-free climate conditions in woodlands within sandy and sandy loam soil (Dlamini & Dube, 2008).

As shown in Figure 1.1, the marula fruit tree is a multipurpose tree mainly appreciated for its fruits, cosmetic oils from seeds and the medicinal properties derived from the bark and leaves (Mutshinyalo & Tshisevhe, 2003; Hiwilepo-van Hal et al., 2014). The trees bear fruits that are small, plum-sized, round or oval, with a thick, pale-yellow peel, a white and highly aromatic, sweet-sour, juicy pulp, and a woody endocarp covering the seed (Borochov-Neori et al., 2008; Kugedera, 2019). The fruits are eaten raw, and the edible part of the fruit is small compared to the fruit's size. The fleshy fruit has a tart taste that is strong and distinctive with a turpentine flavour, while being sweet and refreshing resulting from a low pH of 4.2 to 4.4 with a sugar content of 11 °Brix (Mojeremane & Tshwenyane, 2004; DAFF, 2010; Hiwilepo-van Hal et al., 2012; Maroyi 2013).

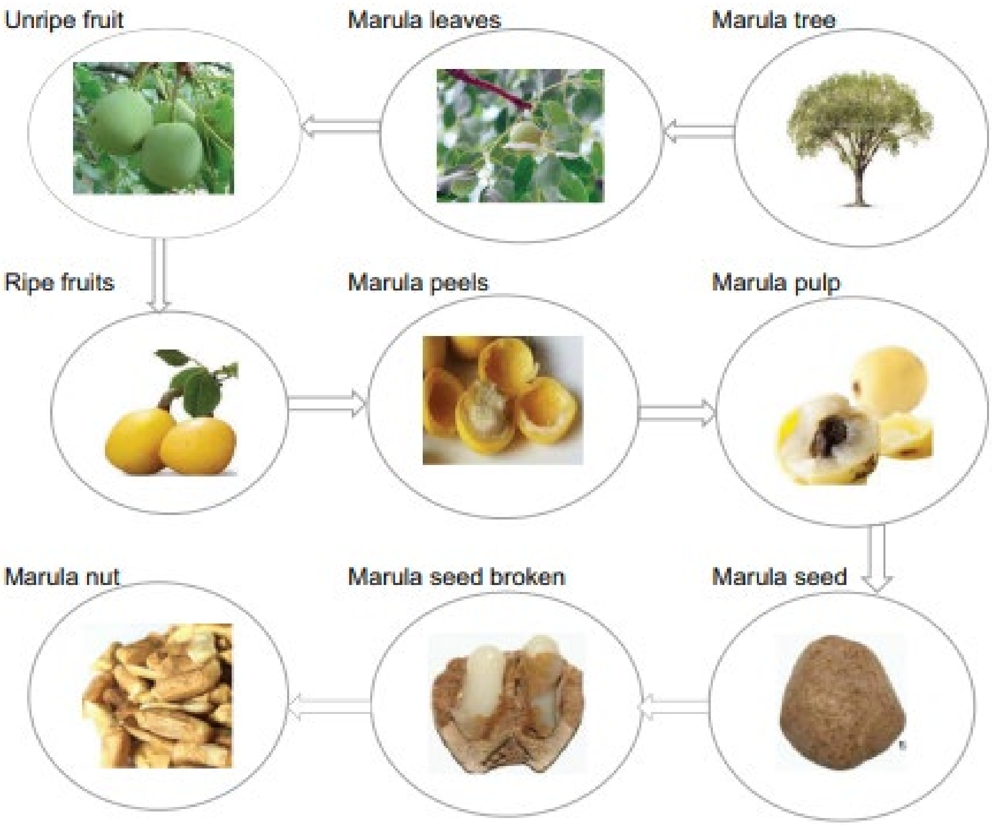

Figure 1.1: Marula tree and its fruit parts (Mashau et al., 2022)

Nutritionally, the fruit is mainly appreciated for its pulp that has a high vitamin C content and its edible nuts (Maroyi, 2013; Hamidou et al., 2014). According to Akinnifesi et al. (2008), Marula fruits has a vitamin C content higher than that of known commercial fruits such as lemon, orange and mango. The fruit pulp can also be further processed into value-added products such as jam, juice, jelly and alcoholic beverages (Hiwilepo-van Hal et al., 2012; Kudera, 2019; Lekhuleni et al., 2024). The resulting kernels can either be consumed after roasting or processed into oil for cooking and cosmetic products (Schreckenberg et al., 2006).

Extracts from the marula bark, roots and leaves provide antidiarrheal, antidiabetic, anti-inflammatory, antiseptic, antimicrobial, antiplasmodial, antihypertensive, anticonvulsant and antioxidant benefits (Ojewole, 2003; Ojewole et al., 2010; Mokgolodi et al., 2011). Thus, they are used to treat a wide range of medical conditions including malaria and fevers, diarrhea and dysentery, stomach ailments, headaches, toothache, backache and body pains, infertility, schistosomiasis, epilepsy and diabetes mellitus (van Wyk et al., 2002; Maroyi, 2013; Hiwilepo-van Hal et al., 2014).

According to the Food and Agricultural Organisation (FAO), the availability and access to nutritionally balanced foods are fundamental principles necessary to maintain life (FAO, 1996). Malnutrition is typified by deficiency either involving macronutrients (carbohydrate, protein and fats) or micronutrients (vitamins, minerals and beneficial phytochemicals) (Chivandi et al., 2015). However, micronutrient deficiency affects over two billion people globally and its effects can lead to poor health or even death (Hodge, 2016; Fortin, 2018; Omotayo & Aremu, 2020). The role of micronutrients in the body is to facilitate physiological reactions as co-enzymes and to contribute to the natural antioxidant pool so that these antioxidants can help the body to increase its resistance to infection (Nkengfack et al., 2012; Falowo et al., 2014). In addition, micronutrient deficiencies can hold back socioeconomic development, particularly in rural communities.

Based on existing literature, the marula fruit tree provides underutilised indigenous fruits that have the potential to contribute to nutrition (Shackleton et al., 2002; Phofuetsile et al., 2002; Mokgolodi et al., 2011). Considering its nutritional composition, the fruit makes a valuable contribution to the recommended nutrient intake for rural communities, thus serving an important role in the dietary needs of communties due to the abundance in the fruit of biochemical constituents required daily by humans (Mokgolodi et al., 2011; Pfukwa et al., 2020).

Since the fruit could be consumed fresh or be used as a raw material to develop value-added products such as fruit juice, consumption of the fruit and its products benefit human health and can aid in mitigating malnutrition (Omotayo & Aremu, 2020). Understanding the protocols related to post-processing quality preservation is essential, particularly for fruit juice products. Emphasizing these protocols in the food industry is crucial, as they help prevent nutritional loss and extend product shelf life.

With the need to develop safe, nutrient-dense food products, the industrial use of chemical preservatives such as ascorbic acid, sodium benzoate, potassium sorbate and sulphur dioxide is important to maintain the nutritional and other properties of food products (Talasila et al., 2015; Nwachukwu & Ezeigbo, 2013; Abiola et al., 2018). In support of this, Mesquita et al. (2003) reported that the quality and shelf life of cashew apple juice was enhanced by the use of a treatment that combined the use of preservatives (ascorbic acid, sodium benzoate, and sulphur dioxide) together with pasteurisation (mild heat treatment). In addition, Sarkar et al. (2014) reported on the effects of storage and preservatives on the antioxidant content of orange, apple, black grapes, sweet lime, litchi, pomegranate, and mango fruit juices.

Another study conducted by Abiola et al. (2018) evaluated the influence of chemical preservatives on the quality attributes of orange juice and found that chemical preservatives in orange juice do not negatively influence consumer acceptability of the product. Nevertheless, there seems to be scant literature on the use of different preservatives on the biochemical and storage stability of marula fruit juice. Therefore, the current study sought to fill a gap in the literature by investigating the effect of using different preservatives that may delay fermentation of processed marula fruit juice that is subjected to variation in pasteurisation and storage under various temperatures.

1.2 Research problem statement

Marula fruit is an important source of vitamin C and minerals, especially for rural communities, and its products have potential commercial value in southern Africa and other parts of the world (Shackleton et al., 2002; Phofuetsile & O'Brein, 2002; Mokgolodi et al., 2011). The demand for marula fruit is gradually increasing due to the natural antioxidants and other biochemical constituents within the fruit (Mokgolodi et al. 2011). Since marula fruits grow naturally in the wild, they are freely available to the wider rural and semi-urban population, and so further processing of the fruit products offers an opportunity for the potential commercialisation of marula fruit value-added products, such as juice, thus creating potential income for poor rural households and improving their well-being (Hiwilepo-van Hal et al., 2013).

During the fruiting season, fruit processing into value-added products such as juice without the use of suitable preservatives to delay fermentation results in significant waste (Lekhuleni et al., 2024). In most rural communities, marula fruit juice is commonly produced. However, during the processing of marula juice, preservatives are not used to prevent the juice from fermenting so that within three to four days of storage, the fruit juice undergoes biochemical reactions that lead to fermentation and becomes an alcoholic beverage. During the biochemical events involved in the fermentation of marula juice, chemical transformations occur that significantly alter the nutritious constituents of the juice (Hiwilepo-van Hal et al., 2013). Several studies have been undertaken regarding the composition of marula fruit but very little is known about the different preservatives used to delay the fermentation of marula fruit juice and their impact on the biochemical constituents' quality and microbial activities in the juice.

To address the issue of fermentation, this study explored suitable preservatives that can prevent fermentation while maintaining the quality of marula fruit juice stored under various conditions. Additionally, combining preservatives with optimal storage methods presents opportunities for the commercialisation of Marula fruit juice in South Africa and globally. This research also provides a chance to educate the community members involved in collecting and processing marula fruit as to preserving the resulting juice, ultimately promoting the utilisation and consumption of indigenous fruits for food and beverages. This initiative can contribute to efforts to eradicate hunger and poverty in rural communities.

Moreover, the development of an appropriate scientific juice protocol might extend marula fruit juice availability and lead to nutritional security since communities can more effectively preserve fruit juice and consume it even out of season. In addition, establishment of suitable marula juice production and quality protocols may unearth a great potential for rural communities and established food companies to commercialise the juice that they produce and sell, resulting in generation of revenue to support their livelihoods and enhance nutrient accessibility, which is one of the main focus areas of SDG 1 (no poverty) and SDG 2 (zero hunger).

1.3 Rationale of the research

According to Maluleke et al. (2021), indigenous fruits are those that are endemic to a specific geographic area and provide sustenance for populations who, due to their financial situation, have limited access to food. The many benefits of indigenous fruits include a high nutritional content, resistance to most pests and diseases, a shorter maturation period, and availability for processing and consumption in the majority of local populations where the fruit trees are commonly found (Mashile et al., 2019). By 2030, the first two SDGs are aimed at eradicating all forms of hunger and malnutrition and ensure that everyone has access to sufficient, nutrient-dense food throughout the year. This entails fostering environmentally friendly markets, technology, and agriculture (Tuckeldoe et al., 2023).

An ongoing quest for "new" crops that may be added to the food supply to improve nutrition, and potential commercialisation is essential to addressing the world's food and malnutrition challenges (Mwangi & Kimathi, 2006). This highlights the need to introduce alternative food products that provide nutrients which will assist in the fulfilment of the recommended daily intake (RDI) in all communities, globally (Lekoba et al., 2024). In recent years, studies on climate change and adaptation have particularly focused on the production and use of underutilised food crops and their value-added products, and these include wild, edible fruit species (Maluleke et al., 2021).

Marula fruit is used as a raw material by rural and semi-urban communities to create goods with added value, such as juice and alcoholic beverages (Mashau et al., 2022). According to Sibiya et al. (2021), a rapid fermentation process is the reason why the juice doesn't have a long shelf life. Thus, community members who do not consume alcohol are excluded from enjoying marula juice. In addition, due to the rapid fermentation rate, communities are denied business opportunities that could help reduce poverty through the sale of marula juice and foster economic emancipation, subsequently hastening SDGs such as Zero Hunger.

Currently, there are few publications describing the preservatives that may be used for marula fruit juice, which is a major bottleneck for developing it for market purposes. Because there is limited information on the suitable preservatives used for delaying marula juice fermentation and quality preservation, most food manufacturing firms and communities will avoid processing the fruit for juice purposes because they are afraid of losing money.

In studying the use of varying suitable preservatives to improve quality and possibly delay fermentation in marula fruit juice, it is critical to evaluate the quality and biochemical properties of the marula juice subjected to different preservatives and storage conditions. The fruit's intrinsic characteristics, such as pH, total soluble sugar (TSS), titratable acidity (TA), vitamins, flavonoids, phenols, macro- and micronutrients and primary metabolites of the fruit can be compared to those of the juice, and these properties have a positive or negative impact on the overall quality of the marula fruit juice. Therefore, the data generated can be used as a benchmark in the potential development and commercialisation of marula fruit juice. Furthermore, providing scientific evidence of how different preservatives and storage conditions affect the quality parameters of marula fruit juice will help marula juice local producers and potential agro-processing companies preserve the product, ensuring susitanable supply and availability throughout the year.

1.4 Research questions

This research study addressed the following research questions:

- What are the physicochemical characteristics and antioxidant activity of marula fruit and its potential role in human health and nutrition?

- Do different preservatives and storage conditions affect the physicochemical and biochemical composition of marula fruit juice?
- Do preservatives and storage conditions affect the mineral composition of marula fruit juice?
- What types and levels of microbial activity, including bacteria, mould, and yeast, are present in preserved marula fruit juice?

1.5 Aim and objectives

1.5.1 Aim

The primary aim of this study was to investigate the most suitable preservatives and storage conditions for processed marula fruit juice with the goal of delaying fermentation, so that a comparative quality analysis could be undertaken for the purpose of future commercialisation of marula fruit juice.

1.5.2 Objectives

The objectives of the study were:

- To evaluate the physicochemical and antioxidant activity of marula fruit.
- To evaluate the effect of preservatives and storage conditions on the physicochemical and biochemical composition of marula fruit juice.
- To determine the mineral composition of marula fruit juice.
- To assess microbial activity, including bacteria, mould, and yeast, in marula fruit juice.

1.6 Reliability and validity

The techniques and equipment used to generate information and analyse data to answer the research questions of interest determine the degree of credibility in scientific research and should be reliable and validated (Creswell, 2014). In this research for instance, it was critical to employ reliable, legitimate, and fair methodologies, supervise experiments, and, most importantly, accurately record results. Creswell (2014) stated that reliability is the consistency and trustworthiness of instruments and procedures used to assess certain parameters in an experiment. For this study, determination of biochemical qualities and optimum preservatives for quality preservation and fermentation delay of marula juice processed from fruits harvested from different regions was undertaken following the proven methods with minor modifications. The maximum allowable quantities of preservatives in various types of food set in numerous nations have been considered in this study Dorothy et al. (2023).

The simultaneous examination of preservatives was therefore done simply and accurately to ensure food safety. Due to their effectiveness in defining and quantifying preservatives, chromatographic techniques such as capillary electrophoresis, gas chromatography, and high-performance liquid chromatography were adopted as previsiously utilised by authors such as Moyo et al. (2018), Maluleke et al. (2022) and Lekoba et al. (2024), with slight modifications. To enrich the target analytes and clean up the sample matrices, an appropriate sample pre-treatment technique analysis was employed because food matrices are typically diverse and complicated. To determine the optimum preservatives to prevent marula juice from fermentation and evaluate physiochemical, nutritional, and volatile compounds and their changes under different storage conditions of marula fruit juice, a randomised design procedure was used in the experiments. The data generated was analysed using appropriate statistical procedures to achieve individual objectives.

1.7 Bias

When conducting experiments, it was paramount to employ practical measures to reduce bias (Creswell, 2014). This author stated that bias is an inaccuracy in the design or execution of an experiment that is skewed in one direction due to non-random circumstances. For this study, the experimental error in each objective was decreased by increasing the number of blocks, replications, and randomisation (Mouton, 2013).

1.8 Significance of the study

Marula fruit is one of the underutilised fruits that possesses essential nutrients and minerals. Notwithstanding this, studies on its fruit juice, and preservation methods and quality composition are limited (Stadlmayr et al., 2013; Ngwemaku et al., 2017). The nutritional and functional characteristics of the fruit is vital to promoting and expanding the utilisation of this indigenous fruit, thereby enhancing food nutritional security and rural economy development (Omotayo & Aremu, 2020). In studying the use of different preservatives and storage conditions to delay fermentation in processed marula fruit juice, it was crucial to evaluate the physicochemical, nutritional and microbial properties, and the overall acceptability of the marula juice.

Therefore, providing scientifically proven findings could allow communities and food manufacturing companies to development highly nutrient-dense and safe marula fruit juice for potential commercialisation and ultimately leading to a reduction in dificiency symptom diseases such as ulcers, skin challenges, bleeding gums, poor night vision, muscle cramps, abnormal heart rhythms, and lost of taste, which are associated with a low vitamins, micro- and macronutrient content in the human diet (Maluleke et al., 2021). Moreover, research findings will assist rural communities and food manufacturers in making production decisions for commercial purposes, since they rely on scientifically proven information to make calculated financial investments.

1.9 Thesis overview

The thesis framework includes an outline of the research study's methodology and activities, and the findings are presented in a methodical and comprehensive manner. The titles of different chapters and the summaries of their contents are described below:

Chapter 1: Introduction

This chapter begins with a brief introduction to emphasise the role of indigenous fruit crops as a source of nutrition for rural, semi-urban and urban communities. It then goes on to detail the study problem statement, research questions, aim and objectives.

Chapter 2: Literature review

The literature review provides and highlight aspects on perspectives in the utilisation and processing of indigenous fruits and to introduce the use of chemical preservatives in marula fruit juice. In this study, the literature was focused on the background of the fruit juice industry, the use of chemical preservatives and nutritional composition of the fruit for juice production and quality parameters. This chapter concludes by identifying gaps in the literature and efforts to close the gaps.

Chapter 3: Methodology

Outlines the different research methodology processes applied to achieve the objective of the research study:

Objective/experiment 1: Physicochemical properties bioactive compounds of marula fruit (*Sclerocarya birrea*) collected in varying regions and its potential role in human nutrition

Focused on this experimental work investigates the physicochemical properties and antioxidant activities of marula fruit collected from varying regions and its potential role in human nutrition. Gathered data were statistically analysed, interpreted, and discussed to reach factual conclusion.

Objective 2/experiment 2: Characterisation of marula fruit (*Sclerocarya birrea*) juice and the effect of storage conditions and preservatives on its biochemical properties

This experimental work examines the effect of preservatives and storage conditions on the physicochemical and biochemical properties of marula fruit juice. Data gathered are statistically analysed, interpreted and discussed to reach factual conclusions.

Objective 3/experiment 3: Effect of preservatives and storage conditions on the mineral composition of marula (*Sclerocarya birrea*) fruit juice

This experiment investigates the mineral composition in marula fruit juice and its potential role in human nutrition. Their potential role in human health and nutrition was estimated by comparing with the recommended daily intake and gathered data were statistically analysed to reach factual conclusions.

Objective 4/experiment 4: Influence of geographical region, storage conditions and preservatives on the microbiological quality of marula (*Sclerocarya birrea*) fruit juice

This experimental section investigated the effect of preservatives and storage condition on the microbial activity of bacteria, mould and yeast on marula fruit juice processed from fuits harvested from varying regions. The gathered data are analysed, interpreted and analysed to reach detailed conclusions.

Chapter 4: Results and discussion

This chapter included the results and discussion of data that have been collected, presented, and analysed from the experimental work carried out in chapter 3. It encompasses several examined variables and the methodology employed to determine the outcome. The collected results were analysed, discussed, and interpreted.

Chapter 5: General conclusions and future work

This concluding chapter of the thesis encapsulates the principal research findings and delineates the significant conclusions derived from the investigation. Suggestions about the prospective commercialisation of marula fruit juice are provided. Additional, future investigation are also recommended.

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CHAPTER 2: Literature review

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2. Overview

In many countries, different methods are employed during the processing and utilisation of fruits to create and add value. The objective of this chapter is to review relevant published literature and to highlight aspects on perspectives in the utilisation and processing of fruits and also to introduce the use of chemical preservatives and storage conditions in marula fruit juice. In this study, the literature focused on the background of the fruit juice industry, juice production and quality parameters, the use of chemical preservatives and the nutritional composition of the fruit used as raw material to generate this value-added product.

2.1. Indigenous fruit as a potential ingredient in the juice industry

2.1.1 Overview of indigenous fruit juice

Indigenous fruit trees of Southern Africa are regarded as a variety of species that are genuinely traditional or have originated from the region and grow naturally in the wild without a constant supply of agricultural inputs such as irrigation and fertilizers (Schreckenberg et al., 2006; Maluleke et al., 2021). Fruits from these trees play an important role as a source of food and nutrients in many rural and semi-urban communities in African countries (Leakey & Akinnifesi, 2008).

These fruits contain essential macro- and micronutrients such as vitamin A and C, fibre, and minerals; phytochemicals such as flavonoids and phenols, whilst others are rich in essential oils and proteins, and some are also used for medicinal purposes (du Preez et al., 2012; van Wyk, 2011). Additionally, indigenous fruits also offer a source of income for various communities who sell and generate monetory profits derived from such sales (Shackleton, 2004). In terms of utilisation, the fruit pulp, especially that of Marula (Sclerocarya birrea); Kei Apple (Dovyalis caffra); Num-num (Carissa macrocarpa), African mangosteen (Garcinia livingstonei); (Engelerophyytum magalismontanum); Baobab (Adansonia digitata) and Mobola Plum (Parinari curatellifolia) has been reported as raw material for development of a variety of by-products (du Preez et al., 2013; Rampedi & Olivier, 2013; van Wyk, 2011). These fruits are mostly consumed fresh or the pulp is used to process different products such as traditional wines/beer, juice and jams (Shackleton, 2004; Saka et al., 2008; Rampedi & Olivier, 2013).

As highly valued species, further processing of these fruits contributes to the reduction of postharvest losses, since they are preserved for later use, while providing more palatable products that add financial value, and allows the extraction of nutrients that are mostly beneficial in combating malnutrition (Saka et al., 2008; van Wyk, 2011). Loots et al. (2006) reported on the polyphenol composition and antioxidant capacity of Kei Apple juice in comparison to various commercial juices. These authors showed that the total polyphenol content of the Kei Apple juice was approximatively twice as high as that of strawberry juice and approximatively four times higher than that of grape and orange juice.

Tembo et al. (2017) investigated the effect of thermal treatment and storage on bioactive compounds, organic acids and the antioxidant activity of Baobab fruit pulp from Malawi. Findings from the study showed that pasteurisation retained a reasonable amount of vitamin C, total phenolic compounds and antioxidant activity during storage when compared to other treatments. Currently, marula fruit juice is available in rural communities and is also classified as an inferior product, due to its rapid rate of spoilage due to fermentation. This is caused by lack of knowledge with regards to the use of pasteurisation, preservatives and optimal storage temperature for quality improvement.

There are many techniques used to preserve fruit juices including freezing, heating (hot fill, aseptic, blanching, pasteurisation, drying) and non-thermal (chemical preservation, sterile filtration) processes (Kapoor et al., 2014; Talasila & Shaik, 2015). Additional focus has been on the development of novel preservation methods that can be used in the food industry including the use of filtration membranes, ultrasonics, irradiation, technologies involving high hydrostatic pressure and pulsed electric fields, and new packaging systems (modified atmosphere packaging and the use of natural preservatives) (Akpan & Kovo, 2005). With the rapid growth and development of new products in the food industry, chemical preservatives such as ascorbic acid, sodium benzoate, potassium sorbate and sulphur dioxide have been used extensively to maintain the nutritional and other properties of food products (Talasila et al., 2012; Nwachukwu & Ezeigbo, 2013; Abiola et al., 2018).

Juice processors utilise various methods to preserve the quality of juice products, which may include the use of chemical preservatives (Walker & Phillips, 2008). However, the preservation methods used for a particular type of fruit juice depend mainly on the biochemical constituents, quality and composition of the fruit, as well as the type of spoilage associated with it (Walker & Phillips, 2008). The study conducted by Mesquita et al. (2003) focused on quality preservation of Cashew Apple juice subjected to chemical preservatives and the authors reported that the quality of the juice was improved by reducing water activity and pH, the addition of ascorbic acid, sodium benzoate and sulphur dioxide as well as mild heat treatment, which appeared to efficiently preserve the juice for a period of four months at 28 °C.

According to Beebi (2009), the use of chemical preservatives such as sodium benzoate, sodium metabisulphite, potassium metabisulphite, benzoic acid and citric acid alone cannot improve the shelf life. To support this claim, a study conducted by Sarkar et al. (2014) investigated the combined effect of storage conditions and preservatives on the antioxidant content of orange, apple, black grape, sweet lime, litchi, pomegranate and mango fruit juices. The authors reported variation in quality of fruit juice subjected to these tretaments. Another study carried out by Mehmood et al. (2008) investigated the impact of pastuarisation and preservatives on the biochemical constituents of apple juice. They found that the use of this combination had a positive impact in terms of quality preservation and shelf life stability of the apple juice. Nonetheless, there appear to be few published articles describing the effect of preservatives and storage conditions on the biochemical and nutritonal stability of marula fruit juices. Therefore, this study sought to fill the gap in the literature by determining the optimum preservative and storage conditions suitable to retain product quality and delay fermentation of marula fruit juice thereby increasing the potential commercialisation prospects for this fruit juice.

2.1.2 The role of preservatives in the fruit juice industry

2.1.2.1 Chemical preservatives

Preservatives are substances, either organic or chemical, that are added to food products to extend their shelf life by reducing or preventing decomposition caused by microorganisms such as bacteria and mould, as well as undesired chemical reactions such as oxidization (Pandey & Upadhyay, 2012). These additives function by suppressing microbial growth or slowing decomposition, thereby preserving and improving food quality, safety, and freshness (Ayub et al. 2010). The application of preservatives in food, particularly in South Africa, is regulated by "Regulation 965 of 3 June 1977," which oversees the utilization of preservatives and antioxidants in food products. The key aspects of the “Regulation 965 of 3 June 1977” includes, (i) permitted additives, (ii) application, (iii) mixture compliance and safety limits, and adeherence to this regulation, especially when creative food products to be consumed by citizen is mandotary.

Within the fruit juice industry, the use and role of preservatives is essential as they assist in preventing microbial spoilage, suppress chemical changes such as oxidation, and lengthen shelf life, all of which contribute to product safety and quality (Tribst et al., 2009; Talasila et al., 2012). In addition, preservatives are responsible for preserving the juice’s ideal taste, color and texture, while reducing food waste by allowing for longer storage and transportation, resulting in a more stable and cost-effective supply chain (Shahnawaz et al., 2023). Common examples include chemical preservatives such as potassium sorbate and sodium benzoate, as well as organic alternatives such as citric acid and rosemary extract (Hooshyar et al., 2020).

Abiola et al. (2018) and Zahan et al. (2024) identified several factors to be considered when utilising preservatives for enhancing the quality of fruit juice, including (i) the type of juice and its acidity, (ii) the desired shelf-life and storage conditions, (iii) consumer preferences, and (iv) the appropriate preservative dosage to ensure the quality and safety related to product consumption. Well-known preservatives such as sodium benzoate, potassium sorbate and sodium metabisulfite have been used to extend the shelf life of most commercial juices, including apple, orange, blackberry, watermelon, strarwberry and grapes, due to their efficacy in preventing deterioration and improving nutritional quality (Ayub et al., 2010; Alam et al., 2012; Castro-López et al., 2016; Bat et al., 2018; Mandha et al., 2023).

There is an abundance of literature concerning the suitable preservatives utilised for the conservation of biochemical, nutritional, and mineral quality in diverse commercial juices. Nonetheless, literature describing the effects of various preservatives on the quality preservation of juices derived indigenous fruits remains scarce. To fill the void, the current study assessed the effects of varying preservatives (sodium benzoate and sodium metabisulfite) on the biochemical quality retention of marula fruit to determine the suitable preservatives for enhancing juice quality, particularly during storage. This initiative was carried out to expedite the commercialization of the juice, thereby contributing to the achievement of SDGs 1 and 2, which focus on eradicating hunger and poverty in communities irrespective of their economic conditions.

2.1.2.2 Pasteurisation

Pasteurisation, which defined as a thermal treatment process that entails heating products to designated temperatures and subsequently cooling them rapidly, primarily aimed at eradicating harmful microorganisms (pathogens) in food and beverages, thereby preventing disease and prolonging shelf life without substantially altering nutritional value or flavour (Tobolková et al. 2024). The advantage of pasteurisation has been explained by authors such as Sampedro et al. (2013) and Shah et al. (2016) as being (i) reduce regulatoey compliance expenses, (ii) enhanced supply chain efficiency due to extended shelf life without chemicals and (iii) improvement of human health by eradicating dangerous pathogens in food and beverages. While microbial reduction is the main objective of pasteurisation, with the aim to improve the shelf life of all types of juices, the physical, chemical, and sensory changes that take place are largely dependent on the particular composition of the juice, including its pH, sugar profile, carotenoids, and enzyme levels, as a result, different types of juices react differently to the treatment (Mena et al. 2013; Vegara et al. 2013).

The impact of different pasteurization techniques on the bioaccessibility of antioxidants in orange fruit juice was examined in a study by Etzbach et al. (2020). When compared to alternative treatments, the authors found that ultrasound treatment improved levels of substances like beta carotene and total carotenoids. Another study conducted by Vollmer et al. (2020), which looked at the physical traits, microbial loads and phytochemical composition of pineapple juice. Authors discovered that the pressure change technology treated resulted in better retention of physical attributes including colourand phytochemical such as vitamins, total phenols, while reducing the microbial load in the juice.

The reviewed literature delineates extensive research on juices extracted from many exotic fruit species, including commercially available crops such as orange and pineapple. However, knowledge concerning the application of pasteurisation on juice extracted from indigenous fruit species, such as marula fruit, remains sparse. The present study utilised pasteurisation treatment as a positive control to evaluate the effects of chemical preservatives (sodium benzoate and sodium metabisulphite) on the phytochemical, mineral, and microbial activities of juice extracted from the underutilised marula fruit, native to Africa, including South Africa.

2.1.3 The role of storage conditions in the fruit juice industry

Storage conditions refer to controlled environmental factors such as temperature, humidity, light exposure and packaging, all of which are essential for preserving the quality, stability, freshness, and safety of food products over their whole shelf life (Kaddumukasa et al., 2017). Critical sensory qualities including flavor, aroma, color and texture can only be maintained under certain storage conditions, which may also affect the rates of physical and chemical degradation (Brugnoni et al., 2013).

