EFFECT OF *MORINGA OLEIFERA* LEAF POWDER EXTRACTS ON THE BROWNING, NUTRITIONAL AND STORAGE QUALITIES OF DRIED GRANNY SMITH APPLE SLICES

by

SIHLE BIYONGO (216235146)

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Supervisor: Prof V.A Jideani
Co-supervisor: Prof Ahmed A. Hussein Mohammed

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DECLARATION

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ABSTRACT

This study aimed to determine the effect of the *Moringa oleifera* leaf powder (MOLP) extracts on the browning, nutritional, and storage qualities of dried granny smith apple slices. The MOLP extracts were prepared by maceration with the assistance of ultrasound extraction to ensure maximum bioactive compounds using methanol (MOME), ethanol (MOET), water (MOAQ) and acetone (MOAC). The obtained extracts were freeze-dried and subjected to bioactive compound profiling using liquid chromatography-mass spectrometry (LC-MS). The extracts were also evaluated for antioxidant activity in terms of FRAP, DPPH, ORAC and ABTS. Thereafter, the anti-tyrosinase activity of MOLP extracts with PEG-6000 as a hydrophilic carrier was examined for IC_{50} . The IC_{50} of all MOLP extracts was used to determine the concentration of the treatment complex for the dried apple slices. For the anti-browning treatments, each extract (MOME, MOET, MOAQ, MOAC) was separately prepared at upper (0.3%), mid (0.03%) and low (0.003%) concentrations in combination with 2% citric acid to determine the effectiveness of each extract on the colour attributes of the dried apple slices. Thereafter, the pre-treatment solution with a strong IC_{50} effect on the colour of the dried apple slices was selected for treatment of the dried apple slices to determine its impact on the proximate composition, and physicochemical properties. The storage stability of the dried apple slices treated with the 0.003% MOAQ anti-browning mixture was investigated through the analysis of physicochemical properties such as water activity, moisture, extensibility, and colour. The pre-treated dried apple slices were subjected to 35°C accelerated conditions and the first order reaction kinetics was used to determine the shelf-life with an assumed Q_{10} of 3. The MOLP extracts showed 23 bioactive compounds such as phenolic compounds, flavonoids, amino acids, quinones and fatty acids. The MOME extract showed a higher number of phenolic compounds, especially flavonoids than other MOLP extracts. A dose-dependent antioxidant activity was observed for all the extracts; however, the MOME and MOET extracts had a significantly ($p = 0.001$) higher antioxidant activity than the MOAQ and MOAC extracts. Furthermore, 34.66 (MOAQ) to 71.29 µg/ml (MOET) was observed for IC_{50} of all the MOLP extracts against tyrosinase. The MOAQ was significantly ($p = 0.001$) stronger (IC_{50} = 34.66 µg/ml). Moreover, the samples treated with the 0.003% MOAQ anti-browning mixture were significantly ($p = 0.001$) lighter, yellower, and greener than the control and other MOLP anti-browning mixtures. The ash, vitamin C and total fat content of the MOAQ-treated samples was higher than the control. The MOAQ-treated apple slices demonstrated an increase in extensibility ($p = 0.001$). Furthermore, the estimated shelf life of MOAQ-treated apple slices was 153 days compared to the control (126 days) at 25°C.The finding of this study suggest that the *Moringa oleifera* leaf powder aqueous extract (MOAQ) is a promising preservative in dried fruits by highlighting its potential for reducing browning, maintaining acceptable colour attributes, and preserving the dried apple slices.

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DEDICATION

Dedicated to the memory of my dear father, Mbonisi Biyongo (†24.06.2020) whose wisdom and love continues to inspire me every day.

GLOSSARY

Terms/Acronyms/Abbreviations	Definition
ANOVA	Analysis of variance
DAD	Diode array detector
DMSO	Dimethyl sulfoxide
DPPH	2, 2- diphenyl-1-picrylhydrazyl
FDA	Food Drug Administration
HPLC	High-pressure liquid chromatography
MO	*Moringa oleifera*
PPO	Polyphenol oxidase
SO_2	Sulphur dioxide
UV	Ultraviolet
MOME	*Moringa oleifera* leaf methanol extract
MOAQ	*Moringa oleifera* leaf aqueous extract
MOAC	*Moringa oleifera* leaf acetone extract
MOET	*Moringa oleifera* leaf ethanol extract
MOLP	*Moringa oleifera* leaf powder
FRAP	ferric reducing antioxidant power assay
ABTS	2,2'-azino-di-3-ethylbenzthialozine sulphonate
ORAC	oxygen radical absorbance capacity
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
LC-MS	Liquid chromatography – mass spectrometry

CHAPTER 1:
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

The undesirable enzymatic browning, caused by enzymatic activities in the processing of fruits, is a significant challenge in manufacturing. This is because it results in the elevation of softening and browning at cut surfaces, consequently reducing the texture consumer appeal, and decreasing the nutritional and shelf quality (Chen *et al.*, 2000:4997; Olivas, 2005:657; Oms-Oliu *et al.*, 2010: 139).

The demand for dried fruit products such as dried apple slices has increased due to their convenience to consumers and ease of transportation (Kahraman *et al.*, 2021a:1). Manufacturing these products involves cutting, trimming, coring, and other processing techniques that affect the fruit tissue, consequently causing the fruits' browning, softening, microbial spoilage (Ansah *et al.*, 2018:1). Therefore, several practices are needed to control these processing effects to maintain the quality of the dried fruit products. These practices include the treatment of the products with anti-browning agents such as citric acid, ascorbic acid, and sulphites to inhibit the formation of melanin (Janovitz-klapp *et al.*, 1990:927; Oms-Oliu *et al.*, 2006:216; Otwell & Iyengar, 1992:255). Studies have been done over the years to compare these agents, and citric acid is less effective in controlling browning when compared to other carboxylic acids (Moon *et al.*, 2020:6; Tsouvaltzis & Brecht, 2017:2). It is widely used because of its safety for human consumption, acceptable acid taste, water solubility, and compatibility with other anti-browning agents (Iyengar & McEvily, 1992:60). Moreover, Sulphites are added as anti-browning agents and preservatives in the form of sodium metabisulphite before drying fruits to prevent browning reactions and improve texture. However, Sulphur dioxide changes fruit taste and is a health hazard resulting in dermatitis, diarrhoea and mental confusion for specific individuals (García-Gavín *et al.*, 2012:261). In response, alternative treatments are needed to maintain the quality of dried fruits. The development of alternative browning inhibitors will reduce the health effects linked to the consumption of dried fruits treated with sulphites.

Moringa oleifera leaf is an acknowledged crop sub-Saharan Africa, in Western Africa (Rufai *et al.*, 2016:209). The leaves of the Moringa plant are edible and are of essential value as food (Umerah *et al.*, 2019:125). Its leaves are a good source of natural antioxidants, protein, iron, vitamins and minerals (Sreelatha & Padma, 2009: 304). Previous research studies have reported on the potent antioxidant and antimicrobial activity of Moringa, which is due to its high contents of flavonoids and phenolics found in the leaves (Kshirsagar *et al.*, 2017:11; Ma *et al.*, 2020:41). Nonetheless, there is limited information on the anti-browning effect of *Moringa oleifera* on the nutritional and physicochemical properties of foods.

1.2 Statement of the Research Problem

Colour is one of the most important quality parameters in fruits and it is used to determine the ripeness and consumer acceptability (Planeta *et al*., 2020:276). Sodium metabisulphite is used as a preservative in dried fruits to prevent browning, microbial and chemical deterioration because of its effectiveness and ease of application. However, it is important to reduce the application of the preservative because it has undesirable health effects such as hypotension, diarrhoea, abdominal pains and headache (do Nascimento *et al*., 2020:616). Multiple studies have been conducted to investigate the effectiveness of citric and ascorbic acid on the browning and storage quality of dried fruits. It was reported that ascorbic acid was less effective at inhibiting browning and preservation of dried tomatoes, banana, and peach samples (Dipersio *et al*., 2006:47). Similarly, Sun *et al*. (2020:5772) reported that dried potato samples pre-treated with citric acid had an improved browning colour and retained the highest Vitamin C content and improved colour after dehydration. Furthermore, 0.1% of *Moringa oleifera* leaf powder extract reduced the discolouration of dried apple slices (Arendse & Jideani, 2022:12). However, there is limited information on the effect of *Moringa oleifera* leaf powder extracts against tyrosinase and browning of dried fruits. Additionally, no study has reported on the combination of citric acid and *Moringa oleifera* leaf powder extracts at concentrations of 0.3, 0.03, and 0.003% determined from the IC_{50} against tyrosinase. Therefore, it is of interest to investigate the IC_{50} of the acetone, aqueous, ethanol and methanol extracts of *Moringa oleifera* leaf powder extracts in combination with citric acid to determine its effectiveness on the anti-tyrosinase activity, storage stability, colour, and texture of the dried apple slices.

1.3 Research objectives

1.3.1 Broad objective

The study aims to determine the anti-browning effect of *Moringa oleifera* leaf extract on the nutritional, physicochemical and storage quality of dried apple (*Malus domestica*) slices.

1.3.2 Specific objectives

The specific objectives were to:

1. Characterize and determine the phenolic composition of the acetone, aqueous, ethanol and methanol *Moringa oleifera* leaf extracts based on the structure and functionality.
2. Determine the antioxidant activity and establish the 50% inhibitory concentration (IC_{50}) of the *Moringa oleifera* leaf powder extracts (acetone, aqueous, ethanol and methanol) against tyrosinase.
3. Establish the effectiveness of the most effective *Moringa oleifera* leaf extract and citric acid as an anti-browning agent in dried apple slices.

4. Establish the physicochemical, nutritional, and shelf-life stability of dried apples treated with the most effective anti-browning mixtures.

1.4 Hypothesis

It was hypothesized that:

1. The occurrence of flavonoids and other phenolic compounds in *Moringa oleifera* leaf powder extracts in combination with citric acid mixtures will reduce the browning of the dried apple slices.
2. The physicochemical and nutritional properties of the dried apple slices treated with the anti-browning mixture will differ from the untreated slices.
3. The shelf-life and storage quality of the dried apple slices treated with the *Moringa oleifera* leaf extracts (anti-browning mixture) will differ from the untreated slices.

1.5 Delineation

Only ultra-sound assisted extraction was used to prepare the *Moringa oleifera* leaf extracts. The extraction of bioactive compounds was done using four solvents, namely acetone, ethanol methanol and distilled water. Lastly, only granny smith apples were used to make the dried apple slices.

1.6 Significance of the Research in South Africa

The growth of the human population and the demand for fruit consumption for human food increased the pressure on the preservation of fruits (Putnik *et al.*, 2017:56). Concurrently, consumers are more concerned about their eating habits, healthy lifestyles, and convenient food products.

The successful development of an anti-browning treatment from *Moringa oleifera leaf* components will promote this crop's use. This will result in the improvement of the social development of the country and create more opportunities for new and existing moringa growers in the South Africa. Dried fruits are less vulnerable to spoilage caused by the growth of microorganisms and moulds supported by high water activity (Nowak & Lewicki, 2005:832). Moreover, dried apple slices will be suitable for individuals with no refrigeration and other resources to keep fresh fruits, such as travellers. The ingestion of foods containing sulphites has been reported to promote clinical effects such as flushing, life threatening asthmatic reactions, diarrhoea (Vally et al., 2009:1643). Skypala *et al.* (2015:2) also reported that 3.9% of 203 individuals with steroid dependent asthma were sensitive to sodium metabisulphite. Thus, the dried fruits will be ideal for people who have asthma and other people who are allergic to sulphites.

Increasing the use of Moringa and its constituents will increase the farming and production of the crop in South Africa. This may involve other countries, attract investors,

and improve the country's economy. Completing this study will provide additional information about *Moringa oleifera* and its magical potential, called the magical tree.

1.7 Thesis Overview

This thesis consists of five chapters. It was written in a journal article format where the layout of each chapter is like a manuscript. The thesis overview is shown in Figure 1.1. Chapter one introduces the study, which demonstrates the design, motivation, and background. It also highlights the research hypothesis, limitations, significance and expected outcomes. The second chapter is the literature review, which clarifies the research background and the significant sub-topics connected to the topic. Chapter three, the first research chapter, shows the production of the *Moringa oleifera* leaf extracts, phytochemical analysis, and the vitro applications of the extracts. The fourth chapter investigates the effect of the best solvent extract on the quality of dried apple slices. It also shows the impact of the extracts on the storage quality and the physicochemical properties of the dried apple slices during storage. The sixth chapter summarises the research findings and outlines the study's conclusions.