Various authors have extensively reported on the utilisation of storage conditions to preserve biochemical quality in fruit juice. For instance, Okudu and Ene-Obong (2015) investigated the effect of different storage durations and temperatures on the physicochemical parameters of Monkey Kola fruit juice. These authors showed that a low temperature of 12°C successfully increased the preservation of biochemical elements, including total soluble sugars and pH, when compared to other treatments such as ambient (29 to 32°C) temperature and extended storage time. Thus, these authors recommended that appropriate conditions and duration of storage be determined before introducing a new product to the market.

Mohamad Salin et al. (2022) evaluated the physicochemical characteristics and phytochemicals of watermelon juice subjected to varying storage temperatures. From their investigation, these authors reported that phytochemical constituents including total soluble sugars, and total phenols were stable when stored under cold refrigerated condition, while there was a noticeable decrease of the same compounds when the juice was stored at ambient room temperature. They further recommended the use of cold storage for nutrient retention and maintenance of quality of watermelon juice during storage.

Most of the literature describes the use of preservatives in fruit juices, particularly that of juice derived from commercial fruits such as apple, orange, and watermelon. To fill a gap in the current literature, the current study assessed the influence of different storage temperatures on the biochemical properties of marula fruit juice, which is currently classed as an underutilised fruit product due to a lack of proven baseline scientific data. Furthermore, this investigation was also conducted to speed the commercialization of marula fruit juice, so assisting in the achievement of SDGs 1 and 2, which advocate for the eradication of hunger and poverty in communities regardless of economic level.

2.1.3 Microbial activities in fruit juice

From a food science standpoint, microbial activity in fruit juice includes spoilage organisms such as yeasts, molds and acid-tolerant bacteria that degrade product quality, as well as pathogenic bacteria (Mandha et al., 2023). Despite the inherent acidity of the fruit juice, these bacteria represent food safety hazards (Pinto et al., 2017). Such an acidic pH is a natural barrier that is, unfortunately, insufficient to counteract acid-stress reactions in pathogens. Other important factors impacting microbial development include water activity, hygienic methods used during juice extraction and processing, and storage temperatures, particularly for unpasteurised juices (Kaddumukasa et al., 2017).

Preventing microbial activity in fruit juice is crucial for both food safety by eradicating pathogens as well as preservation of fruit juice quality, which increases shelf life and prevents spoilage of the juice (Brugnoni et al., 2013). This is performed via pasteurisation and other heat treatments that kill bacteria and deactivate spoilage enzymes, as well as non-thermal methods like as high-pressure processing and UV irradiation. Maintaining sanitary processing conditions, using appropriate chilling, and controlling parameters such as acidity all help to limit microbial growth and retain the juice's fresh flavor and nutritional value (Raccach & Mellatdoust, 2007).

In their study, Mandha et al. (2023) evaluated the the microbial activities of various juices subjected to varying storage conditions. These authors discovered that microbial activities are linked to the juice type and storage duration. Furthermore, the study noted low microbial activities (yeast and molds) in mango fruit juice that was pastuarised and stored for nine days in comparison to pineapple and watermelon juice under the same treatments. However, the microbial activities in all juices increased as storage duration increased. These authors further highlighted that fruit juice producers and consumers should be mindful of the pasteurisation time and storage duration in order to successfully minimise the development of moulds and yeasts in fruit juice, which pose a threat to the quality and safety of the product.

The current and existing literature appears to cover microbial activities in relation to preservation and storage conditions of commercial juice, which is currently dominated by juices derived from exotic plant/crop species. However, information is relative scarce regarding the microbial activities of juice derived from indigenous fruits such as Marula. To fill the void in the literature, the current study determined the microbial activities associated with marula juice that was subjected to varying preservatives and storage conditions. This was performed in order to promote the safety associated with consumption of marula fruit juice subjected to varying preservatives and storage conditions.

2.1.4 The role of recommended daily intake in human nutrition

The Recommended Daily Intake (RDI) is vital for human health as it offers critical nutrient standards necessary for maintaining bodily functioning, preventing chronic diseases (such as diabetes and heart disease), and ensuring adequate growth (Dang et al. 2001). Adhering to recommedended daily intake criteria prevents dietary shortages, enhances immunological function, and maximises long-term physical and mental health (Nielsen 2000).

In research by Leet (2001), the authors assessed the significance of various minerals, including calcium, copper, iron, potassium, magnesium, sodium, and zinc, as contributors to human daily dietary requirements. Researchers found that although most food products can fulfill dietary requirements, no single food can satisfy all nutritional needs; therefore, it is essential for individuals to consume a diverse array of foods to adequately meet their dietary needs, as food varies in nutritional composition.

Another study conducted by Quann et al. (2015) assessed whether the use of dairy products would help fulfill daily recommended intake levels and diminish the prevalence of insufficient micronutrients. The study found that while the regular consumption of dairy products aids in meeting recommended daily intakes for humans, its effects differ between age groups. They additionally proposed that diverse age demographics can satisfy their daily nutritional requirements by using supplementary items abundant in minerals like calcium, magnesium, and vitamins, as no food products can entirely match the human recommended rietary Allowances.

2.1.4.1 Recommended daily intake of different phytochemicals and minerals for fullment of daily nutritional needs

2.1.4.1.1 Phytochemicals

Phytochemicals are non-nutritive, bioactive molecules present in plant-based meals, including fruits and their value-added products like juice, jam, and powder, which significantly contribute to human nutrition by offering health advantages that extend beyond mere caloric requirements (Lekoba et al. 2024). Although they are vital for immediate survival, they are imperative for long-term health, disease prevention, and physiological regulation (Almodaifer et al. 2017). Well-known phytochemicals include flavonoids, phenols, tannins, and vitamnis, are reported to be abundant in plant-based food (Maluleke et al. 2025). However, the source and storage conditions of the plant-based food determine the constituents of these phytochemicals (Lekhuleni and Maluleke 2025).

Klimczak et al. (2007) evaluated the impact of storage duration (6 months) and temperature (18, 28, and 38˚C) on the levels of vitamin C and phenolic compounds in orange juice. Although the combination of these factors (storage duration and temperature) seems effective in the initial months (first month) under lower temperature of 18˚C, there was a marked decline in phytochemicals such as vitamin C and total phenols, underscoring the importance of optimizing these factors during post-storage to enhance nutrient retention.

2.1.4.1.2 Minerals constituents

Minerals, as shown in Table 2.1, are vital inorganic minerals that the human body cannot synthesize and must obtain daily through diet to sustain optimal physiological function, growth, and health maintenance (Awuchi et al. 2020). Minarels are classified into two groups, with macro-minerals being regarded as those required by humans in a larger quantity, while while micro-nutrients are required in a small quantity (Barrett et al., 2010). Dietary minerals consistently create concerns within the food business, health, and nutrition, directly influencing consumer interactions with specific foods (Meijer et al. 2020). While some scholars, including Singh and Sharma (2017), contend that the mineral constituents of plant food-based products, such as juice, remain stable during storage. Conversely, Bhardwaj and Mukherjee (2011) found that the mineral content of fruit juice can fluctuate during post-process storage, although these changes typically occur at a slower rate than those of phytochemicals like vitamins.

In a study by Mandha et al. (2023), the authors assessed the qualitative features of different fruit juices subjected to pasteurization and variable storage durations for the purpose of optimization. Researchers identified differences in the mineral composition of the juice, with watermelon juice exhibiting the highest levels of calcium, copper, iron, manganese, magnesium, potassium, sodium, and zinc, in comparison to other juices such as pineapple and mango.

Another study conducted by Atif et al. (2021) assessed the impact of different pasteurization methods and the application of preservatives on the quality of carrot juice throughout various storage periods. Research findings demonstrated that the application of preservatives was crucial in preserving beta carotene levels over a two-month period; however, a significant reduction in retention was observed when the storage duration extended to four to six months.

Despite some degree of similarity and contradictory findings among various authors regarding the impact of preservatives and storage duration on the quality of fruit juice—specifically that derived from watermelon, pineapple, mango, and carrots, which are of exotic origins—there appears to be a paucity of research on the treatment combinations of different preservatives and storage conditions. As a result, to address the gap in the current literature, this study examined the effects of different preservatives (sodium metabisulphite and sodium benzoate) on marula fruit juice under various storage conditions (4, 6, and 10˚C), enabling a comparative analysis aimed at determining optimal storage conditions for potential commercialisation of the juice.

Table 2.1: Reccommended daily intake of various phytochemicals and minerals importance for human health and nutrition

Phytochemical	Main role in human health and nutrition	ARDI (mg/100g)	Reference
Total tannins	Promote cardiovascular, gut and metabolic health	200	Baba and Malik (2015) & Lekhuleni and Maluleke (2024)
Total flavanoids	Improve vascular fucntion and lower cardiovascular disease risk	750	Moyo et al. (2018) & Maluleke and Thobejane (2025)
Total phenols	Reduce oxidative stress, enhancing immune function and improving metabolism	1050	Moyo et al. (2018) & Maluleke and Thobejane (2025)
Vitamin C	Improve immune function, antioxidant protection and neurotransmitter	82.5	Maluleke et al. (2024) & Mogale and Maluleke (2025)
Calcium	Oxygen transport	1116	Lekoba et al. (2024)
Iron	Oxygen transport and improve metabolism	14.2	Lekoba et al. (2024) & Mogale and Maluleke (2025)
magnesium	Muscle, nerve function, energy	370	Achaglinkame et al. (2019) & Lekhuleni et al. (2024)
Potassium	Fluid balance and heart health	2760	McLean & Wang (2021) & Maluleke et al. (2024)
Zinc	Immunity and would ghealing.	10.5	Kanwar & Sharma (2022) & Lekoba et al. (2024)

Note: ARDI= Average recommended daily intake.

Average recommended daily intake formula: RDI Average = **(Sum of all values)/(Number of values)** = Equation 2.1

2.2 Marula fruit as a research topic

2.2.1 Marula (*Sclerocarya birrea subsp. caffra*) fruit

Marula (Sclerocarya birrea subsp. caffra) is a medium to large tree, belonging to the Anacardiaceae family, which is mainly classified as tropical and subtropical evergreen, deciduous trees, shrubs and wood plant (Chirwa & Akinnifesi, 2008). It is one of the most utilised indigenous wild fruit trees in the southern African region and has a variety of common names. For instance, it is called marula (English), moroela (Afrikaans), umGanu (isiZulu), nkanyi (Xitsonga), morula (Sepedi) and mfula (Tshivenda) according to authors such as Shackleton et al. (2002), and Bille et al. (2013;). In South Africa, this fruit tree is generally found in the lowveld regions areas such as Limpopo, Mpumalanga, Eastern Cape and KwaZulu Natal (Viljoen et al. 2008). However, in the Limpopo province, it is more dominant in Giyani and Phalaborwa, and in Bushbuckridge in Mpumalanga (Wynberg & Laird, 2007; DAFF, 2010; Nitcheu Ngemakwe et al., 2017).

The tree is deciduous, with its height ranging from 1 to 7 metres. It is considered as a large, tree that is drought resistant, that naturally thrives under warm, frost-free climate conditions in woodland or bushveld regions (Mokgolodi et al., 2011). Regarding soil and rainfall preferences, the tree grows well in sandy and sandy loam soil, with a mean annual rainfall of 200 mm to 1500 mm (Shackleton et al., 2002; Dlamini & Dube, 2008; Mokgolodi et al., 2011). A marula tree has a single round stem with branches ranging about three to four meters above the ground. It normally produces flowers from September to November and bears fruit from November to April.

In the middle of the rainy season (February to April), the ripe marula fruit starts to drop from the trees in large quantities (Jama et al., 2008; Hiwilepo-van Hal et al., 2012). The marula tree is a multipurpose tree where all parts of tree are used, with the fruit being the most utilised part of the plant due to its cultural and economic benefits, especially in southern Africa where it is prevalent (Hall, 2002; Shackleton et al., 2004; Mokgolodi et al., 2011). The fruits, which are normally used as a raw material for a variety of value-added products are small, round or oval in shape with a diameter of 30 to 40 mm with a thick leathery peel (exocarp), that is translucent, white. The highly aromatic sweet-sour pulp (mesocarp) and a woody endocarp protect the seed

(Mojeremane & Tshwenyane, 2004; Hiwilepo-van Hal et al., 2012). Unripe fruits are characterised by their pale green colour which further change to pale yellow upon ripening.

The fruit is considered ripe when the colour changes from green to yellow, and its sugar content has been reported to be approximatively 11 °Brix with strong, distinctive and turpentine flavour (Chirwa & Akinnifesi, 2008; DAFF, 2010; Suarez et al., 2012). On average, the fruit contains approximately 85% moisture content (Chirwa & Akinnifesi, 2008). The marula fruit is considered a climacteric fruit, since is ripens on the ground after it has abscised (Emongor & Tautsagae, 2016). Marula fruits are generally consumed either as fresh or used for the creation of value-added products such as jam, juice and jelly (Shackleton et al., 2002). Regarding the juice processing and preservation, there is a paucity of literature on the effect of different preservatives and storage conditions for quality improvement and possible delay of fermentation of fruits and fruit juice. Therefore, the current study assessed the influence of different preservatives and storage conditions on the improvement in quality and delay in fermentation of marula fruit juice processed from fruits harvested in differing regions of South Africa.

2.2.2 The effect of primary metabolites on the fruit pulp and juice quality

Plant metabolites are biological substances crucial for plant growth and development, directly influencing biochemical contents (Sarker et al., 2020). Particularly for fruit crops, the biochemical makeup of the crop is directly impacted by the environment to which it is exposed (Hadid, 2016).

Maluleke (2022) investigated the metabolite profile of African horned cucumber fruit grown in different environments. The study findings showed that environmental conditions were the main factor affecting the metabolite profile and quantity of the fruit. These metabolites not only affect the fruit but also impact the biochemical and nutritional content of value-added products such as jam and juice, food products that are derived from the fruit (Bermejo et al., 2016).

Primary metabolites such as sugars and organic acids are the principal factors influencing the taste, sweetness, and acidity of fruit juice (Bat et al., 2018). Other principal metabolites such as amino acids also influence flavor, nutritional value, and antioxidant capacity, with their quantities fluctuating according to fruit type, variety, and environmental conditions (Forner-Giner et al., 2023). The equilibrium of these metabolites affects the overall flavor profile and quality of fruit juice (Veberic et al., 2010).

Tavarini et al. (2008) evaluated the impact of harvesting time and storage on the nutritional quality of Kiwi fruit and showed that variation in biochemical constituents such as vitamin C and the phenolic content of the fruit is linked with the harvesting period and storage conditions. They further recommended that fruit and food producers should consider these factors so as to maximise the quality of the products derived from the fruit. Another study conducted by Bat et al. (2018) assessed primary and secondary metabolites as a cause for variation in apple juice processed from fruits harvested in differing geographical regions. These authors found variation in metabolites in the apple juice processed from fruits sourced from varying regions, and linked temperature and rainfall as the contributing factors to the variation in metabolites.

Published information regarding their impact on indigenous fruit such as marula and its juice appear to be scanty. However, since marula fruit is prevalent in the lowveld regions (Bushbuckridge, Giyani and Tzaneen) with distinct climatic conditions, the metabolite profile derived from distinct climates and their impact on the juice quality would assist satart up farmers and food producers as to quality considerations regarding the marula fruit and juice.

2.2.3 Biochemical composition of fruit, juice and the role in human health and nutrition

The fruit and juice sector have a direct impact on food and nutrition security since thay can fulfil the dietary needs of societies by supplying essential daily vitamins and minerals needed by humans (Oluwole et al., 2023). In the era of Sustainable Development Goals, these industries are pivotal to households and food sectors through the income generated from the sale of fruits and value-added products such as juice (P´erez-Escamilla, 2017). By facilitating access to nutritious, nutrient-dense foods such as fruits and juice, the sector can directly combat hunger and enhance health, especially among vulnerable communities, subsequently addressing the SDG goal 1 (no poverty) and 2 (zero hunger) (Esturo et al. (2023).

In respect of the nutritional contribution of marula fruit, the pulp contains essential phytochemicals such as total flavonoids, total phenols; minerals such as calcium, magnesium, iron and vitamin C (Mdluli & Owusu-Apenten, 2003). Regarding the nutritional and mineral content of the marula value-added products such as pulps and jam, Lekhuleni et al. (2024) reported a valuable amount of beta-carotene, flavonoids, phenols, vitamins, and minerals in these products. However, there is a paucity of literature regarding the impact of geographical regions on the marula fruit and its juice and so the current study aimed to fill the gap in the literature by determining the impact of varying geographical regions with distinct climates on the biochemical constituents of marula fruit and its juice.

2.2.3.1 The effect of varying preservatives and storage conditions on the total flavonoids content of fruit juice

As indicated in Figure 2.1, plant flavonoids are phytochemicals present in fruits that are known for their antioxidant and anti-inflammatory qualities, which protect cells and lower the risk of chronic diseases (Hoensch & Oertel, 2015). Such phytochemicals are linked to improved cardiovascular health, a lower risk of metabolic illnesses such as diabetes and obesity and have potential anticancer properties (Tsanova-Savova et al., 2018). However, factors such as storage conditions, the use of preservatives and environmental conditions play a significant role on the quantity in a fruit juice product.

In a study conducted by Igual et al. (2011), the authors evaluated changes in the flavonoid content of grape juice subjected to pastuarisation and storage conditions. These authors reported a variation in the biochemical constituents of grape juice, with the use of a microwave as a pastuarisation method exhibiting higher retention of flavonoids when compared to other treatments. In other published research, Sentandreu et al. (2007) assessed the effect of storage conditions on the flavonoid content of citrus juice. A lower storage temperature assists with flavonoid retention, as a decrease in flavonoid content was noticeable in the fruit juice that was stored at room temperature for longer period when compared to other treatments.

The available literature demonstrated the need for using storage conditions and pasteurisation for preserving the quality of juice derived from exotic fruits such as grapes. However, research on the use of preservatives and storage conditions to improve the quality characteristics of fruit juice derived from indigenous fruit species such as marula remains limited. As a result, the current study filled a gap in the literature by assessing the combined influence of preservatives and storage conditions on the quality improvement and retention of marula fruit juice.

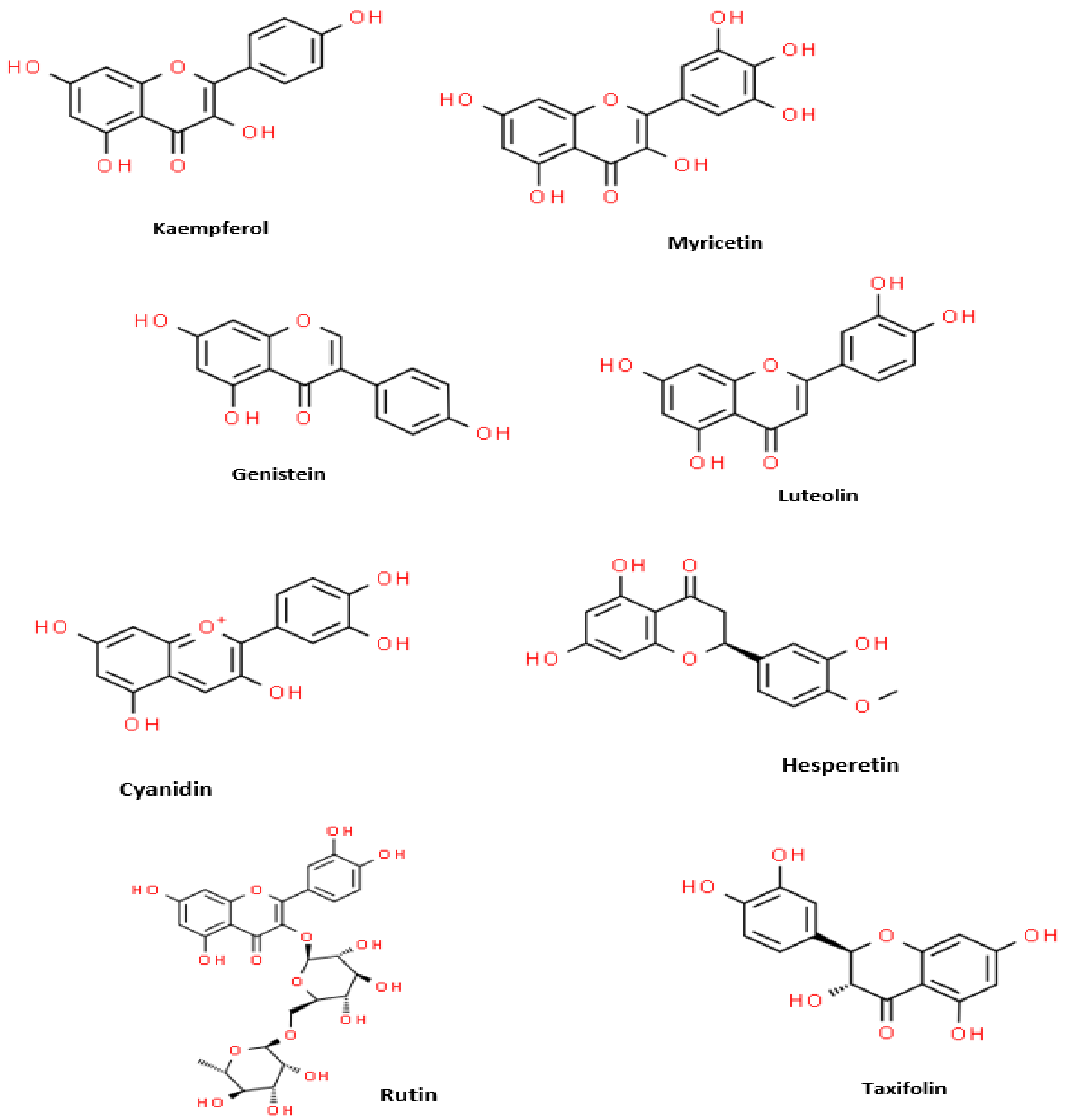

Figure 2.1: Various flavonoids determined in marula fruit and its juice. Adopted from Lekhuleni et al. (2024).

2.2.3.2 *The effect of varying preservatives and storage conditions on the total phenolic content of fruit juice*

Figure 2.2 indicates some of the phenolic compounds identified in marula fruit and extracted juice. The phenolic content in fruit juice is crucial for human health, where these compounds function as antioxidants, mitigating the risk of chronic diseases such as cardiovascular ailments and specific malignancies, and provide anti-inflammatory, antiviral, and anti-allergic properties (Guiné & Barroca 2014). The role of these compounds is to safeguard cells from oxidative damage, enhance cognitive function, and promote overall well-being. Nonetheless, while 100% fruit juices serve as a supplementary source, entire fruits are still advised as a healthy, dietary mainstay (Lekhuleni et al., 2024).

Ahmed et al. (2021) investigated the impact of preservatives on the physicochemical properties of blackberry juice during storage. These authors reported that that the use of preservatives such as sodium benzoate and potassium sorbate promoted the retension of more phenonic compounds when compared to those of the control treatment without preservatives. Authors further recommended that the use of preservatives should be considered since it is a practical method that could assist in nutritional quality preservation for juice products during storage.

Another investigation carried out by Mandha et al. (2023) characterised the nutritional quality of varying fruit juices subjected to differing pasteurisation times and storage duration. These authors found that varying pasturisation times have a direct impact on the nutritional quality of fruit juice. For instance, a treatment consisting of a combination of pasteurisation of more than 10 minutes and above 9-day storage led to a drastic reduction in the total phenolic content of watermelon juice when compared to other juices subjected to similar treatment.

Current literature indicates that preservatives and storage conditions influence the overall phenolic content of most fruit juices. Nevertheless, the existing literature on the effects of these treatments (preservatives and storage conditions) on the nutritional content of juice from indigenous fruit species, such as marula, remains sparse. As a result, the current study assessed the influence of preservatives and storage conditions on the total phenolic content of marula fruit juice, aiming to establish baseline data to assist the juice processors in preserving juice quality for year-round availability. This will help guarantee food and nutritional security among communities, as the juice can be kept for an extended period without diminishing quality.

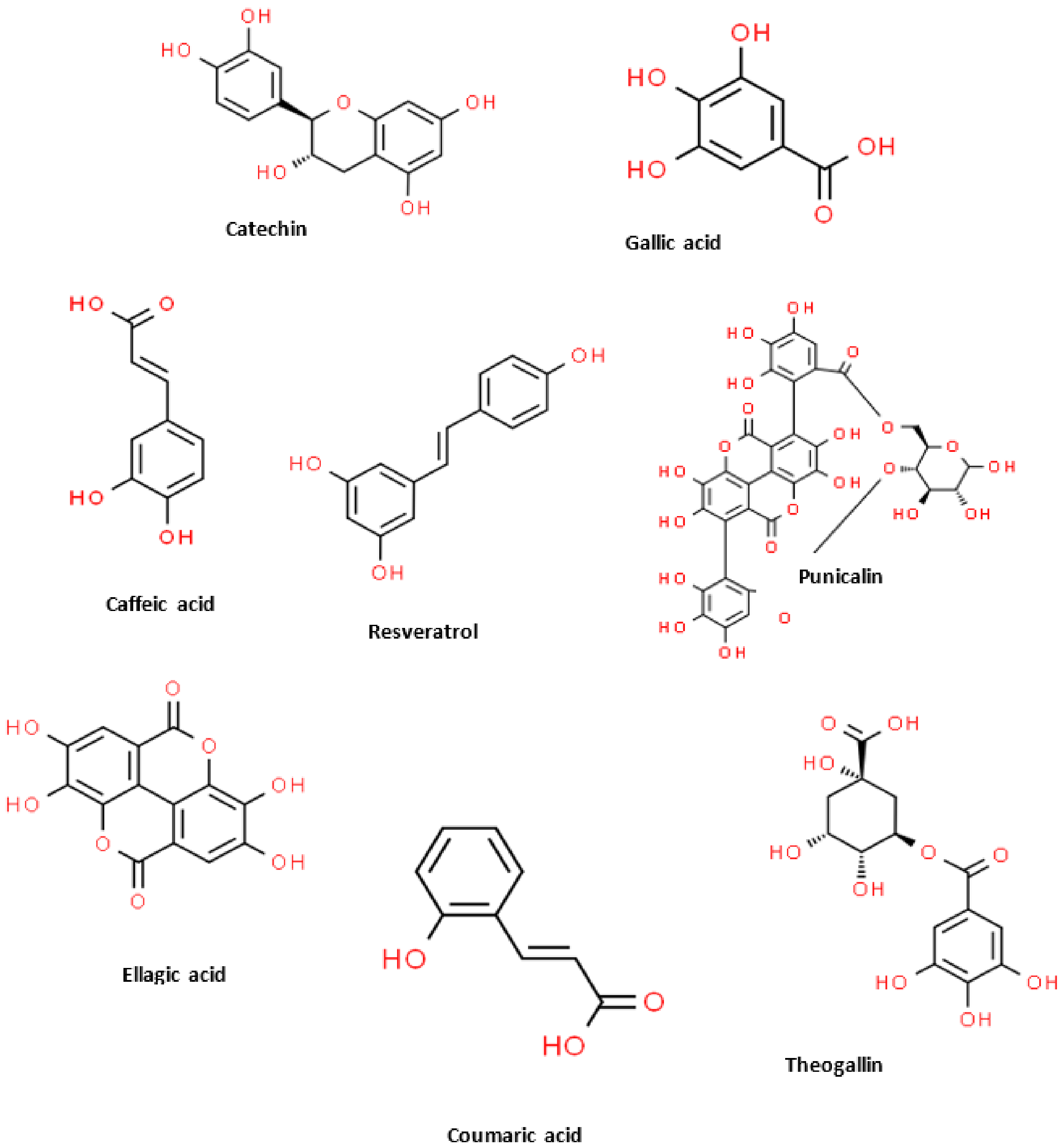

Figure 2.2: Various phenolic compounds of marula fruit and its juice. Adopted from Lekhuleni et al. (2024).

2.2.3.3 *The effect of varying preservatives and storage conditions on the vitamin content of fruit juice*

Plant vitamins are crucial for human health functioning as necessary cofactors for enzymes, facilitating metabolism, and serving as antioxidants to safeguard cells (Iqbal et al. 2004; Lykkesfeldt 2020). They are acquired through a diverse plant-based diet and are essential for functions like bone health, blood coagulation, vision enhancement, and immune system support (Lee & Kader, 2000). Consumption of a varied variety of fruits and their value-added products derivatives, such as juice, can contribute to the nutrional quality diet, which aids in the prevention of chronic diseases and promotes general health (Stadlmayr et al. 2013). Various vitamins are listed in Table 2.1.

El-Ishaq and Obirinakem (2015) investigated the vitamin C content of different juices extracted from varying fruits that were stored under ambient room temperature. Their investigation reported that pineapple juice had a superior vitamin C content compared to other juices, despite all juices being subjected to a similar preparation treatment. In their investigation, Abiola et al. (2018) assessed the quality attributes of orange juice subjected to varying preservatives for three weeks. From their studies, these authors reported that the use of chemical preservatives such as sodium benzoate (SB), sodium metabisulphite (SM), potassium sorbate (PS) and their combination (AP) preserved vitamins in orange fruit juice compared to untreated juice. These authors further recommended that a combination of pre-treatment and the use of preservatives should be considered to preserve the vitamin content in fruit juice.

According to the existing literature, the use of preservatives combined with storage conditions, has a direct impact on the quality of fruit juices, including pineapple and orange juice, which are all derived from exotic plant species. There is a paucity of published literature regarding the use of preservatives and storage conditions as to shell-life improvement of fruit juice derived from indigenous fruits such as marula. Thus, the current study examined the combined impact of preservatives and storage conditions on the vitamin content of marula fruit juice to make a contribution towards the incorporation of value-added products from indigenous fruit species in the food industry.

Table 2.2: Vitamins of marula fruit pulp and nut**.**

Vitamins	**Functions**	**References**
Vitamin A	Promotes normal growth and formation of skin and bone, immune function, reproduction.	FDA 2020
Vitamin B_3	Assists in digesting and converting cholesterol in food into energy.	FDA 2020
Vitamin C	Acts as an antioxidant and in the formation of connective tissue and collagen.	FDA 2020
Carotene	Is a precursor of Vitamin A assists in vision function.	FDA 2020
Vitamin E	Promotes immune function, blood vessel formation and act as an antioxidant.	FDA 2020

2.2.3.4 The effect of varying preservatives and storage conditions on the tannin content of fruit juice

Tannins, whose chemical structure is indicated in Figure 2.3 are naturally occurring compounds available in fruits, vegetables, cereals, and seeds and have wide applications in human health, plant defence, development and plant growth (Erb & Kliebenstein, 2020; Fraga et al., 2019). These compounds are present in vascular plants and have an important role in human health by reducing chronic disease risk due their antioxidant activities, anti-inflammatory and cardioprotective benefits (Fraga et al., 2019).

Talasila et al. (2012) evaluated the links between juice quality and shelf life of Cashew Apple juice subjected to different preservatives. In their investigation, these authors discovered that the use of preservatives and low storage temperature resulted in a reduced tannin loss particularly following the use of a treatment combination involving citric and benzoic acid at 0.1 g/L as preservative and storage under 4°C for a period of 4 months. They further encouraged food and, especially, juice producers to consider the treatment combination of preservatives and storage conditions to maximise quality of their products during storage, thereby increasing food safety associated with juice in order to protect consumers.

Current literature mainly examines the effects of various preservatives and storage conditions on the tannin concentration of fruit juice from exotic species, whereas information regarding indigenous fruits remains limited. To address the gap in literature, the present study assessed the combined impact of preservatives and storage conditions on the tannin content of marula fruit juice. This was undertaken to encourage the incorporation of value-added products from indigenous fruit species such as marula, thereby transforming the food system and expediting the commercialisation of the juice.

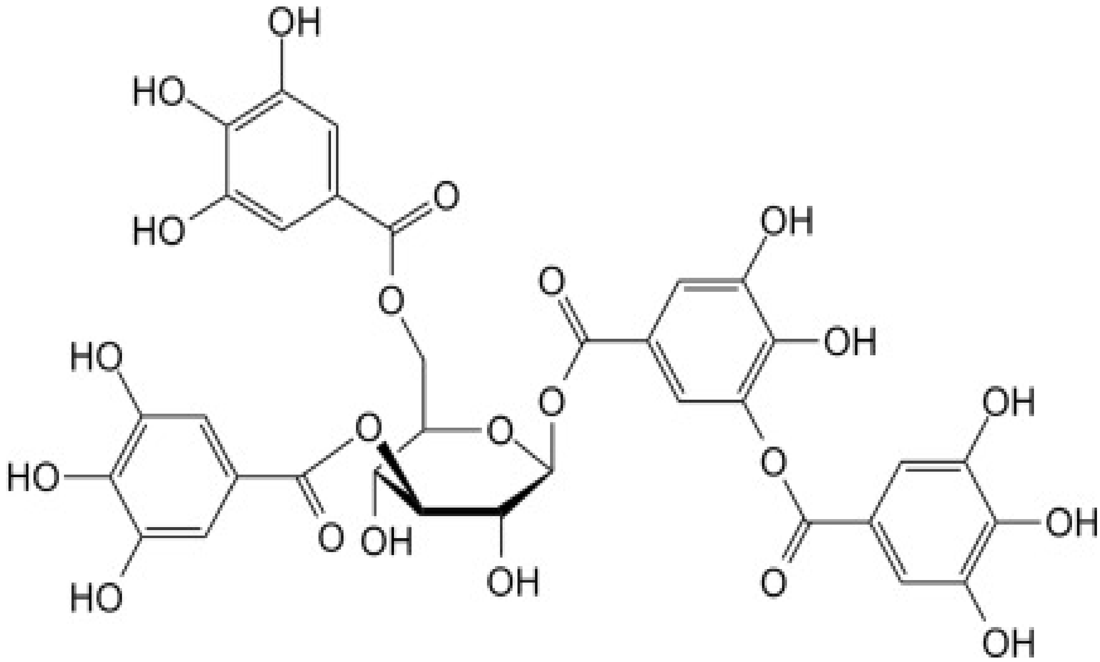

Figure 2.3: Chemical structure of tannins. (Adopted from Fraga et al. (2019).

2.2.3.5 The effect of varying preservatives and storage conditions on the antioxidant activity of fruit juice

Antioxidant activity is determined by chemical structures capable of inhibiting or preventing oxidation by neutralising deleterious free radicals and reactive oxygen species (ROS), which can inflict damage on cells and molecules (Tambunan et al., 2018). In human health, antioxidants contribute to protection against cellular damage and the onset of disorders linked to oxidative stress, hence preserving general cellular health (Ames et al., 1993; Dragsted, 2003; Glevitzk et al., 2008; Guo 2025).

In an investigation conducted by Castro-López et al. (2016), the authors evaluated the fluctuations in antioxidant activities of fruit beverages subjected to varying storage conditions over a 20-day period. In their investigation, these authors noted that pomegranate juice retained a superior level of antioxidant activity (DPPH) under storage conditions of between 4°C and 8°C for 10 days, but there was a notable reduction in activity following this storage duration. They further recommend that treatment of fruit juice with preservatives combined with low temperature storage conditions maximises the quality of fruit juices during storage.

While published research is available as to how preservatives and storage conditions affect the quality of juice processed from exotic species, including the antioxidant activities of the juice, there appears to be limited research on the effects of these treatments (preservatives and storage conditions) on the tannin content of marula fruit juice. Consequently, the current study addressed this gap in the literature by quantifying the tannin content of marula fruit juice subjected to various preservatives and storage conditions, thereby establishing the baseline data requisite for quality preservation of the juice.

2.3.2.6 Effect of preservatives and storage conditions on the macro minerals of fruit juice

Macro-minerals are essential substances required by the human body in substantial quantities, playing a crucial role in health and nutrition by regulating numerous metabolic processes and participating in nearly all body functions (Taşğın, 2017). These minerals are predominantly found in fruits and their value-added products, such as juice, however their mineral concentration degrades during storage (Rashid et al., 2014).

Nur-A Kabir et al. (2019) examined the mineral composition and retention in tomato juice treated with sodium benzoate and potassium metabisulphite preservatives stored for a period of 60 days to enhance shelf life. These researchers discovered that the juice preserved with sodium benzoate exhibited enhanced content and preservation of macrominerals, specifically magnesium and potassium, compared to the potassium metabisuphite treatment. They recommended that food processors, particularly juice producers, should consider the use of preservatives to minimize mineral loss during storage.

Existing literature addresses the impact of preservatives and storage conditions of the macromineral content of juice derived from exotic species but published research concerning the effects of these treatments on juice extracted from indigenous fruits such as marula remains limited. The current study addressed this gap in the literature by assessing the combined impact of preservatives and storage conditions on the macro-mineral content of marula juice, by conducting a comparative analysis to identify optimal treatments for enhancing the quality of marula fruit juice during storage. Additionally, this initiative was also directed at enhancing the incorporation of value-added products derived from indigenous fruits, such as marula, which is regarded as being underutilised by food producers and manufacturers due to the absence of validated scientific baseline data.

2.3.2.7 Effect of preservatives and storage conditions on the microminerals of fruit juice

Details of microminerals are listed in Table 2.2. Microminerals are essential biochemical components required by the human body in minimal amounts to facilitate physiological processes such as growth, development and overall health (Maluleke et al., 2021). Moreover, they are crucial for numerous key biological activities, including immune system support, cell creation, hormone regulation, and illness prevention (Lekoba et al., 2024). Due to the body's limited capacity to synthesize them, microminerals are mostly acquired via fruits and their value-added products, such as juice, or through supplements (Lekhuleni et al., 2024).

In an investigation carried out by Durrani et al. (2010), whereby authors evaluated the use of preservatives (citric acid, sodium benzoate and potassium metabisulphite) for quality preservation on the physicochemical properties of apple pulps. They discovered that the use of preservatives such as sodium metabisulphite had a positive impact on retention of microminerals including iron, when compared to other tretaments. The authors further highlighted that pastuarisation should be used together with preservatives in order to improve the apple fruit pulp and its juice.

Although there is published literature on the effect of preservatives on the iron content of fruit pulp and juice, it focuses on pulps and juices from commercial species, such as apples. There is currently little research on how treatments (preservatives and storage conditions) affect the the micromineral content of fruit juice derived from native fruits such as marula. Therefore, the current study assessed the impact of different preservatives and storage conditions on the micromineral content of marula fruit and fruit juice to close this gap in the literature. The establishment of suitable preservatives and storage conditions for shelf-life improvement of marula juice will assist in accelerating efforts to commercialise marula juice and also to help achieve UN SDGs 1 and 2.

Table 2.2: Minerals of marula fruit pulp and nut

Minerals	Functions	References
	Macrominerals	
Sodium	Regulates fluid, acid-base balance and blood pressure.	Muhammard et al., (2015)
Potassium	Help heart and nervous system and muscle contraction.	(Glew et al., 2004)
Calcium	Assists in blood clotting, formation of bone and teeth and promotes hormone secretion, nervous system.	Aganga & Mosase (2001)
Magnesium	Regulates blood sugar, blood pressure and formation of protein.	Wairagu et al., (2013)
	Microminerals	
Copper	Acts as an antioxidant promotes bone formation and iron metabolism.	FDA, (2020)
Manganese	Helps in metabolism of protein, carbohydrates, cholesterol and formation of bone cartilage.	Borochov-Neori et al., (2008)
Iron	Asssist in the formation of red blood cells and promotes immune function.	Oyeleke et al., (2012)
Zinc	Promotes hormone secretion, nervous system, growth and development.	Aganga & Mosase, (2001)

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CHAPTER 3: Methodology

3.1 Introduction

The chapter explains the experiments that were conducted at Agricultural Research Council - TSC in Mbombela.

3.2 Physicochemical properties and bioactive compounds of marula (*Sclerocarya birrea*) fruit harvested from diverse geographical regions and its potential contribution to food and nutritional security

The Sustainable Development Goals (SDGs) set forth by the United Nations (UN) aim to eradicate hunger, malnutrition and guarantee that all people have access to sufficient nutrient-dense food by 2030. The introduction of underutilised indigenous fruit species such as marula fruit (*Sclerocarya birrea*), which is reported to be abundant in vitamin C and minerals needed by humans for the fulfilment of their recommended daily diet seems to be a reliable strategy to achieve these goals. This study aimed to investigate the effect of varying geographical locations on the physicochemical properties and bioactive compounds of the marula fruit and its juice. Fruits utilised for the analysis of physicochemical properties, such as fruit mass, pH, total titratable acidity and total soluble solids; bioactive compounds such as total flavonoids, and vitamin C, were collected from varying regions with distinct climates (Bushbuckridge, Giyani, and Tzaneen) during the summer of 2024.

3.2.1 Study area and sample collection

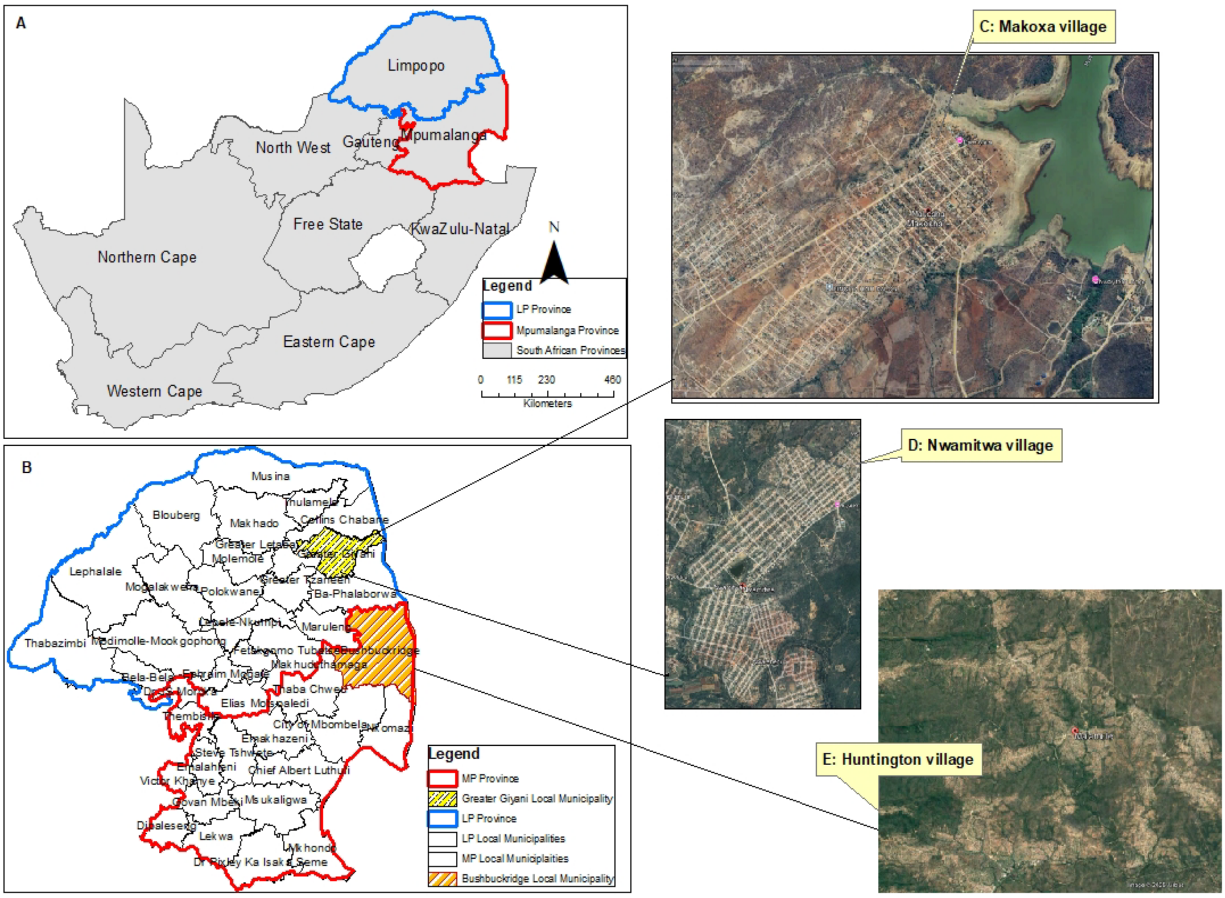

Figure 3.1: Map of study areas (Bushbuckridge, Giyani and Tzaneen) where marula fruit samples were collected.

As shown in Figure 3.2, the study was conducted in three different locations (Bushbuckridge in Mpumalanga Province, and Giyani and Tzaneen in Limpopo Province), areas classified as lowveld regions in South Africa with distinct climate (Table 3.1). The sampling of botanically identical marula trees were conducted in accordance with the procedure established by Lekoba et al. (2024), with minor adjustments. A cluster randomised sampling technique was carried out in three locations of lowveld region experiencing distinct climatic conditions, namely (i) Bushbuckridge (-24.91648,31.41572), (ii) Giyani (-23.31748,30.72255), and (iii) Tzaneen (-23.73398,30.36925). The prevalence and usage of marula fruit trees in these locations dictated the selection of sampling areas.

The fruits were collected during the summer of 2024 in February. A total of 200 kilograms of marula fruits were collected from trees spaced at a minimum of one (1 km) kilometre apart in each location. Marula trees (Specie/ID number: 360) were identified by a qualified botanist, Dr R. Munyai, a researcher at the University of South Africa, and verified at the National Herbarium at Pretoria National Botanical Gardens (SANBI). All specimens gathered for this study are preserved in the University of South Africa Herbarium, Florida Science Campus (S 26° 9.501 E 27° 54.113).

The fruit colour chart developed by Nambi et al. (2015) and adopted by Yap et al. (2017) and Lekoba et al. (2024) was used for this study. Mature, ripe, fresh marula fruits were collected from trees naturally growing in the wild. The 200 kg of marula fruits collected from each region yielded a total of 600 kg of fruits that were then transported to the Agricultural Research Council Institute for Tropical and Subtropical Crops (ARC-TSC) in Mbombela, Mpumalanga Province (-25.8990023 S 28.2152523 E.) for further processing and analysis.

In addition, soil samples collected from the three geographical regions were analysed as to mineral composition at the Agricultural Research Council (ARC), Pretoria, following the procedure described by Tuckeldoe et al. (2023), adopted by Maluleke et al. (2021). Results of this soil analysis are shown in Table 3.1. The experiment was conducted strictly in compliance with the University of South Africa (UNISA), College of Agriculture and Environmental Sciences Research and Higher Degree Committee (Ethical number/Reference #: 2022/CAES HREC/104), and other relevant local and global regulations.

Meteorological data noting the average temperature and rainfall of the three geographical regions (Bushbuckridge, Giyani, and Tzaneen) where fruits were collected was obtained from the South African Weather Services. It is noteworthy that an analysis of various parameters, including fruit color, fruit mass, fruit length, fruit width, pH, titratable acidity, and total soluble sugars, was conducted on the same day the fruits were harvested. Furthermore, it is worth noting that other analysis, including DPPH, total flavonoids, total phenols, tannins, and vitamin C, were were carried out on juice extracted from fruit that had been stored overnight at 10˚C prior to extraction.

Table 3.1: Meteorological data of the study areas (Bushbuckridge, Giyani and Tzaneen).

	Tmax (˚C)	Tmin	Rainfall (mm)
	Bushbuckridge		
Month			
September	32	11	26.2
October	34	17	99.1
November	32	18	83.3
December	34	20	143.3
January	33	21	176.5
	33	**17.4**	**528.4**
	Giyani		
September	27	15	15
October	36	19	20
November	37	19	55
December	38	18	123
January	34	20	133
	34.4	**18.2**	**346**
	Tzaneen		
September	28	14	35
October	32	15	62
November	31	17	119
December	32	16	163
January	34	19	167
	31.4	**16.2**	**546**

Tmin= means minimum temperature. ***Tmax***= means maximum temperature. Materological data was soruced from South African Weather Services.

The soil mineral composition of the three geographical regions as shown in (Table 3.2), was performed by the Agricultural Research Council (ARC) following the procedure of Tuckeldoe et al. (2023), adopted by Lekoba et al. (2024). The ICP-OES 9800 Series (Shimadzu, Japan) was utilised to analyse the soil mineral composition such as calcium, iron, magnesium, potassium, phosphorus, sodium, manganese, and zinc (Table 3.2).

Table 3.2: Soil mineral composition ($mg \cdot kg^{-1}$) of the three study areas.

Minerals	Fe	Mn	Zn	Na	Ca	P	Mg	K
Bushbuckridge	35	10	11,4	7,9	1922	46,4	258	361
Giyani	42	18	16	9,3	1896	49	220	251
Tzaneen	37	41	13,2	19	183	51	1489	347

3.2.2 Juice extraction procedure

Marula fruit juice was extracted manually by punching the fruit with a fork before removing the peel from the pulp and seed. The peel and seed were then hand-squeezed to separate the pulp from the seed. The pulp was then transferred to a juicer machine (Milex 1200 W, Johannesburg, South Africa) to separate the juice from the solids. The marula juice was further analysed for bioactive compounds (Figure 3).

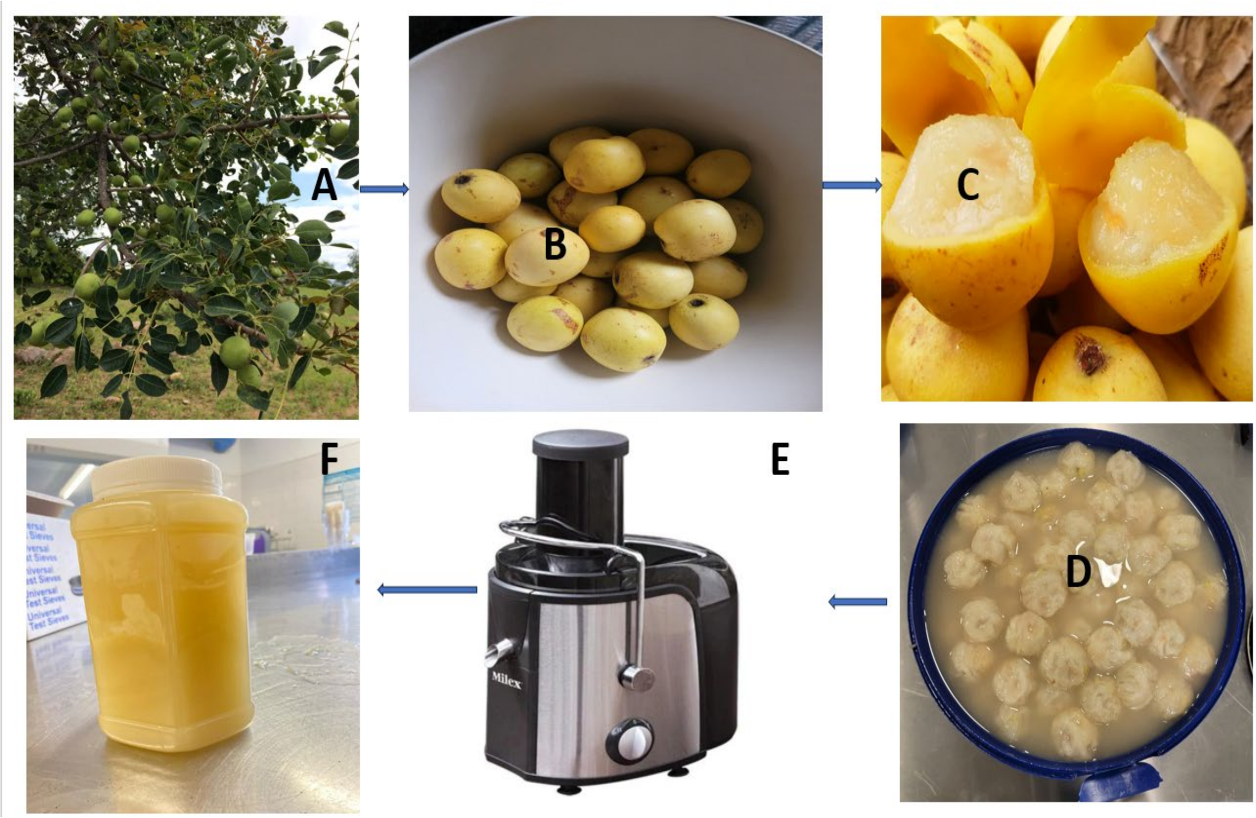

Figure 3.2: Process to extract marula juice. Marula tree with fruits **(A)**. Ripe marula fruits **(B)**. Half-peeled marula fruit **(C)**. Deskinned marula fruit with pulp **(D)**. Juicer machine used for marula pulp juicing **(E)**. Extracted marula juice **(F)**.

3.2.3 Physicochemical properties and bioactive compounds

3.2.3.1 Fruit Colour determination

For colour attributes, a sample of twenty fresh fruits of the uniform size and without physical damage harvested in varying geographical regions (Bushbuckridge, Giyani and Tzaneen) was used for the colour(external) profile using a colorimeter (Lovibond® LC100, Japan) in CIELAB (L*, a* and b*). The parameter L* indicating lightness of the colour, a* the hue range of colours red (+) and green (-) and b* the hue range of colours yellow (+) and blue (-). From the colour values chroma (C) and hue angle (H) were calculated according to equations 1 and 2 developed by Yap et al. (2017):

$$Chroma\ (C) = \sqrt{a^2 + b^2} \quad (3.1)$$

$$Hue\ angle\ (H) = \tan^{-1}(\frac{b}{a}) \quad (3.2)$$

3.2.3.2 Fresh weight determination

The fruits were individually weighed on a calibrated laboratory weighing balance (ScalTec SBA 61). A clean weighing boat was placed on the weighing balance and tared before each measurement was taken. All measurements were recorded in grams (g).

3.2.3.3 Fruit size determination

A vernier calliper was used to measure the diameter and length of each fruit following the method used by Tebeila (2022). The length was measured from the proximal (adjacent to the stalk) end to the distal end of the fruit and the diameter was determined between the opposite smooth ends of the fruit. The values were presented in millimetres (mm).

3.2.3.4 Total Soluble Sugars (TSS) determination

Total soluble sugars were measured by using a hand refractometer (HI 96801 Refractometer, USA) and values were expressed as B (°Brix). An aliquot of 0.03 ml of marula juice was placed onto the aperture of the refractometer and readings were taken immediately, following the method of Maluleke et al. (2021). The refractometer aperture was rinsed between different juice samples with distilled water and dried with a soft paper towel.

3.2.3.5 Titratable acidity (TA) and pH determination

The TA and pH were determined using Prime-Flash Automatic Titrator (Steroglass Srl AS24) according to the method adopted by Lekoba et al. (2024) with modification. Briefly, approximately 10 ml of juice in 50 ml of distilled water were auto-titrated against 0.1 N NaOH solution to reach an endpoint pH of 8.2. Results for TA was expressed in terms of g/L citric acid. Regarding pH content determination, at room temperature, a pH meter (Crison, Barcelona, Spain) was dipped into juice contained in a glass beaker for 3 seconds and readings were taken immediately. The pH meter was then rinsed in between different juice samples with distilled water and dried with a soft paper towel.

3.2.3.6 Vitamin C determination

The method described by Dorothy et al. (2023) was adopted and the vitamin C content of marula fruit juice was determined by using the AOAC (2005) method with slight modifications. Marula fruit juice sample was titrated with 2.6-dichloro-indophenol (DCPIP) dye. Each added drop was counted until the colour changed from blue to the faint pink endpoint. Analyses were conducted in in triplicate. The amount of Vitamin C in fruit juice was calculated using the formula (3) and values were expressed as mg /100 ml of marula fruit juice.

$$Vitamin\ C = \frac{V1 \times C \times 100}{V2} \quad \ldots\ldots(3.3)$$

Where V1 = volume of DCPIP used for sample
C = mg of ascorbic acid
V2 = volume of juice sample used

3.2.3.7 Total tannin content determination

The tannin content of marula fruit juice was determined following the method described by Baba and Malik (2015) with slight modification. For extraction, 5 ml of 80% methanol was added to 1 ml of marula juice. The mixture was then incubated overnight and thereafter centrifuged at 6000 rpm for 5 minutes. Approximately 1 ml of an aliquot from each extract was diluted with 4 ml of distilled water, and 250 µl of Folin Ciocalteu reagent was added to each mixture and vortexed to ensure thorough mixing. Then 500 µl of 35% Na_2CO_3 was added to each mixture, before 10 ml of distilled water was added to each, gently shaken and the mixture was incubated for 60 minutes in the dark at room temperature. The absorbance was measured at 726 nm. A blank sample solution was prepared without adding the extract, following the same procedure as the test solutions. Gallic acid was used as a standard for the calibration curve, and total tannin content was expressed as milligrams of gallic acid equivalence per millilitre of extract (mg GAE/ml).

3.2.3.8 Total flavonoid content determination

The total flavonoid content of marula fruit juice was determined following the method described by Moyo et al. (2018) and Baba and Malik (2015), as adopted by Tuckeldoe et al. (2023) with modification. The aluminium chloride colorimetric method was used to determine the total flavonoid content. For extraction, 5 ml of 80% methanol was added to 1 ml of marula juice. The mixture was then incubated overnight, before being centrifuged at 6000 rpm for 5 minutes. To a 500 µl aliquot of each extract was added 1.5 ml of 80% methanol, 100 µl of 10% aluminium chloride ($AlCl_3$) and 100 µl of 10% Na_2CO_3 and the mix was made up to 5 ml by adding 2.8 ml of distilled water. Each mixture was gently shaken before being incubated for 30 minutes at room temperature until a pale milky colour was observed. The absorbance was measured at 430 nm. Quercetin was used as a standard for the calibration curve, and total flavonoid content was expressed as milligrams of quercetin per millilitre of extract (mg QE/ml).

3.2.3.9. Total phenolic content

The total phenolic content of marula fruit juice was determined following the method of Moyo et al. (2018) with modifications. For extraction, 5 ml of 80% methanol was added to 1 ml of marula juice. The mixture was then incubated overnight and thereafter centrifuged at 6000 rpm for 5 minutes. Then, a 500 µl aliquot of sample was mixed with 2.5 ml of 10% Folin Ciocalteu reagent and 2 ml 1N N_2CO_3. The solution was then incubated for 60 minutes at room temperature before the absorbance was measured at 726 nm. Gallic acid was used as a standard for calibration curve and total phenolic content was expressed in milligram garlic acid equivalents per millilitre of extract (mg GAE/ml).

3.2.3.10 DPPH radical scavenging activity

The DPPH radical scavenging activity of the marula fruit juice samples was determined using the method outlined by Apea-Bah et al. (2014) with modifications. Samples were measured in triplicate. To 1 ml of marula juice was added 5 ml of 80% methanol. The mixture was then incubated overnight and thereafter centrifuged at 6000 rpm for 5 minutes. A 1 ml aliquot of sample was combined with 2 ml of 2.2-Diphenyl-1-pirolhydrazyl (DPPH) solution and incubated for 30 minutes in the dark at room temperature. The absorbance was measured at 516 nm, and the results were expressed as percentage RSA.The standard curve for total flavonoids and total phenolic content is depicted in Figures 3.4A and 3.4B. A UV-visible spectrophotometer (Model: 7315, Brand: Jenway, United Kingdom) was used for detection.

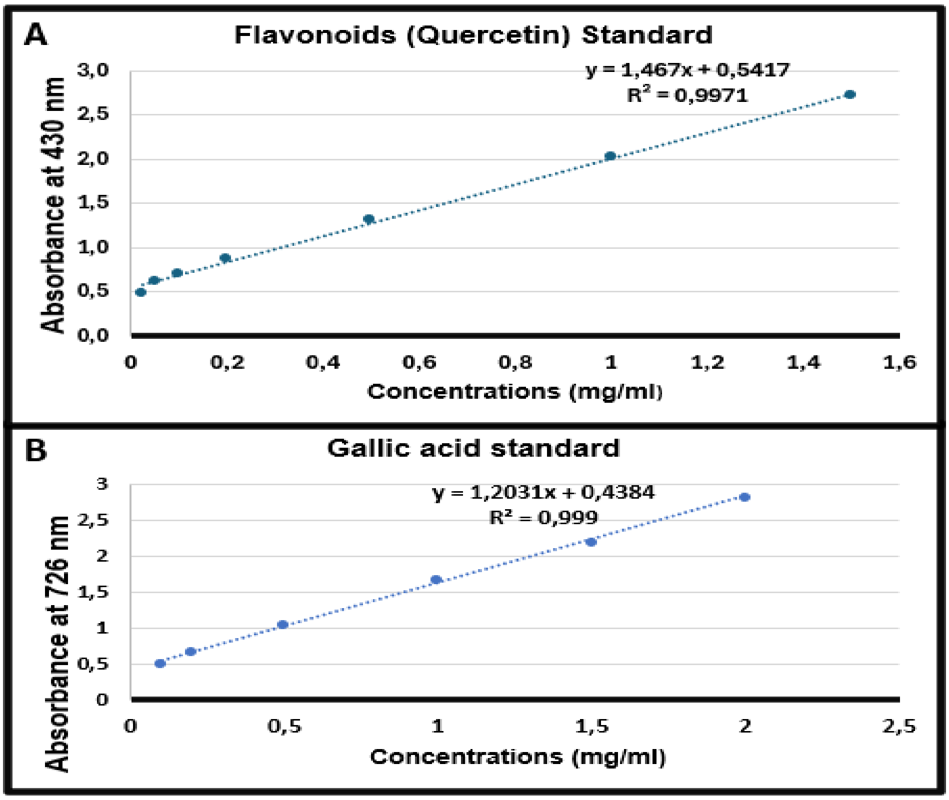

Figure 3.3: Standard curves of antioxidants. Total flavonoids **(A)** and Total phenols **(B)**.