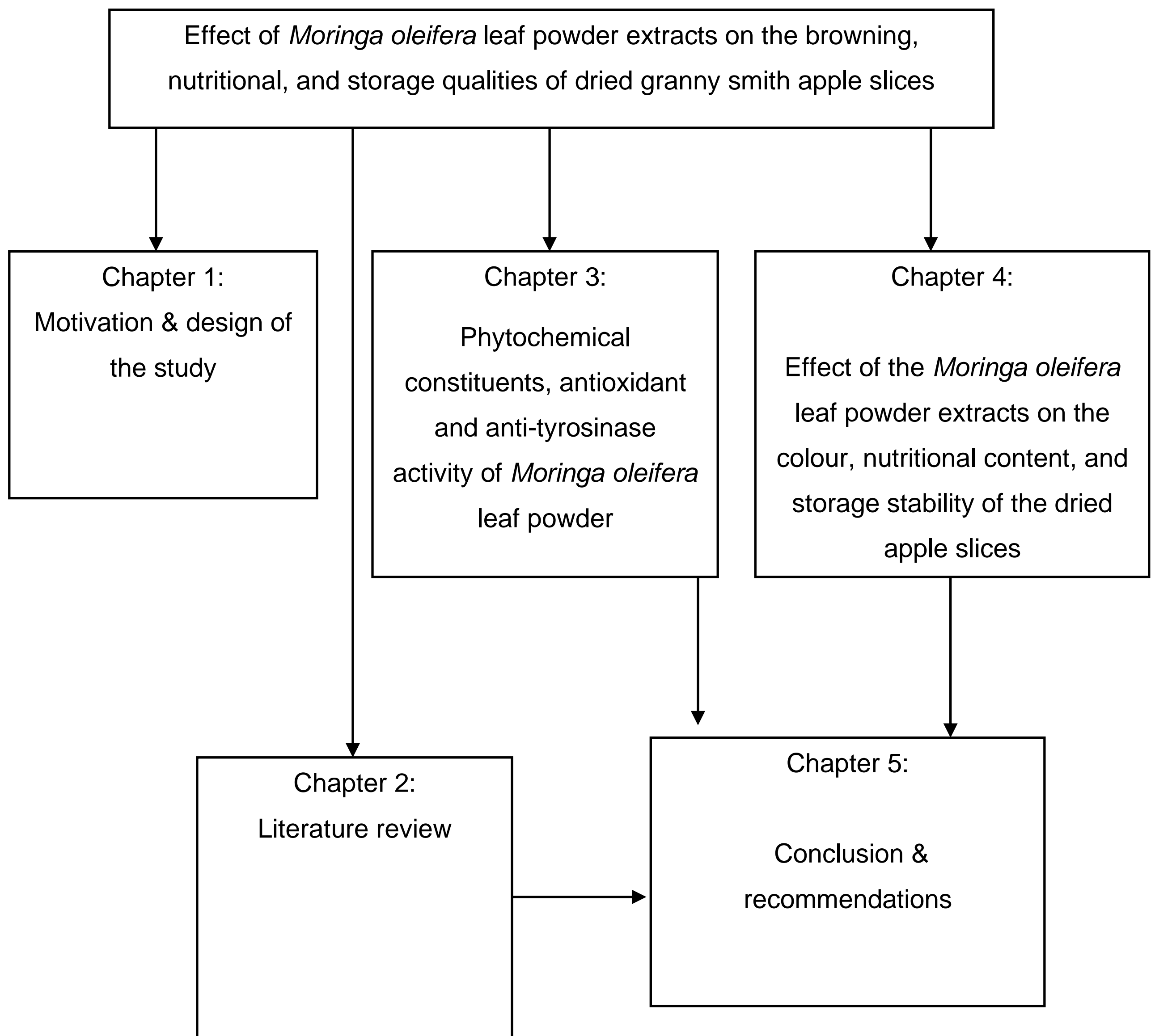

Figure 1.1 Thesis overview

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CHAPTER 2:
LITERATURE REVIEW

2.1 Background of *Moringa oleifera* leaf

Moringa oleifera, the drumstick tree, is an indigenous Asian plant that has been cultivated in many parts of the world in warm countries such as Malaysia and other sub-tropical countries (Mallenakuppe *et al.*, 2019:2323; Harcourt, 2015:3905; Sohaimy *et al.*, 2015:188). The plant was first established in India and the South of the Himalayan mountains. At the beginning of the 20th century, *Moringa oleifera* was introduced to Eastern Africa and is now cultivated throughout the tropical belt (Mallenakuppe *et al.*, 2019:2323). The plant belongs to the class *Magnoliopsida* and family *Moringaceae,* which consists of several species, such as *Moringa borziana, Moringa concanensis, and Moringa drouhardi,* that are cultivated internationally are cultivated throughout the world (Saucedo-Pompa *et al.*, 2018:437–438). *Moringa oleifera* (MO) is well known by different names in various areas of the world. Some authors refer to MO as the miracle tree, ben oil tree, horseradish, mother's best friend, and the miracle tree because of its outstanding nutritional, environmental, and medicinal properties (Daba, 2016:1). When the Moringa tree is grown, it requires extremely little water, all parts of the plant can be consumed once it matures, which makes it unique and thus called the magic tree (Ruchita & Sharda, 2017:967).

One tree can be a source of nutrition for humans and livestock because every part of the tree is edible (Pinto *et al.*, 2015:3). Its seeds can be used for water purification and seed oil extraction, while the wood can be used to construct a shelter (Mahima *et al.*, 2014:284). The flowers can be ingested as a herb or used to prepare tea with a high mineral content (Koul & Chase, 2015:692). MO and its products fight malnutrition in infants and breastfeeding women (Siddhuraju & Becker, 2003:2145). Moreover, the leaves are a source of calcium, potassium, and fatty acids (El-absy *et al.*, 2019:3). All parts of the plant contain beneficial biochemical complexes, namely ascorbic acid, carotenoids, flavonoids, and other phenolic compounds (Khairulmazmi & Tijjani, 2019:454; Koul & Chase:697, 2015; Kou *et al.*, 2018:3). Furthermore, the tree is well known for its medicinal value because it exhibits anti-cancer, anti-diabetic, and antifungal properties (Ganga *et al.*, 2019:145).

Moringa oleifera leaf plays a considerable role in the semi-arid regions of Africa and is mainly grown for its economic value (Mashamaite *et al.*, 2021:5). It grows to a maximum height of 7 m and is naturally on hillsides, river basins, and pasture lands. Moringa is known as an everlasting tree for its ability to develop well in arid climates and its resilience in the face of unfavourable environments. Its growth and development are advantageous amongst other crops because it can be grown in hot, dry tropical, subtropical, and damp soil (Koul &

Chase, 2015:689–690). The long tuberous taproot of MO expands deeply into the ground to extract minerals and water enabling it to survive drought conditions (Mashamaite et al., 2021:3). Mallenakuppe *et al.* (2019:2324) also confirmed that its ability to penetrate deep into the soil enables MO trees to endure severe conditions throughout their life. Thus, the nick name everlasting tree outlines its multiple benefits, resilience and longevity making MO a valuable plant in nutrition, food technology, and sustainable agriculture. The useful structural parts of the MO plant include the stem, branch leaves, flowers, and fruits with seeds (Taiwo *et al.*, 2014:92). Figure 2.1 elaborates on all parts of the drumstick tree.

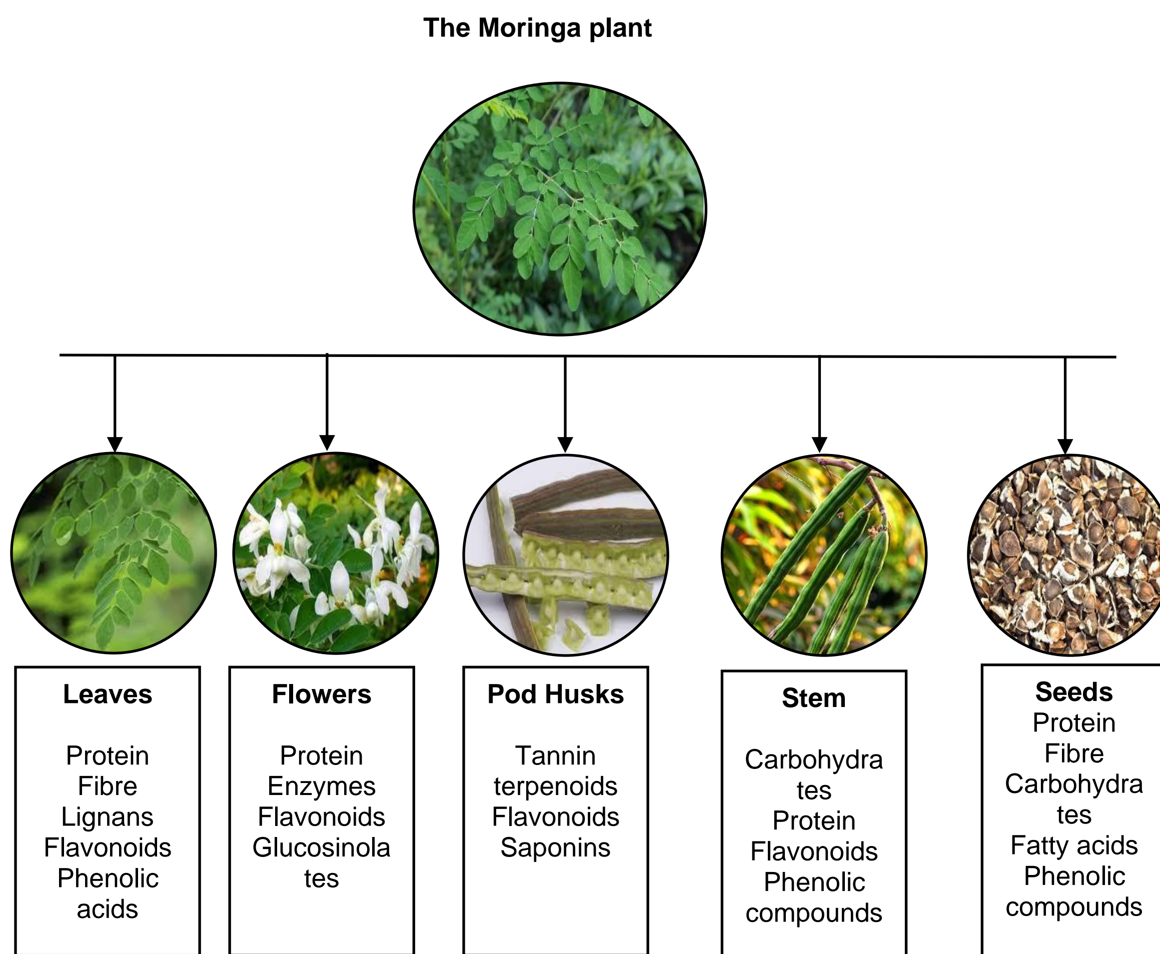

Figure 2.1 Different morphological parts of the *Moringa oleifera* tree

Adapted from Milla *et al.* (2021:2)

2.2 Nutritional characteristics of *Moringa oleifera*

The nutritional value of *Moringa oleifera* leaf is indicated in **Table 2.1**. Bamishaiye *et al.* (2011:235) reported on the proximate composition of *Moringa oleifera* leaf. However, there may be differences in the nutritional value due to the variations in the cultivator and location. MO is well known for its high protein content due to the availability of essential

amino acids in the leaves. According to Su & Chen (2020:4), the *M. oleifera* leaves contain essential amino acids such as leucine, histidine, isoleucine, phenylalanine, lysine, and phenylalanine. They also reported that MO leaves contain sulphur-rich amino acids, such as cysteine and methionine, found in some legumes in minimal quantities. Its leaves have about 10.74-29.4 g of protein/100 g dry weight compared to other vegetables, which mainly contain these nutrients in their seeds. Moreover, Sánchez-Machado *et al.* (2010:178) reported that *M. oleifera* leaves have 44% amino acids, while the pods contain 31% per 100 g dry weight. The protein content of the dried leaves was confirmed by Teixeira *et al.* (2014:53–54), who reported the crude protein content of dried *M. oleifera* leaves to be 28.7 mg of protein/100 g. According to Gopalakrishnan *et al.* (2016:51), the *M. oleifera* pods have 20.66% protein and 46.78% fibre content. Moreover, Govender & Siwela (2020:14) reported that bread fortified with *M. oleifera* leaf powder contained about 13.5% protein, and consumption of a serving would provide more than 50% of the estimated average requirement of protein for vulnerable households. Therefore, *M. oleifera* can be declared a relevant protein source (Valdez-Solana *et al.*, 2015:3).

Table 2.1 Nutritional composition of dried *Moringa oleifera* leaf

Nutrient	**Dried leaves**	**Reference**
Carbohydrates (g/100g)	38.2	Rathnayake *et al.* 2019:257–256
Fibre (g/100 g)	19.2	
Protein (g/100 g)	10.74-29.4	
Fat (g/100 g)	2.3	
Magnesium (mg)	42-368	Zaku *et al.*, 2015:458
Phosphorus (mg)	204	
Potassium (mg)	1 324	
Vitamin A (mg)	6.78-16.3	Zaku *et al.* 2015:458; Valdez-Solana *et al.* 2015:3; Gopalakrishnan *et al.* 2016:51; Khalid Abbas *et al.* 2018:57
Vitamin B1-thiamine (mg)	0.06-2.64	
Vitamin B2-riboflavin (mg)	0.05-20.5	
Vitamin C (mg)	17.3-220	
Calcium (mg)	99.1 - 2.003	
Zinc (mg)	0.16-3.29	Singh *et al.* 2017:105–106

All values are presented in 100 g per plant material.

The miracle tree is a vital room of essential nutrients and anti-nutrients. Therefore, incorporating *M. oleifera* leaves into the human daily diet may increase the availability of essential nutrients that the body cannot synthesise independently. The amount of ash was previously found at 8.05-10.38% in *M. oleifera leaves*, which generally refers to the total

inorganic residues such as minerals available after combustion of the organic matter in food products. Therefore, *M. oleifera* leaves are rich in minerals like iron, calcium, potassium, phosphorus, zinc, and copper. All parts of the tree, namely, the flowers, leaves, and pods, are rich in beta-carotene of vitamins A, folic acid of vitamin B, Vitamin C, D and E. Amongst the essential minerals for growth, the *M. oleifera* leaves have been found to possess about 1000–4000 mg of calcium. According to Falowo *et al.* (2018:319), the MO *leaves* contain four times more calcium and two times more protein than milk. In living organisms, calcium is essential for the primary development of the teeth, bones and maintaining the nervous system (Beto, 2015:2). Therefore, *M. oleifera* can fight malnutrition in nursing mothers and infants in underdeveloped countries. Its leaves were also reported to have 28 mg of iron and 25.5–31.03 mg of zinc per kg (Barminas *et al.*, 1998:32).

The leaves contain three times more potassium and iron than spinach and banana (Cabanac *et al.*, 2021:1). Iron is a significant component of haemoglobin, a protein that conveys oxygen from the lungs throughout the body. It is essential for the body's biochemical reactions and assists muscles to utilise and store oxygen. Moreover, enough iron encourages growth in living organisms such as animals, mainly in the presence of zinc. For instance, the supplementation of *Moringa oleifera* leaf powder in children from Tanzania significantly reduced severe anaemia. This nutritional disorder affects children younger than 2 years and is mainly caused by iron deficiency (Shija *et al.*, 2019:2592). Therefore, when used in formulas and fortified food products, children can ingest *Moringa oleifera* leaf powder to address anaemia among children or infants.

The dried MO leaves also comprise about 38.2-44.36 g of carbohydrates/100 g and a moisture content of 6.0-6.3 g/100 g (Bamishaiye *et al.*, 2011:235; Sodamade *et al.*, 2013:48). Researchers reported on dried leaves' fibre content, ranging from 18.1 to 21.1 g/100g (Umerah *et al.*, 2019:128; Rathnayake *et al.*, 2019:263). Thus, we can conclude that the plant has an incomparable nutrient value in one plant species.

2.3 Phytochemical Compounds and Bioactive components in plants

Aromatic and medicinal plants such as those mentioned in ancient text have taken various uses and forms throughout history. Most of the population from Asian and Indian the population has been depended on traditional medicine, phytomedicine or botanicals (Petrovska, 2012:4). Traditional plants can serve as initial medicinal and pharmaceutical research materials due to their health benefits and advantageous properties. The main components of herbal medicine include active plant parts such as seeds, leaves, roots, and flowers. Most recently, the demand, limited availability and possible depletion of natural plants encourage knowledge, research and development, utilisation, and production for sustainability (Agrawal *et al.*, 2017:473). Medical prescriptions with plant-based formulations and botanically derived components are used to treat chronic disorders. With

the increasing resistance of microorganisms against antibiotics, there is an ongoing search for the development of safe and cost-effective anti-microbial compounds.