3.2.4 Statistical analysis

Data gathered in this research study were used to statistically analyse the effect of the different regions where the marula trees were growing as to the physicochemical properties and antioxidant activity in marula fruit and juice. The one-way ANOVA was used to determine the effect of a single independent variable, geographical region (Bushbuckridge, Giyani or Tzaneen), on the dependent variables: colour, weight, length, width, pH, total soluble sugars, titratable acidity, vitamin C, total flavonoids, total phenolics, total tannin and DPPH (RSA). Significant differences at $P \leq 0.05$ (95% confidence level) for one factor (location) on studied dependent variables was considered and reported. The least significant difference (LSD) of means was reported per variable. Statistica v. 10, (StatSoft, USA) was used for all statistical analyses.

3.3 Characterisation of marula fruit (*Sclerocarya birrea*) juice and the effect of storage conditions and preservatives on its biochemical properties

Characterisation, preservation and storage are crucial processes in fruit juice processing. The aim of the study was to evaluate the chemical and biochemical properties of chemically preserved and stored marula fruit juice. The study involved manual extraction of marula fruit juice from fruit collected from trees growing in Limpopo (Giyani and Tzaneen) and Mpumalanga (Bushbuckridge), provinces in South Africa. Three samples were developed by treating marula juice with 0.05% sodium benzoate (SB) and sodium metabilsulphite (SM). The untreated samples were used as a control (C). The juice samples were subjected to varying storage temperatures at 4°C, 6°C, and 10°C for a duration of 20 days. The pH, titratable acidity, total soluble solids, biochemical properties and antioxidant activity were determined using standard methods.

3.3.1 Juice extraction procedure

The marula fruit juice was extracted manually by punching each fruit with a fork to then separate the peel from the pulp and seed. Thereafter the peel and seed were hand squeezed to separate the pulp from the seed. The pulp was transferred into a juicer machine where the juice was separated from the solids. A flow diagram of this process is indicated in Figure 4.2. Three batch juice samples were subjected to varying preservatives namely: sodium benzoate and sodium metabisulphite. These two different preservatives were added into the juice at a concentration of 0.05% (w/v) in accordance with the the method followed by Okokon and Okokon (2019). The juice samples that were pasteurised but not treated with preservatives were used as a control to benchmark for the other treatments. Juice samples were filled into 2000 ml glass bottles under room temperature, was then pasteurised at 85°C for 5 minutes in a water bath (GFL, Germany). The juice samples were then allowed to cool at 5°C. The cooled juice samples were then transferred into 50 ml plastic sterilised containers and then stored at different storage conditions of 4°C, 6 °C and 10°C for a duration of 20 days. This is indicated in Figure 4.3. Measurements of biochemical and antioxidant properties were conducted on day 0, day 10 and day 20 in order to determine the suitable storage conditions (temperature and preservatives) of marula juice for potential large-scale production. A determination of the optimal preservative and storage temperature of the juice would be reflected in the analysis of the kinetics of maintenance of the beneficial nutrients in the marula juice over time. It is worth to note that only the juice derived from the fruits harvested in the Giyani region was used to characterisation of marula fruit juice and the effect of varying preservatives and storage conditions on its biochemical properties due to its abundance in vitamin C, titratable acidity and the overall analytic costs.

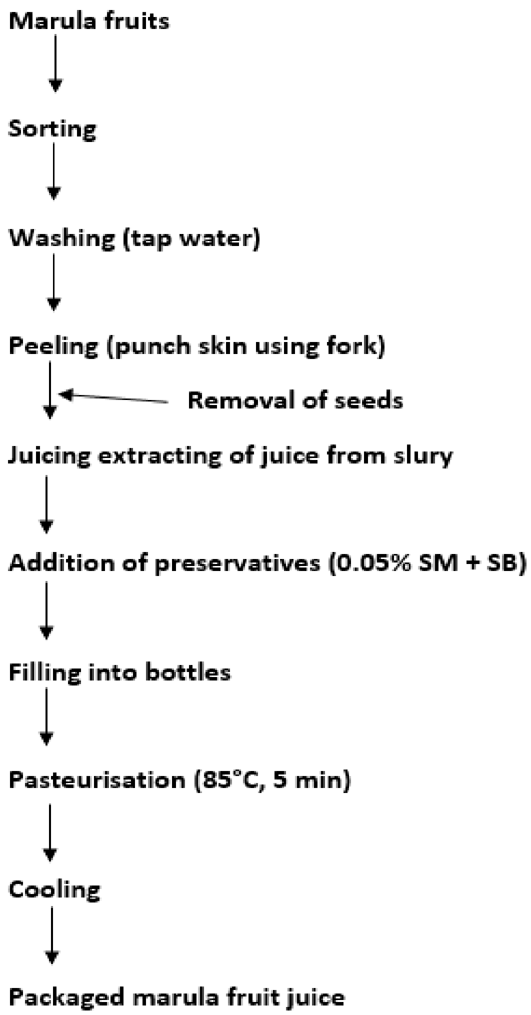

Figure 3.4: marula fruit juice process flow. Sodium metabisulphite (SM), Sodium benzoate (SB)

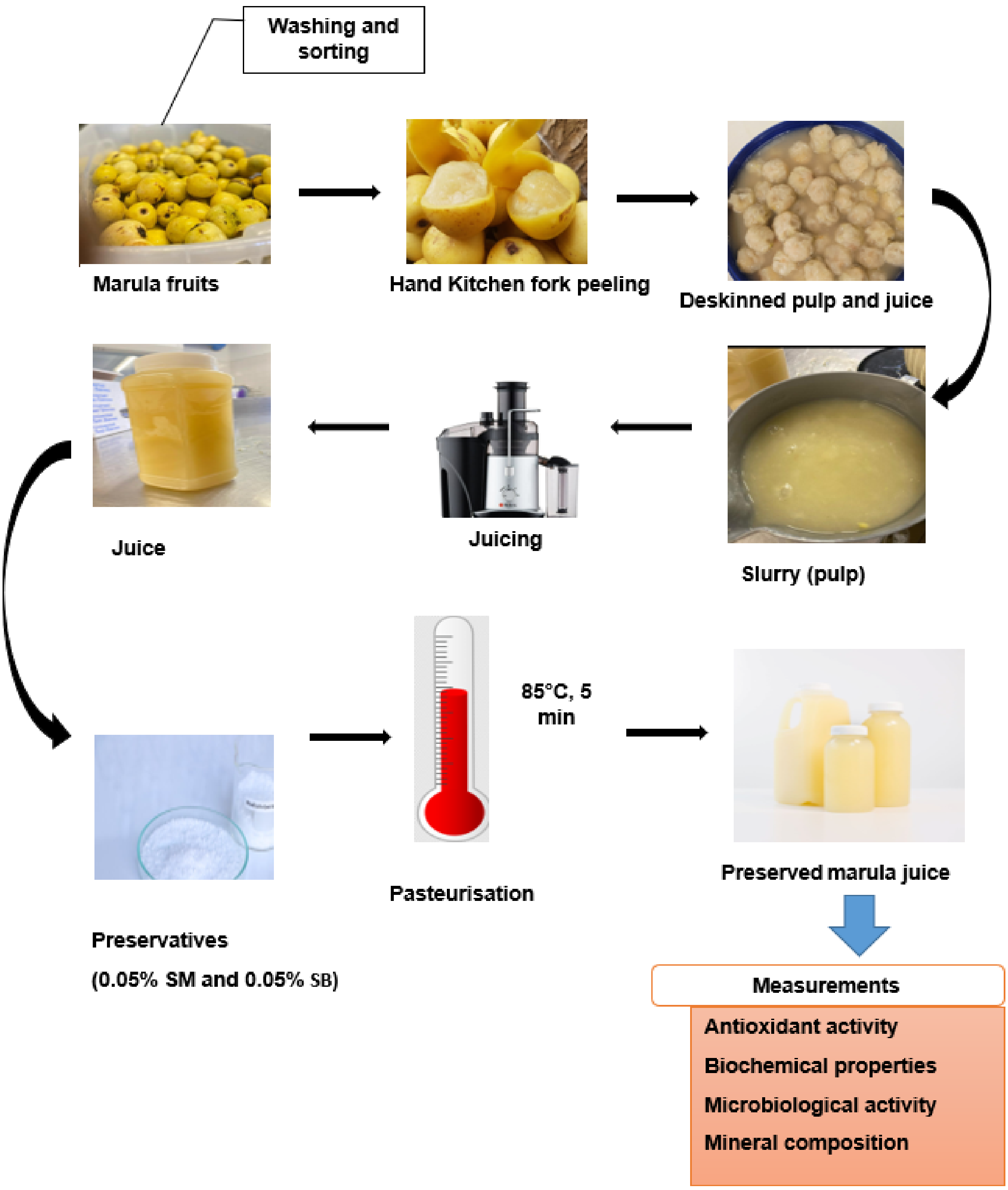

Figure 3.5: marula juice extraction, processing, and treatment. Note: SM: sodium metabisulphite, SB: sodium benzoate, Min: minutes.

3.3.2 Statistical analysis

Data gathered in this research were used to statistically analyse the effect of storage conditions and preservatives on the physicochemical properties and antioxidant activity of stored, preserved marula fruit juice. The two-way ANOVA was used to determine the effect of two independent variables (storage conditions and preservatives) on dependent variables such as pH, total soluble sugars, titratable acidity, vitamin C, DPPH (%RSA), total flavonoid content, total phenolic content, and total tannin content. Significant differences at $P \leq 0.05$ (95% confidence level) on studied variables was considered and reported. The least significant difference (LSD) of means was reported per variable. For all statistical analysis, Statistica v. 10 (StatSoft, USA) was used.

3.4 The effect of preservatives and storage conditions on the minerals stability of marula (*Sclerocarya birrea*) fruit juice

The fruit juice industry is regarded as one of the most important players in nutritional security and the economy because it provides sustainable access to important minerals required by humans daily, which in turn creates job opportunities since manufacturers can generate revenue through juice product sales. Most indigenous fruits of Southern Africa including marula have been utilised as raw materials for value-added products such as juice, which is claimed to be rich in minerals needed for sustenance of human health and nutrition. However, research and knowledge on optimal post-processed storage treatments for mineral retention appear to be scanty. The objective of the current study was to assess the effect of different preservatives and storage temperatures on the mineral composition of marula juice. Marula fruit juice was subjected to varying preservatives (sodium benzoate and sodium metabisulphite) and storage temperatures (4°C, 6°C and 10°C) for 20 days. Juice samples were subjected to microwave-assisted acid digestion (HNO_3), and minerals were determined using inductively coupled plasma optical emission spectrometry (ICP-OES).

3.4.1 Mineral analysis

The method used to determine the mineral content of marula fruit juice involved microwave-assisted acid digestion and inductively coupled plasma optical emission spectrometry ICP-OES, as described by de Souza et al. (2022). Sampling of marula juice was done after 20 days of storage. Each marula juice bottle was shaken manually to homogenise the bottle contents. Then, a 10 ml aliquot of nitric acid was added to each 5 ml sample of marula fruit juice. The samples were then introduced into the microwave oven and digested using a four-step temperature program. Firstly, the temperature was linearly increased from room temperature to 90ºC by increasing the power up to 1000 W for 2.5 minutes. The temperature was then elevated to 140ºC in 6 minutes (power 1000 W). Finally, the temperature was increased to 180ºC in 5 minutes (800 W), before maintaining these conditions for 15 minutes. The digestion procedure was carried out in triplicate for each sample. In addition, a sample blank digestion was prepared for each series of digestions using distilled water. The digested samples and blanks were diluted to 50 ml with distilled water prior to analysis using ICP-OES.

3.4.2 Statistical analysis

A two-way analysis of variance (ANOVA) was used to analyse the effect of independent variables (preservatives and storage conditions) on the mineral retention of marula fruit juice. The dependent variables measured were calcium, magnesium, potassium, iron and zinc. Fundamental statistical analysis including the least significant difference (LSD) was considered for all variables. Statistica (USA) version 10 was utilised for all statistical analyses.

3.5 Influence of geographical region, storage conditions and preservatives on the microbiological quality of marula (*Sclerocarya birrea*) fruit juice

The purpose of the current study was to evaluate the microbiological quality of chemically preserved marula fruit juice during storage. The study involved manually extracting marula fruit juice from fruit collected from trees growing in Limpopo (Giyani and Tzaneen) and Mpumalanga (Bushbuckridge), provinces in South Africa. Samples for analysis were developed by treating the marula juice with 0.05% preservative sodium benzoate (SB) and sodium metabisulphite (SM). Untreated juice samples were used as a control. Chemically preserved and unpreserved marula juice were packaged in 50 ml plastic containers and stored at refrigeration temperatures of 4°C, 6°C and 10°C for 20 days. Microbiological evaluation was determined using the spread plate count method. After incubation, enumeration of the colony forming units (CFU) and species identification were performed. Microorganisms were identified using the 16S rRNA gene sequences.

3.5.1 Microbiological analysis of fruit juice

The number of microorganisms present in the fruit juice was determined using the spread plate count method described by Ekanem and Ekanem (2019), with slight modification. Six, tenfold serial dilutions of juice samples were prepared using sterile Ringer's solution before 100 µl of each dilution was evenly spread with a sterile glass spreader on duplicate nutrient agar plates, for bacterial growth, and full-strength potato dextrose agar plates (Sigma, South Africa), for fungal growth. All plates were incubated using Sure-Temp (Meck, South Africa), at both 25°C and 37°C for 24 - 48 hours. The average count of the two plates for each dilution was used as an estimate of the number of viable microorganisms per dilution. Plates containing 30 - 300 colonies per plate were counted. At this colony number, the number counted is high enough to have statistical accuracy, yet low enough to avoid mistakes due to overlapping colonies.

3.5.2 Identification of microbial isolates

The microorganism responsible for juice spoilage was identified using 16S rRNA gene sequence analysis to study bacterial phylogeny and taxonomy, and ITS sequencing to study fungal phylogeny and taxonomy. Prior to the sequencing, DNA was extracted from single colonies using DNA extraction kit. A specific region of the 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR). The DNA was cleaned up to remove any impurities and it was quantified and normalised. The prepared DNA library was subjected to 16S ribosomal RNA (rRNA) sequencing in order to identify and analyze the genetic material of bacterial isolates. The Internal Transcribed Spacer (ITS) sequencing instrument used to identify and analyse the genetic material of fungi (mould and yeast cultures).

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Chapter 4: Results and discussion

4.1 Physicochemical properties and bioactive compounds of marula (*Sclerocarya birrea*) fruit harvested from diverse geographical regions and its potential contribution to food and nutritional security

4.1.1 Meteorological data

The average temperature and rainfall of the three geographical regions (Bushbuckridge, Giyani, and Tzaneen) where fruits were collected are presented in Table 4.1. Climate conditions, especially minimum temperature obtained on this study showed variations among all geographical regions. For instance, the Tzaneen region, at 16.2°C, exhibited the lowest minimum temperature compared to the lowest temperature for the Bushbuckridge (17.4°C) and Giyani (18.2°C) regions. Regarding maximum temperature, the Giyani region, at 34.4°C, exhibited a higher maximum temperature, when compared to the other geographical regions at Bushbuckridge (33°C) and Tzaneen (31.4°C). In terms of rainfall, data revealed that it ranged from 346 mm to 546 mm. The Giyani region, at 346 mm, showed the lowest rainfall, followed by the Bushbuckridge region at 528.4 mm and the Tzaneen region, at 546 mm.

The influence of climate on fruit quality has been thoroughly documented by numerous researchers. According to Bacelar et al. (2024), climatic change can profoundly affect the physical quality, productivity, and nutritional value of fruit. Temperature fluctuations can result in delayed or accelerated fruit ripening, leading to rapid alterations in fruit colour development and biochemical composition (El-Bassiony et al., 2014). Conversely, rainfall directly influences fruit quality (Patanè et al., 2011). Both reduced and excessive rainfall affect fruit parameters such as size, flavour, texture, and the quality of biochemical constituents by altering the soil's water content. However, consistent moderate rainfall or water is deemed optimal for superior fruit quality (Pérez-Pérez et al., 2009; Volschenk, 2021). According to the findings of this study, climatic conditions could directly impact fruit quality. Hence, it was necessary to comprehensively investigate the quality of marula fruit harvested in various geographical regions to provide baseline data regarding climatic impact on fruit quality. Such data will assist potential plant breeders in understanding the climatic impact on fruit quality, allowing them to develop varieties that are both nutrient dense and stable under varying climatic conditions.

Table 4.1: Meteorological data of the study areas (Bushbuckridge, Giyani and Tzaneen).

	Tmax (˚C)	Tmin	Rainfall (mm)
	Bushbuckridge		
Month			
September	32	11	26.2
October	34	17	99.1
November	32	18	83.3
December	34	20	143.3
January	33	21	176.5
	33	**17.4**	**528.4**
	Giyani		
September	27	15	15
October	36	19	20
November	37	19	55
December	38	18	123
January	34	20	133
	34.4	**18.2**	**346**
	Tzaneen		
September	28	14	35
October	32	15	62
November	31	17	119
December	32	16	163
January	34	19	167
	31.4	**16.2**	**546**

Note: Tmin means minimum temperature and Tmax means maximum temperature

4.1.2 Physical properties of marula fruit

The physical properties of marula fruit mass, length, width, and colour (L*, a*, b*, C and hue) harvested in varying geographical regions are depicted in Table 4.2. In terms of fruit mass, study results showed that there was substantial ($P \leq 0.01$) variation. The study results showed that fruit mass ranged from 36.5 g to 44.6 g. Moreover, results showed that fruits from the Giyani region had the lowest mass (36.5 g), while those from the Bushbuckridge region showed the highest fruit mass (44.6 g). The difference between the lowest and highest fruit masses (8.1 g) represents an 18.2% reduction in fruit mass. According to Remberg et al. (2010), temperature and the mineral element calcium are known to accelerate most biological processes in plants, including the size and development of fruit. Since these factors directly impact fruit size, they can dictate how large and heavy the fruit develops and when it will become available for consumption and processing for value-added product development (Nambi et al., 2015). The temperature difference between different geographic areas, particularly between Bushbuckridge (31.4°C) and Giyani (34.4°C), is 3°C, while the differential in soil mineral (calcium content) between Bushbuckridge (1922) and Giyani (1896) varied by 26 mg/kg. Climatic factors, temperature and soil, appear to have contributed to the variation in fruit weight of marula growing in differing regions. These findings could imply that marula fruit size potentially increases between the average temperatures of 17.3°C and 32.9°C when combined with calcium-rich soil, as shown in Tables 3.1 and 3.2. These findings are consistent with those of Adams et al. (2001), who reported variation in fruit weight on tomato fruit grown under varying temperature ranges and calcium-rich substrates.

For fruit length, study results depicted significant ($P \leq 0.01$) variation among marula fruit harvested from the three geographical regions (Bushbuckridge, Giyani, Tzaneen). These results a shown in Table 4.2 and indicate that fruit length ranged from 27.8 mm to 44 mm. In addition, results showed that fruit harvested from the Bushbuckridge region were short, at 27.8 mm, while those harvested from the Tzaneen region were longer (44.1 mm). The difference in length between the shortest and longest fruit (16.3 mm) implies up to a 37% reduction in fruit length amongst geographical areas.

A combination of soil minerals, especially potassium, temperatures and water directly impact the ability of the fruit to develop tissue cells, subsequently affecting their shape and size (Cieslak et al., 2016). The soil from the Bushbuckridge region had a higher potassium content (361 mg/kg) than that from Tzaneen (347 mg/kg), a difference of 14 mg/kg. The higher rainfall in the Tzaneen region (546 mm), combined with the relatively high soil mineral potassium concentration (347 mg/kg), appear to directly contribute to fruit being longer in the Tzaneed area. These findings agree with those of Guichard et al. (2005), who reported varying lengths in fruits subjected to varying potassium and rainfall levels.

In terms of fruit width, the study results revealed substantial variation ($P \leq 0.01$) among fruits harvested in different geographical regions. According to study results indicated in Table 4.2, marula fruit width ranged from 23.5 mm to 40.11 mm. Moreover, study results revealed that fruit harvested from the Bushbuckridge region had the lowest width (23.5 mm). In comparison, those harvested from the Tzaneen region had superior width (40.1 mm), implying that fruit width varied by up to 41.4% relative to the widest fruit. As with fruit length, the soil mineral element potassium and water supply play a pivotal role in fruit development, shape and width (Fontes et al. 2000). The variation in the fruit width of marula can be attributed to different soil mineral content and water supply through rainfall. The soil in the Tzaneen region at (347 mg.kg-1) had a relatively high potassium content and experienced the highest rainfall (546 mm) of the three regions (Tables 4.1). These findings correlate with those of Liu et al. (2011), who reported varying sizes of fruit grown under substrates subject to differing irrigation deficits and potassium levels.

According to Nambi et al. (2015), marula fruit ripeness is determined by factors such as colour, pH level, titratable acidity, and total soluble sugar content. In addition, colour is also regarded as one of the important indicators of fruit quality together with, amongst others, maturity and nutritional value (Opara et al., 2007; Liu et al., 2011; Salehi, 2020). Lightness in colour is represented by the letter L* (Table 4.2), which ranged from 72 to 76.5. The study results showed that the fruits harvested from the Giyani region had the lightest colour, at 76.5, followed by those from the Tzaneen region at 74.8 and those from the Bushbuckridge region at 72.

Table 4.2: Physical properties of marula fruits harvested from different regions

Location	Fruit mass (g)	Fruit length (mm)	Fruit width (mm)	L*	a*	b*	Chroma	Hue
Bushbuckridge	44.6(3.6)	27.8(1.3)	23.5(1.9)	72.0(1.1)	3.95(0.8)	39.7(1.8)	39.94(1.7)	84.4(1.5)
Giyani	36.5(2.1)	41.4(0.8)	38.9(1.2)	76.5(1.8)	5.17(1.7)	50.9(1.5)	51.28(2.8)	84.3(3.6)
Tzaneen	41.6(2.5)	44.1(1.4)	40.1(1.1)	74.8(1.1)	2.76(2.7)	41.4(2.1)	41.97(3.3)	86.4(3.2)
Grand mean	40.89	37.76	34.19	74.42	3.96	44.17	44.40	85.01
LSD	2.18	0.96	1.13	1.99	0.99	2.16	2.21	1.16
Pvalue	**<0.001**	**<0.001**	**<0.001**	**<0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Note: The standard deviations of the mean are shown by numbers enclosed in brackets. L* = lightness, a* = red (+) and green (-), b* = yellow (+) and blue (-). Lower than 0.05 P values are in bold. In terms of mean difference, LSD0.05 is the least significant.

For the yellowness, (b*) as shown in Table 4.2, represent the ripeness of the fruit. Results showed significant variation ($P \leq 0.01$). Furthermore, results showed that it ranged from 39.7 to 50.9. Moreover, study results showed that fruits harvested from Giyani at 50.9 had the highest ripeness, followed by those from Tzaneen (41.4) and the Bushbuckridge region (39.7). Mohammed et al. (2021) claimed that temperature is one of the environmental factors affecting fruit ripening rate. These authors also pointed out that different fruits have varied ideal temperatures. For example, certain fruits will ripen slowly in an extremely cold environment and more quickly in a hotter environment. According to the fruit colour chart, there was an 11.2 ripening rate difference between the highest (50.9) and the lowest (39.7) in this investigation. This indicates that the ripening rate varied by 11.2, almost 20%, across all regions. The difference in temperature between the Giyani region (34°C) and Tzaneen (31.4°C) was 2.6°C, indicating an 8% variation. Based on the findings of this study, fruit colour is primarily influenced by temperature variations. These results are consistent with those of Haokip et al. (2020), who noted that fruits grown in different locations with different temperatures ripened at different rates.

For colour intensity, results of this study evinced substantial (P ≤ 0.01) variation among fruit harvested from varying geographical regions. The colour intensity, Chroma (C), ranged from 39.8 to 51.3. Furthermore, study results revealed that fruits harvested from the Giyani region had the highest colour intensity (C) at 51.3, followed by those from the Tzaneen region (41.4) and Bushbuckridge (39.9). Ntsoane et al. (2019) reported that the colour intensity of fruits is mainly linked to climatic temperature. This study revealed a colour intensity difference of 11.3 between the highest (51.3) and the lowest values (39.9) obtained across the regions. The difference in maximum temperature between the Giyani region (34°C) and Tzaneen (31.4°C) was 2.6°C, indicating a 7.6% variation. Therefore, variation in the colour intensity of the fruit was significantly influenced by temperature variations across the geographical regions. These findings are in line with those of Dorothy (2023), who noted that climatic conditions, especially temperature and radiation, directly impact the physical attributes of the fruit, especially colour intensity.

Regarding the hue anle, study results illustrated significant (P ≤ 0.01) variation among fruit harvested from differing geographical regions. The mean values of the hue angle ranged from 84.3 to 86.4. Study results showed that fruits harvested from the Tzaneen region had the highest hue angle at 86.4, followed by those from the Bushbuckridge region at 84.4 and the Giyani region at 84.3. This means that the hue angle values from the Tzaneen region (86.4) showed to be 3.6 degrees closer to the 90-degree angle of the yellow colour (b*) than that of fruits from the Giyani region, which was 5.7 degrees closer to the 90-degree hue angle. Therefore, the hue angle values varied by 2.05 across all regions. Kortei and Akonor (2015) reported that the hue angle is a reliable method to evaluate the colour quality of fruits. These results are in line with those of Mohammed et al. (2021), who noted that climate conditions are the main contributing factors to the physical attributes of fruits, especially the colour quality (Hue angle).

4.1.3 Chemical properties and antioxidant activity of marula fruit juice and their potential role in human nutrition

4.1.3.1 pH, titratable acids and total soluble solids

The impact of varying geographical regions on the chemical properties of marula fruit juice (pH, titratable acids, and total soluble solids) is shown in Table 4.3. Study results revealed substantial ($P \leq 0.01$) variation in the chemical properties among fruits harvested in the three regions. The pH levels in the marula fruit ranged from 3.5 to 4.2. Fruit harvested from the Bushbuckridge region had the lowest pH value at 3.5, followed by those from the Giyani region at 4.1 while the highest pH value was found in fruit harvested from the Tzaneen region at 4.2. For titratable acid, results showed that it ranged from 22.3 g/l to 48.7 g/l. Study results evinced those fruits harvested from Bushbuckridge had the highest titratable acidity at 48.7 g/l, followed by those from Giyani, at 28.8 g/l, and in fruit harvested from the Tzaneen region, at 22.3 g/l. It is well known that mineral elements, particularly calcium, directly affect the pH of plants, particularly fruits. It improves the aggregation of soil particles, which in turn encourages water and mineral absorption by plant roots (Kader & Lindberg, 2010).

Kader and Lindberg (2010) cited that the primary variables influencing pH control in plants are a mixture of different environmental conditions, including temperature, water supply, and soil minerals. In this study, the variation between the highest pH in fruit (Tzaneen at 4.2) and the lowest (Bushbuckridge at 3.5) was 0.7. This shows that the pH in juice from fruits harvested in differing regions varied by 16.7%. The difference between the highest titratable acidity (Bushbuckridge at 48.7) and the lowest (Tzaneen at 22.3) was 26.4 g/l. This shows that the titratable acidity among fruits harvested in different regions varied by 54.2%. Furthermore, the variation between the highest soil calcium content (Bushbuckridge at 1 922 mg/kg) and the lowest (Tzaneen at 183 mg/kg) was 1 739 mg/kg, while the temperature varied by 3°C between the highest (Giyani at 34.4°C) and the lowest (Tzaneen at 31.4°C). This suggests that temperature and soil minerals, especially calcium, directly attributed towards the pH and titratable acidity of marula juice from fruits growing in the three geographical regions. These findings are in harmony with those of Neina (2019) who reported varying quality parameters in fruits subjected to different temperature ranges and calcium levels in soils.

Concerning the total soluble sugars (TSS), study results showed that it ranged from 10.6 °Brix to 12.9 °Brix (Table 4.3). Additionally, study results delineated those fruits harvested from the Tzaneen region at 12.9 °Brix had the highest total soluble sugars, followed by those of the Giyani region at 12.3 °Brix and those from the Bushbuckridge region (10.6°Brix). It is well established that temperature affects sugar accumulation of several plants, especially fruit crops (Gautier et al., 2008). According to Cordenunsi et al. (2005), plants exposed to high temperatures for long periods accumulate more sugar than plants exposed to low temperatures. In addition, the soil mineral elements, especially potassium, are responsible for assisting plants in accumulating the sugar content across all organs (Ganeshamurthy et al., 2011). The difference between the highest temperature (34.4°C in the Giyani region) and the lowest (31.4°C in the Tzaneen region) was 3°C, which means that there was 4% variation. The difference between the highest potassium content (Tzaneen region 347 mg/kg) and the lowest (Giyani region 251 mg/kg) was 96 mg/kg), which amounted to 18% variation. Therefore, varying temperatures and soil potassium content across the three regions directly attributed to the variation in total soluble sugar content of marula fruit juice. These findings are in line with those reported by Maluleke et al. (2021) who found variation in the fruit sugar content of plants grown under different temperatures and potassium levels.

In human nutrition, total soluble sugars are known to be responsible for regulating signals throughout the body and affect the expression of many genes involved in growth and metabolism (Rosa et al., 2009). It also provides vital internal organs such as the brain with their primary energy supply, making it a critical factor in human survival (Lopez et al., 2002). Therefore, consumption of marula fruit may assist in curbing conditions such as irregular heartbeat, sweating, shakiness, and constant fatigue, which are symptoms linked to low sugar intake in the human diet. These results are in line with those reported by Tuckeldoe et al. (2023), who found that a variety of underutilised edible fruits had acceptable soluble sugar contents that may have dietary uses for human sustainability.

Table 4.3: Chemical properties of marula fruit juice from varying regions

Location	**pH**	**TA g/l Citric acid)**	**TSS (% Brix)**
Bushbuckridge	3.5(0.1)	48.7(4.1)	10.6(0.1)
Giyani	4.1(0.0)	28.8(1.2)	12.3(0.1)
Tzaneen	4.2(0.1)	22.3(1.5)	12.9(0.1)
Grand mean	3.93	33.27	11.96
LSD	0.075	2.919	0.532
Pvalue	**<0.001**	**<0.001**	**<0.001**

Note: The standard deviations of the mean are shown by numbers enclosed in brackets. Lower than 0.05 P values are in bold. In terms of mean difference, LSD0.05 is the least significant. pH = percentage hydrogen. TA = titratable acid. TSS = total soluble sugars.

4.1.4 Phytochemicals properties of marula fruit juice and their potential role in human health and nutrition

The bioactive properties of marula juice extracted from fruits harvested in varying geographical regions are depicted in Table 4.4. Study results indicated a significant ($P \leq 0.01$) variation in antioxidants (DPPH radical scavenging activities, tannins, total flavonoids, total phenols). However, there was no substantial ($P < 0.07$) variation in the vitamin C content of marula fruit juice extracted from fruits harvested in the three geographical regions.

Results showed that DPPH radical scavenging activities ranged from 72.1% to 76.7% (Table 4.4). Moreover, results indicated that marula fruit juice extracted from fruits harvested from the Bushbuckridge region had superior DPPH radical scavenging activities at 76.6%, followed by those from the Tzaneen region at 72.2% and the Giyani region, at 72.1%. The variation between the highest scavenging activities (Bushbuckridge at 76.7%) and the lowest (Giyani at 72.1%) was 4.6%. It has been established that water supply and irrigation directly affect most plants' antioxidant content, particularly in fruit crops (Tuckeldoe et al., 2023). Osmotic pressure, which is known to facilitate the transport of nutrients throughout various plant organs, influences radical scavenging activities. Osmotic pressure is one of the processes that can be adversely impacted by an excess or insufficient water supply to plants (Bartolini et al., 2015). For this study, the difference between the highest rainfall (Tzaneen region, 546 mm) and the lowest (Giyani, 346 mm) was 200 mm, showing a 37%

variation in annual rainfall. As a result, water supply or rainfall intensity was a main contributor towards the variation in radical scavenging activities in marula juice extracted from fruits harvested in varying geographical regions. These findings are in line with those of Jin et al. (2022) who found variations in antioxidants in fruits harvested from plants subjected to varying water supplies.

Regarding human health and nutrition, most chronic health problems, such as cancer, inflammation, and cardiovascular diseases, are linked to damage caused by free radicals (Herrling et al., 2008). In addition, antioxidants assist by preventing tissue damage from free radicals through scavenging, inhibiting, or promoting the breakdown of radicals (Carvalho et al. 2010). Therefore, the results from this study could support the assertion that the consumption of marula fruit juice may assist in curbing conditions such as cancer, kidney malfunctioning, obesity, liver malfunction, inflammation, and low immune system, which are symptoms linked to low antioxidant intake in human diet.

In regard to tannins, study results showed that they ranged from 0.54 to 0.58 mg GAE/ml FW (Table 4.4). In addition, study results indicated that marula juice extracted from fruits harvested in the Giyani and Tzaneen regions had a lower tannin content, at 0.54 mg GAE/ml, than fruits harvested in the Bushbuckridge region, which had a tannin content of 0.58 mg GAE/ml. The variation between the higher (0.58 mg GAE/ml) and lower (0.54 mg GAE/ml) tannin content was 0.04 mg GAE/ml. According to Kumar et al. (2017), temperature is one of the most important factors affecting plants' antioxidant activity. The initiation processes are usually accelerated or slowed down by, respectively, higher or lower temperature ranges, which in turn affects the antioxidant activities already existing in plant organs (Reyes-Carmona et al., 2005). The variation between the highest temperature (34.4°C in the Giyani region) and the lowest (31.4°C in the Tzaneen region) was 3°C. This implies that temperature variation across geographical regions directly contributes towards differences in the tannin content of marula fruit juice. These findings agree with those of Gautier et al. (2008), who reported variation in antioxidants extracted from fruits grown under differing regions experiencing distinct temperatures.

In terms of human health and diet, water-soluble polyphenols, such as tannins, are known to be present in a variety of plant foods, including fruits (Santos-Buelga & Scalbert, 2000). Although, tannins are not deemed essential nutrients for human health, as the body does not require them for functioning or survival. They are bioactive phytochemicals with significant health advantages, including potent antioxidant, anti-inflammatory, and antibacterial properties that may contribute to the prevention of chronic diseases such as cancer and cardiovascular disorders (Sharma et al., 2021). The variation between the highest tannin content of marula juice (0.58 mg GAE/ml) and recommended daily intake (200 mg GAE/ml) is 199.42 mg GAE/ml. This suggests that about 344.8ml quanitity of marula fruit juice would be needed to contribute roughly 100% of the ARDI for tannins requiredy humans.Therefore, the consumption of marula fruit juice may potentially assist in curbing conditions of chronic diseases such as blood circulation problems and cancer, which are symptoms linked to low tannins in human health (Santos-Buelga & Scalbert, 2000).

For the total flavonoid content of marula fruit juice, study results showed that it ranged from 0.78 mg QE/ml FW to 1.4 mg QE/ml FW (Table 4.4). Juice extracted from marula fruit harvested in the Tzaneen region had the highest total flavonoid content at 1.4 mg QE/ml FW, followed by Giyani at 1.3 mg QE/ml FW. The lowest total flavonoid content (0.78 mg QE/ml FW) in marula juice was observed in fruit harvested in the Bushbuckridge region. Temperature and rainfall (water supply) are climatic factors controlling how flavonoids are synthesised and accumulated by plants (Kumari & Jain, 2012). In addition, flavonoids play a crucial role in defending plants against abiotic stressors such as heat, UV light, and drought (Mierziak et al., 2014). The variation between the highest temperature (34.4°C in the Giyani region) and the lowest (31.4°C in the Tzaneen region) was 3°C, amounting to around a 10% difference. The difference in annual rainfall between Tzaneen (546 mm) and Giyani (346 mm) is 200 mm. This implies that temperature and rainfall differences across regions caused plants to release varying metabolites to survive specific environmental stresses within a certain geographical region. Therefore, temperature and rainfall variation among varying geographical regions directly impacted the total flavonoids content of marula juice extracted from fruits harvested in varying geographical regions. Findings from this study are in line with those of Taylor and Grotewold (2005) who reported variation in the antioxidant quality of marula juice extracted from fruits harvested in differing geographical regions with distinct temperatures and annual rainfall.

In relation to human nutrition and health, flavonoids are a class of chemicals with different phenolic structures known for their antiviral, anti-inflammatory, antioxidant, and anticancer properties (Rao et al., 2018; Maluleke et al., 2024). In the current study, the variation between the highest flavonoid content (1.4 mg) and the recommended daily intake (750 mg) is 748.6 mg. This suggests that 535 ml of marula fruit juice would be required to provide 100% of ARDI of total flavanoinds needed by humans daily. Although values obtained from this study are low compared to the average recommended daily intake, consumption of marula fruit juice may potentially assist in the prevention of ailments such as frequent colds, infections, excessive bruising, and nose bleeding, which are linked to a low flavonoid content in the human diet (Li et al., 2015).

Concerning total phenols, results from the current study showed that it ranged from 2.02 to 2.99 mg GAE/ml FW (Table 4.4). Moreover, results illustrated that juice extracted from fruits harvested in the Bushbuckridge region had the highest total phenolic content at 2.99 mg GAE/ml FW, followed by the Tzaneen region at 2.08 mg GAE/ml FW while the lowest total phenolic content (2.02 mg) was observed in juice extracted from fruits harvested in the Giyani region. According to Hiwilepo-van Hal et al. (2014), climatic factors such as temperature are known to have a direct impact on the total phenolic content of plants due to their sensitivity towards heat. Therefore, climatic variations during different growing seasons, such as the different air temperatures and rainfall rates, have been reported as some of the factors contributing towards the total phenolic content of fruits (Cordenunsi et al., 2005). The temperature difference between Bushbuckridge (33°C) and Giyani (31.4°C) is 1.6°C, indicating a 5% variation between the two regions. In this study, temperature as an environmental factor can be categorised as the primary factor that caused variation in the total phenolic content of the juice extracted from marula fruit harvested in different geographical regions. This is because temperature differences among geographical regions caused plants to release different metabolites that allowed them to adapt to varying geographical regions, which in turn affected their phenolic content. These findings are in line with those reported by Baba and Malik (2015), Arena et al. (2021)

and Tuckeldoe et al. (2023) who reported variation in the total phenolic content of fruits and juice extracted from fruits grown under different climatic conditions.

With respect to human health and nutrition, phenols are micronutrients found in many different fruits and vegetables and are appreciated for their health-promoting qualities (Tuckeldoe et al., 2023). In addition, due to their ability to lower inflammation, enhance blood sugar regulation, improve heart health, and lower the chance of developing cancer, they are essential for human nutrition and health (Schwartz et al., 2009; Maluleke et al., 2024). The variation between the highest phenolic content (2.99 mg GAE/ml) and the average recommended daily intake (28.6 mg GAE/ml) is 25.7 mg GAE/ml. This means that 10 ml of marula fruit juice would be required for provision of 100% of ARDI needed by humans. Even though values obtained from this study are lower than the recommended daily intake, consumption of marula fruit juice could potentially assist in the reduction of various conditions such as cardiovascular diseases, diabetes, cancer, and hypertension, which are linked to low phenolic intake in the human diet (Lekoba et al., 2024).

For vitamin C, study results illustrated that there was no substantial ($P < 0.07$) variation in marula juice extracted from fruits harvested in varying geographical regions (Table 4.4). Although there was no statistical difference, results indicated that it ranged from 50.4 mg/100 ml FW to 83.9 mg/100 ml FW. According to Dorward (2015), plants produce their own vitamin C in the mitochondria when they experience environmental stressors such as high temperatures and poor water supplies (Fenech et al., 2019). Furthermore, vitamin C enters other cell organs, including chloroplasts, where it participates in metabolic processes as a coenzyme and antioxidant to help shield the plant from environmental abiotic stress (Xu et al., 2015). The variation between the highest vitamin C content in marula fruit juice extracted from the Giyani region and the lowest from Bushbuckridge is 37 mg/100 ml GAE FW. There was a 1.4 °C variation in temperature between the Giyani and Bushbuckridge regions, while the rainfall intensity varied by 182.4 mm (Table 4.1). This suggests that a combination of climatic factors such as temperature and rainfall intensity directly contributed to the variation of vitamin C content in the juice extracted from marula fruit harvested in distinct regions. These attributes are linked to plants releasing primary metabolites that enable them to deal with environmental stress, especially in the Giyani region where there was a higher temperature range combined with lower rainfall intensity than in other regions. These findings are in agreement with those of Lekoba et al. (2024), who reported varying vitamin C contents in plants subjected to varying environmental stresses such as water and climates.

In relation to human nutrition and health, the development and maintenance of tissues in every area of the human body depend on vitamin C (Pullar et al., 2017). In addition, vitamin C helps create collagen, a protein crucial for the formation of blood vessels, tendons, ligaments and skin. Vitamin C is also regarded as a crucial component in accelerating the development of scar tissue and wound healing (Lykkesfeldt et al., 2020). The variation between the highest vitamin C content (83.9 mg/100 ml) extracted from marula fruit and the recommended daily intake (82.5 mg/100 ml) is 1.4 mg/100 ml. This could mean that 0.9 ml of marula fruit juice would be needed to provide 100% of ARDI required by humans. Moreover, findings from this study could imply that the consumption of juice extracted from marula fruit could assist in curbing conditions such as poor wound healing, excessive bruising, skin-related challenges and constant fatigue, symptoms associated with low vitamin C intake in the human diet (Gautier et al., 2008).

Table 4.4: Antioxidant properties of marula fruit juice from varying regions

Location	**DPPH (% RSA)**	**Tannins (mg GAE/ml juice)**	**Total Flavonoids content (mg QE/ml juice)**	**Total Phenol content (mg GAE/ml juice)**	**Vitamin C (mg/100ml juice)**
Bushbuckridge	76.7(1.4)	0.58(0.12)	0.78(0.1)	2.99(0.1)	50.4(3.7)
Giyani	72.1(0.6)	0.54(0.8)	1.28(0.1)	2.02(0.1)	83.9(2.9)
Tzaneen	72.2(0.6)	0.54(0.3)	1.4(0.2)	2.08(0.1)	70.8(5.8)
Grand mean	73.7	0.56	1.15	2.36	68.32
LSD	1.267	0.003	0.303	0.151	28.444
Pvalue	**<0.001**	**0.02**	**0.010**	**<0.001**	0.073

Note: The standard deviations of the mean are shown by numbers enclosed in brackets. Lower than 0.05 P values are in bold. In terms of mean difference, LSD0.05 is the least significant.

4.1.5 Conclusion

The study demonstrated that the physicochemical qualities of marula fruit are directly influenced by differing climatic circumstances, leading to nutritional disparities in their native regions. For instance, fruits from the Bushbuckridge region exhibited greater mass than those from other regions. In comparison to other sample collection sites, the total soluble sugars and flavonoids were found to be greater in fruits from the Tzaneen region. Fruits from the Giyani region exhibited a significantly higher quantity of vitamin C compared to those from other regions. This supports the argument that underutilised indigenous fruit trees, such as marula, may hold a potential solution for future food security in Africa due to its abundant nutritional content. Value-added products produced from marula fruit may also help mitigate hunger and malnutrition. The physicochemical constitution of marula fruit has demonstrated a potential role in human nutrition, and it aligns with the average recommended daily intake, rendering it a viable crop for adoption as a potential mainstream agricultural crop. Consequently, the findings of this study recommend that prospective plant breeders take climatic factors into account when selecting accessions to develop varieties that are nutritionally dense. This will help to establish baseline knowledge about the crop's cultivation requirements, which is considered a bottleneck preventing most commercial farmers and the food processing industry from adopting it for large-scale farming and creating value-added products such as alcoholic beverages, juice, jam and nuts. Lastly, further exploration of aspects such as cultivation requirements, metabolite profiling, and the development of value-added products can yield considerable economic and social benefits. Such advancements can facilitate a consistent provision of fruits, nutrients, and related products to consumers, sellers, and processing industries, thereby influencing the economic and health sectors within the regions, particularly near where the crop is prevalent. Nonetheless, genetics, age of the tree, and seasonal variability remain as the potential limitations that may directly affect the nutritional and biochemical contents of the fruits and its juice.

4.2 Characterisation of marula fruit (*Sclerocarya birrea*) juice and the effect of storage conditions and preservatives on its biochemical properties

4.2.1 Effect of storage condition and preservatives on the chemical properties of marula fruit juice

The effect of storage condition and preservatives on the pH, titratable acidity and total soluble solids content of marula fruit juice is shown in Table 4.5. The results indicated that there was no discernible ($P \leq 0.05$) variation in the pH levels according to the temperature used to store the samples. However, the pH level ranged from 3.49 to 3.99 respectively according to the treatment and the duration of storage. The observed trend revealed that there was an increase in pH level as the storage days and temperature increased, irrespective of the preservative used. The maximum pH level (pH 3.99) at the end of the experiment was observed in SB (10°C) treated juice, while the minimum level of (pH 3.73) was found in SM (6°C). The pH level appeared to have increased under the treatment combination of SB stored at 10°C for 20 days, whereas the treated combination of SM stored at 6°C retained the lowest pH level of pH 3.73. Moreover, study results illustrated that there was 6.2% increase from day 0 to day 20 in treatment combination SM at 6°C. pH is critical in food quality, particularly in taste, texture and microbiological stability where it affects chemical and enzymatic processes that influence factors such as colour, flavour and consistency (Andrés-Bello et al., 2013). Findings from this study may suggests that the slight increase in pH may be due to degradation of organic acids in the juice sample. Marula juice is high in ascorbic acid (vitamin C) and this acid undergoes oxidation over time to converts into dehydroascorbic acid and to eventually lose its carboxylic acid moieties (Tebeila, 2022; Mandha et al., 2023). These study findings concur with those of Ayub et al (2010) and Wang et al. (2022) who reported a significant increased pH in apple and strawberry juice as storage days increased.

In terms of titratable acidity (TA), Table 4.5 indicates that there was a significant ($P \leq 0.05$) difference among treatments. The TA ranged from 16.33 g/L to 22.06 g/L citric acid, respectively. Additionally, results illustrated a downward trend in TA as the storage days and temperature increased. After 20-day storage, a minimum TA content was observed in marula juice subjected to a treatment combination of SM at 10°C, while the maximum content (20.10 g/L) was observed under the treatment combination of SB at 6°C. Moreover, study results demonstrated that the use of treatment combination SM at 10°C after 20 days reduced the titratable acidity content of marula fruit juice so that, from day 0, the value observed ranged from (22.06 g/L) to (16.33 g/L), indicating a 26% retention capacity, when compared to other treatments. The maximum TA content of 20.10 g/L was retained in the treatment combination of SB stored at 6°C, indicating an 8.8% retention capacity on TA from day 0 to 20, when compared to other treatments. The study findings suggests that the descrease in TA may be due to slow enzymatic metabolism and complex formation with phenolic compounds. Marula is rich in phenolics, so acid-phenolic interactions may reduce the amount of free titratable acids.Therefore, values obtained from this study may have revealed that the combination of SB and 6°C for 20 days storage is the most effective method for preserving the titratable acidity of marula juice, as both factors could have been responsible for inhibiting microbial and enzymatic activities, thereby prolonging the retention of the original content compared to alternative treatments. These findings are in line with those reported by Pareek et al. (2011) and Wang et al. (2022). These authors reported a downward trend (decrease) of TA in apple juice, when the storage duration increases. Findings of the current study revealed that preservatives sodium metabisulphite (SM), sodium benzoate (SB), as well as the control (C), significantly affected the TA content, as the storage days progressed. Thus, denoting that the treatment combination of SB and stored temperature (6°C) appear to be the most effective conditions for maintaining the pH of marula juice over a 20-day storage period.

In most food products, including juice, titratable acidity, is a measure of total acid concentration that includes both free and bound hydrogen ions, and is critical in determining quality and flavour of a product (Tyl & Sadler, 2017). Moreover, titratable acidity is a predictor of how organic acids in the food impact flavour. Juice acidity plays a key role in its sensory acceptability by consumers (Mandha et al., 2021, 2022).

Regarding the effect of preservatives and storage conditions on the total soluble sugars (TSS) of marula fruit juice, results showed a significant ($P \leq 0.05$) difference among treatments. The TSS ranged from 11.20 °Brix to 13.70 °Brix. Additionally, the observed trend revealed that there was a decline in TSS as the storage days and temperature increased. The minimum TSS content of 11.20 °Brix at the end of 20 days of storage was observed from the treatment combination SM and 10°C storage condition, whereas the maximum TSS content of 12.71 °Brix was recorded in the treatment combination of SB and 4°C storage condition. Furthermore, study results indicated that on storage at 4°C for 20 days, following treatment with SB retained the highest TSS content (12.71) TSS, whereby a 7% decrease on TSS was noted from day 0 to day 20. When compared to other treatments, the lowest TSS content, of 11.20, was retained in the treatment combination of SM and storage at 10°C, indicating a 17% decrease from day 0 to day 20.

Therefore, the study findings revealed that marula juice preserved with sodium metabisulphite (SM), sodium benzoate (SB), and control (C) significantly affected TSS content retention over the storage period. The decrease in TSS during storage may be attributed by the slow enzymatic degradation of sugars. Enzymes that are involved in carbohydrate metabolism may have catalyse the breakdown of sugars involved, hence there was a decrease. These findings are in line with those reported by Mandha et al. (2023) who linked a decrease in TSS of mango juice to longer storage conditions. Similar observations were reported by Ampofo-assiama and Quaye (2019), who found that a decrease in TSS of pasteurised soursop juice was associated with longer storage duration and higher temperature. The study suggests that the treatment combination of SB and 4°C appears to be the most suitable combination for retaining TSS content of marula fruit over a 20-day storage period.

Table 4.5: Effect of storage conditions and preservatives on the pH, total acidity and total soluble solids of marula fruit juice.

Days	Treatment	pH			TA g/l Citric Acid			TSS %Brix		
		Storage Temperature								
		4°C	**6°C**	**10°C**	**4°C**	**6°C**	**10°C**	**4°C**	**6°C**	**10°C**
0	C	3.50	3.50	3.50	20.64	20.64	20.64	13.63	13.63	13.63
	SM	3.50	3.50	3.50	20.12	20.12	20.12	13.53	13.53	13.53
	SB	3.49	3.49	3.49	22.06	22.06	22.06	13.70	13.70	13.70
10	C	3.65	3.72	3.80	19.69	20.96	19.03	12.17	12.51	11.89
	SM	3.90	3.80	3.87	19.05	19.93	18.84	11.79	12.22	12.22
	SB	3.82	3.91	3.86	20.22	21.47	20.94	11.67	12.76	11.94
20	C	3.91	3.88	3.83	17.55	18.23	17.05	12.06	12.00	12.12
	SM	3.95	3.73	3.97	17.71	16.93	16.33	12.06	11.94	11.20
	SB	3.92	3.75	3.99	19.53	20.10	19.03	12.71	11.87	11.58
Grand mean		3.73			19.67			12.57		
$LSD_{0.05}$		0.26			1.64			0.396		
Pvalue		0.221			**<0.001**			**<0.001**		

Note: C = Control. SM = Sodium Metabisulphite. SB = Sodium Benzoate. Lower than 0.05 P values are in bold. The least significant difference between means, or LSD0.05, was used.

4.2.2 Effect of storage condition and preservatives on the biochemical and antioxidant properties of marula fruit juice

4.2.2.1 Total flavonoid content

The total flavonoid content of preserved marula fruit juice (preservatives and storage conditions) is presented in Figure 4.1. These results show that there was no discernible ($P \leq 0.05$) difference amongst treatments. However, the total flavonoid content showed a notable behaviour during storage, which ranged from 1.31 to 3.17 mg QE/ml. A prominent decrease was observed from the initial analysis on day 0 to 10, thereafter an increase was noted from day 10 to 20 across all treatments. On day 0, the highest flavonoid content (3.17 mg QE/ml) was observed in SB-treated juice samples stored at 4°C, 6 °C and 10°C. However, on day 20, the highest total flavonoid content (2.49 mgQE/ml) were noted in juice samples subjected to SB and stored at 10°C as compared to other treatments. The variation between the initial flavonoid content (3.17 mg QE/ml) observed on day 0 and the maximum retained content (2.49 mg QE/ml) after day 20 was 0.68, indicating a 21% decrease in total flavonoids content across all treatments (C, SB and SM) and stogae temperatures (4°C, 6°C and 10°C). The observed trend in the current study demonstrated that as the storage conditions progressed, there was a decrease in total flavonoid content across all treatments. The treatment of SB and 10°C appeared to be the most effective combination for total flavonoid retention of marula fruit stored for a 20-day period. The elevation of storage temperature (10°C) and prolonged duration may have contributed to a substantial decrease in flavonoid content due to their heightened sensitivity to temperature, which typically results in degradation as elevated temperatures facilitate the hydrolysis of glycosidic bonds, rendering the remaining aglycones less stable.These results concur with those reported by Baltacioğlu et al. (2011) and Igual et al. (2011), who observed a significant reduction in the total flavonoid content of grape and rowanberry fruit juice subjected to varying treatments and storage conditions.

Increased in storage temperature (10°C) and extended durarion may have attributed to higher reduction in falavonids content due to its higher sensitivity towards temperature, which normally leads to degradation as higher temperature promotes the hydrolysation of glycosidic bonds, which makes the remaining aglycones less stable.

Regarding human nutrition, flavonoids provide beneficial effects such as free radical scavenging, strong antioxidant activities in preventing oxidation and anti-inflammatory actions (Hoensch & Oertel, 2015; Lekhuleni et al., 2024). In addition, they are important health-promoting components in fruit juices (Shourove et al., 2020). The difference between the highest retained recorded total flavonoid content (2.49 mg QE/ml) observed in the treatment combination (SB and 10°C) and the average recommended daily intake (60.3) is 57.81 mg QE/ml, indicating that SB-treated fruit juice stored at 10°C for a period of 20 days may potentially contribute about 4.1% of total flavonoids required by humans daily. This could imply that marula fruit juice subjected to the treatment combination (SB and 10°C) is sufficient to offer 24.2 ml, which is considered the required quantity for the provision of 100% ARDI of total flavonoids required by humans. As a result, the study findings may suggest that the treatment of SB and storage at 10°C for 20 days appeared to be a suitable combination to retain the highest total flavonoid content compared to other treatment combinations. Therefore, the current study findings could mean that consumption of marula fruit juice subjected to the treatment combination of SB and stoage at 10°C for 20 days may assist in preventing cardiovascular disorders, neurology, urology, immunology and gastroenterology challenges, which are conditions linked with low flavonoid intake in the human diet (Hoensch & Oertel 2015; Tsanova-Savova et al., 2018).

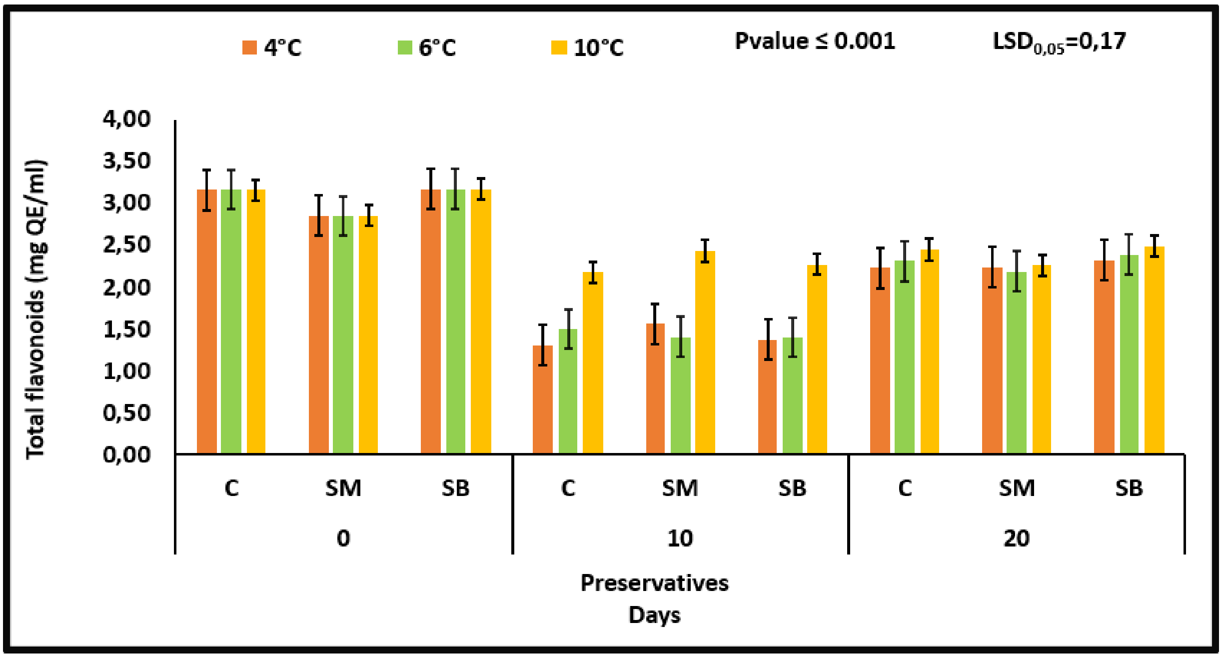

Figure 4.1 Total flavonoid content of marula fruit juice. Note: C= Control. SM= Sodium Metabisulphite. SB= Sodium Benzoate.

4.2.2.2 Total Phenolic content

The results of the analysis of the total phenolic content of preserved (preservatives and storage conditions) marula fruit juice is presented in Figure 4.2. The study results indicate that preservatives and storage conditions showed a significant ($P \leq 0.05$) difference on the total phenolic content. In addition, the total phenol content ranged from 2.9 mg GAE/ml to 3.81 mg GAE/ml. The observed trend revealed that there was an increase in total phenolic content as the storage days and temperature increased. On day 0, the highest phenol content (3.04 mg GAE/ml) in marula juice was observed in the treatment combination of control at all storage conditions (4°C, 6°C and 10°C). However, on day 20 the highest total phenolic content (3.81 mg GAE/ml) was observed in the treatment combination of SM and storage at 10°C. This suggests that the difference between the highest phenolic content obtained from the treatment of combination SM and 10°C on day 20 and day 0 (2.75 mg GAE/ml) was 1.06 mg GAE/ml. This indicates that there was a 28% increase in phenolic content respectively. Furthermore, the study revealed that marula juice preserved with sodium metabisulphite (SM), sodium benzoate (SB), and control (C) significantly affected the total phenolic content as the storage days progressed. Thus, denoting that treatment combination of SM and 10°C is effective in maintaining the total phenolic content of marula juice over a 20-day storage period. The increase in total phenolic content may be attributed by enzymatic and cellular structure reactions, which normally lead to the breakdown of compounds into smaller phenolic molecules, resulting in increased phenolic concentration over time (Tarazona-Díaz & Aguayo, 2013). During storage, the cellular structure of the juice particles may deteriorate, facilitating the release of phenolic chemicals from cell wall polysaccharides, starch, or pectin into the juice. These molecules undergo oxidation and subsequently react to generate new polymeric compounds that retain antioxidant properties, resulting in a perceived increase in overall phenolic content. Hence, there was a significant rise below 10 degrees compared to other temperatures, irrespective of the preservative utilised. These findings are in line with those of Tchuenchieu et al. (2018), who reported an increase of total phenolic content in fruit juices subjected to varying pasteurisation temperature.

In human nutrition, phenolic compounds act as natural antioxidants that have the potential to improve health, lowering inflammation and lowering the risk of chronic illnesses (Mphahlele et al., 2016; Lekoba et al., 2024). Furthermore, they also play a paramount role in defensive mechanisms such as anti-ageing (Mphahlele et al., 2016). The variation between the highest recorded total phenolic content (3.81 mg GAE/ml) of marula juice, obtained from treatment combination (SM and 10°C) and the average recommended daily intake (28.6 mg GAE/ml) is 24.79 mg GAE/ml. This suggests that 7.5 ml of marula fruit juice treated with the combination (SM and 10°C) would suffice to meet 100% of the ARDI of total phenols required by humans.Therefore, findings could mean that consumption of marula fruit juice subjected to SM and 10°C stored for 20 days may help prevent conditions such as hypertension, metabolomic challenges and incendiary infections, which are symptoms associated with low phenolic intake in human diet (Achaglinkame et al., 2019; Lekhuleni et al., 2024).

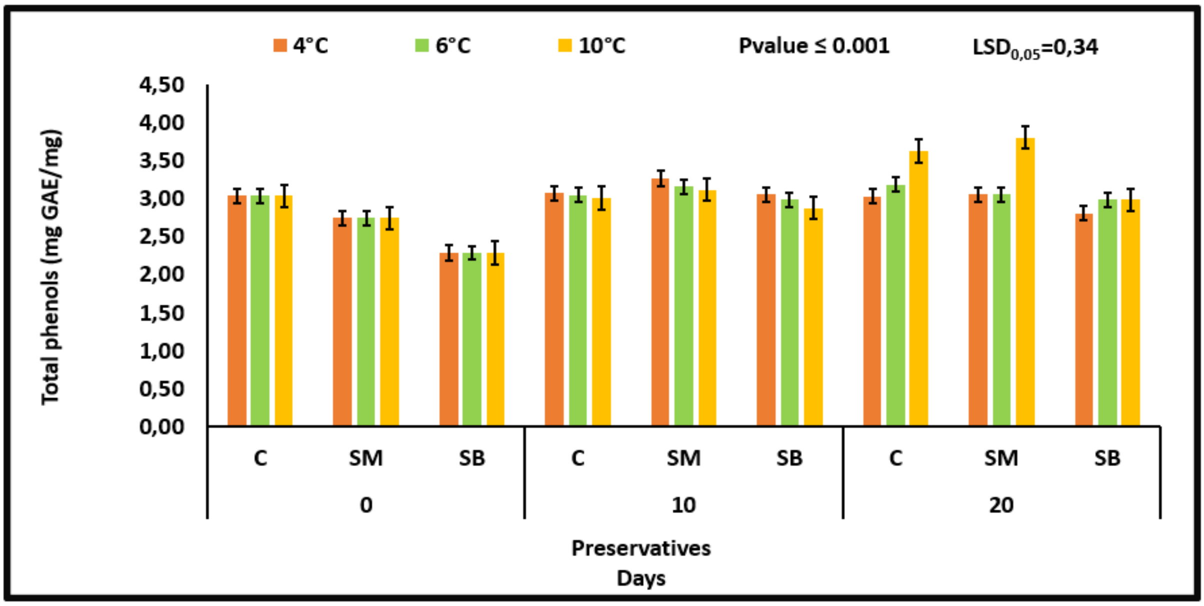

Figure 4.2: Total phenolic content of marula fruit juice. Note: C= Control. SM= Sodium Metabisulphite. SB= Sodium Benzoate.