Phytochemicals are secondary metabolites primarily found in plants at high concentrations, even though they have a limited role in the development and growth of the plants. They are found in most plants and have been used in ancient times as medication for curing and preventing various diseases (Riaz *et al.*, 2023:2503). Even now, about 70-80% of the world's population in developing countries uses phytochemicals as traditional health care medicine. Plant phytochemicals are classified into 5 classes based on their chemical structure: alkaloids, polyphenols, carotenoids, and other sulphur-containing compounds (Riaz *et al.*, 2023:2514). According to Hassan *et al.* (2021:6), MO contains most of these phytochemicals; hence, both the disease-preventative ability and biological activities are hugely considered to be related to their presence. They further reported that it consists of the major phenolic compounds such as phenolic acids, flavonoids, and glycosides. Polyphenols are one of the primary phytochemicals identified by phenolic rings in their chemical structure. Phenolic acids can further be recognised by one phenolic ring, while flavonoids have more than one phenolic ring in their chemical structure (Tsao, 2010:1232).

Table 2.2 Polyphenol compounds from different parts of *Moringa oleifera*

Sr. No	Plant part	Extract	Phytoconstituents
1	Leaves	Aqueous and alcoholic	Niazirin and Niazirinin – nitrile glycosides,4-[(4'-O- acetylalpha- L-rhamnosyloxy) benzyl isothiocyanate, Niaziminin A, and Niaziminin B, three mustard oil glycosides, niaziminin, a thiocarbamate,4-(alpha-1- rhamnopyranosyloxy)-benzylglucosinolate, quercetin-3-O-glucoside and quercetin-3-O-(6"- Malonyl- glucoside),Niazimicin. Pyrrole alkaloid (pyrrolemarumine 400-O-a-L-rhamnopyranoside) and 40- hydroxyphenylethanamide(marumoside A and B) 4.alpha and gamma-tocopherol.2
2	Seeds	Aqueous and Hydro-alcoholic	Methionine, cysteine, 4-(alpha-L- rhamnopyranosyloxy) benzylglucosinolate, Moringine, benzylglucosinolate, niazimicin niazirin.
3	Pods	Hydro-alcoholic	Isothiocyanate, nitrites, thiocarbamates,O-(1heptenyloxy) propyl undecanoate, O-ethyl-4-(alpha-L-rhamnosyloxy) benzyl carbamate, methyl- p-hydroxybenzoate, beta- sitosterol .
4	Bark	Alcoholic	4-(alpha-L- rhamnopyranosyloxy) benzylgiucosinolate.
5	Flowers	Hydro-alcoholic	D-glucose, quercetin, isoquercetin, kaemopherol, kaempferitin and ascorbic acid, protein, D-mannose.
6	Root	Alcoholic	Moringine, moringinine, spirachin, 1,3-dibenzyl urea, alpha- phellandrene, p-cymene, Deoxy-niazimicine, 4-(alpha-L-rhamnopyranosyloxy)benzylglucosinolate.
7	Stem	Aqueous and Hydro-alcoholic	4-hydroxyl mellein, vanillin, octacosonoic acid, beta- sitosterone and beta- sitosterol.

Sourced from Paikra *et al.* (2017:196)

2.3.1 Flavonoids of *Moringa oleifera*

Dietary flavonoids are secondary metabolites with a phenolic structure widely found in fruits and vegetables. They are dispersed in plants in their unsaturated form or linked to sugars as glycosides. Flavonoids are also commonly found in their free form as aglycones or attached as disaccharides or monosaccharides by O-glycosylation (Falcone Ferreyra *et al.*, 2012:11). The degree of saturation and the carbon of the C ring on which the B ring is attached qualifies them for division into various groups. The B ring structure of the isoflavones is linked to the third position of the C ring, while neoflavanoids are related to the fourth position (Jung *et al.*, 2020:1608). The flavonoids in which the B ring structure is linked to the second C ring are divided into multiple groups such as anthocyanins, chalcones, flavonols, catechins, anthocyanins, flavanonols, flavones, flavanones (Figure 2.2) (Falcone Ferreyra *et al.*, 2012:2; Jung *et al.*, 2020:1606–1607). Flavonoids are extensively found in beverages and foods of plant origin, such as cocoa, tea, and wine. About 5000-6000 flavonoids contribute to the biological activities of animals, plants, and microorganisms. These secondary metabolites play a considerable role in the colour and aroma and are synthesised in specific sites of the plants (Amalesh *et al.*, 2011:12). Similarly, vegetables and fruits use flavonoids to defend against plaque and maturity, which assists with spore and seed germination during the development of seedlings (Panche *et al.*, 2016:2).

Flavonoids relate to a wide range of remedial healing effects and are essential in medicinal, nutraceutical, pharmaceutical, food, and cosmetics applications. Due to their anti-inflammatory, antioxidant, anti-carcinogenic, and anti-mutagenic properties linked to their critical enzyme-modulating function (Kaur, 2014:1). These compounds can interfere with proteins or nucleic acids, resulting in anti-microbial properties. Therefore, flavonoids can be used as potent preservatives, antifungal, antibacterial, and pesticides in agricultural applications and food science (Kakkar & Bais, 2014:7; Xu *et al.*, 2018:749). They are also famous for their capacity to regulate crucial cellular enzyme functions and as potent enzyme inhibitors such as tyrosinase, xanthine oxidase, phosphoinositide, and lipoxygenase. Shah & Smith (2020:6–7) evaluated the plant flavonoids in depth and reported on their agricultural applications. Hashim *et al.* (2021:9) reviewed the activity of MO flavonoids against tyrosinase, while Natsir *et al.* (2018:1) reported on their action against alpha-glucosidase. The interest in these compounds has been aligned to their ability to scavenge and reduce free radicals. The highly reactive hydroxyl group of flavonoids reacts with oxygen radicals to form more stable radicals and less reactive radicals. Panche *et al.* (2016:8–10) reported that rutin and epicatechin's powerful radical scavenging ability may be related to their anti-enzyme activity.

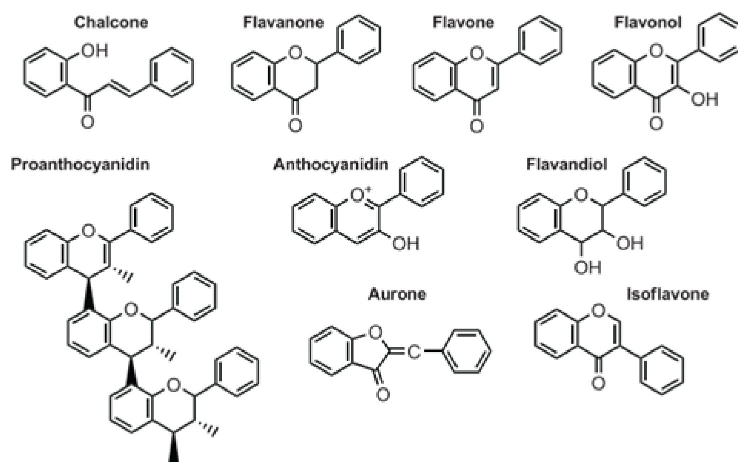

Figure 2.2 The different classes of flavonoids
Sourced from Falcone Ferreyra *et al.* (2012:2).

Furthermore, quercitrin, myricetin, and rutin are very productive in modulating lipid peroxidation and producing superoxide radicals (Wang *et al.*, 2010:16; Patil *et al.*, 2013:91). This review discusses current research and development trends of flavonoids in terms of their trends, applications as dietary, health benefits, and broad classification and future research directions. Therefore, the flavonoids' antioxidant functions and enzyme inhibitory potential can assist in reducing the synthetic antioxidants used in the pharmaceutical and food industries.

Similarly, *M. oleifera* leaf extracts have potent antioxidant activity in vivo and in vitro due to abundant flavonoids and phenolic acids (Rodríguez-Pérez *et al.*, 2015:252–253). *Moringa oleifera* has been studied by multiple authors for the existence of flavonoid compounds in the leaves, stem, roots, and pods (Nobossé *et al.*, 2018:2195–2196; Makita *et al.*, 2016:118). Flavonoids are considered the main phenolic compounds in *M. oleifera* plants. The total quantity of flavonoids and overall nutrient composition in *M. oleifera* differs with the cultivar type and location (Gopalakrishnan *et al.*, 2016:49–50). They concluded that the Moringa tree grown in Nigeria was less nutritive than *M. oleifera* tree grown in India because of extremely high temperature in the Savannah region.

Similarly, 6 kaempferol-O-glycosides were identified in *M. oleifera* from South Africa, while only 3 out of the similar compounds were identified in *M. oleifera* leaves sourced from Namibia (Huber *et al.*, 2017:124–125). The major flavonoids in *M. oleifera* and derivatives identified include rutin, kaempferol, 3',4'-diOMe quercetin, and quercetin with a comparatively potent antioxidant activity than ascorbic acid (Gopalakrishnan *et al.*, 2016:50; Abd El-Hack *et al.*, 2018:4). Similarly, Koul & Chase (2015:692) reported that quercetin can be found as quercetin-3-O-glucoside and is a robust immune system modulator. Therefore, this suggests that *M. oleifera* and its products can be incorporated in food formulations and processes worldwide in different edible products as functional foods to improve their nutritional value significantly.

2.3.2 Phenolic acids of *M. oleifera*

Phenolic acids are a group of secondary metabolites, also called compounds, obtained from naturally occurring hydroxybenzoic and hydroxycinnamic acids in plants (Rashmi & Negi, 2020:1). The solid biological activities of these compounds and their availability in various food products such as grains, beverages, fruits, and vegetables have drawn abundant attention among scientists. These compounds contribute to food's flavour, colour, and organoleptic properties, such as flavour, colour, and astringency (Shahidi & Ambigaipalan, 2015:834). These compounds have gained popularity due to their health benefits and capabilities as antioxidants, anti-allergic, and anti-cancer properties (Lopez-Corona *et al.*, 2022:1-3). Phenolic acids are associated with functionalities like anti-microbial, cardioprotective, anti-diabetic, and immunoregulatory properties (Milla *et al.*, 2021:1; Vergara-Jimenez *et al.*, 2017:3-5; Robbins, 2003:2867-2868). Alternatively, phenolic acids are applied in food products as additives for the retention of colour and controlling microbial growth. Various phenolic acids have been used as preservatives in food products by inhibiting lipid oxidation in oil and bakery products (Martinengo *et al.*, 2021:19).

Plant researchers mainly focus on the leaves because of their health benefits and applications. For example, the MO leaves have chlorogenic gallic acid, ellagic acid, vanillic and Ferulic acid, and other phenolic acids (Zhu *et al.*, 2020:3). Furthermore, Nascimento (2017:3) reported that the total phenolic acid content in MO leaf extracts was about 170.07 ± 0.43 mg /100 g gallic acid dry weight. The range of these findings was consistent with results from other researchers on the phenolic content of *Moringa oleifera* leaves (Rocchetti *et al.*, 2020:5–7; Zhao *et al.*, 2019:11; Stohs & Hartman, 2015:797). It has been suggested that the antioxidant property of the MO leaves may be associated with their abundant phenolic acid concentration. The antioxidant properties of phenolic acids are related to eliminating free radicals (Kumar & Goel, 2019:3). Therefore, it is connected to inhibiting enzymes and chelating trace metals that produce free radicals (Yener *et al.*, 2020:3). The constant rise in interest between traditional utilities and scientific information on phenolic acids shows that the study of the health benefits of phenolic acids from food products and their applications needs further investigation.

The above section highlights that *M. oleifera* is of essential nutritional value with bioactive compounds such as phenolic acids and flavonoids. These bioactive components have various health benefits which means they can be applied in the food technology processing and various industries. Phytocompounds, particularly flavonoids have been identified to have a strong antioxidant and potent anti-enzyme activity. The *M. oleifera* plant is also reported to have potential anti-tyrosinase activity, an enzyme connected to the enzymatic browning of vegetables and fruits. Thus, the importance of the *M. oleifera*

bioactive compounds can also be related to its potential of inhibiting tyrosinase and reduce browning.

2.4 Tyrosinase / Polyphenol oxidase (PPO)

Tyrosinase, also referred to as polyphenol oxidase, cresolase, diphenol oxidase, or catechol oxidase, is a copper-containing monooxygenase that plays a role in the synthesis of melanin (Figure 2.3) (Zolghadri *et al.*, 2019:280). The enzyme is found in many organisms, including plants, animals, bacteria, and fungi. Polyphenol oxidase has two copper atoms in its active site and catalyses two separate reactions in the presence of molecular oxygen (Singh *et al.*, 2018:66). Firstly, it catalyses the slow hydroxylation of monophenols to diphenols, which results in colourless compounds. At the same time, the second reaction involves rapid oxidation of diphenols to O-quinones, resulting in dark-coloured products or pigments such as melanin (Muñoz-Muñoz *et al.*, 2013:17). The substrates for the enzymes are found in vacuoles, while the enzymes are in the cytoplasm, which means that the reactions can only occur when the components are combined in the presence of oxygen (Ioannou & Ghoul, 2013:310). Over and above, cutting, slicing, and other post-harvesting techniques trigger browning reactions, resulting in flavour alterations. This enzyme is commonly distinguishable in animal or plant tissues, such as avocados, pears, apples, bananas, eggplant, guava, and mushrooms. Thus, the activity of polyphenol oxidase is vital for controlling browning; likewise, essential factors such as the plant tissues, polyphenol involved, pH, and oxygen presence are targeted to prevent enzymatic browning (Tinello & Lante, 2018:73; Shrestha *et al.*, 2020b:11).

The techniques applied to prevent browning are classified into chemical and physical processes. The famous physical methods involved in browning control include blanching, elimination of exposure to oxygen, storage at lower temperatures, microwave, and irradiation treatments (Ioannou & Ghoul, 2013:310–311; Shrestha *et al.*, 2020b:11). Blanching successfully controls enzymatic browning by heat application because enzymes comprise proteins denatured at conditions outside their optimum. However, the heat treatment involved in browning causes the production of unpleasant colour, flavour, and softening of the tissues; thus, it is hardly used for fruits or vegetables that are ingested without cooking. Xiao *et al.* (2017:106) reported that changes in the cell wall polymers and turgor loss cause softening of the tissues after blanching. They further noted that blanching soybeans at temperatures between 80°C, 90°C, and 100°C decreased the hardness as the blanching temperature increased. Fraeye *et al.* (2009:1378) further found that thermal treatment softened the texture of strawberry fruit tissues. When blanching cannot be used due to its undesirable textural changes, browning is prevented by eliminating oxygen and substituting air with inert gas such as carbon dioxide (CO_2) or nitrogen (N_2). Alternatively, edible coatings such as lipids, polysaccharides, protein, or their combination control browning and extend the shelf

life of fruits and vegetables. Pham *et al.* (2023:521) reported that edible coatings regulate the transfer of gases such as CO_2 and N_2, moisture, lipids, and other compounds. Furthermore, high-pressure processing and ultrasound are effective treatments for inactivating PPO without altering fresh fruits' bioactive compounds and sensory attributes (Silva & Sulaiman, 2022:3). Similarly, Liu *et al.* (2020:2–3) reported on the superior ability of sub-zero temperatures to inhibit deterioration, pathogenic microorganisms, and respiratory rate in fruits and vegetables.

Chemical methods are also used to control PPO activity, including chelating agents, antioxidants, acidification, and natural extracts (Vámos-Vigyázó, 2013:312-316; Lim & Wong, 2018:3002-3005). PPO is sensitive to acidity; this means it shows optimal activity at pH 5-7. Thus, the enzyme can be inactivated by lowering the pH using acidifying agents such as ascorbic or citric acid (Nogales-Delgado, 2021:13). Furthermore, sulphate and its derivatives have been proven to significantly retard the enzymatic activity by acting as an irreversible inhibitor of PPO (Eissa *et al.*, 2009:254). Similarly, antioxidant agents, L-cysteine and ascorbic acid, prevent browning by attaching to the intermediates. Oxalic and citric acids successfully retard the activity of PPO by their copper-chelating activity. Copper chelating agents suppress enzymatic browning by forming complexes with the PPO substrates and copper in the enzyme (Zolghadri *et al.*, 2019: 280–281). The Figure 2.3 below narrates the browning mechanism and how different chemical treatments prevent PPO activity. Figure 2.4 also demonstrate the hydroxylation and oxidation catalysed by PPO (Queiroz *et al.*, 2008:362).

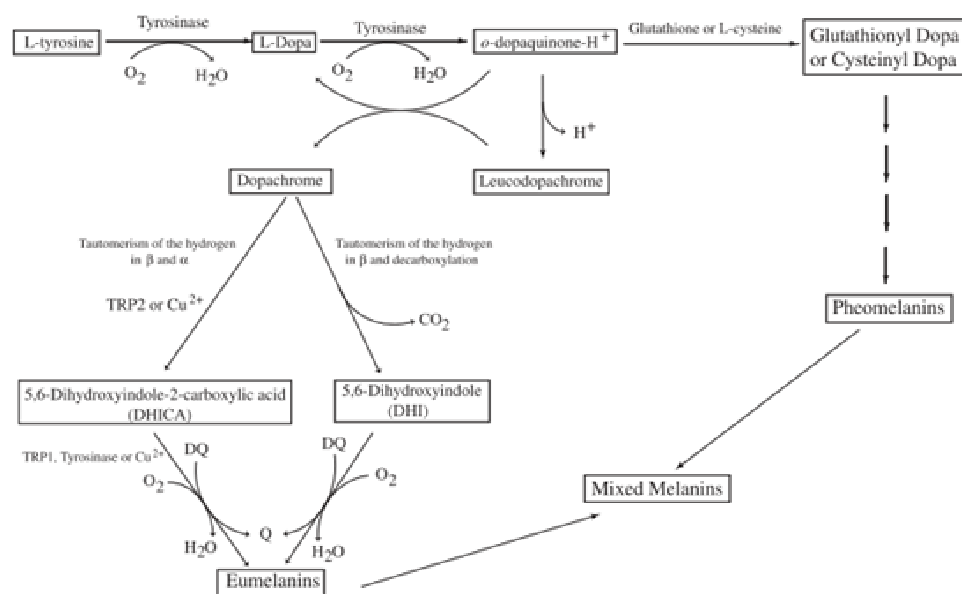

Figure 2.3 The synthesis of melanin pigment from the multifunctional copper-containing enzyme tyrosinase

Sourced from Zolghadri *et al.* (2019:280).

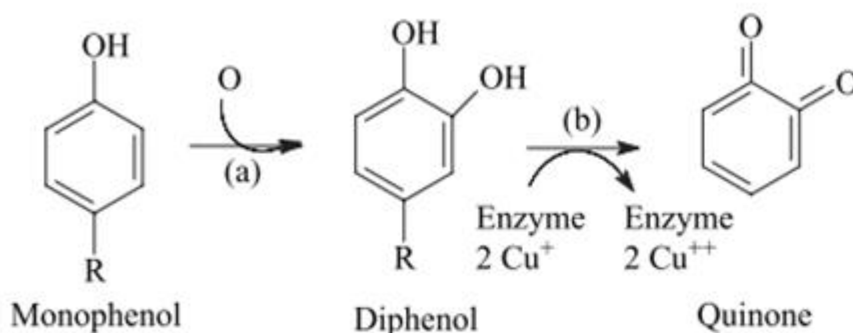

Figure 2.4 Reactions of hydroxylation and oxidation catalysed by PPO.
Sourced from Queiroz *et al.* (2008:362).

2.5 Browning of fruits & vegetables

Browning is the gradual shift of colour of food items from white to brown or dark brown over time (Moon *et al.*, 2020:1). The browning reactions in food are classified into enzymatic and non-enzymatic browning, depending on the mechanism. Non-enzymatic browning reactions produce brown-coloured products through a chemical reaction involving a single compound or several food constituents without enzymes. These include caramelisation, Maillard reaction, and ascorbic acid oxidation (Ibarz *et al.*, 2000:1162; Manzocco *et al.*, 2000:340). Since food comprises complex constituents, non-enzymatic browning reactions occur in clusters rather than different reactions (Hemmler *et al.*, 2018:383).

Conversely, enzymatic browning can be related to polyphenol oxidase, catecholase, tyrosinase, cresolase, and phenolase depending on substrate specificity in a copper-containing enzyme found in a wide range of organisms, including plants, animals, bacteria, and fungi. In plants, the enzyme plays a vital role in chloroplasts' defence mechanisms, flavour enhancement, and oxygen level regulation (Aylward & Haisman, 1969: 4–5; Zhang *et al.*, 2001: 46). The oxidative reaction involves oxidase and peroxidase, where browning is observed during post-harvesting technologies such as transportation, preparation of fresh-cut fruits, drying, freezing, and storage. Enzymatic browning is beneficial in some foods such as cookies, bread, soy sauce, black tea, and raisins to produce unique colours (Karadeniz *et al.*, 2000:5343; Rada-Mendoza *et al.*, 2004:167–168). However, enzymatic browning somehow induces unwanted variations in appearance, taste, and nutritional value, which can compromise the quality of the product (Panadare & Rathod, 2018:431).

Fruit and vegetables possess high health benefits for consumers because of their high concentration of fibre, vitamins, and antioxidant compounds (Slavin & Lloyd Beate, 2012:506). However, enzymatic browning and microbial attacks may occur during cutting, drying, freezing, and storage. These mechanical processes and temperature fluctuations result in damaging the vascular tissues. In response to the tissue damage, the exposure of polyphenol oxidase and other phenolic compounds to oxygen promotes the oxidation of phenols into quinones (Oms-Oliu *et al.*, 2010:142–143). After that, an insoluble brown pigment, melanin, is formed from the polymerisation of quinones and their derivatives through multiple reactions. In this context, the primary oxidative response is enzymatic browning, which involves polyphenol oxidase and peroxidase (Hithamani *et al.*, 2018:4356–4357; Jiang *et al.*, 2016:153).

2.6 The mechanism of browning control

2.6.1 Acidulants

Acidulants such as phosphoric, citric, malic, and ascorbic acids retard browning by decreasing the pH value to create acidic conditions. The optimum pH for tyrosinase is from 6 to 7; therefore, a decrease in the pH results in inactivation of the enzyme (Chang, 2009:2444). The anti-browning effect of ascorbic, citric, and cysteine in apples, bananas, and mushrooms at pH 3.5, 4, and 4.5 improved the browning during storage (Arpita *et al.*, 2010:550). Ascorbic acid successfully reduced the pH of high hydrostatic pressure-processed mango puree at pH 3.5 (Guerrero-Beltrán *et al.*, 2006:582). Similarly, after treatment, the anthocyanin content, texture, ascorbic acid retention, and colour of frozen strawberries combined with 0.4% citric and 1% Ca lactate dip (Abd-Elhady, 2014:74). Above all, chemical treatments control enzymatic browning by different mechanisms, so acidulants can be combined with chelating or reducing agents to improve their anti-browning properties (Hithamani *et al.*, 2018:4359).

2.6.2 Reducing agents

Reducing agents, also called antioxidants, can suppress browning by reacting with oxygen. These compounds can also break the chain reaction, significantly inhibiting the formation of melanin by responding with the intermediate products of the response. Environmental factors such as light, atmospheric composition, pH, and temperature influence the success of reducing agents as tyrosinase inhibitors (Singh *et al.*, 2018:70-71). The mechanism of reducing agents involves the chemical reduction of dopaquinone. Thereupon, reducing agents such as ascorbic acids retard melanogenesis because they reduce o-dopaquinone to dopa, thus retarding melanin and dopachrome formations (Chang, 2009:2444). Antioxidants widely studied for preventing plant browning include ascorbic acid, cinnamic acid, erythorbic

acid, glutathione, and N-acetyl cysteine (NAC). Ascorbic acid is an anti-browning agent whose mechanism depends on its reducing ability.

In contrast, ascorbic acid does not directly interact with the enzyme but reduces the number of oxidised substrates to prevent browning. Ascorbic acid at 0.2% m/v significantly reduced the formation of the brown colour in potatoes after cooking (do Nascimento & Canteri, 2020:380). Furthermore, Tan *et al.* (2015:631) reported that adding ascorbic acid reduced the browning of thermally processed green coconut water. The individual use of N-acetyl cysteine or glutathione at 1% (w/v) resulted in the lowest relative PPO activity in fresh-cut Fuji apples during storage for 14 days (Rojas-Graü *et al.*, 2008:267). N-acetyl cysteine, a sulphur-containing polypeptide, was also found to have more significant anti-browning activity in minimally processed pear slices than calcium propionate, ascorbic, and citric acid.

2.6.3 Chelating agents

Chelating agents reduce browning by reacting with PPO or its substrates to form complexes, causing a decrease in enzymatic browning. The effectiveness of chelating agents is related to their ability to form complex structures with Copper (Cu) in the enzyme's active sites. Citric acid, the primarily used chelating agent in processing fruits and vegetables, inactivates the enzyme's activity by reducing the pH and chelating Cu at the active sites of PPO. Additionally, among all reported chelating agents, kojic acid is a famous chelating agent extracted from secondary fungal metabolites such as *Penicillium ssp* and *Aspergillus spp.* For example, Shah *et al.* (2017:156–158) reported that treating litchi fruit with 6 mmol L^{-1} kojic acid maintained the activity of antioxidative enzymes and delayed browning. However, kojic acid is hardly used in the food industry because its large-scale production is difficult and costly. Similarly, oxalic, cinnamic, benzoic acids and other carboxylic acids can also form complex structures with copper at the PPO active sites because they bond with copper ions. Carboxylic acids have been reported to have anti-browning properties because of their pH-lowering and metal-chelating ability (Moon *et al.*, 2020:6).

According to He & Luo (2007:4), combining an acidulant, a chelating agent, and a chemical acidulant results in a successful anti-browning agent for fresh-cut fruits and vegetables. Likewise, among a group of anti-browning compounds, namely ascorbic acid kojic acid, cysteine, oxalic acid, oxalacetic acid, and NAC, oxalic acid and ascorbic acid showed a synergistic effect against browning in apple slices (Son *et al.*, 2001:23). Özŏlu & Bayndrl (2002:215) investigated the impact of chemical anti-browning agents in cloudy apple juice. The effect of sorbic acid, cinnamic acid, ascorbic acid, L-cysteine, sorbic acid, and b-cyclodextrin on the browning and enzyme activity of cloudy golden apple juice was examined after storage at room temperature for one day. It was concluded that the combination of L-cysteine, cinnamic, and ascorbic acid resulted in optimum inhibition of browning activity.

Ethylenediaminetetraacetic acid (EDTA) and its salts are chelating agents in food processing. EDTA and phosphates have been studied for their anti-browning activity as chelating agents (Vámos-Vigyázó, 2013:313; He & Luo, 2007:3–4). A model system was used to investigate the ability of tyrosinase to catalyse the conversion of L-dopa to dopachrome by observing the potent absorption at 475 nm in the presence of organophosphorus compounds. It was reported that the compounds were significantly more active against tyrosinase than ascorbic acid, even though the mechanism of action was dependent on multiple factors (Darroudi *et al.*, 2020:119). A study was conducted to evaluate the potent tyrosinase inhibition by Triazole through green nano catalyst (TMS-EDTA). It was concluded that the derivatives of thiazolidine-triazole derivates had a promising activity against tyrosinase with IC_{50} values ranging from 5.90-9.81µM. However, chelating agents are generally incorporated with other anti-browning compounds to control browning successfully.

2.6.4 Complexing agents/Mixed type Inhibitors

Numerous compounds show many mechanisms for anti-browning effects even though anti-browning agents are established according to their functioning characteristics. These compounds show various mechanisms for their anti-browning effects by interacting with the deoxy form of PPO in the absence of substrates; hence, a decrease in enzyme activity is noted. However, in the presence of substrates, complexing agents bind onto the met structure at the active sites of PPO, subsequently as enzyme inhibitors. For instance, Cyclodextrins have 6, 7, and 8 glucopyranose units linked to α glycosidic bonds, a hydrophobic cavity, and can form complexes with enzymes. The water molecules in the enzyme are repositioned from the hydrophobic cavity, resulting in apolar-apolar solid bonding between the enzyme and cyclodextrin. Therefore, the bond between PPO and cyclodextrin reduces the ability of the substrate to interact with the enzyme, causing inhibition of PPO (Casado Vela *et al.*, 2006:634–635). Researchers also investigated the synergistic effect of cyclodextrins and ascorbic acid in a kinetic model of apple juice, where López-Nicolás *et al.* (2007:1165) reported that maltosyl-β-CD enhanced the natural antioxidant capacity of ascorbic acid as a secondary antioxidant hence improving the colour of apple juice.

Furthermore, Singh *et al.* (2015:469) reported that β-Cyclodextrin showed mixed-type PPO inhibition and had a significant anti-browning effect in pineapple, potato, pear, and apple fruit juices. Research has also focused on extending the shelf life of fresh-cut fruits and vegetables from natural antioxidants. Edible coatings such as chitosan assist with preserving vegetables and fruits because of their ability to control moisture, oxygen, and Carbon dioxide. It was reported that chitosan coating successfully retarded the PPO monophenolase and diphenolase activity of PPO with pyrogallol and hydrochloride substrate, respectively, in pomegranate stored at cold temperatures (Sajedi, 2013:372–373). On the other hand, Maclurin, a yellowish phenolic compound, elevated the antioxidant effect and resulted in

long-term browning termination in potato supernatant by forming aromatic interactions and hydrogen bonds with the binding pockets (Mi Moon *et al.*, 2019:345).