4.2.2.3 Antioxidant activity (DPPH)

The effect of preservatives and storage conditions on the antioxidant activities (DPPH) in marula fruit juice is presented in Figure 4.3. These study results show that the antioxidant activity of the marula juice did not differ significantly ($P \leq 0.05$) amongst treatment combination. However, the antioxidant activity ranged from 40.32% RSA to 76.28% RSA. In addition, the observed trend illustrated that as the storage days progressed, there was a downward trend, showing a decrease on the antioxidant activity irrespective of preservatives and storage conditions. The study results showed that on day 0, the highest antioxidant activity of (76.28% RSA) was observed under the treatment combination of SM and storage conditions (4°C, 6°C and 10°C), whereas the lowest (40.32% RSA) was observed in juice subjected to the treatment combination of SB at 10°C for 20 days. Moreover, the study further revealed that on at the 20-day storage, the treatment combination of SM and 10°C retained the highest antioxidant activity of 50.04% RSA when compared to other treatments. This suggests that the difference between the highest retained antioxidant activity in treatment combination (SM and 10°C) on day 20 (50.04% RSA) and day 0 (76.28% RSA) was 26.24% RSA.

Ascorbic acid, widely known as vitamin C, serves as a significant antioxidant in juice and is especially susceptible to elevated storage temperatures and oxygen exposure. Elevated temperatures, in conjunction with oxygen present in the headspace or dissolved in the juice, catalyze the aerobic oxidation of ascorbic acid into dehydroascorbic acid, which then degrades into acids and other compounds, resulting in a loss of its antioxidant efficacy. Although there was notable decrease in all treatments, the combination of sodium metabisulphite and 10°C inhibited the proliferation of fungi and bacteria that degrade bioactive compounds in fruit juice, which are directly involved in disrupting the oxidative chain reaction of phenolic compounds, resulting in superior retention within this treatment compared to others. These findings are co-related to those of Ampofo-Asiama and Quaye (2019), who reported a reduction in total antioxidant capacity of soursop juice stored under differing temperature and durations.

In human health, antioxidants are essential for human health and nutrition as they safeguard cells from damage inflicted by free radicals, unstable molecules that may lead to various diseases (Yahia, 2017; Baliyan et al, 2022). By neutralizing these free radicals, antioxidants diminish the risk of conditions associated with cell damage (Carvalho et al., 2010). Therefore, the ability of treatment combination of SM and 10°C to retain some antioxidant activities could be beneficial to human health and nutrition, since conditions as heart disease, cancer related diseases, and neurogenerative disorders can be prevented, which are associated with low antioxidant content in human nutrition (Carvalho et al., 2010).

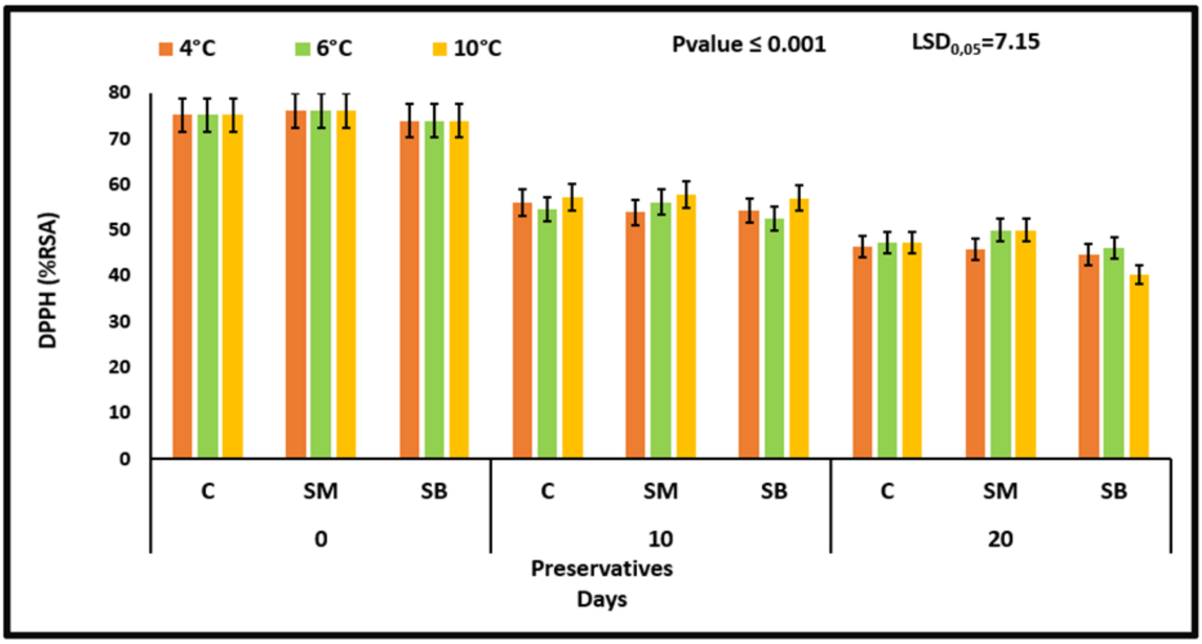

Figure 4.3: DPPH of marula juice. Note: C= Control. SM= Sodium Metabisulphite. SB= Sodium Benzoate.

4.2.2.4 Total tannin content

The total tannin content of marula fruit juice subjected to different preservatives and storage conditions is shown in Figure 4.4. The study results indicata that preservatives and storage conditions had a significant ($P \leq 0.05$) influence on the total tannin content in marula juice. Moreover, results showed that total tannin content ranged from 1.52 mg GAE/ml to 2.48 mg GAE/ml. Based on the observed trend, the tannin content showed an upward and downward trend over the 20 days storage period. On day 0, the highest tannin content of (2.48 mg GAE/ml) was observed in the treatment combination of Control and storage conditions (4°C, 6°C and 10°C), whereas the lowest tannin content of 1.52 mg DAE/ml was observed in the treatment combination SB and 4°C for 20 days. Furthermore, results showed that on a 20-day storage, treatment combination of control and 6°C retained the highest total tannin content (2.0 mg GAE/ml), when compared to other treatments. The variance observed between the highest total tannin content (2.00 mg GAE/ml) in the treatment combination of control and 6°C on day 20 and day 0 (2.48 mg GAE/ml) was 0.48 mg GAE/ml, suggesting that there was a 20% decrease over a 20-day storage duration. The depleted rate of total tannin content was observed in the treatment combination SB and 4°C for a period of 20 days.

Pasteurisation is a practice that is known to slow down enzymic reactions in most food products such as juice (Tobolková et al. (2024). In addition, it has been established that the combination of treatments (pasteurization and low storage temperature) is effective in preserving various compounds in fruit juice during storage. This method primarily inactivates enzymes that degrade polyphenolic compounds, while the low temperature inhibits microbial activity, thereby stabilizing the overall chemical composition of the juice. Therefore, findings from this study could mean that the use of pasteurisation combined with storage at low temperature (4°C) could have contributed to preventing the activeness of enzymes that are responsible for degradation of antixodents compounds, hence theres was improved retention of total tannin content when compared to other treatments. These findings align with those reported by Aneja et al. (2014), who asserted that the combination of pasteurisation and appropriate storage temperature is crucial for enhancing the quality of fruit juice.

In respect to human health and nutrition, tannins, which are a class of polyphenolic chemicals present in plants, have several functions including antioxidant anti-inflammatory, as well as antibacterial effects (Oluwole et al., 2022; Cosme et al., 2025). The difference between the highest retained total tannin content (2.00 mg GAE/ml), observed in marula juice subjected to the treatment combination of control and 6°C and the average recommended daily intake (200 mg GAE/ml) is 198.00 mg GAE/ml. This could suggest that, from the treatment combination of (positive control and 66°C), about 100 ml of marula fruit juice would be required to provide 100% of ARDI of tannins required by humans. Therefore, the study findings may imply that consumption of marula fruit juice subjected to the treatment combination of control (pasteurised) and 6°C may potentially assist in preventing conditions such as cardiovascular disease, cancer, and diabetes, which are symptoms linked to low tannins content in humans (Fraga et al., 2019; Kitabatake et al., 2021).

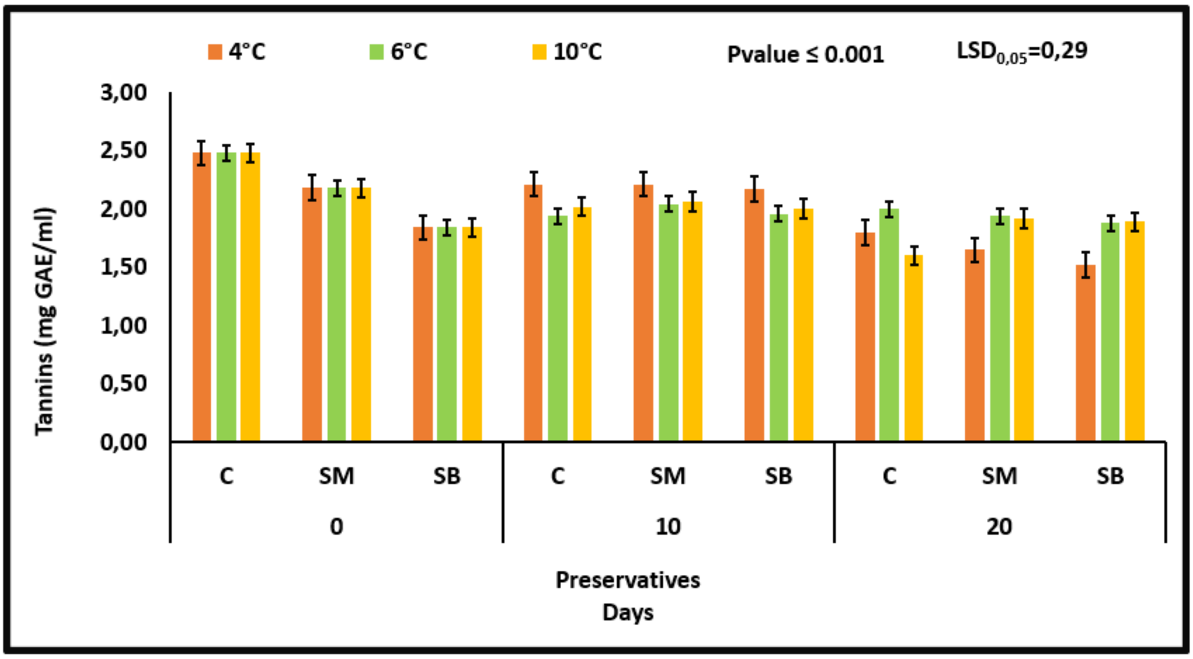

Figure 4.4: Total tannin content of marula fruit juice. Note: C= Control. SM= Sodium Metabisulphite. SB= Sodium Benzoate.

4.2.2.5 Vitamin C

The effect of different preservatives and storage conditions on the vitamin C content of marula juice is presented in Figure 4.5. The study results demonstrated that preservatives and storage conditions had a significant ($P \leq 0.05$) effect on the Vitamin C content in marula juice. In addition, the vitamin C content ranged from 81.66 mg/100 ml to 85.55 mg/100 ml respectively. Study results further illustrate that as the length of storage days increased, there was generally an increase in vitamin C content on day 0 to 10, however a noticeable decrease was observed from day 10 to 20. On day 0, the highest vitamin C (85.55 mg/100 ml) content was observed from the treatment of SM, whereas the lowest content (83.45 mg/100 ml) was observed from treatment combination of SB and 4°C. Interestingly, on day 20, the highest vitamin C content (83.26 mg/100 ml), was observed in the treatment combination controlled (pasteurised) and 10°C and the lowest figure (81.66 mg/100 ml) was observed in the treatment combination of SM and 10°C as compared to other treatments.

The variation between the highest retained vitamin C content (83.26 mg/100 ml) obtained from the treatment combination of control and 10°C on day 20 and day 0 (84.01) was 0.75, meaning that there was a 1% decrease over a 20-day storage period. It has been established that vitamin C content in different juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light access (Kabasakalis et al., 2000; Zerdin et al., 2003; Akinola et al., 2017). Despite a significant reduction across all treatments, the treatment combination of pasteurisation and the utilisation of low temperature (10°C) on marula fruit juice seem to have been crucial in inactivating heat-sensitive enzymes that are known for accelerating vitamin C degradation by causing enzymatic browning. Thus, the current study supports the assertion that the treatment combination of control (pasteurised) stored at 10°C is effective in retaining the vitamin C content of marula juice over a 20-day storage period. These findings are in agreement with those of Klimczak et al. (2007) and Akinola et al. (2017), who reported a decrease in vitamin C content of preserved orange juice subjected to varying preservatives and storage duration.

Concerning human health and nutrition, vitamin C (ascorbic acid), which is a water-soluble vitamin, plays an important role in several vital processes such as energy production, cell growth and immune function (Padayatty et al., 2003; Pullar et al., 2017) in the human body. The difference between the highest retained vitamin C content (83.26 mg/100 ml), observed from marula fruit juice subjected to the treatment combination (control - only pasteurised) and storage at 10°C and the average recommended daily intake (82.5 mg/100 ml) is 0.76 mg/100 ml. The study findings could suggest that 0.9 ml of marula fruit juice, treated with pasteurization at 10°C and stored for 20 days, would meet 100% average recommended Dietary Intake (RDI) for of vitamin C required by humans. As a result, findings from this study could suggest that consumption of marula juice subjected to the treatment combination of control (only pasteurised) and 10°C stored for a period of 20 days, may assist in curbing conditions such as bleeding gums, constant fatigue, joint pain, dry skin, and poor wound healing, which are symptoms associated with low vitamin C intake in the human diet (Padayatty & Levine, 2016).

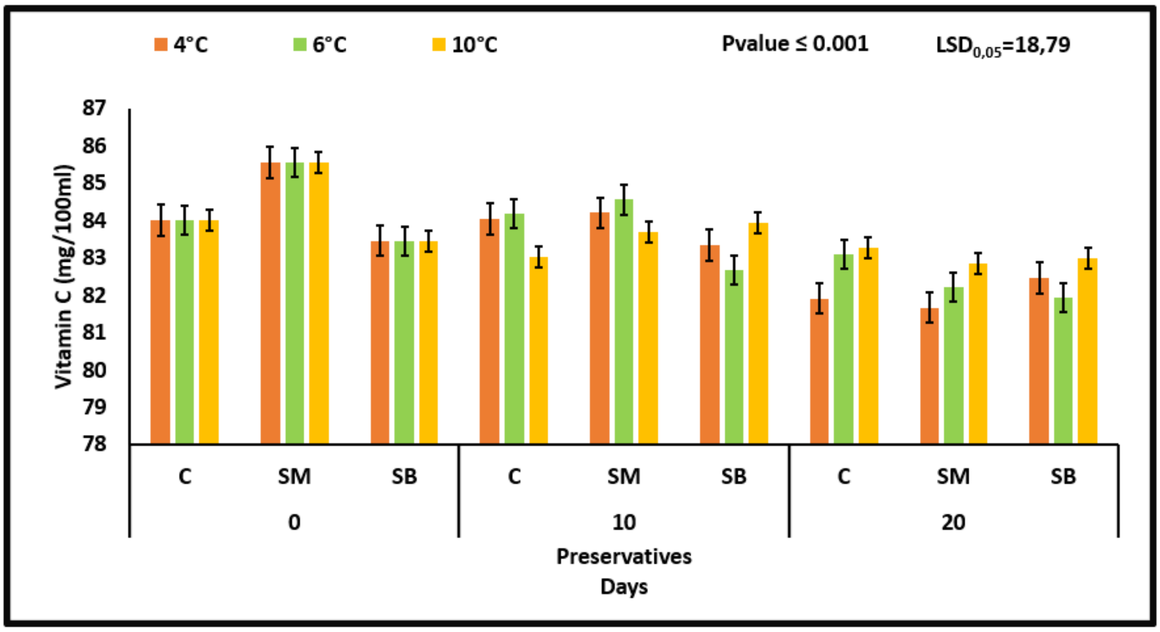

Figure 4.5: Vitamin C content of marula fruit juice. Note: C= Control. SM= Sodium Metabisulphite. SB= Sodium Benzoate.

4.2.3 Conclusion

The study investigated the effect of different preservatives and storage conditions of the biochemical constituents of pure marula juice. Findings from the study demonstrated that the use of sodium metabisulphite and sodium benzoate as preservatives influenced the biochemical properties and antioxidant activity retention in marula fruit juices during storage. Thus, the use of a treatment combination of preservatives (sodium benzoate and 4°C) exhibited suitable efficiency in retention of titratable acidity and total soluble sugars. Antioxidants such as total flavonoids and DPPH were highly retained under the treatment combination of sodium metabisulphite and storage at 10°C. Vitamins C was favourably retained under the treatment combination of control (only pasteurised) and storage at 10°C. Therefore, the biochemical retention capabilities provided in this study is evident that these treatments have a potential to reasonable retain biochemical constituents in marula juice in relation to the recommended daily intake. These findings provide a persuasive argument for converting marula juice from local use to commercial production, which has the potential to contribute to SDGs 1 and 2, no poverty and zero hunger, respectively. As the juice can be preserved for extended periods, this ensures the availability of important health-associated nutrients to consumers throughout the year. However, toxicological studies on marula juice remain a limitation that was not evaluated in this study. As a results, future studies should be conducted to identify allergies associated with consumption of marula fruit and its juice. This will increase the food safety component of product consumption, hence improving public food safety within communities and among marula fruit juice consumers.

4.3: The effect of preservatives and storage conditions on the minerals stability of marula (*Sclerocarya birrea*) fruit juice

4.3.1 Mineral elements and their potential role in human health and nutrition

4.3.1.1 Calcium

Figure 4.6 illustrates the impact of treatments combination (preservatives, storage temperature) on the calcium concentration in marula fruit juice. The results indicated a substantial difference ($P \leq 0.05$) across the treatments. The observed trend showed that the calcium content decreased as the storage duration and temperature increased. Moreover, study results indicated that the calcium concentration ranged between 44.3 mg/L to 101.2 mg/L. Furthermore, in comparison to the negative control juice (fresh, untreated, and unpreserved juice) with a calcium content of 101.2 mg/L, the use of treatment combination (positive control, which consists of pasteurised juice without preservatives stored at 10°C) exhibited a significantly reduced calcium retention at 44.3 mg/L. However, the use of treatment combination of sodium benzoate and storage at 4°C resulted in enhanced calcium retention (81.3 mg/L) in processed marula juice compared to other treatments. A decrease in marula juice may be due to physiochemical changes that occurs in the juice during prolonged storage. Wherby the calcium ions interact with pectin sustances that are present in the juice. Pectin binds with calcium to form calcium-pectate complexes (Huang et al., 2023; Zhang et al., 2019).

It has been established that storing fruit juice at lower temperatures combined with the use of preservatives helps retain the mineral content due to their ability to slow down enzyme reactions and bacterial growth, subsequently preserving the quality of the juice (Alam et al., 2012; Ahmed et al., 2021). The difference in calcium content between the baseline fresh, untreated juice (101.2 mg/L) and the one subjected to treatment combination of sodium benzoate and 4°C (81.3 mg/L), which is the highest among other storage treatment, is 19.7 mg/L. Meanwhile, the variation between the baseline fresh untreated juice (101.2 mg/L) and the one subjected to the treatment combination of positive control (pasteurised) and storage at 10°C (44.3 mg/L) is 56.9 mg/L. These findings illustrate that preservative (sodium benzoate) combined with lower temperature (4°C) positively contributed to an increased calcium retention in the marula juice since there was minimal enzymic activities and bacterial growth within this treatment. Similar findings were reported by Abiola et al. (2018), who found higher nutrient retention in orange juice subjected to chemical preservatives and lower temperature.

In terms of human health and nutrition, scholars such as Lekoba et al. (2024) asserted that the human body requires calcium for muscle movement and for nerve signal transmission. Calcium facilitates the circulation of blood through blood vessels, support heart function and the secretion of hormones that influence many bodily activities (Maluleke & Thobejane, 2025). The difference between the maximum calcium content (81.3 mg/L) in marula juice treated with sodium benzoate and storage at 4°C and the average recommended daily intake (1116 mg/L) is 1034.7 mg/L. This indicates that 13.6 ml of marula fruit juice, from the treatment combination (SB and 4°C) would be required to fullfill 100% ARDI of calcium required by humans. Despite the values derived from this study being below the recommended daily intake, the consumption of marula juice preserved with sodium benzoate at 4°C may help alleviate conditions such as muscle cramps, arrhythmias, and respiratory difficulties (Cormick & Belizán, 2019; Shlisky et al., 2022).

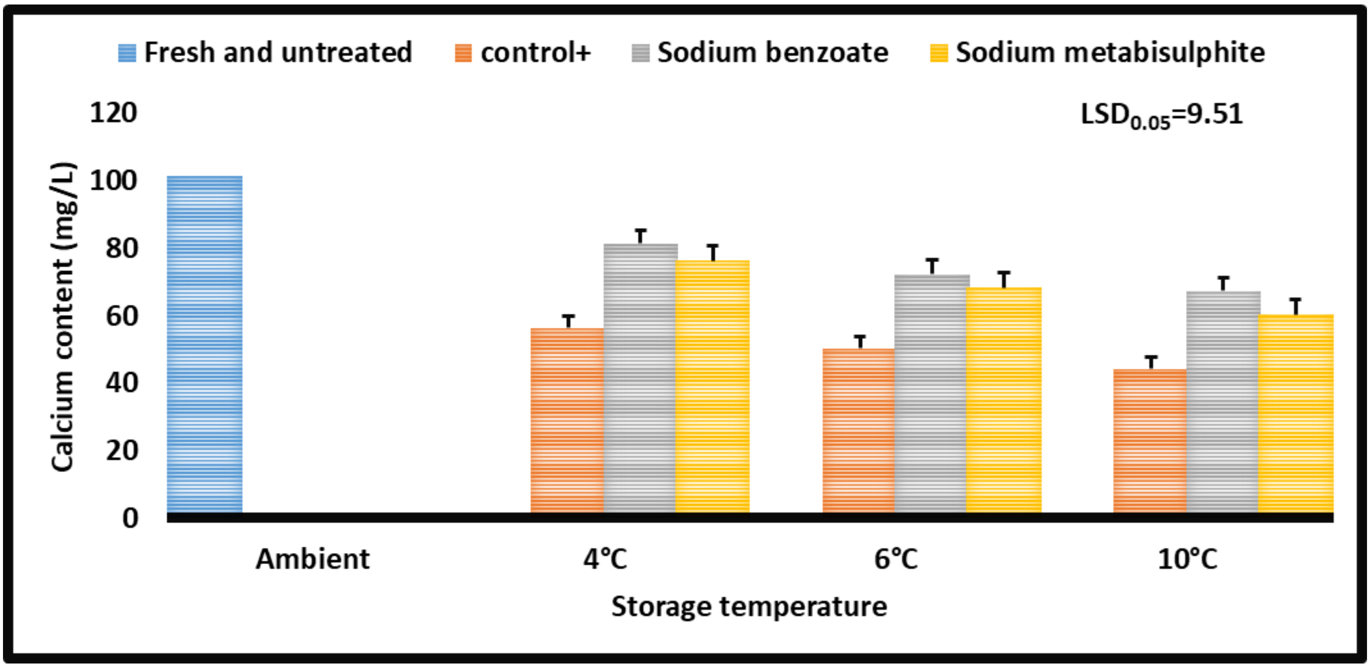

Figure 4.6: Calcium content of preserved marula juice subjected to varying preservatives and storage temperature. LSD = Least significant difference.

4.3.1.2 Magnesium

The effect of varying treatments (preservatives, storage temperature) on the magnesium content of marula fruit juice is presented in Figure 4.7. The results showed that there was significant ($P \leq 0.05$) difference amongst treatments. The observed trend illustrated that an increase in storage temperature causes a reduction in magnesium content of the marula fruit juice regardless of the preservatives applied. Moreover, results indicated that the magnesium content ranged from 180.8 mg/L to 201 mg/L. Regarding the preserved juice, the treatment combination of sodium metabisulphite and storage at 10°C resulted in lower magnesium retention, whereas the combination of sodium benzoate and storage at 4°C demonstrated superior magnesium retention when compared with other treatments. The decreased magnesium content may be due to the fact that magnesium interacts with organic acids and pectin subtances present in the juice. Naturaly marula juice contains organic acids which may react with magnesium to form less soluble salts (Fennema, 2017).

The use of preservatives and storage temperature is largely intended to suppress microbial development, thereby increasing shelf life, and maintaining quality, and preventing nutrient loss in processed food products such as fruit juice (Ayub et al., 2010; Wisal et al., 2013; Prisacaru et al., 2023). The variation between the baseline fresh untreated juice (201 mg/L) and the experiment juice treated with sodium benzoate stored at 4°C (180.8 mg/L), which had the highest retention for processed preserved juice, is 20.2 mg/L. Meanwhile, the difference between the baseline fresh untreated juice (201 mg/L) and the experimental juice treated with sodium metabisulphite and stored at 10°C (124.9 mg/L), showed the lowest magnesium retention, at 85.1 mg/L. Consequently, the findings of this study demonstrate that the combination of treatments (sodium benzoate and storage at 4°C) positively influenced magnesium retention in marula juice compared to other treatments, owing to its capacity to inhibit microbial growth and enzymatic activities, thereby enhancing nutrient retention, particularly magnesium, in marula juice.

Magnesium is important in human health and nutrition because it can act as a cofactor in a variety of enzymatic activities (Nielsen, 2015; Schwalfenberg & Genuis, 2017). It also helps with muscle and neuron function, blood sugar control, blood pressure regulation, and energy production (Alawi et al., 2018; Fiorentini et al., 2021). The variation between the highest magnesium content (180.8 mg/L), observed from the preserved marula juice and the average recommended daily intake (370 mg/L) is 189.2 mg/L. This could imply that from the treatment combination (sodium benzoate and storage at 4°C), 2.1 ml of marula fruit juice would be required to provide 100% ARDI of magnesium required by humans. Furthermore, the results of this study may indicate that consuming marula fruit juice stored under these treatment conditions may help to prevent conditions such as constant fatigue, muscle cramps, and weakness, all of which are symptoms of low magnesium intake in the human diet (Grzebisz, 2011; Lonsdale 2015).

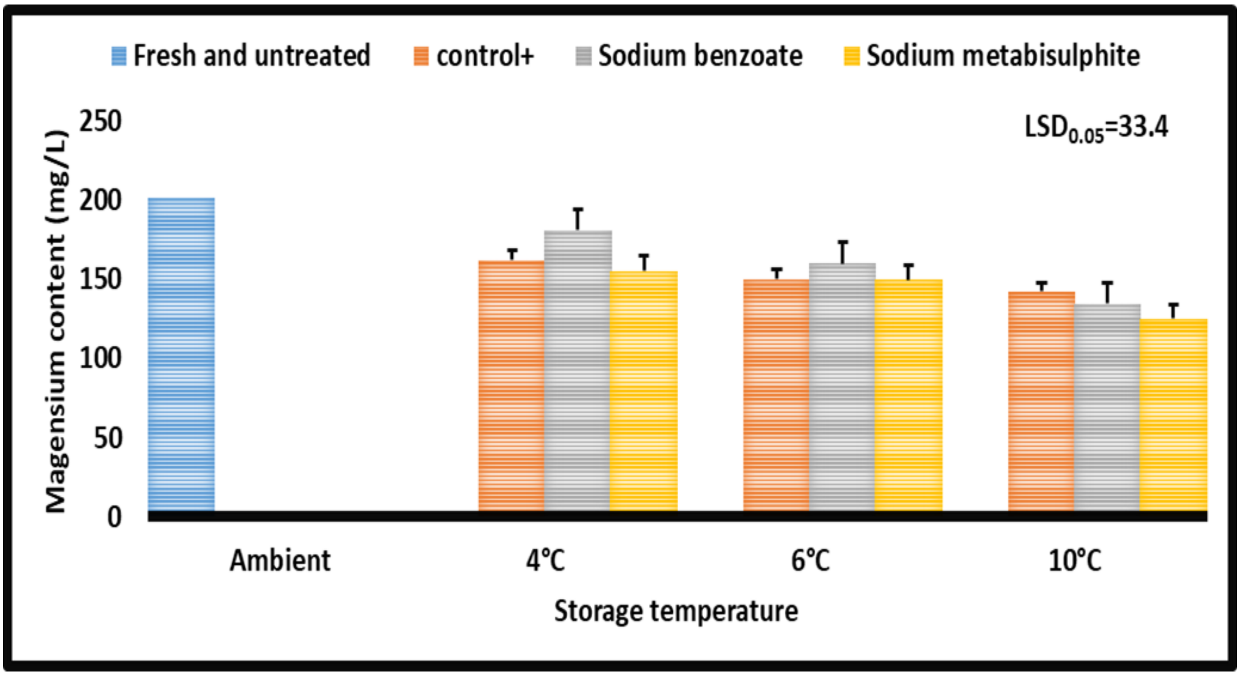

Figure 4.7: Magnesium content preserved marula juice subjected to varying preservatives and storage temperature. Note: LSD = Least significant difference.

4.3.1.3 Potassium

The effect of different treatments (preservatives and storage temperature) on the potassium content of marula fruit juice is shown in Figure 4.8. Results demonstrate that there was a significant ($P \leq 0.05$) difference amongst the treatments. The potassium content ranged from 925.2 mg/L to 1765.5 mg/L. The observed trend revealed that nutrient retention is higher under treatment combination of preservatives and lower temperature. However, an increase in temperature revealed a relative decrease in nutrient retention, regardless of the preservatives used. In terms of preserved marula fruit juice, study results illustrated that the treatment combination of sodium metabisulphite and storage at 10°C resulted in relatively low potassium retention of marula fruit juice, whereas the treatment combination of positive control and storage at 4°C exhibited superior potassium content retention when compared to other treatments. It was established that storage temperature and pasteurisation significantly influence the nutritional retention of fruit juice (Panigrahi et al., 2021). For example, lower storage temperature and the application of pasteurisation are essential in the preservation of minerals, since they effectively inhibit microbial proliferation and enzymatic activity (Kabir et al., 2019). Conversely, higher temperatures are known to

accelerate nutrient degradation by enhancing microbial activity, which may result in nutrient depletion and subsequent fermentation (El-Ishaq & Obirinakem, 2015). The difference is 192.4 mg/L between the baseline fresh and untreated juice (1765.5 mg/L) and that treated with a combination of positive control and storage at 4°C (1573.1 mg/L), which has the highest potassium content for preserved juice. Meanwhile, the difference between the baseline fresh and untreated juice (1765.5 mg/L) and treatment combination of sodium metabisulphite and storage at 10°C (925.2), which had the lowest retention for preserved juice, is 840.3 mg.
The combination of pasteurisation and reduced temperature directly contributed to potassium retention in fruit juice by blocking microbiological and enzymatic processes that damage the juice matrix, hence reducing the leaching and degradation of the compound during storage. While potassium as a mineral does not typically degrade, its stability in juice is associated with the overall preservation method and physical structure of the juice, which inhibits settling or the formation of unstable byproducts. Hence, the combined treatment of pasteurisation and 10°C proved to be more effective than other methods. This could suggest that marula fruit juice preserved under the treatment combination of positive control and storage at 4°C (1573.1 mg/L) positively contributed towards preservation of the potassium content of marula fruit juice due to its ability to discourage microbial growth and enzymic activities, subsequently improving the shelf life of the juice. Similar findings were reported by Bhattacherjee et al. (2011) and Mandha et al. (2023), who reported variation in fruit juice quality subjected to varying pasteurisation duration and storage temperature.

In human health and nutrition, potassium is an electrolyte that influences fluid balance, neuron and muscle function, and blood pressure management (Mohamed et al., 2025). It also aids in the movement of nutrients into cells and waste materials out of cells, so mitigating the effects of sodium on blood pressure (Weaver, 2013). Potassium is also essential for heart and bone health, and it may help prevent kidney stones (Sharma et al., 2013; McLean & Wang, 2021). The variation between highest potassium content (1523.1), obtained from the juice preserved from the treatment combination of positive control and storage at 4°C and the recommended daily intake (2760 mg/L) is 1236.9 mg/L. The study's findings imply that 1.8 ml of marula fruit juice, derived from the treatment combination of positive control and 4°C, is needed to fulfill 100% ARDI of potassium required by humans. Furthermore, the results of this study suggest that consuming marula fruit juice preserved from a treatment combination of positive control and 4°C may help to alleviate conditions such as muscle weakness, constant fatigue, digestive problems, and irregular heartbeat, all of which are symptoms associated with low potassium intake in the human diet (Grzebisz, 2011; Gupta & Gupta 2014).

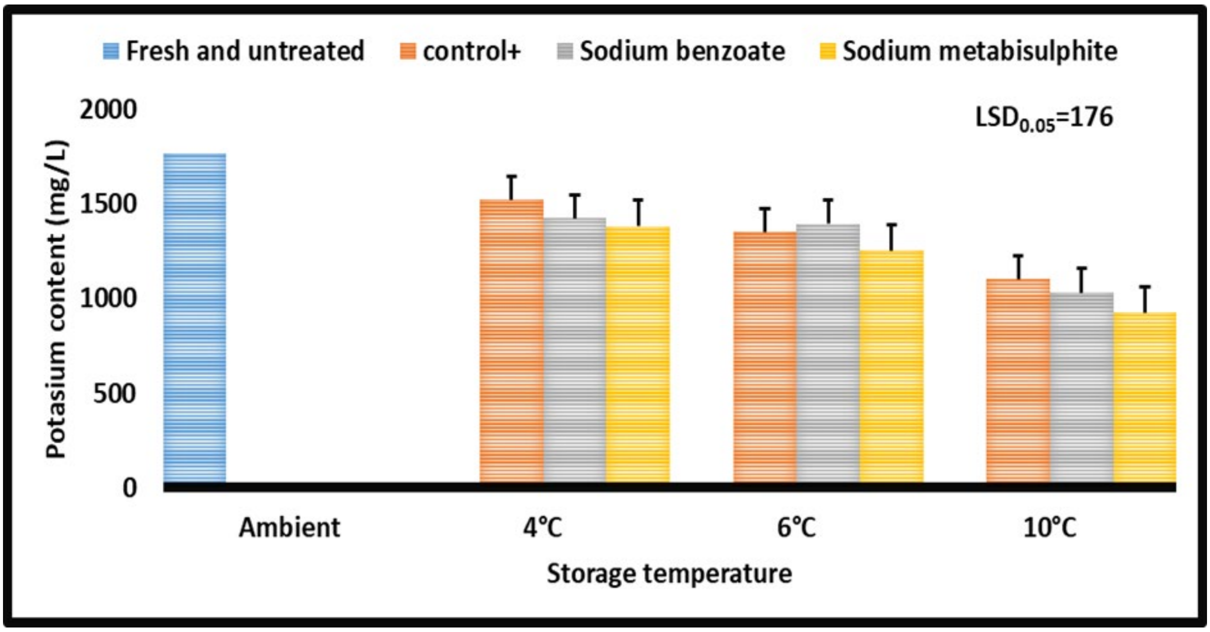

Figure 4.8: Potassium content marula juice subjected to varying preservatives and storage temperature. Note: LSD = Least significant difference.

4.3.1.4 Iron

The effect of different treatments (preservatives and storage temperature) on the iron content of marula fruit juice is shown in Figure 4.9. The results illustrated that there was a significant ($P \leq 0.05$) difference amongst the considered treatments. The observed trend illustrated that the use of sodium benzoate regardless of the storage temperature significantly increased iron retention when compared to the other treatments. Furthermore, study results revealed that there was a decrease in iron retention as the temperature increases and that there was an iron content in marula juice ranging from 4.3 mg/L to 37.8 mg/L. In terms of the preserved juice, study results illustrated that the treatment combination of positive control (pasteurised juice) and storage at 10°C led to the lowest iron content retention of 4.3 mg/L, whereas the use of sodium benzoate combined with storage at 4°C demonstrated superior iron retention at 33.4 mg/L. Storage temperatures and preservatives significantly influence the nutritional retention of food items by either enhancing or inhibiting microbial and enzymatic activities, particularly in fruit juice (Morales-de la Peña et al., 2016; Siow & Wong 2017).

In this investigation, the difference between the baseline fresh and untreated juice (37.8 mg/L) and the juice with the maximum retention (33.4 mg/L), derived from the treatment combination of sodium benzoate and storage at 4°C, is 4.4 mg/L. Meanwhile, the difference between the baseline fresh and untreated juice (37.8 mg/L) and the juice with the lowest iron retention (4.3 mg/L), which was obtained from the treatment combination of positive control (pasteurised juice) and storage at 10°C, is 33.5 mg/L. The heightened storage conditions, irrespective of the preservatives utilized, may have been the actual cause of the reduction in juice stored at elevated temperatures due to accelerated chemical degradation, enhanced oxidation, and the formation of insoluble iron complexes, resulting in the precipitation of iron from the liquid solution. As a result, the findings of this study show that preservatives and low storage temperature are the primary contributors to iron retention in processed marula fruit juice. These findings are consistent with those of Durrani et al. (2010), Tiencheu et al. (2021) and Mwaijibe and Vicent (2025), who found variations in quality parameters of fruit juice subjected to r different preservatives and storage settings.

In terms of human health and nutrition, iron is essential due to its capacity to facilitate oxygen flow within the body (Briguglio et al., 2020). It is crucial for red blood cell production and the synthesis of haemoglobin, a protein that transports oxygen from the lungs to tissues (Lal, 2020). In addition, iron facilitates immunological function, hormone synthesis and energy production (Mesías et al., 2013). The variation between the highest preserved iron (33.4 ml) and average recommended daily intake (14.4 mg) is 19. Study findings would imply that 0.43 ml of marula fruit juice from the treatment combination (sodium benzoate and stored at 4°C) would be needed to fulfil 100% ARDI of iron required by humans. Furthermore, study findings may imply that consumption of marula juice preserved with this treatment combination could assist in curbing conditions such as constant fatigue, constant weakness, skin and respiratory challenges, which are symptoms associated with low iron intake in the human diet (Lynch, 2011; Saini et al., 2016).

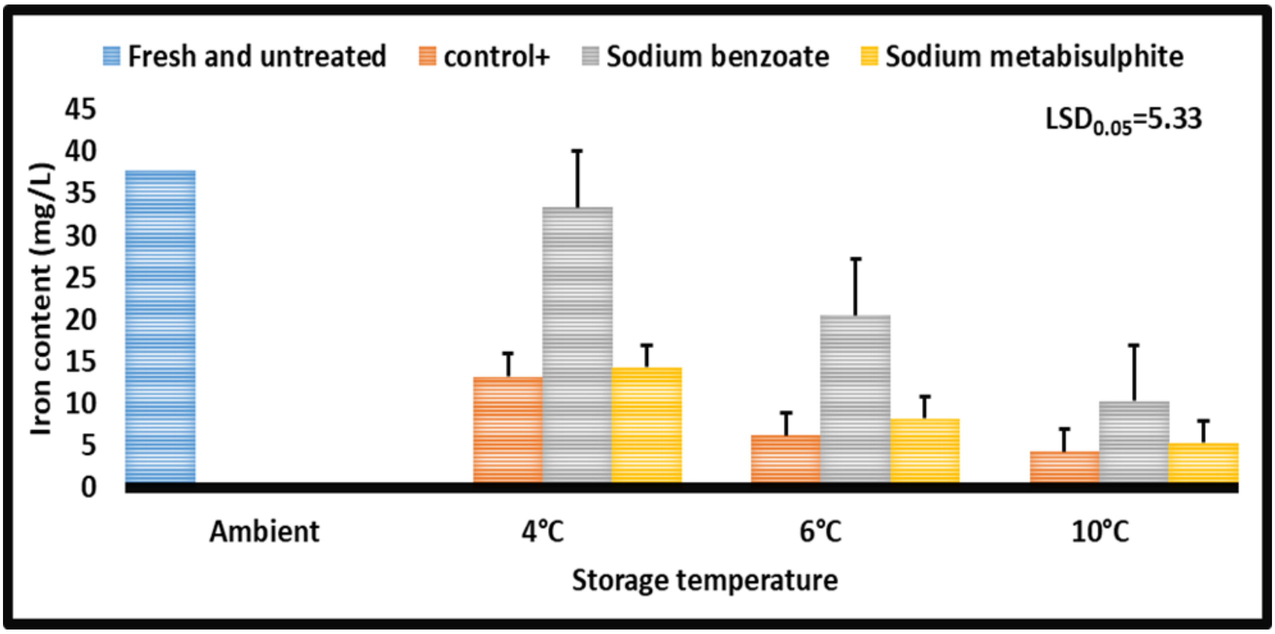

Figure 4.10: Iron content of marula juice subjected to varying preservatives and storage temperature. Note: LSD = Least significant difference.

4.3.1.5 Zinc

The effect of varying treatments (preservatives storage temperature) on the zinc content of marula fruit juice is shown in Figure 4.11. Results show that there was a significant ($P \leq 0.05$) difference amongst treatments. The observed trend indicated that zinc content retention is greater at lower storage temperatures, while it diminishes with rising storage temperatures, irrespective of the preservatives used. Study results showed that the zinc content of marula juice ranged from 3.2 mg/L to 15.6 mg/L. Concerning the preserved juice, results illustrated that the treatment combination of positive control (pasteurised juice) and storage at 10°C showed the lowest zinc retention (3.2 mg/L), while the treatment combination of sodium benzoate and 4°C demonstrated superior zinc content retention when compared to the other treatments. The application of preservatives alongside storage temperature is considered one of the most successful methods for quality preservation in the food sector, particularly for value-added products like fruit juice (Alam et al., 2012). Preservatives and low storage temperatures are known for their critical function in minimizing microbial proliferation, enzymatic activities, and bacterial growth (Shakoor et al., 2024). For this study, the difference between the baseline fresh untreated juice (15.6 mg/L) and the preserved juice that retained the most zinc concentration (13.8 mg/L), which involved treatment with sodium benzoate and storage at 4°C, is 1.8 mg/L. Meanwhile, the difference between the baseline fresh untreated juice (15.6 mg/L) and the juice with the lowest zinc retention (3.2 mg/L), which was the positive control (pasteurisation) followed by storage at 10°C, is 12.4 mg/L, which varied by a significant margin. Under warm storage, irrespective of the preservatives used, rapid deterioration and structural alterations transpire in the juice particles and suspended particulates. This indicates that the zinc bound to insoluble fiber or other cell wall constituents is detached or leached, resulting in its loss from the liquid phase, hence causing a minor reduction in the juice content. As a result, the findings of this study demonstrated that the use of preservatives (sodium benzoate) and a low storage temperature (4°C) had a beneficial impact on the preservation of processed marula fruit juice, particularly regarding the retention of zinc. These findings are consistent with those of Tiencheu et al. (2021), who showed variations in juice quality metrics under different storage temperatures and preservatives.

In human health and nutrition, zinc is considered an essential mineral that is responsible for synthesising cells, repairing damaged cells, and boosting both testosterone levels and the immune system (Allouche-Fitoussi & Breitbart, 2020; Hu et al., 2022). The difference between the zinc content of preserved marula fruit juice (13.8 mg/L), which was obtained from the treatment combination involving sodium benzoate and storage at 4°C) and the average recommended daily intake (9.5 mg/L), is 4.3 mg/L. Therefore, study findings could imply that 0.6 ml of marula fruit juice from treatment combination (sodium benzoate at storage at 4°C) would be required to provide 100% ARDI of zinc required by humans. Thus, consuming such processed marula fruit juice may help to alleviate conditions such as a poor digestive system, skin-related problems, poor wound healing, and a weakened immune system, all of which are symptoms of low zinc intake in the human diet (Gibson, 2012; Chasapis, 2012; Prasad, 2014; Allouche-Fitoussi & Breitbart, 2020; Kanwar & Sharma, 2022). The above findings concur with those reported by Kaur and Aggarwal (2014) and Prestes et al. (2023), who reported variation in mineral retention of fruits and vegetable juice preserved under varying preservatives and storage temperature.

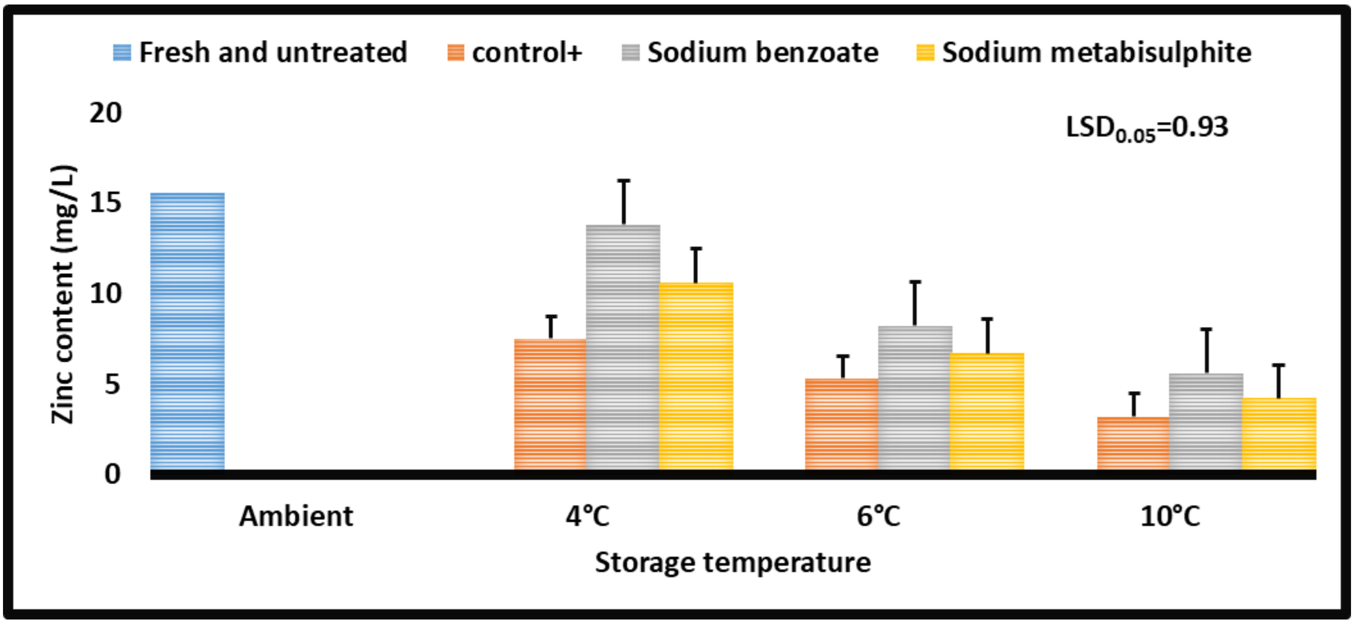

Figure 4.11: Zinc content of marula juice subjected to varying preservatives and storage temperature. Note: LSD = Least significant difference.

4.3.2 Conclusion

This study investigated the combined impact of varying preservation methods and storage conditions on the mineral retention of processed marula fruit juice. Study findings revealed that the use of treatment combination of sodium benzoate and lower storage temperature (4°C) positively influenced the retention of various minerals including calcium, magnesium, potassium, iron, and zinc of processed marula fruit juice. The attribution of these combined treatments (sodium benzoate and 4°C) appear to play a pivotal role not limited to mineral retention, but also ensure that the preserved juice is aligned the recommended dietary needs for human consumption, which is linked to SDG 2 (zero hunger), an initiative of the United Nations that advocates that everyone should have access to quality food that is nutrient-dense. Therefore, with the information provided in this study, it is evident that processed marula fruit juice that is subjected to suitable treatment and storage conditions could be relied on as a stable value-added product that would contribute to the eradication of malnutrition and nutrients deficiencies in regions where the fruits are abundant, subsequently addressing food security within local communities. However, due to factors such as seasonal availability and lack of facilities such as processing and storage equipments, the possibilities of mass production are limited. Nonetheless, this untapped business opportunity could be a potential solution for malnutrition and job creation in the lowveld regions of Southern Africa and other part of the continent.

4.4 Influence of geographical region, storage conditions and preservatives on the microbiological quality of marula (*Sclerocarya birrea*) fruit juice

4.4.1 Bacterial population counts

The effect of geographical region, preservatives and storage temperatures on the bacterial counts of marula fruit juice throughout the storage period is summarised in Table 4.6. The results of the study showed no (nil) viable counts of bacterial contamination across all the treatments of juice of fruit harvested from Bushbuckridge. The bacterial contamination ranged from 9.4 x 10^2 to 3.3 x 10^5. The study results showed that juice of fruit harvested from Giyani untreated (control) had viable bacterial contamination of 1.5 x 10^5 cfu/ml and 1.7 x 10^5 cfu/ml when incubated at 27 and 35°C for 20 days at 10°C, respectively. The highest bacterial growth (3.3 x 10^5 cfu/ml) was also observed on juice of fruit harvested from Tzaneen treated with sodium benzoate when incubated at 27°C while a colony count of 3.0 x 10^5 cfu/ml was noted from juice incubated at 37°C and stored at 10°C for 10 days. The lowest viable bacterial counts (9.4 x 10^2 cfu/ml) were observed on juice of fruit harvested from Tzaneen untreated (control) stored at 4°C for 20 days. The fact that the bacterial growth was restricted to two juice samples originating in Tzaneen (TC and TSB) may suggest handling error incurred during the preparation of the juice samples.

Colonies growing on agar plates are indicated in Figure 4.12 and identified using 16S ribosomal RNA (rRNA) sequencing. Of the plated preserved juice samples (n=63), only 4 showed bacterial growth, which is equivalent to a 6.3% contamination rate. As indicated above and in Table 4.6 and Figure 4.12, these Bacillus spp. were only isolated from GC, TSB and TC juice samples, whilst BC, BSB, BSM juice samples showed no bacterial contamination. Bacteria of Bacillus spp. are part of the plant pathogens that are mostly found on raw plant material.

Table 4.6: Bacterial count of marula fruit juice persevered using different preservatives and stored at different temperature.

Treatment	Day	Storage temperature	Bacterial count (CFU/ml) Incubation T° 27°C	Incubation T° 35°C	Bacterial spp.
BC	0				
BSB	0				
BSM	0				
BC	10	4	-	-	
BSB	10	4	-	-	
BSM	10	4	-	-	
BC	10	6	-	-	
BSB	10	6	-	-	
BSM	10	6	-	-	
BC	10	10	-	-	
BSB	10	10	-	-	
BSM	10	10	-	-	
BC	20	4	-	-	
BSB	20	4	-	-	
BSM	20	4	-	-	
BC	20	6	-	-	
BSB	20	6	-	-	
BSM	20	6	-	-	
BC	20	10	-	-	
BSB	20	10	-	-	
BSM	20	10	-	-	
GC	0	-	-	-	
GSB	0	-	-	-	
GSM	0	-	-	-	
GC	10	4	-	-	
GSB	10	4	-	-	
GSM	10	4	-	-	
GC	10	6	-	-	
GSB	10	6	-	-	
GSM	10	6	-	-	
GC	10	10	-	-	
GSB	10	10	-	-	
GSM	10	10	-	-	
GC	20	4	-	-	
GSB	20	4	-	-	
GSM	20	4	-	-	
GC	20	6	-	-	
GSB	20	6	-	-	
GSM	20	6	-	-	
GC	20	10	$1.5x10^5$	$1.7x10^5$	*Bacillus spp..*
GSB	20	10	-	-	
GSM	20	10	-	-	
TC	0	-	-	-	

TSB	0	-	-	-	
TSM	0	-	-	-	
TC	10	4	-	-	
TSB	10	4	-	-	
TSM	10	4	-	-	
TC	10	6	-	-	
TSB	10	6	-	-	
TSM	10	6	-	-	
TC	10	10	-	-	
TSB	10	10	$3.3x10^5$	$3.0x10^5$	*Bacillus spp.*
TSM	10	10	-	-	
TC	20	4	$9.4x10^2$	$1.6x10^3$	*Bacillus spp.*
TSB	20	4	$1.0x10^5$	$1.2x10^5$	*Bacillus spp.*
TSM	20	4	-	-	
TC	20	6	-	-	
TSB	20	6	-	-	
TSM	20	6	-	-	
TC	20	10	-	-	
TSB	20	10	-	-	
TSM	20	10	-	-	

Note: BC: bushbuckridge control, BSM: bushbuckridge sodium metabisulphite, BSB: bushbuckridge sodium benzoate, GC: giyani control, GSM: giyani sodium metabisulphite, GSB: giyani sodium benzoate, TC: tzaneen control, TSM: tzaneen sodium metabisulphite, TSB: tzaneen sodium benzoate, IT°: incubation temperature.

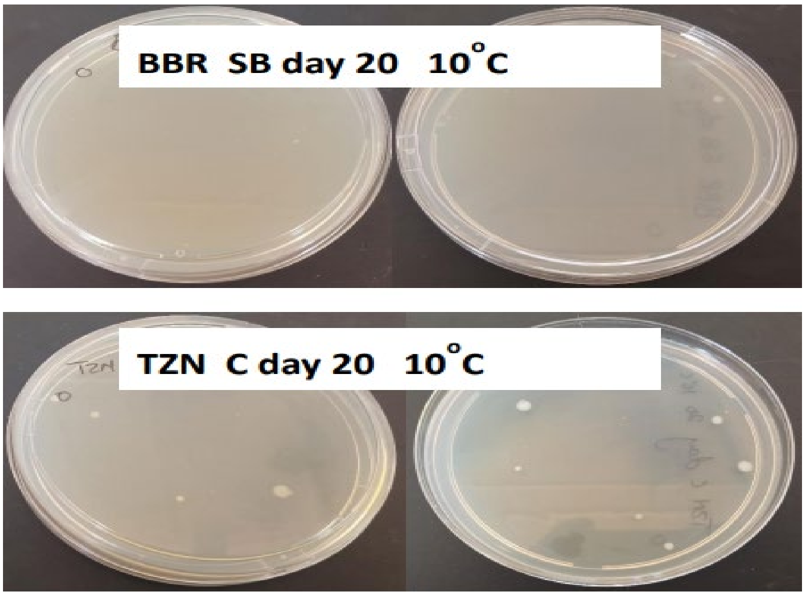

Figure 4.12: Microbial colony growth on agar plates by day 20. Note: BBR SB: bushbuckridge sodium benzoate, TZN C: tzaneen control.

Control juice samples showed high levels of bacterial counts when compared with juice treated with SB and SM. From the above results, the microbial load data obtained indicates that the SM preservative was effective in prolonging the shelf-life of the marula fruit juice irrespective of storage temperature and storage duration. The results are similar to those described by Chukwumalume (2012) who studied the microbiological assessment of preservative methods for African Star Apple juice. The juice sample was pasteurised and preserved with 0.1% SB at ambient and refrigeration temperature for six weeks. Chukwumalume (2012) reported that the combination of pasteurisation, use of sodium benzoate, and storage at refrigeration temperature for six weeks gave the best storage stability with a minimum microbial load of African star apple. Similarly, the use of chemical preservatives such as sodium metabisulphite and pasteurisation showed lower microbial load in soursop juice stored at ambient temperature for two weeks (Nwachukwu & Ezeigbo 2013).

The GSO 1016/2015 microbiological regulation criteria define the acceptability of a product, a batch of foodstuff or process, based on the absence, presence or number of microorganisms and on the quantity of their toxins/ metabolites per unit(s) of mass (GSO 2015). The criteria are divided into two types namely: (1) food safety criteria – which deal with the presence of microorganisms in the food, that represent a risk to human health, and (2) process hygiene criteria - dealing with microorganisms that can be used as indicators of the level of hygiene present in the food processing. The acceptable microbial level for pasteurised fruit juice and drink (including concentrates) is as follows: (1) acceptable if the total plate count was between 1 x 10^2 and 1 x 10^4 cfu/ml and (2) if the yeast and mould plate count is between 1 x 10^2 and 1 x 10^3 cfu/ml. If the values are exceeding these criteria, then the product is deemed unacceptable.

The study results show that of the four juice samples that showed bacterial contamination, three of these four (75%) juice samples had a bacterial count that exceeded the acceptable microbial limits. Unacceptable bacterial counts were observed on GC, TC and TSB juice samples. The microbial count in juice sample GC was 1.5 x 10^5 and the sample was incubated at 27°C, while the second sample had a count of 1.7 x 10^5 and was incubated at 35°C for 20 days at 10°C, a sample TSB showed a count of 3.3 x 10^5 and was incubated at 27°C) (3.0 x10^5 incubated at 35°C) stored for 10 days at 10°C, and TSB (1.0 x10^5 incubated at 27°C) (1.2 x10^5 incubated at 35°C) stored for 20 days at 4°C were comparatively higher than the acceptable limits (1 x10^4). According to GSO1016/2015 guidelines for the microbiological quality of pasteurised fruit juice and drink, it is quite evident from the results, as is indicated in Table 6.1, that the bacterial count for 95% of the preserved samples were within microbial acceptable limits for human consumption.

4.4.2 Yeast population counts

The yeast population of marula fruit juice throughout the storage period is shown in Figures 4.13 and 4.14. The yeast count of the preserved samples ranged from nil (0) to 7.7 x10^3 cfu/ml. The results showed that the highest yeast count of 7.7 x 10^3 cfu/ml when incubated at 27° was observed on BSB juice samples on day 20 at 6°C (Figure 6.4). The BSM juice samples showed yeast growth (3.3 x10^3) on samples stored for 10 days at 10°C. On day 20, an increased yeasts count (7.7 x10^3) on BSB samples stored at 6°C was also observed. The lowest count of 3.2 x 10^2 cfu/ml were observed on GSM juice samples stored at 10°C for 20 days (Figure 6.5). The GC samples showed an increased yeast growth (4.9 x10^2) after storage at 10°C for 20 days. This might be due to the high storage temperature and prolonged storage days which perpetuated the emergence of yeasts.

Similar studies were conducted by Osakuade et al. (2023) on the effect of storage condition and preservatives on the microbial, physicochemical and sensory quality of cucumber juice and carrot juice. Their results revealed that the microbial (yeast) count for cucumber and carrot juice stored at refrigerated temperature were less than that of juice at ambient temperature stored for 6 days. In adddition, Akinola et al. (2018) studied the influence of chemical preservatives (0.03% sodium benzoate, sodium metabisulphite, potassium sorbate and their combination) on the quality attributes of orange juice. Their results revealed that the total bacteria and yeast count increased as the weeks of the storage increased.

Interestingly, in the current study, there were no (nil) yeasts growth on all TC, TSB and TSM juice samples. Yeasts were predominant in BSB and BSM, and GC and GSM juice samples. The use of different preservatives in marula fruit juice prevented yeast proliferation throughout. In the TZN juice samples, there was no growth on all in the preserved and control samples over a storage period of 20 days, irrespective of storage temperatures.

In this study, the yeasts present in juice samples GC, GSM and BSM were identified as *Candida parapsilosis*, however on sample BSM the yeast species was not identified. Among the yeast cultures, one isolate was not determined (ND) in BSB juice samples stored at 6°C on day 20 (Figure 4.15). This non-identification may be due to the fact that ITS regions can exhibit substantial variability both within and among yeast species. Some yeast species have highly variable ITS regions, which can make it difficult to match the sequences obtained with a reference database (Lucking et al., 2021).