2.6.5 Browning inhibitors from Plant extracts

Plant polyphenols are classified as secondary metabolites with multiple biological activities. They are responsible for the red/blue colour characteristics and protection against herbivores, pathogens, and UV radiations. According to Paudel *et al.* (2020:1), secondary metabolites in plant extracts are safer than chemical additives used in controlling the browning of beverages and food products. However, Lim *et al.* (2019:2) reported that synthetic inhibitors had a slightly higher anti-browning effect in sweet potatoes than natural browning inhibitors extracted from fruits and vegetables. Research has been conducted to demonstrate the relationship between the structure of polyphenols identified from plants and their inhibitory activity against PPO (Putnik *et al.*, 2017:2; Siddhuraju & Becker, 2003:2151; Moon *et al.*, 2020:10; Niu *et al.*, 2020:9–10). Tyrosinase accepts multiple polyphenols as substrates depending on the position or presence of an additional subsistent polyphenol that may act as an inhibitor (Chang, 2009:2444–2445). According to Zolghadri *et al.* (2019:284), several plant polyphenols such as flavonoids, phenolic acids, terpenoids, quinone derivatives, and anthocyanidins have the potential to prevent browning activities in food products synergistically. These compounds can also be considered natural food preservatives because they have anti-browning, antioxidant, and anti-microbial properties (Carocho *et al.*, 2014:380–382).

Since phenolic compounds are widely spread in plants, researchers have focused on determining the anti-tyrosinase combinations from plant extracts. For instance, the anti-tyrosinase of multiple plant extracts has been investigated (Taherkhani, 2017:743–744; Abdillahi *et al.*, 2011:500–502; Lall *et al.*, 2016:5955–5956). Their results suggest that the phenolic compounds found in extracts of 4 different *Podocarpus* species used to treat fever in South Africa successfully retarded tyrosinase activity dose-dependently. They further showed that *Artemisia aucheri* oil and *Greyia radlkoferi Szyszyl* extracts showed antioxidant and anti-tyrosinase activity, respectively. The studies show that the diverse plant extracts responsible for anti-tyrosinase activity are sources of steroids, alkaloids, and phenolic acids with similar structures. Among all polyphenols, the major and minor groups of flavonoids and their derivatives have been established to be potent tyrosinase inhibitors. Şöhretoğlu *et al.* (2018:168–169) found that the A and C rings of the flavonoids' structure play a significant role in their competitive inhibition against tyrosinase. They further reported that quercetin, kaempferol, and their derivatives successfully retarded browning.

Furthermore, catechin, a flavone extracted from an ethanolic extract of *Distylium racemosum*, showed a high IC_{50} of 30.2 mg/mL (Ko *et al.*, 2011:1454). The anti-tyrosinase activity of two curcuminoids, desmethoxycurcumin and curcumin from the *Artocapus altilis*

methanolic extract, showed significantly higher activity against tyrosinase than kojic acid. Therefore, this section indicates that natural compounds in plants have an exceptional potential as natural tyrosinase inhibitors. Figure 2.5 demonstrates some of the natural tyrosinase substrates.

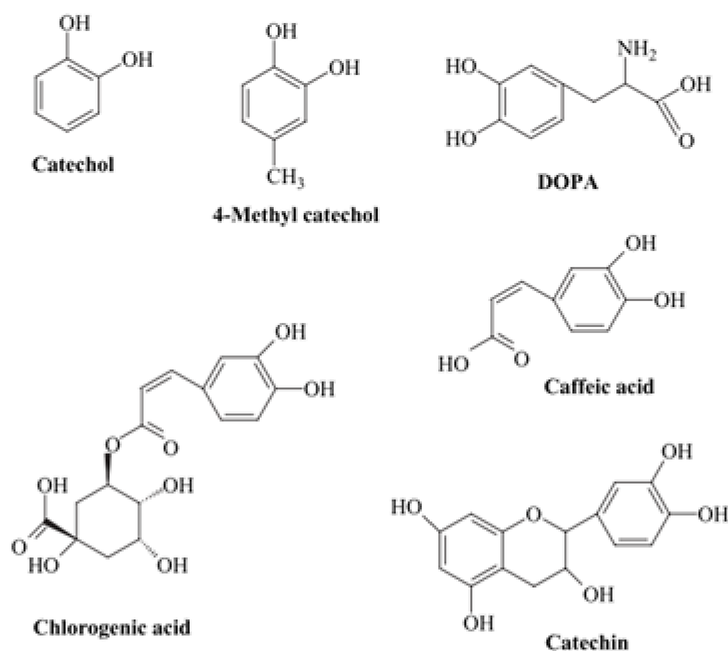

Figure 2.5 Structures of PPO natural substrates
Sourced from Queiroz *et al.* (2008:363)

2.7 Importance of Apples in human nutrition

There has been an increased understanding and appreciation of the connection between human health and consuming fruits and vegetables. Researchers have shown interest in the bioactive compounds in fruits and vegetables, such as phytochemicals (Karasawa & Mohan, 2018: 339–341; Dhalaria *et al.*, 2020:6–16). These plant-based bioactive components are likely to regulate the occurrence of infections such as pulmonary disorders, cardiovascular disease (Wang *et al.*, 2014:1), diabetes, and cancer. According to Frankowska *et al.* (2019:109), consuming fruits is crucial for essential macronutrients and prevents malnutrition. They also stated that fruits can lessen the risks of premature deaths and chronic illnesses. Therefore, consumers need to prioritise the consumption of fruits and vegetables to prevent chronic diseases and sudden death.

Apples are one of the fruits in high demand because of their seasonal availability and the widespread of the fruit geographically. The consumption of apples has been linked to reduced risks of cardiovascular, coronary heart diseases, and stroke because of their high fibre content (Hyson, 2011:413). Furthermore, apples have been reported to influence human blood pressure, lipid content, and vascular functioning. Apples' cardioprotective effect

is associated with high fibre and polyphenol content (Bondonno *et al.*, 2017:243). The macro composition of apples mainly consists of carbohydrates, water, fructose, and other sugars. The micronutrients reported in apple cultivators (*Malus domestica*) by researchers include polyphenols, minerals, and vitamins C & E. These nutrients are also available in apple secondary products such as apple cider vinegar, apple juice, and pomace (Schieber *et al.*, 2001:270). Table 2.2 represents polyphenols identified in apple pomace samples using high-performance liquid chromatography (HPLC) (Schieber et al., 2001:270).

Table 2.2 Polyphenol compounds from apple pomace

Compound	Commercial pomace	Apple pomace cv. Jonagold		Apple pomace cv. Elstar	
		Air dried (110°C)	Lyophilized	Air dried (110°C)	Lyophilized
5-HMF	220	27	–	21	–
Procyanidin B1	Trace	–	–	–	–
Catechin	14	7.8	9.4	12	14
p-Coumaroyl glucose[b]	10	–	–	–	–
Chlorogenic acid	450	79	54	40	33
p-Coumaroyl quinic acid[b]	34	6.2	4.7	9.0	7.9
Procyanidin B2	47	120	160	93	130
Caffeic acid	8.2	–	–	–	–
Epicatechin	77	160	190	140	160
p-Coumaric acid	3.0	–	–	–	–
Quercetin-3-galactoside	360	200	210	260	280
Quercetin-3-glucoside	130	31	31	75	81
Quercetin-3-xyloside	160	99	100	76	93
Quercetin-3-rhamnoside	230	350	340	110	120
Phloridzin	910	390	350	400	390
Quercetin	67	17	19	23	3.5
Phloretin	Trace	0.7	1.0	1.4	1.1

[a] Sample preparation and HPLC determination were performed in duplicate; –, not detectable.
[b] Tentatively identified; calculated as coumaric acid.

Sourced from Schieber *et al.* (2001:270).

2.8 Preservation of Fruits

Preserving fruit is vital to minimise spoilage and organic waste and ensure availability by extending the shelf-life. Multiple preservation methods are practised in the food industry, such as canning, juicing, freezing and modified atmospheric packaging, and hot air drying (Barrett & Lloyd, 2012:8). Drying can also be used to make vegetable snacks, condiments, and instant foods to balance the demand and supply of agricultural products throughout the year. Moreover, water extraction from farm products prevents enzymatic browning and microbial decay (Wang *et al.*, 2021:2). Conventional drying is commonly used to preserve fruits by reducing the water activity. It involves removing a concurrent amount of moisture via heat transfer and decreasing the water activity. Drying fruits reduces the chances of deterioration from chemical and microbiological reactions, making the products available all year round (Mounir & Allaf, 2008:454). The preservation of dried fruits is because of low water activity and pH. Therefore, dried fruits are declared as foods that can be stored at ambient temperatures (Alp & Bulantekin, 2021:1334). Hot-air drying is commonly used

because of its simplicity and low operating costs. However, hot air drying has some side effects, such as an extended drying time, and it degrades the nutritional and physicochemical qualities of food products (Pérez-González *et al.*, 2023:5). Alternatively, freeze-drying prevents changes in the nutritional value and physicochemical properties such as shrinkage, colour, and texture of fruits. However, freeze-drying is expensive, and the dehydration process takes an extended period (Ai *et al.*, 2022:2). To achieve high-quality products with the desired nutritional value, multiple drying techniques, such as microwave and solar drying technologies, are used. The composition of foods, relative humidity, volatile compounds, drying temperature, and air velocity influence the drying method (Thamkaew *et al.*, 2021:1764). Therefore, the drying parameters are essential for choosing an appropriate drying mechanism and obtaining a good quality product.

2.9 Convective Hot air drying

Hot air drying is one of the commonly used preservation techniques for extending the shelf-life of fruits while retaining the nutritional value of fruits because of their profitable benefits. The process involves applying hot air to reduce the water activity to a percentage that retards the enzymatic, non-enzymatic reactions and microbial growth. It is profitable because dried fruits have an extended shelf life with minimal handling, packaging, and transportation requirements. Some benefits of hot-air drying include using non-toxic, hot air to produce hygienic products more rapidly than sun drying (Inyang *et al.*, 2017:48–50). In contrast, hot air drying causes some food products unpleasant organoleptic, nutritional, physical, and mechanical modifications. Onwude *et al.* (2022:176) revealed that 70% of vitamin C, phenolic compounds, and carotenoids in dried fruits and vegetables were reduced by hot air drying.

Similarly, Dukare *et al.* (2022:3901–3905) reported that an increase in the drying temperature of mango peels significantly decreased their pH, total phenolic, ascorbic acid, and antioxidant activity. They noted that the drying process preserved the mango peels by maintaining an acceptable microbial account and storage properties at ambient temperatures with the potential of being used as a functional food all year round. Zhu *et al.* (2022:1005) reported combining hot hair drying and CO_2 puffing drying improved apple chips' sensory and physical properties. Therefore, efficient results can be obtained from hot-air drying in combination with other treatments to get quality dried fruits and vegetables.

Hot-air drying is an energy-intensive process, but it is flexible and straightforward to operate and construct (Geng *et al.*, 2022:1). Mechanical dehydrators are popular in convectional drying where drying parameters such as the nature of the product, process of drying including the pre-treatment technology, size of fruits, air velocity, time, and temperature are controlled. Depending on the size or type mechanical dehydrators are equipped with cabinets, belts, tunnel dryers, and chambers depending on the type of dryer and size.

2.10 Freeze drying

The principle of freeze-drying, also known as lyophilisation, uses the conversion of frozen water into water crystals to preserve heat-sensitive fruits and vegetables (Joseph *et al*., 2015:517). The bioactive components and nutrients remain in huge quantities during this process because freeze-drying is done at low temperatures. Moreover, freezing water during the procedure reduces shrinkage by preserving the original structure while retaining the aroma, texture, flavour, vitamins, and minerals in tropical fruits (Marques *et al*., 2009:1232). The significant advantage of the preference for freeze-drying compared to hot air drying and other conventional methods is the vast mass reduction for convenient handling and storage. Freeze-dried products have other unique properties, such as adding water for reconstitution and the high yield of final products after drying, saving money and time (Ciurzyńska & Lenart, 2011:166).

Regardless of the benefits, freeze-drying is expensive because it requires frequent maintenance and high energy. It was reported to use almost double the power of hot air drying to remove 1 kg of water from a product. However, the operational costs can be reduced by combining freeze-drying with other technologies, such as freezing fruits before freeze-drying, to reduce the processing time and energy. Furthermore, the Pulse electric field (PEF) significantly reduced the freeze-drying time of apple slices when compared to untreated samples. Multiple authors have investigated the effect of freeze drying on the quality of fruits and to determine the suitable treatment parameters (Izli *et al*., 2017:605–606; Chen *et al*., 2007:1007–1009; Athmaselvi *et al*., 2014:8). Suherman *et al*. (2023:182) reported that freeze-drying mango resulted in typical moisture content, colour, texture, rehydration capacity and total phenolic content. They further concluded that freeze-drying lasts 13 hours while retaining mango quality and structural properties. Similarly, it was reported that freeze drying is an effective treatment for pre-treated banana slices, resulting in reduced moisture content, lower microbial activity, and decreased enzymatic browning (Milani *et al*., 2020:3769). Freeze dryers have 4 primary components: the condenser, a source of heat, a vacuum pump, and a drying chamber (Garcia-Amezquita *et al*., 2016:105) (Figure 2.6).

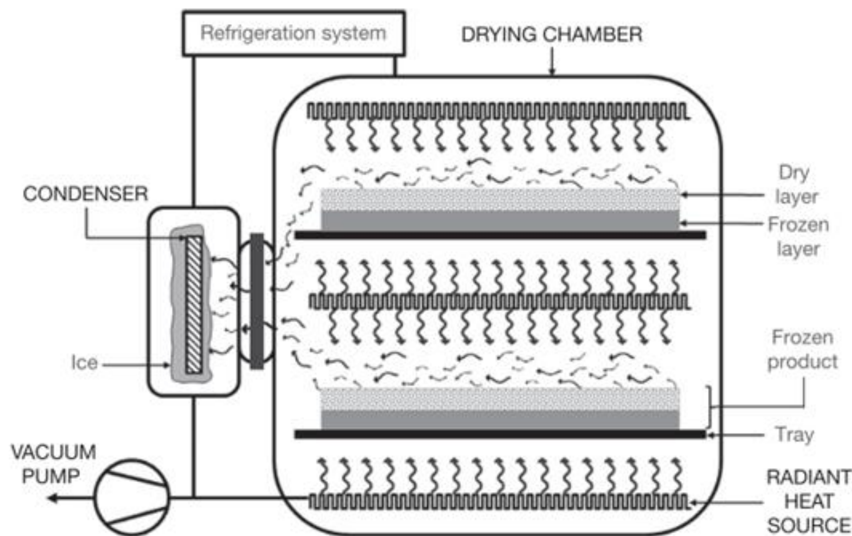

Figure 2.6. The essential components of a freeze dryer
Adapted from (Garcia-Amezquita *et al.*, 2016:105).

Each element and operation is essential to ensure maximum freeze-drying results for all products. The pre-frozen product is placed in the drying chamber that is always vacuum tightened in shelves designed to allow heating and cooling inside the chamber. In addition, the vacuum pump assists with reaching the recommended vacuum level required to remove gases that cannot changed into solid or liquid phase from the drying chamber (Garcia-Amezquita *et al.*, 2016:104–105; Gallé, 2001:1467–1468). Once the frozen product reaches the high vacuum state, the heating sources supply heat for sublimation (Di Matteo *et al.*, 2003:267–268). The heating source provides heat at temperatures between 30 - 150°C, depending on the moisture content and nature of the product undergoing lyophilisation. Since the principle of freeze drying is based on sublimation, the condenser collects the water released from the product in the form of gas to allow sufficient cooling (Joseph *et al.*, 2015:353). The gas is converted into ice crystals to facilitate water removal from the system. The concept and mechanism of freeze-drying have been proven to be an alternative to hot air drying by drying foods to extend the shelf-life without altering the quality. Thus, knowledge of foods' principles and drying characteristics can open new channels for freeze drying and obtaining quality products with a decent shelf life.

2.11 Microwave drying

Microwave drying involves the connection of microwave energy with food materials, leading to the generation of heat through molecular activation. This drying technique is also referred to as microwave convection because it involves passing air over the surface of foods (Guiné *et al.*, 2011:491-492). The mechanism of microwave drying is based on two major

interactions namely ionic conduction and dipolar rotations. It is considered a power-efficient convection because the temperature of the air passing through the products can be adjusted, resulting in shorter drying periods (Çelen, 2019:4). Furthermore, the temperature adjustments of the air are dependent on the nature and composition of the food product. Some have demonstrated the benefits of using microwave-assisted drying of fruits and vegetables (Lv *et al*., 2019:59-60; Zhang *et al*., 2006:524). The reduction in the falling rate during the drying of fruits and vegetables is the most beneficial of microwave drying when compared to other drying methods. Some of the advantages of microwave drying involve higher drying rates and consumption of less energy which means lower operating costs. Özcan *et al*. (2020:233) also reported that using the microwave oven to dry fruits resulted in better quality fruits than other conventional drying methods. Özcan *et al*. (2020:233) investigated the nutritional value of kiwi after using conventional and microwave ovens. They reported that samples dried with microwave treatment had the highest antioxidant activity than conventionally dried and fresh fruits. Thus, these findings imply that microwave drying is more useful than conventional drying methods. Tepe & Tepe (2020:3047) also compared the effect of conventional and intermittent microwave drying of apple slices. They reported that hot air drying resulted in longer drying time, less effective moisture diffusivity and slower drying times when compared to intermittent microwave drying. The effect of pre-treatments and different drying methods of button and oyster mushrooms was investigated (Walde *et al*., 2006:108). They reported that the drying time for mushrooms to reach 2% moisture was significantly lower for microwave drying than vacuum, fluidised bed and cabinet moisture drying. Thus, microwave drying seems to be a promising method for drying fruits and vegetables for lower drying times and good quality.

2.12 Solar drying

The concept of solar driers is based on the sun's energy in various ways to develop thermal energy. The conversion is achieved through the major component of any solar system, solar energy collectors (Fernandes & Tavares, 2024:15-17). Solar energy collectors absorb solar radiation, converts it into heat fluids such as air, water, and oil that normally flows through the collector. Thereafter, the collected solar energy in heat fluid is transferred into a thermal energy storage container which can be drawn during operation of driers depending on the design and functionality of equipment (Kalogirou, 2004:240). This technology aims at removing moisture from food products' interior through evaporation by conduction or convection depending on the configuration and design of the system. This method is a solution to pollution, climate change and depletion of fossil fuels as an efficient way to alternative power sources. Given the environmental challenges and energy supply in the planet, solar energy is used to replace fossil fuels because it is a renewable, clean, and inexhaustible source of energy (Ebhota & Jen, 2020:103-104). Solar driers are widely used in

the agricultural industry for dehydration of foods without destroying the environment. One of many advantages of solar drying is that this preservation method does not require any addition of preservatives in food products which allows ingestion without allergic reaction concerns. Furthermore, solar drying is a more efficient alternative for rural areas where power is not supplied regularly (Elicin & Sacilik, 2005:207). Solar energy has been used to dry fish, grains, fruits, and vegetables in rural areas because of it is an efficient source of power. It can be coupled with other pre-treatments prior drying to retain the physicochemical, nutritional and sensory characteristics of dried foods. Da Silva *et al.* (2022:1-2) investigated the use of solar drying after pre-treatment by immersion in 1% citric acid, water and 10% lemon juice. They reported solar drying as an economical sustainable drying method to extend the shelf-life of fruits. Previously, (Sarsilmaz *et al.*, 2000:117) tested the ability and performance of a rotary column cylindrical dryer supported with solar energy for drying apricots. The authors reported that the collaboration of the rotary column cylindrical dryer with an air solar collector reduced the rotation of drying chamber rate, drying time while increasing the drying rate. Thus, the incorporation of the air solar collector in fruit driers could gain manufacturers gain on both labour and time. Furthermore, Guiné *et al.* (2011:55) explored different methods of drying pears such as solar dryer, stove and a tunnel dryer. The findings of the study showed a moisture reduction of about 60% when both the solar stove and drier was used. Therefore, solar drying of fruits is widely used as a time and energy saving drying method in the food industry.

2.13 Storage of dried fruits

Drying is one of the ancient methods of preserving food products by reducing the water activity. Maintaining the initial water activity during storage is essential to preserve the quality. Even though drying is an excellent preservation method, dried fruits have an active surface and are very sensitive oxidation humidity during storage (Amit *et al.*, 2017:8-10). Thus, it is important to ensure the packaging and storage conditions are carefully considered to prevent nutritional and physicochemical changes. Modified internal atmospheric conditions and impermeable packaging is mostly used to preserve food products until consumption (Miranda *et al.*, 2019:1). Researchers has been done with various fruits to determine the drying conditions, preservation, packaging, and storage of dried fruits to increase the shelf-life (Akharume *et al.*, 2018:61; Elmaci *et al.*, 2008:147; Miranda *et al.*, 2014:566; Chavan *et al.*, 2010:381). The packaging used for dried fruits must be impermeable to gases, volatiles and a good barrier against water or vapour. For these to be controlled, a packaging system that includes oxygen scavengers, carbon dioxide emitters and time-temperature monitoring devices. They further include microbial growth indicators and biosensors to ensure that packaging is proven to sustain food products until end of life (Amit *et al.*, 2017:34-38). The most important active packaging systems involve the use of oxygen scavengers, humidity

and ethylene absorbers, carbon dioxide and ethanol emitters, and antimicrobial active packaging systems. Intelligent packaging devices include biosensors, as well as time–temperature, oxygen, carbon dioxide, and microbial growth indicators (Nayik & Muzaffar, 2014:34-36). Thus, the packaging and storage condition of dried fruits is important to maintain the quality until end of life.

Korese *et al.* (2022:1) investigated the shelf-life of dried fruit powder in different single laminated paper bags with low-density, high-density polyethylene as their primary material at ambient temperatures. They reported that the water activity, pH, microbial count, and nutritional value of the powdered fruits increased. Their microbiological growth findings also suggested that the product could be stored at ambient temperatures for 3 months without an acceptable loss in the quality. Thus, drying improves the shelf-life by reducing the microbial load and this preservation method required suitable storage conditions for minimal fungal loads and maintaining the quality of the food products.

2.14 Conclusion

This chapter started with exploring the importance of the *Moringa oleifera* leaf plant and its structural parts. It highlighted the structural parts, versatility, medicinal, nutritional, and phytochemical composition. The context of this chapter was based on defining important concepts related to this study. Polyphenol oxidase (tyrosinase) and its importance on browning was also explored. The tyrosinase activity which results to browning can be controlled by both chemical and physical methods. Emphasis was placed on the most common anti-browning measures such as reducing agents, acidulants, inert gas and application of edible coatings. Moreover, Literature from various authors has linked phytochemical rich plants such as flavonoids to potential anti-tyrosinase, and anti-oxidant activity because of their activity against pathogenic microorganisms. Furthermore, plants that are rich in phytochemicals are promising because of their antioxidant activity. Multiple studies demonstrated the effectiveness of phytoconstituents such as phenolic acids and flavonoids which connects them with natural preservation of foods. In this context, the phytochemicals were considered as potential natural preservatives in dried fruits coupled with drying. Fruits such as apples are essential in promoting health and preventing chronic diseases, hence it is important to preserve these fruits. The literature in this section highlighted the importance of preserving apples by exploring different drying techniques like convective, solar, microwave, solar and storage of the dried fruits. Overall, this chapter was based on understanding the phytoconstituents of *M., oleifera*, their potential in controlling the activity of tyrosinase and how its extracts can be used as natural anti-browning agents. Chapter three focuses on the research methodology and related aspects.

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CHAPTER 3:

PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITY OF *MORINGA OLEIFERA* LEAF POWDER EXTRACT

ABSTRACT

Moringa oleifera, a naturally growing plant in South Africa, has been reported to have antioxidant properties, bioactive chemical constituents, and outstanding nutritional value. This chapter aims at characterising and determining the phenolic composition of the acetone, aqueous, ethanol and methanol *Moringa oleifera* leaf powder extracts based on the structure and functionality. It also aims at determining the antioxidant activity and establishing the 50% inhibitory concentration (IC_{50}) of the *Moringa oleifera* leaf powder extracts (acetone, aqueous, ethanol and methanol) against tyrosinase enzyme. The *Moringa oleifera* leaf powder aqueous extract (MOAQ) was obtained by maceration in boiling water at room temperature to allow maximum extraction of polyphenolic compounds. The extraction of the *Moringa oleifera* leaf powder ethanol (MOET), methanol (MOME) and acetone (MOAC) extracts were facilitated by ultrasound extraction after macerating in individual solvents for 4 hours. A liquid chromatograph-mass spectrometry (LC-MS) was used to characterise the phytocompounds of the MOAC, MOAQ, MOET, and MOME extracts from the *Moringa oleifera* leaf powder (MOLP). The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ferric-reducing antioxidant power assay (FRAP), 2,2'-azino-di-3-ethylbenzthialozine sulphonate (ABTS) and oxygen radical absorbance capacity (ORAC) assays were explored to determine the antioxidant activities of the MOLP extracts. The anti-tyrosinase activity of the MOLP extracts was determined using a microplate reader (Spectrostar nano, Lasec) with kojic acid used as a positive control. A total of 23 phytochemicals such as flavonoids, phenolic acids, amino acids, quinones and fatty acids were identified from all the MOLP extracts. There was a high content of flavonoids in particular such as rutin (231.6 mg/L), isoquercitrin (216.6 mg/L), hyperoside (217.5 mg/L) and others. The MOET and MOME extracts showed significantly (p = 0.001) higher antioxidant activity than the MOAC and MOAQ extracts. The IC_{50} for all MOLP extracts ranged from 34.66 (MOAQ) to 71.29 µg/ml (MOET). The MOAQ showed a significantly (p = 0.001) stronger inhibition against tyrosinase. The anti-tyrosinase activity of the MOLP extracts may be associated with the presence of phenolic compounds such as flavonoids. Therefore, *M. oleifera* is a potent anti-browning agent and can be used in various dried fruits as a preservative.

3.1 Introduction

Tyrosinase enzyme, also known as polyphenol oxidase (PPO), catalyses the hydroxylation of monophenols and customising them into diphenols, thereafter catalysing the oxidation of *o*-diphenols to bring about *o*-quinones (Di Petrillo *et al.*, 2016:4). PPO belongs to the third class of the copper protein family, with two copper ions separately linked to a different set of three histidine residues with active sites (Si *et al.*, 2012:999). The polymerisation of the highly reactive *o*-quinones produces black, brown, or red coloured pigments, which cause enzymatic browning (Muñoz-Pina *et al.*, 2020:7966). The enzyme catalyses the production of melanin pigmentation in the skin, browning in fruits and vegetables, and the formation of melanin pigment in plants and animals (Kim & Uyama, 2005:1708).

The produced melanin plays a significant part in UV protection and is a critical component of the skin's defence mechanism against hazardous substances (ElObeid *et al.*, 2017:516). However, the overproduction of melanin may cause several skin disorders, such as ephelides, melasma, senile lentigos, and post-inflammatory hyperpigmentation (Kim & Uyama, 2005:1707; Di Petrillo *et al.*, 2016:2). In this context, this reaction is necessary and beneficial in some food products, such as chocolate and tea; however, browning is undesirable in fruits and vegetables with high polyphenols (Ioannou & Ghoul, 2013:311; Akharume *et al.*, 2018:61). Additionally, the tyrosinase activity results in enzymatic browning, altering food products' colour, organoleptic and nutritional value. Browning is crucial in food processing as the appearance and colour of the food products mainly determine the consumers' acceptance.

Consequently, tyrosinase is responsible for enzymatic browning, and its inhibitors have become more essential targets for the food industry (Hamdan *et al.*, 2022:2). However, the currently available tyrosinase inhibitors, such as sulphites and heat treatment, may destroy sensitive nutrients such as vitamins and be hazardous for asthmatic individuals (Moscetti *et al.*, 2019:403). Therefore, there is an ongoing search for improved inhibitors derived from natural sources devoid of adverse side effects. The search for natural products that can face the rising necessities of food in the context of food preservation is a significant challenge in Africa and the food industry. Several studies have been conducted to address nanotechnological solutions to identify a variety of naturally occurring compounds in higher plants that have antioxidant and other protective biochemical functions (Oyaizu, 1986:17–18; Zheng *et al.*, 2008:1535; Süntar *et al.*, 2011:74–75; Brilhante *et al.*, 2017:622; Muñoz-Pina *et al.*, 2020:7970; Maamoun *et al.*, 2021:6).