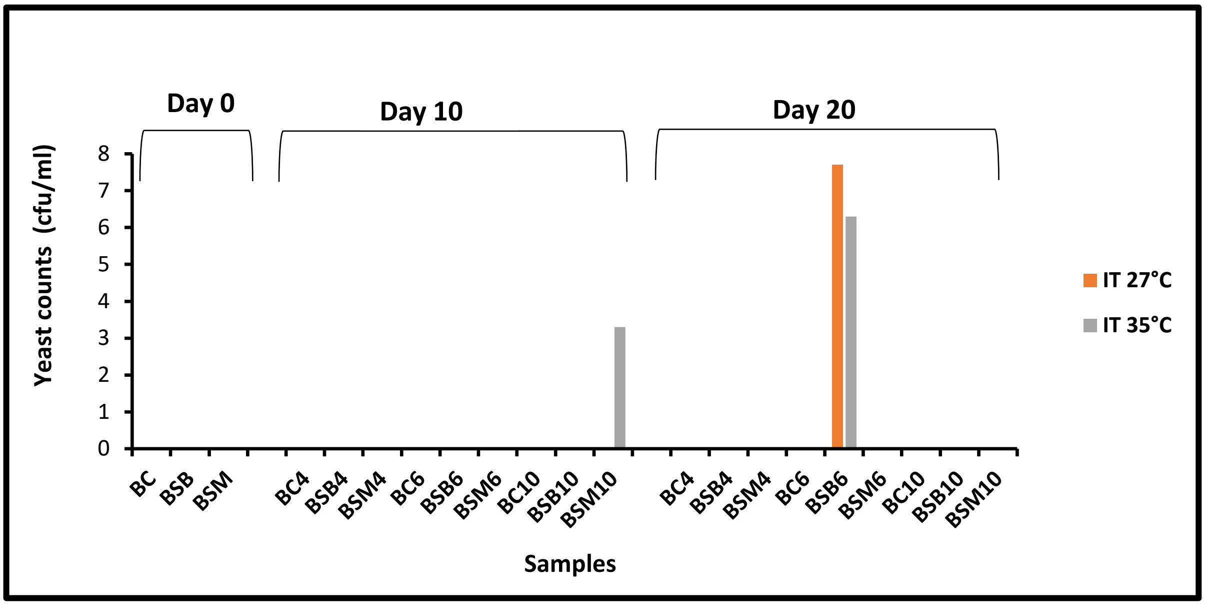

Figure 4.13: Yeast population counts (10^3) on BBR samples during storage. Note: BC: bushbuckridge control, BSM: bushbuckridge sodium metabisulphite, BSB: bushbuckridge sodium benzoate, GC: giyani control, GSM: giyani sodium metabisulphite, GSB: giyani sodium benzoate, TC: tzaneen control, TSM: tzaneen sodium metabisulphite, TSB: tzaneen sodium benzoate, IT°: incubation temperature.

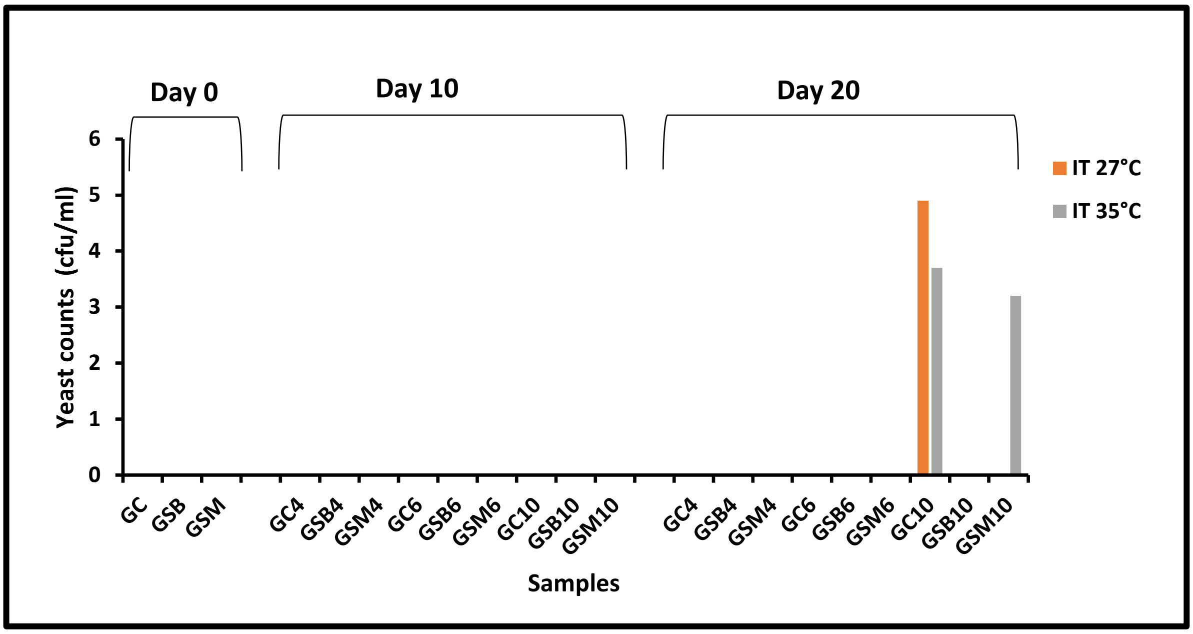

Figure 4.15: Yeast population counts (10^2) on GYN samples during storage. Note: BC: bushbuckridge control, BSM: bushbuckridge sodium metabisulphite, BSB: bushbuckridge sodium benzoate, GC: giyani control, GSM: giyani sodium metabisulphite, GSB: giyani sodium benzoate, TC: tzaneen control, TSM: tzaneen sodium metabisulphite, TSB: tzaneen sodium benzoate, IT°: incubation temperature.

According to GSO 1016/2015, the acceptable value of mould and yeast in beverages and juices is between $1x10^2$ to $1x10^3$ cfu/ml and exceeding this level would indicate potential health hazard and imminent spoilage. In the current study, the results revealed that out of 63 juice samples, only 4 has shown yeast proliferation, meaning that there was a 6.3% contamination rate. All 4 contaminated samples had a yeast count that exceeded the acceptable yeast count limit. The present study indicates that viable yeasts from the few contaminated juices ranged from 3.2×10^2 cfu/ml to 7.7×10^3 cfu/ml and these juices are considered not safe for human consumption. These few yeasts growth in marula juice indicate a lack of adherence to good manufacturing practices (GMPs) during preparation and handling of these samples.

Non-hygienic practices during preparation of the juice could add substantial numbers of microorganisms, especially yeasts and bacteria (Alam et al., 2019). Basar and Rahman (2007) and Mahale (2008) stated that the presence of yeast in juice samples could reflect the quality of the raw materials, processing equipment, environment, packaging materials and the training of the personnel in the production process. Although, the study findings revealed that the use of preservatives on marula fruit juice did not have a superior effect on preservation of marula juice when compared to control juice during the period of storage. It is recommended to subject marula fruit juice to treatment combination of preservatives and storage conditions as these factors play an essential role in prolonging the shelf-life of the juice. With regards to GSO1016/2015 guidelines for the microbiological quality of pasteurised fruit juice and drinks, it is evident from the results, as is indicated in Figures 4.13 and 4.15 that the yeast count for 94% of the preserved and control samples were within acceptable limits for human consumption.

4.4.3 Conclusion

Food preservation is important in preventing food spoilage while maintaining the quality and nutritional attributes of the food. The microbiological quality of fruit juices is one of the most important aspects to be taken care of by the manufacturers. Likewise, the consistency of temperature while preserving and subsequent storing of the juice is vital. The findings of this study indicate that an acceptable marula juice can be stored effectively for 20 days when preserved with 0.05% sodium benzoate, sodium metabisulphite and stored at 4°C, 6°C and 10°C. In 93% of juice samples, the microbial growth counts were within the acceptable level and safe for human consumption. The acceptable microbiological criteria of total plate count is between 1 x 102 and 1 x 104 cfu/ml while the trotal plate count for yeasts and moulds is between 1 x 102 and 1 x 103 cfu/ml. The bacterial and yeast count present in marula juice sample were identified as Bacillus spp. and Candida parapsilosis. Therefore, utilisation of the preserved marula fruit juice should be encouraged as sustainable food and nutritional security within communities. Above all, preservation of marula fruit juice is important because of the seasonal availability which will make it abundantly available during its season and off season. However, this study focused on the use of two preservatives and was limited to a 20-day storage period. Therefore, future studies should explore different preservatives, ratios and longer storage duration.

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CHAPTER 5: General conclusions and future recommendations

5.1 General conclusion

In South Africa there are numerous fruit trees that have been recognised, studied, and protected under the National Forest Act of 1998 (Act No. 84 of 1998). Marula (*Sclerocarya birrea*) is of great revelance in this study, mainly because it is one of the underutilised African indigenous fruit trees. However, the tree is considered valuable due to its socio-economic and cultural significance among the African population. Over the years, several products from various sections of the tree have been sold to local communtities where the trees are prevalant. These value-added products include jelly, jam, nuts, and marula fruit beer are available mainly for local sale while the marula alcohic beverage is available in most liquor store interprises in southern Africa. Of all the marula products, the production of marula juice remains underexplored, particularly in the lowveld regions of South Africa, where the marula tree is predominantly found. This may be attributed to a deficiency in adequate baseline information and resources necessary for conducting such investigations. The processing and preservation of marula fruit juice is of keen interest in this study due to its abundant nutritional content such as vitamin C and antioxidant activity. Thus, marula juice has the potential to become the first 100% juice made from African indigenous fruits. This could help achieve some of the sustainable development goals, especially within communities who may not be able to afford nutrient-dense foods. While research has been conducted regarding the composition of marula fruit, little is known about the use of preservatives and storage conditions aimed at delaying fermentation in pure marula fruit juice, nor the nutritional propeties and stability of the juice. The current study focused on generating knowledge on the characterisation of the fruit, identifying the most suitable preservatives and storage conditions, and establishing comprehesive information on the microbial stability and nutritional properties of the preserved marula juice.

The first objective included the evalution of the physicochemical and antioxidant activity of marula fruit harvested from three regions with varying climates. This was motivated by the fact that marula fruit is considered a significant source of various biochemical constituents such as vitamin C, total flavonoids, total soluble sugars, calcium, magnesium, iron and zinc, all of which are required by humans. However, the nutrient composition of the fruit is affected by various environmental factors such as volume rainfall, and variation in temperature and soil types. Overall, the study findings showed that geographic locations with distinct environmental factors (rainfall, soil fertility and temperature) influenced the physicochemical properties and biochemical compounds (including total phenols and flavonoids, and antioxidant activity (DPPH) of marula fruit. These results are heartening as they make it feasible for the attaining of the SDG 2 initiated by the United Nations, which mandates that everyone should have access to nutritious food and not go hungry. The findings of this study will assist plant breeders in selecting indigenous marula trees with minimal climatic impact that serve as sources of nutrients and antioxidants, while also providing fruit and other value-added products.

The second objective focused on evaluating the effect of preservatives and storage conditions on the physicochemical and biochemical composition of marula fruit juice while comparing them with recommended daily intake (RDI). This investigation was motivated by role of preservatives in maintaining overall quality and nutritional attributes of juice while preventing spoilage. Findings from the study demonstrated that the use of sodium metabisulphite and sodium benzoate as preservatives influences the biochemical properties and antioxidant activity retention in marula fruit juices during storage. The biochemical retention capabilities provided in this study is evident that these treatments have a potential to reasonable retain biochemical constituents in marula juice in relation to the recommended daily intake. These findings provide a persuasive argument for converting marula juice from local use to commercial production, which has the potential to contribute to Sustainable Development Goals 2 (No Poverty and Zero Hunger), as the juice can be preserved for extended periods, thereby ensuring its availability to consumers throughout the year.

The third objective of this study was to assess the effect of different preservatives and storage temperatures on the mineral composition of marula juice. Marula fruit is claimed to be rich in minerals needed for sustaining human health and nutrition. Various macro- and microminerals including calcium, magnesium, potassium, iron and zinc are present in the marula fruit and its value-added products such as juice. The study findings showed that the use of treatment combinations of sodium benzoate and lower storage temperature (4°C) positively influence the retention of these minerals in the processed marula fruit juice, and these preservatives align the nutritional value of the juices with those of the recommended dietary intake for human consumption.

Lastly, objective 4 of the study explored the antimicrobial efficacy of chemical preservatives (sodium metabisulphite and sodium benzoate) and storage conditions (4°C, 6°C and 10°C) in marula fruit juice, with the goal of extending the shelf life of marula fruit juice. The study findings revealed that marula fruit juice can be stored effectively for at 4°C, 6°C and 10°C for 20 days when preserved with 0.05% sodium benzoate, sodium metabisulphite. The microbial growth counts were within the acceptable level and safe for human consumption. Therefore, utilisation of the preservatives combined with storage conditions quality preservation of marula fruit juice should be encouraged as a solution for sustainable food and nutritional security within communities.

5.2 Recommendation future and perspectives

The current study findings successfully illustrated how differing preservatives (sodium benzoate, sodium metabisulphite) and storage conditions (4, 6 and 10°C) affect the biochemical and nutritional quality of marula fruit juice during storage. As a result, future studies should focus on the following.

- Further studies focusing on the identification and quantification of bioactive compounds associated with the functional properties of marula juice are required to support its utilisation and potential commercialisation.
- Comprehensive analysis of volatile compounds responsible for aroma and flavour characteristics, as well as trained and consumer sensory evaluation, to determine preservative treatments that optimise both quality and acceptability of the product to enable commercialisation.
- Investigation of marula juice blends incorporated with commercially available fruit juices at varying ratios to improve sensory and consumer acceptance.

5.3 Contribution to food sciences research and the scientific body of knowledge

Research into the effects of preservatives and storage conditions on the quality of value-added products, such as marula juice made from indigenous fruit, advances the field of food science by providing scientific evidence in relation to the effect of preservatives and storage conditions on quality, nutritional stability and microbial saefty of value-added products developed from an indigenous African fruit "marula". Additionally, this research will contribute to the transformation of the food industry, as this product will be scaled up from local consumption to commercial production. Lastly, the study will also offer opportunities for community-based enterprises to improve food availability, nutritional quality, and economic resilience.

5.4 Limitation of the study

This study primarily focused on the physicochemical analysis of the fruit, the use of preservatives and storage for quality preservation, mineral retention, and microbial activity of fruit juice processed from fruits harvested in South Africa's Lowveld regions (Bushbuckridge, Giyani, and Tzaneen), where the marula fruit tree is prevalent and dominant. However, marula fruit juice processed from other regions, such as Kwazulu Natal and Northwest province, have not been sampled for this study, due to some logistical costs associated with this investigation. Therefore, the limitation of this study regarding the collection of data from fruits collected in different geographical regions must be addressed to fully comprehend the climatic impact on the overall quality of the juice. Furthermore, the post-process storage time was 20 days, which may not have afforded some microbial development to occur. Lastly, even though the study's conclusions indicated that the preservative and storage conditions could preserve the majority of minerals and other quality parameters, trained and untrained sensory evaluation could significantly advance this research and to support product commercialisation.

APPENDINCES

Appendix I: Mega Project Ambrella Ethical Clearance Certificate

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 26/04/2023

Dear Dr Maluleke

NHREC Registration # : REC-170616-051
REC Reference # : 2022/CAES_HREC/104
Name : Dr MK Maluleke
Staff #: 90190270

Decision: Ethics Approval from 25/04/2023 to 30/04/2026

Researcher(s): Dr MK Maluleke
malulm@unisa.ac.za; 011-471-3838

Prof M Machate
machef@unisa.ac.za; 011-471-2075

Mrs CK Marokane-Radebe
cynthiakwena@yahoo.com; 083-365-1714

Dr A Shabalala
Ayanda.Shabalala@ump.ac.za; 013-002-0178

Working title of research:

Consumption, commercialisation and sustainability of African indigenous fruits in Bushbuckridge, Giyani and Tzaneen, South Africa

Qualification: Non-degree purposes

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for three years, **subject to further clarification and submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 30 April 2024

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150

Appendix II: Current Project Ethical Clearance Certificate

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 10/07/2023

NHREC Registration # : REC-170616-051
REC Reference # : 2023/CAES_HREC/068
Name : Ms ILG Lekhuleni
Student #: 67203507

Dear Ms Lekhuleni

Decision: Ethics Approval from 06/07/2023 to 30/06/2028

Researcher(s): Ms ILG Lekhuleni
lekhuleniil@gmail.com; 083-514-9167

Supervisor (s): Dr MK Maluleke
malulm@unisa.ac.za; 011-471-3838

Dr F Malongane
malonf@unisa.ac.za; 072-599-7272

Working title of research:

Investigating the suitable preservatives to prevent marula (*Sclerocarya birrea*) fruit juice from fermentation and improving shelf-life

Qualification: PhD Life Science

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for five years, **subject to submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

Due date for progress report: 30 June 2024

The progress report is available on the college ethics webpage: https://www.unisa.ac.za/sites/corporate/default/Colleges/Agriculture-&-Environmental-Sciences/Research/Research-Ethics

Appendix III: Language editing latter

John Dewar Tel: +27833210844

PhD, DAHM Email: johndewar65@gmail.com

Dear Drs Maluleke, Malongane, Shabalala and Mphahlele,

This letter confirms that I completed a language and content edit of a thesis entitled: **Investigating the effect of preservatives and storage conditions on quality of Marula (*Sclerocarya birrea* subsp. *caffra*) fruit juice**. This describes a research study that you supervised and that will be presented to the Department of Life and Consumer Sciences at the University of South Africa. The thesis was prepared by Ms Lerato Lekhuleni.

My edit included the following:

- Spelling and grammar
- Vocabulary and punctuation
- Adjusting the references for consistent APA presentation

Text formatting included:

- Suggesting that Chapter 1 should include details on poverty and hunger in South Africa, sustainable development goals and daily food intake.
- Checking the interpretation of study results relative to RDI
- Presenting further study results in the conclusions
- Reformatting some in-text references

Yours sincerely,

John Dewar (Professor)

20th October 2025

Appendix IV: Turnitin Report

PAPER NAME

Lerato Lekhuleni_PhD_Nov 2025_Turnitin .docx

AUTHOR

ISOBEL LERATO GOSH LEKHULENI

WORD COUNT

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Appendix V: Permission Letter (Mopani District Municipality)

MOPANI DISTRICT MUNICIPALITY

Private Bag X9687
Giyani
0826

Tel: +27 15 811-6300
Fax: +27 15 812-4301
E-mail: mathebulabs@gov.za

Office of the Municipal Manager

Date: 06th September 2022
Enq: Mathebula B.S

To: Dr Maluleke M.K
University of South Africa

SUBJECT: REQUEST TO CONDUCT RESEARCH – TOPIC: CONSUMPTION, COMMERCIALISATION AND SUSTAINABILITY OF AFRICAN INDIGENOUS FRUITS IN BUSHBUCKRIDGE, GIYANI, TZANEEN AND PHALABORWA, SOUTH AFRICA

The above matter refers,

Kindly note that your request to conduct research in Mopani District area is herewith approved. You may commence with data collection.

Yours sincerely

06/09/2022

pp Mogano T.J (Mr)
Municipal Manager

Date

Appendix VI: Permission Letter (Bushbuckridge Local Municpality)

R533 Graskop Road.
Opp Mapulaneng DLTC
Bushbuckridge
Co-ordinates: 31°3'59.796"E 24°50'24
Tel: 013 004 0291/92/95

BUSHBUCKRIDGE
LOCAL MUNICIPALITY

Private bag x 9308
Bushbuckridge
1280
Email:info@bushbuckridge.gov.za
Website: www.bushbuckridge.gov.za

Enquiries : Mr. L Mokoena (Waste Manager)
: 083 798 4703

11 February 2025

University of South Africa
P.O Box 392
UNISA
0003

Attention: Unisa (Leeto La-Africa community Project Committee)

PERMISSION FOR REASEARCH EXTENSION

The Municipality is pleased to inform you that an extension of the research has been granted, allowing for the continued conduct of research and related activities on the Waste Management Indigenous Fruit Project. This extension has been approved for the period from 2025 to 2030.

The Municipality recognizes the significance of these projects in addressing environmental sustainability, waste management improvements, and the economic potential of indigenous fruit resources. We believe that extending the research period will further enhance the quality of insights gathered, leading to innovative solutions and sustainable community development.

In light of this, we kindly request that the findings, data, and recommendations from the research be shared with both the Municipality and participating stakeholders. This collaborative exchange of information will help in shaping local policies, fostering economic growth, and empowering communities through improved waste management practices and indigenous fruit utilization.

The Municipality remains committed to supporting research initiatives that contribute to environmental conservation and economic development. We appreciate your efforts and look forward to the valuable contributions that your research will bring to our Municipality and its residents.

DIRECTOR: EDPE
TIMBA FS

Appendix VII: Published Article 1

Discover Food

Review

Quality aspects of marula (*Sclerocarya birrea*) fruit, nutritional composition, and the formation of value-added products for human nutrition: a review

Isobel Lerato Lekhuleni[1] · Ayanda Shabalala[2] · Mdungazi K. Maluleke[1,3]

Received: 24 March 2024 / Accepted: 28 May 2024
Published online: 31 May 2024

Abstract
The review aimed to explore the reported biochemical, nutritional, and quality aspects of marula fruit and its value-added products for enhancement of human nutrition. Marula (*Sclerocarya birrea*) fruit is one of the underutilised indigenous fruits that grows naturally across the northern and eastern regions of Southern Africa. A search on Google Scholar, Scopus, Science Direct, and Web of Science databases was conducted in September and November of 2023. Use the following search "terms" to find relevant literature: "*Marula fruit, underutilised African fruit crops*, "*Nutritional composition of marula fruit*", "*biochemical constituents of marula fruit*, "*marula fruit value-added products, function in human health and nutrition*". Available literature shows that almost every part of a tree, namely the leaves, bark, roots, and fruit, have known nutritional benefits for human health and nutrition. The fruit contains valuable biochemical constituents such as vitamin C, carotene, flavonoids, phenols, calcium, magnesium, iron, and zinc. Its value-added products, such as alcoholic beverages, juice, jam, and nuts, contain biochemical constituents with potential enrichment of human health and nutrition, endorsing a compelling case for potential commercialisation globally. The different nutritional and biochemical properties were compared against the human recommended daily intake to determine their potential role in human health and nutrition. The study reveals that the marula fruit and its value-added products may be vital in providing the required nutrients to meet human nutritional daily needs and could play a pivotal role in accelerating the Sustainable Development Goals 1 and 2 (no poverty and zero hunger). Therefore, there is a need for more research on agroprocessing of marula fruit to create nutritious value-added products, for potential commercialisation. As a result, the objective of the study was to examine the literature on the biochemical components of marula fruit and its value-added products, comparing them to the daily recommended intake, while assessing their possible significance in human nutrition for possible commercialisation.

Keywords Biochemical constituents · Marula fruit · Nutritional composition · Recommended daily intake

Appendix VIII: Published Article 2:

Tittle: Physicochemical properties of Marula (*Sclerocarya birrea*) fruit harvested from diverse geographical regions and its potential contribution to food and nutritional security

Journal: Discover Food

Published year: 2024

Full reference of published article:

Lekhuleni, I. L. G., & Maluleke, M. K. (2025). Physicochemical properties of Marula (Sclerocarya birrea) fruit harvested from diverse geographical regions and its potential contribution to food and nutritional security. *Discover Food*, 6 (11), 1-23. https://doi.org/10.1007/s44187-025-00743-6

Lekhuleni and Maluleke *Discover Food* (2026) 6:11
https://doi.org/10.1007/s44187-025-00743-6

Discover Food

RESEARCH **Open Access**

Physicochemical properties of Marula (*Sclerocarya birrea*) fruit harvested from diverse geographical regions and its potential contribution to food and nutritional security

Isobel Lerato Gosh Lekhuleni[1] and Mdungazi Knox Maluleke[1*]

*Correspondence:
[1]Department of Environmental Sciences, Leeto-La-Africa (CU1600), College of Agriculture and Environmental Sciences, University of South Africa, Tshwane 0002, South Africa

Abstract

The Sustainable Development Goals (SDGs) set forth by the United Nations (UN) aim to eradicate hunger, malnutrition and guarantee that all people have access to sufficient nutrient-dense food by 2030. The introduction of underutilised indigenous fruit species such as Marula fruit (*Sclerocarya birrea subsp. caffra*), which is reported to be abundant in vitamin C and minerals needed by humans for the fulfilment of their recommended daily diet seems to be a reliable strategy to achieve these goals. This study aimed to investigate the effect of varying geographical locations on the physicochemical properties of the marula fruit and its juice. Fruits utilised for the analysis of physicochemical properties, such as fruit mass, total flavonoids, and vitamin C, were collected from varying regions with distinct climates (Bushbuckridge, Giyani, and Tzaneen) during the summer of 2024. Results showed that juice processed from fruits harvested from the Giyani region had higher levels of vitamin C (83.85 mg/100 mL), than those from other regions. In comparison other regions, juice derived from fruits harvested in the Tzaneen region had the highest flavonoids content of 14 CE/mL. Higher fruit mass (44.6 g) was observed on fruit harvested from Bushbuckridge. Findings from this study suggest that geographical regions should be considered when fruits are harvested for various purposes, including sales and the creation of value-added products, since they directly impact the quality of the fruit. Given the observed vitamin C and phenolic contents, marula fruit could contribute to nutrient intake where it is endemic. Regional differences suggest site selection matters for quality, which is relevant for future cultivation and value-addition.

Article highlights

- **Biochemical wealth:** Marula fruit, a source of flavonoids and vitamins, holds significant antioxidants and nutritional potential.
- **Potential market:** Marula is nutritionally dense and can be utilised to produce value-added products.
- **SDG 1 and 2:** Marula fruit can be used to create value-added, nutritional-dense products to be sold for income generation, addressing No-Poverty and Zero-Hunger initiatives.

Appendix IX: Drafted article 1: Characterisation of marula fruit (Sclerocarya birrea subsp. Caffra) juice and the effect of storage conditions and preservatives on its biochemical properties

Characterisation of marula fruit (*Sclerocarya birrea* subsp. *Caffra*) juice and the effect of storage conditions and preservatives on its biochemical properties

Isobel Lerato Gosh Lekhuleni[1], Mdungazi Knox Maluleke[1], Florence Molangoane[1], Rebogile Mphahlele[1]

[1]Leeto-La-Africa, Community Engaged Scholarship (CU_1600), College of Agriculture and Environmental Sciences, Department of Environmental Sciences, University of South Africa, Tshwane, 0002, South Africa

Abstract

The utilisation of indigenous fruit as a raw material for the development of nutrient-dense, value-added products like juice is one of the promising strategies that could be used to achieve Sustainable Development Goals such as Zero Hunger (SDG 2). Marula (*Sclerocarya birrea subsp. caffra*) is considered as underutilised fruit crop of Southern Africa region and is reported to be rich in vitamins and minerals required by humans daily. In terms of utilisation, the fruit is used as raw material for creation of value-added product as a juice. While there is abundance of literature on quality parameters of preserved fruit juice of exotic species such as apple, grapes, mango and oranges, the biochemical constituents of preserved marula fruit juice appear to be scanty. 100% marula fruit juice was subjected to preservatives (sodium benzoate and sodium metabisulphite) and storage conditions (4, 6, and 10 °C) for 21 days. Results of the study revealed that untreated pasteurized (C) marula juice stored at 10°C for 20 days retained the highest vitamin C (83.26 mg/L) when compared to other treatments. Marula fruit juice subjected to the treatment combination of sodium metabisulphite stored at 10°C for 20 days exhibited higher retention of antioxidant activity (76.28 %RSA) and total phenolic content (3.81 mg GAE/ml) when compared to others. Therefore, the use of preservatives and low storage temperature conditions present a compelling case for a suitable preservation combination of marula juice for potential commercialisation.

Appendix X: Drafted article 2: Influence of storage conditions and preservatives on the microbiological quality of marula (Sclerocarya birrea) fruit juice

Influence of storage conditions and preservatives on the microbiological quality of marula (*Sclerocarya birrea*) fruit juice

Isobel Lerato Gosh Lekhuleni[1], Mdungazi Knox Maluleke[1], Florence Molangoane[1], Rebogile Mphahlele[2]

[1]College of Agriculture and Environmental Sciences, Department of Environmental Sciences, University of South Africa, Tshwane, 0002, South Africa

[2]National Department of Agriculture, Private Bag X250, Pretoria 0001, South Africa

Abstract

The purpose of the study was to evaluate the microbiological quality of chemically preserved marula fruit juice during storage. The study was based on manually extracted marula fruit juices collected from Limpopo (Giyani and Tzaneen) and Mpumalanga (Bushbuckridge). Samples were developed by treating the marula juice with 0.05% preservative sodium benzoate (SB) and sodium metabisulphite (SM). The untreated juice sample was used as a control. Chemically preserved and unpreserved marula juice were packaged in 50ml plastic containers and stored at different refrigeration temperature of 4, 6 and 10°C for 20 days. Microbiological evaluation was determined using the spread plate count method. After the incubation period, enumeration of the colony forming units (CFU) and species identification were performed. Microorganisms were identified using the 16S rRNA gene sequences. The results revealed that juice samples from Bushbuckridge did not show any microbial growth up to 20 days of storage. The total viable bacterial counts of fruit juice harvested from Giyani (GYN) and Tzaneen (TZN) ranged from 9.4×10^2 to 3.3×10^5 cfu/ml. Juice of fruit harvested from TZN showed low bacterial counts (9.4×10^2) on untreated samples stored for 20 days at 4°C, when compared with juice of fruit collected from TZN (3.3×10^5) treated with sodium benzoate and stored 10 days at 10°C. Fruit juice treated with 0.05% sodium metabisulphite had low microbial counts of 1×10^3 at the end of storage, indicating that they fall within acceptable level for human consumption. The bacteria present in the juice samples were identified as *Bacillus spp* which was not distinguished at a species level.

Appendix article XI: Drafted article 3: The effect of preservatives and storage conditions on the mineral stability of marula fruit juice and its potential role in human health and nutrition

The effect of preservatives and storage conditions on the mineral stability of marula fruit juice and its potential role in human health and nutrition

Isobel Lerato Gosh Lekhuleni[1]. Nkosikhona Goodman Magwaza[1]. Mandlenkosi George Robert Mahlobo[2]. Nothando Cynthia Shiba[2]. Mumsy Evidence Chibe[1]. Sabelo Shezi[1]. Thanyani Sylvia Ralulimi[1]. Rabelani Munyai[1]. Danisili Leonah Mthombeni[1]. Philix Mnisi[1]. Petunia Mashiane[1]. Yingisani Chabalala[1]. Malongane, Florence[1]. Rebogile Mphahlele[1]. Ayanda Shabalala[1]. Mdungazi Knox Maluleke[1*].

[1]Leeto-La-Africa (CU1600), College of Agriculture and Environmental Sciences, Department of Environmental Sciences, University of South Africa, Tshwane, 0002, South Africa

[2]Department of Chemical Engineering, Florida Campus, University of South Africa, Roodepoort 1710, South Africa.

Corresponding author: malulm@unisa.ac.za

Abstract

Background: The juice industry is regarded as one of the most important players in nutritional security and the economy because it provides sustainable access to important minerals required by humans daily, which in turn creates job opportunities since manufacturers can generate revenue through juice product sales. Most indigenous fruits of Southern Africa including marula have been utilised as raw materials for value-added products such as juice, which is claimed to be rich in minerals needed for sustenance of human health and nutrition. However, the suitable post-processed storage treatments for mineral retention appear to be scanty. The study objective was to assess the effect of different preservatives and storage temperatures on the mineral composition of marula juice extracted from fruit harvested in distinct geographical regions.

Methods: Marula fruit juice was subjected to varying preservatives (sodium benzoate and sodium metabisulphite) and storage temperatures (4, 6 and 10 °C) for 20 days. Juice samples were subjected to microwave-assisted acid digestion (HNO_3), and minerals were determined by inductively coupled plasma optical emission spectrometry (ICP OES).