In traditional medicine*,* the *Moringa oleifera* leaves *are* famous for their high bioactive compounds, such as alkaloids, flavonoids, and tannins, which justify the plant's medicinal properties. The moringa plant has been reported to possess several biomedical applications, such as antioxidant activity (Siddhuraju & Becker, 2003:2145), anti-inflammatory (Saini *et al.*,

2016:5), and anti-cancer (Aja *et al.*, 2014:5), and the treatment of diabetes. The leaves and seeds have been reported to have a broad profile of nutrients and health-promoting compounds such as flavonoids, fatty acids, beta-carotene, and other phenolic compounds. Flavonoids are secondary metabolites dispersed in plant tissues that can exist as glycosides or aglycones (Makita *et al.*, 2016:118). Concerning the presence of these compounds, the seeds of *M. oleifera* have been successfully used in water treatment to eliminate pathogenic bacteria related to food spoilage (Brilhante *et al.*, 2017:622). In addition to the flavour, aroma, and taste that Moringa gives to food, its bioactive components are of significant interest in food preservation and shelf-life extension. Moringa leaves have been used to extend the shelf life of raw beef packed in modified atmospheric packaging, as these leaves are rich in several natural antioxidant compounds such as carotenoids, ascorbic acid, and phenolic compounds (Shah *et al.*, 2015:34). Arendse & Jideani (2022:12) also reported that the flavonoids of *M. oleifera* leaf powder extract influenced the reduced browning activity and low microbial count of the dehydrated apple slices. Based on the hypothesis that the bioactive constituents, specifically flavonoids of *M. oleifera* leaf powder, may inhibit oxidation of L-DOPA by attaching to the active sites of tyrosinase (Maamoun *et al.*, 2021:1). This chapter aims to evaluate the antioxidant activity of the *M. oleifera* leaf powder bioactive components and their influence on tyrosinase in a model system.

3.2 Materials and methods

3.2.1 Source of chemical reagents and equipment

Mushroom tyrosinase (lyophilised powder, 8503 units/mg), L-tyrosine, kojic acid, and dimethyl sulfoxide (DMSO) were supplied by Sigma-Aldrich. Analytical grade acetone, ethanol and methanol were also purchased from Sigma-Aldrich. *Moringa oleifera* leaf powder was supplied by Supa Nutri PTY Ltd. The water used in this study was purified using the milli-Q water purification system (Millipore, Microsep, Bellville, South Africa).

3.2.2 Preparation of the *Moringa oleifera* leaf aqueous extract (MOAQ)

The preparation of *M. oleifera* leaf powder extract was prepared as described by Rocchetti *et al.* (2019:320) with modifications. An amount of 10 g of the plant material was macerated in 100 mL boiling water at room temperature to allow maximum extraction of compounds, and the solution was allowed to cool down to room temperature. The solution was filtered using a Whatman filter paper (16 µm particle retention, 110 mm). The collected filtrate was centrifuged (4000 rpm, 4 °C for 30 minutes) using Avanti® J-E Centrifuge, JSE111330, Beckman Coulter Inc., Brea, CA, USA. The obtained extract was frozen at -23°C and freeze-dried at –60°C using a freeze dryer (benchtop Pro, SP Scientific) to achieve a powdered extract. Lastly, the freeze-dried extract was stored at -4°C and subjected to phytochemical analysis.

3.2.3 Preparation of the acetone (MOAC), ethanol (MOET), and methanol (MOME) extracts of *Moringa oleifera* leaf powder

The ultrasound-assisted extraction method was used to prepare the acetone, ethanol, and methanol extracts from the *M. oleifera* leaf powder as described by Rocchetti *et al.* (2019:320) with some modifications. Approximately 10 g of the powder was weighed separately into 100 ml of each solvent (acetone, ethanol, and methanol), soaked at room temperature for 4 hours to allow maximum extraction, and thereafter sonicated for 20 minutes at 25 °C using an ultrasonic bath (Lasec SA 2510 Branson ultrasound bath 42 kHz ± 6%, USA). After extraction, a Whatman filter (16 µm particle retention, 110 mm) was used to filter to remove residual particles. The resulting supernatant of each extract was concentrated using a rotary evaporator (Heidolph, Germany) equipped with a heating bath (Buchi, B100) and a vacuum pump (V100). The extracts were separately weighed, transferred into vials, and further evaporated to dryness under a fume hood. The extracts were sealed and stored at 4 °C to prevent deterioration until further analysis. Thereafter, the yields of all extracts were calculated based on 100 g *Moringa oleifera* leaf powder.

3.2.4 Extraction of polyphenols and identification of the *Moringa oleifera* leaf powder extracts bioactive compounds

As per the method of Arendse & Jideani (2022:3-4), an amount of 2 g of each extract was accurately weighed into a 50 mL centrifuge tube with a screw cap. An amount of 15 ml aqueous solution of 50% methanol and 1% formic acid was added, and the tubes were tightly capped. After that, the samples were vortexed for 60 seconds, followed by extraction in an ultrasonic bath for 1 h. The sample (2 mL) was withdrawn and centrifuged at 14,000 rpm for 5 minutes. The clear supernatant was then transferred into 1.5 mL glass vials for analysis.

The identification and quantity of the *M. oleifera* leaf components were performed by Liquid chromatography-mass spectrometry. A Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution HPLC-MS analysis. The column eluate first passed through a Photodiode Array (PDA) detector before going to the mass spectrometer, allowing simultaneous collection of UV and MS spectra. Electrospray ionisation was utilised in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimised for best resolution and sensitivity. The data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode and MSE mode. In MSE mode, two channels of MS data were developed, one at low collision energy (4 V) and the second using a collision energy ramp (40−100 V) to obtain fragmentation data. Leucine enkaphalin was used as lock mass for accurate mass determination, and the instrument was calibrated with sodium

formate. The separation was carried out on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column. An injection volume of 2 µL was used, and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 100% solvent A for 1 min and linearly changed to 28% B over 22 min. It then went to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 mL/min, and the column temperature was maintained at 55 °C. The compounds were quantified relatively against a calibration curve established by injecting a range of catechin standards from 0.5 to 100 mg/L catechin.

3.2.5 Preparation of solid dispersion

To improve the solubility of the extracts in water, the method of Tafu & Jideani (2021:3) with minor modifications was used. An amount of 50 g at a ratio of 1:1 for each of the MOLP extracts and polyethylene glycol (PEG-6000) was separately weighed into 500 mL glass beakers. The carrier complex was melted at 25 °C for approximately 5 minutes using a magnetic stirrer. The liquified mixture was thoroughly mixed and stirred for 10 min. The melted mixture was allowed to solidify in an ice bath. The obtained solid mass was finely crushed, stored in centrifuge tubes, and kept in the dark until use for further analysis.

3.2.6 Preparation of extract stock solutions for antioxidant and anti-tyrosinase activity

Approximately 20 mg of kojic acid and each complex (MOME, MOAQ, MOET, & MOAC) were dissolved separately in 1 ml of dimethyl sulfoxide (DMSO) to make up a stock solution of 2000 µg/ml. The working sample solutions were prepared by serial dilution from each stock solution in distilled water at concentrations ranging from 10 000 µg/ml to 31.25 µg/ml in distilled water.

3.2.7 The antioxidant activity of *Moringa oleifera* leaf extracts

Various methods were used for the evaluation of the antioxidant capacity because of different antioxidants modes of action. The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was used to evaluate the potential of the *M. oleifera* leaf powder extracts to neutralize and scavenge free DPPH radicals. Similarly, the ferric-reducing antioxidant power assay (FRAP) assay was used to determine the potential of the phenolic compounds in the extracts (MOET, MOAQ, MOET MOAC) to reduce ferrous ions (Fernandes *et al.*, 2016:453). The oxygen radical absorbance capacity (ORAC) was conducted to evaluate the ability of the extracts to scavenge oxygen radicals. Like ORAC and FRAP, the 2,2'-azino-di-3-ethylbenzthialozine sulphonate (ABTS) was also analysed to provide a quantitative measure of the antioxidant potential *M. oleifera* leaf powder extracts (Falowo *et al.*, 2017:250–251).

3.2.8 1,1-Diphenyl-2-picryl-hydrazyl (DPPH)

The modified DPPH assay was performed to measure the antioxidant scavenging capacity of the *M. oleifera* leaf extracts as described by (Shipp & Abdel-Aal, 2010:12). The DPPH reagent was prepared by weighing approximately 10 mg into 20 ml of absolute methanol, forming a dark purple solution. An aliquot of 25 µL of the *M. oleifera* leaf powder extracts was added to 275 µL of the DPPH reagent in the designated wells. The reaction mixture was incubated at 25 °C for 30 minutes in the dark, and a spectrophotometer (Spectrostar nano, Lasec) was used to measure the absorbance at 517 nm. Trolox standard (6-Hydrox-2,5,78-tetramethylchroman-2-carboxylic acid) was used as a standard, and the antioxidant activity was expressed as the percentage of DPPH radical scavenged by *Moringa oleifera* leaf extracts calculated as described in equation 1.

$$\% \text{ inhibition} = \frac{Blank - Sample}{Blank} \text{ x } 100 \quad (1)$$

Where, Blank = absorbance of the blank

Sample = absorbance of the sample

3.2.9 Ferric-reducing antioxidant power assay (FRAP)

The FRAP antioxidant assay was conducted as described by Azieana *et al*. (2017:3) with some modifications. An amount of 10 µL of the L-Ascorbic acid standards, control, and samples was separately added into the relevant wells of the 96-well plate. A multichannel pipette was used to accurately add 300 µL of the FRAP reagent into the wells to make a total volume of 310 µL, and the reaction mixture was incubated at 37 °C for 30 minutes. A microplate reader (Spectrostar nano, Lasec) measured the absorbance at 593 nm.

3.2.10 Oxygen radical absorbance capacity (ORAC) assay

The ORAC antioxidant assay was performed as described by Prior *et al*. (2003:3274) with slight changes. An amount of 10 µL of the Trolox standard, control, and the MOLF extracts were added into the designated wells of a black 96-microwell plate. In a separate Eppendorf tube, a solution was prepared separately by adding 10 µL of the fluorescein sodium salt stock solution to 2 ml of the phosphate buffer. An amount of 240 µL was further diluted in 15 ml of the phosphate buffer in a screw cap tube. Thereafter, 138 µL of the suspension was added to the wells. An amount of 50 µL of AAPH radical (25 mg/ml) was accurately transferred into the wells using a multichannel pipette to make a total volume of 200 µL in each well. The reaction mixture in the black 96-microwell plate was incubated at room temperature for 2 hours to allow the reaction to occur. Subsequently, a microplate reader (Spectrostar nano, Lasec) was used to measure the absorbance at the extraction of 485 nm

and emission of 530 nm. The result was obtained by measuring the degree and time of inhibition by measuring the area under the curve.

3.2.11 ABTS (2,2'-azino-di-3-ethylbenzthialozine sulphonate) radical cation scavenging assay

The ABTS radical cation scavenging activity of the *Moringa oleifera* leaf extracts was determined as described by (Gómez-Martínez *et al.*, 2020:3) to determine the total antioxidant status of the extracts. Approximately 0.0192 g of ABTS Diammonium salt was dissolved in 5 ml distilled water to a 7 mM concentration. ABTS radical cation was prepared by reacting 5 ml of the ABTS stock solution with 88 µL of potassium-peroxodisulphate (140 mM) and allowing the mixture to stand in the dark for 24 hours before analysis. For the determination of the *Moringa oleifera* leaf powder extracts, approximately 1 ml of the ABTS solution was diluted with 20 ml of ethanol to an absorbance of 2±0.1 at room temperature (25±2°C). A 25 µl aliquot of each extract was added to 300 µl of the ABTS solution, and the absorbance (measured at 734 nm, 25̊C) reading was taken after 30 minutes of incubation using a spectrophotometer (Spectrostar nano, Lasec). Trolox was used as a reference, and the results were given as vitamin C equivalent.

3.3 Anti-tyrosinase Essay

The tyrosinase inhibition model system was conducted as described by De Freitas *et al.* (2016:5) with slight modifications. Approximately 70 µL of each test sample in 50 mM phosphate buffer (pH = 6.5) and 30 µL tyrosinase (500 units/ml) were pipetted into 96-well plates. The stock solutions from different solvents were diluted in distilled water and tested at different concentrations ranging from 10 000 to 31.25 mg/ml. After 30 minutes of incubation at 25 °C, 110 µL L-tyrosine (2 mM) was added and incubated at 25 °C for 30 minutes. A microplate reader (Spectrostar nano, Lasec) was used to measure the optical density of the samples at 490 nm and compared to the control without the inhibitor, showing a linear change in absorbance with time during the 30 minutes of the experiment. The control incubators portrayed 100% activity of the enzyme and were performed similarly by replacing the extracts with distilled water. To eliminate the absorbance produced by the extracts, the phosphate buffer replaced the enzyme solution. A comparison of the enzyme activity in the presence of the evaluated inhibitor was used to assess the inhibitory activity of the extracts. Kojic acid was used as the positive control, and the samples were tested in triplicate to guarantee the significance of the results. The percentage inhibition was calculated using equation 2. Thereafter the 50% inhibitory concentration (IC_{50}) determined using GraphPad prism software where the dose-response data was added and a sigmoidal curve of the data was obtained.

$$\% \, inhibition = \frac{(A-B)-(C-D)}{(A-B)} \times 100 \qquad (2)$$

Where, A = absorbance of control with the enzyme

B= absorbance of the control without the enzyme

C= absorbance of the test sample with the enzyme

D= absorbance of the test sample without the enzyme

3.4 Statistical analysis

The statistical analysis was performed to determine the significant difference between the IC_{50} and antioxidant activities of the extracts using analysis of variance (ANOVA) and Duncan's multiple range test to separate the existing differences in the mean values.

3.5 Results and discussion

3.5.1 Bioactive components of *Moringa oleifera* leaf extracts

The characterisation of the phenolic compounds in the *M. oleifera* leaf extracts was performed due to their bioactive properties, such as possessing potential antioxidant and anti-tyrosinase activity (Rocchetti *et al.*, 2019:320). All extracts were put through LC-MS analysis to identify the specific phytochemical constituents in negative/positive ion mode, and the findings are presented in Table 3.1. The compounds were identified by MSdial and MSfinder, where the retention time retrieved from the base of the peak of the chromatograms is assigned and compared to a possible list of parent molecules. The data on the chromatographic properties, such as retention time, molecular weight, and preliminary determination of the phenolic compounds identified in *M. oleifera* leaf extracts (aqueous, acetone, ethanol, and methanol), are described in Table 3.1. A total of 23 phytocompounds that can be generally classified as flavonoids, quinones, phenolic acids, fatty acids, and amino acids were identified. The chemical structure of the phenolic compounds is classified using the chemical structure, which may be linked to sugar moiety in the form of glycosides or aglycones in their free form (Makita *et al.*, 2016:116).

The specific compounds that were identified and quantified are rutin, hyperoside, isoquercitrin, luteolin-7-*O*-glucoside, iso-orientin, orientin, neochlorogenic acid (3CQA), astragalin 7-*O*-rhamnoside, kaempferol 3-*O*-(6-acetylgalactoside), 4''-*O*-acetylmyricitrin, icariside F2, phenylalanine, 5-*O*-methylembelin, lariciresinol-9-O-galactoside, chlorogenic acid, 1,2,10-trihydroxydihydro-trans-linalyl oxide 7-*O*-glucopyranoside, 3-*O*-*p*-coumaroylquinic acid, Z)-hex-3-en-1-ol-*O*-beta-D-xylopyranosyl-glucoside, 3-*O*-*p*-Coumaroylquinic acid, (Z)-hex-3-en-1-ol-*O*-beta-D-xylopyranosyl-glucoside, lariciresinol-9-O-glucoside, dicaffeoylquinic acid, verbasoside, hydroperoxylinolenic acid, procatechuic acid, aristolich acid derivative respectively. The aqueous and acetone extracts showed comparatively higher polyphenol content than the methanolic and ethanolic extracts. The

bioactive compounds of *M. oleifera* leaf extracts have been substantially analysed by multiple authors (Jideani & Diedericks, 2014:195; Kou *et al.*, 2018:2; Álvarez-Román *et al.*, 2020:404). The findings of this section show peak variances between the solvent extracts of *Moringa oleifera* leaf powder, detailed in the section below.

Table 3.1 Quantification and identification of phytochemical compounds of *Moringa oleifera* leaf powder extracts

Phytocompound	Rt (min)	Formula (MF)	Experimental Mz [M-H]	Quantity of compounds in extracts (mg/L)			
				MOME	MOAC	MOAQ	MOET
Quinones							
5-*O*-Methylembelin	8.98	$C_{18}H_{28}O_4$	365.14	38.8	48.3	5.1	25.8
Phenolic acids							
Dicaffeoylquinic acid	10.26	$C_{25}H_{24}O_{12}$	515.14	14.8	ND	8.3	0.6
Neochlorogenic acid	9.59	$C_{16}H_{18}O_9$	353.09	97.1	7.7	49.4	29.7
Procatechuic acid	5.81	$C_7H_6O_4$	191.05	2.8	1.6	1.2	1.6
3-*O*-*p*-Coumaroylquinic acid	11.25	$C_{16}H_{18}O_8$	337.09	24.9	3.2	11.3	8.2
Flavonoids							
1,2,10-Trihydroxydihydro-trans-linalyl oxide 7-*O*-beta-D-glucopyranoside	14.65	$C_{16}H_{30}O_{10}$	381.18	38	22	16.2	30.9
Chlorogenic acid	12.17	$C_{16}H_{18}O_9$	353.09	38.2	2.3	14.6	10
Iso orientin	19.60	$C_{21}H_{20}O_{11}$	447.09	144.9	64	41.8	93.9
Orientin		$C_{21}H_{20}O_{11}$	448.38	143.3	63.1	45	98.7
Rutin	17.39	$C_{27}H_{30}O_{16}$	609.15	231.6	58	91.2	158.1
Hyperoside	17.93	$C_{21}H_{20}O_{12}$	463.09	217.5	85.2	69.9	147.9
Isoquercitrin	3.03	$C_{21}H_{20}O_{12}$	464.38	216.6	86.4	69.1	149.1

Table 3.2 Quantification and identification of phytochemical compounds of *Moringa oleifera* leaf powder extracts continued

Phytocompound	**Rt (min)**	**Formula (MF)**	**Experimental Mz [M-H]**	**Quantity of compounds in extracts (mg/L)**			
				MOME	MOAC	MOAQ	MOET
Luteolin-7-*O*-glucoside	20.10	$C_{22}H_{22}O_{12}$	477.10	145.4	63.1	43	96.4
Icariside F2	13.39	$C_{18}H_{26}O_{10}$	401.15	51.5	26.2	28.2	38.8
Verbasoside	9.01	$C_{20}H_{30}O_{12}$	461.18	11.8	5.5	5.1	7.4
4''-*O*-Acetylmyricitrin	18.82	$C_{23}H_{22}O_{13}$	505.10	55.2	8.5	39.7	19.1
Astragalin 7-rhamnoside	19.02	$C_{27}H_{30}O_{15}$	593.15	86.9	36.4	38.9	74.9
Lariciresinol-9-*O*-glucoside	16.90	$C_{26}H_{34}O_{11}$	521.20	18.9	16.4	6.8	15.1
Kaempferol 3-(6-acetylgalactoside)	20.78	$C_{23}H_{22}O_{12}$	489.10	86.4	16.5	45.4	32
Fatty acids							
(Z)-hex-3-en-1-ol-*O*-beta-D-xylopyranosyl-glucoside	16.16	$C_{17}H_{30}O_{10}$	393.17	20.6	12.5	8.6	17.6
Hydroperoxylinolenic acid	7.98	$C_{18}H_{30}O_4$	307.19	6.7	10.7	1.7	4.9
Amino acids							
Phenylalanine	2.38	$C_9H_{11}NO_2$	166.09	49.6	4.4	19.1	18.9
Aristolich acid derivative		$C_{17}H_{11}NO_7$	341.28	2.4	12.4	39.4	9.2

Compounds of *Moringa oleifera* leaf powder aqueous (MOAQ), acetone (MOAC), ethanol (MOET), and methanol (MOME) extracts. Rt = Retention time, Mz = Experimental weight

Rocchetti *et al.* (2019:320–321) evaluated the effect of different extraction methods, such as ultrasound-assisted, homogeniser-assisted extraction, maceration with 100% methanol, and hydroalcoholic methanol as solvents on moringa polyphenols. They reported that homogeniser-assisted extraction with 100% methanol and methanol: water 50:50, v/v showed a significantly (p = 0.001) higher polyphenol content than the other extraction methods. Dahiru *et al.* (2006:73) also confirmed the presence of flavonoids and tannins in *M. oleifera* leaf extract that was used to screen its antiulcerogenic effect in humans. According to Bhalla *et al.* (2021:6920), the methanol extract showed a comparatively higher polyphenol content than other extracts. Thus, these phytochemicals suggest that *M. oleifera leaves* have potent antioxidant activity. They further indicate that *M. oleifera* leaf can be incorporated into nutrient-dense food products as a functional food to add these essential healthy compounds while improving the physicochemical properties of the food products (Sohaimy *et al.*, 2015:189). The discussion in the sections below further explains the presence of different phytocompounds in the moringa extracts.

a) Flavonoids of the *Moringa oleifera* leaf powder extracts

Multiple flavonoid compounds were identified and quantified in all the *M. oleifera* extracts (Table 3.1). Different classes of flavonoids were present, determined by the attachment of the B ring structure to the carbon of the C ring, oxidation, and the unsaturation of the C ring structure (Panche *et al.*, 2016:2). The identified flavonoids and derivatives include 1,2,10-Trihydroxydihydro-trans-linalyl oxide 7-O-beta-D-glucopyranoside, chlorogenic acid, iso orientin, orientin, rutin, hyperoside, isoquercitrin, luteolin-7-O-glucoside, icariside F2, verbasoside, 4''-O-Acetylmyricitrin, astragalin 7-rhamnoside, lariciresinol-9-O-glucoside, kaempferol 3-(6-acetylgalactoside). Authors have supported the presence of flavonoids such as kaempferol, kaempferol-3-O-glucoside, quercetin-3-O-(600-malonyl) glucoside and myricetin in *M. oleifera* leaves symptoms (Zaku *et al.*, 2015:458; Mahmud *et al.*, 2020:917; Meireles *et al.*, 2020:498). The presence of different classes of flavonoids, such as O-glycosyl compounds agrees with findings by (Arendse & Jideani, 2022:11) on the secondary metabolites identified in *M. oleifera* leaf powder extract. Similarly, Fidrianny *et al.* (2021:10777) reported kaempferol and quercetin as the main flavonoids obtained through ethanol and methanol extraction. Leone *et al.* (2015:12807) identified rutin in *M. oleifera* 80% methanolic extract at concentrations of 0.39 – 1.674 mg/g. Vergara-Jimenez *et al.* (2017:2) reported kaempferol, quercetin, and myricetin had 7.57, 5.8, and 0.207 mg/g, respectively, as the major flavonoids in *M. oleifera* leaves. Similar findings have been reported where the ethanolic and methanolic extracts of dried *M. oleifera* leaves contained a higher concentration of flavonoids. The results are consistent with Vongsak *et al.* (2013:571), where the effect of 70% ethanol and distilled water on the total flavonoid content was determined using different extraction methods. The authors concluded that maceration in 70% ethanol

was the most satisfactory extraction method for dried *M. oleifera* leaves than distilled water. These findings may be related to the high dissolution efficiency of flavonoids in ethanol or methanol than in water. Bamishaiye *et al.* (2011:235) conducted a study on the antioxidant activity and proximate and phytochemical composition of *M. oleifera* leaves at different stages of maturation. They concluded that the presence of flavonoids at all stages of plant growth was responsible for the plant's medicinal properties. Additionally, the authors concluded that the potent antioxidant activity of the flavonoids influenced their superoxide anions, hydroxyl radicals, and lipid peroxy radical scavenging ability. Antioxidant compounds assist in delaying and inhibiting lipid oxidation to minimise rancidity and the formation of toxins. Additionally, these compounds help increase the shelf life of food products and maintain the nutritional quality (Lako *et al.*, 2007:1728). Thus, we speculate that the variance in the content of flavonoids may be due to genetic variances, maturity of the moringa plant and growth environment.

b) Phenolic acids of the *Moringa oleifera* leaf extracts

Four phenolic acids were identified in this study, as demonstrated in Table 3.1. The identified phenolic acids were dicaffeoylquinic acid, neochlorogenic acid, procatechuic acid, and 3-0-p-Coumaroylquinic acid. The concentration of these compounds differs from the solvent used to extract the polyphenols. As expected, the phenolic acids in the MOME extract were comparatively higher than in all other solvent extracts due to its comparatively high polarity than acetone, ethanol, and water. The acetone extract showed a relatively low concentration of some phenolic acids, while dicaffeolylquinic acid was not identified. Phenolic acids have been highly researched for their anti-cancer, antioxidant, and anti-inflammatory properties (Zaku *et al.*, 2015:457–458; Gupta *et al.*, 2018:4–6). Vergara-Jimenez *et al.* (2017:4–6) reported on the glucose, cholesterol-lowering effects, and enzyme-inhibitory activity of chlorogenic acid.

Similarly, Leone *et al.* (2015:12798) reported phenolic acids in *Moringa oleifera* leaves where different propagation methods in leaves with other morphological properties were studied. Singh *et al.* (2009:1112) previously identified similar phenolic acids in dried *M. oleifera* leaf aqueous extract. Although some phenolic acids were not identified in the *M. oleifera* leaf extracts, similar structural phenolic acids were identified in the current study. The solvents used for extraction, growth conditions, and genetic variances influence the phenolic acid concentration in the leaves. Therefore, the findings of this study demonstrate that the different solvents affect the composition and yield of the bioactive compounds in the *M. oleifera* leaf powder extracts. The polarity, and solubility of solvents has an influence on the separation of phenolic compounds from plant material which affects the phenolic compounds in solvent extracts (Baeshen *et al.*, 2023:2). The findings of his study agree with (Ng *et al.*, 2020:3-4) where the yield of phenolic compounds in different solvent extracts was presented.

They reported that the decrease in phenolic compounds from medicinal plant foods was directly proportional to the decrease in polarity of solvents such as methanol, ethyl acetate and distilled water. Therefore, these findings can be used to justify the significantly high phenolic compounds found in the methanolic extract. Similarly, Pkm & Malarivizhi (2022:89) reported that the hydro-methanol extract of *Azolla microphylla* had the most number of secondary metabolites when compared with other solvents. Since the polarity of plant polyphenols ranges from polar to non-polar, the best extractions are obtained in polar solvents because of the interaction between hydrogen from solvent and polar sites of the phenolic acids. In contrast, the yield of phenolic acids in the water extract is dependent on the nature and polarity (Brglez Mojzer *et al.*, 2016:4-7). All phenolic compounds, including phenolic acids are bonded with other molecules like carbohydrates and protein. Efficient solvents can break the interactions such as hydrogen bonds between these molecules, however, water cannot break the bonds due to its polarity and its polarity needs to be adjusted with organic solvents to facilitate efficient extraction from plants (Almohasin *et al.*, 2023:3-7). Thus, these findings clearly demonstrate that the extracted yield of phenolic acids is dependent on the polarity of solvents used in the extraction process.

c) Fatty acids of the *Moringa oleifera* leaf extracts

The following fatty acids were identified in the *M. oleifera* leaf extracts (Table 3.1) Z-hex-3-en-1-ol O-beta-D-xylopyranosyl-glucoside and hydroperoxylinolenic acid. The acetone extract demonstrated comparatively higher concentrations of hydroperoxylinoleic acid than the methanol, ethanol, and aqueous extract. The fatty acid profile of *Moringa oleifera* leaves has been studied by many researchers (Castillo-López *et al.*, 2017:165; Moyo *et al.*, 2011:12929–12931). Castillo-López *et al.* (2017:165) reported 62.72% of polyunsaturated fatty acids, such as linoleic acid, in the methanolic moringa extract. Polyunsaturated fatty acids are associated with the development and health of the human immune system (Moyo *et al.*, 2011:12929). *M. oleifera* is a good source of essential fatty acids, and its oil can be incorporated into the human diet for essential fatty acids.

d) Amino acids and quinones of the *Moringa oleifera* leaf extracts

The amino acids identified in this study include phenylalanine and acid derivatives, as indicated in Table 3.1. The MOME extract showed a higher concentration of amino acids and quinones than the other *M. oleifera* leaf extracts. Moreover, phenylalanine has the highest concentration, while aristolich acid derivative was identified at relatively lower concentrations. Natsir *et al.* (2019:5) identified essential amino acids such as phenylalanine at 17236.01 ppm. They further reported that amino acids have high inhibitory activity against DPPH. Thus, amino acids in *M. oleifera* leaves have a high antioxidant potential due to the presence of essential amino acids. The human body cannot produce essential amino acids, which can

only be obtained by ingesting amino acid-rich food products or supplements (Adeyeye *et al.*, 2019:1–2). Similarly, Brilhante *et al.* (2017:622) reported the presence of essential and non-essential amino acids in moringa seeds and leaves. The identified main amino acids were also identified by Moyo *et al.* (2011:12917) in *M. oleifera* leaves of the South African ecotype. They further reported that amino acids bond to form protein, influencing the quality and quantity of protein. Given these points, *M. oleifera* leaves can be incorporated into the human diet as a protein supplement to assist with detoxifying hazardous compounds. Likewise, (Jain *et al.*, 2019:2093) found that *M. oleifera* can be effectively used as an alternative source of protein for human consumption because of its essential amino acid concentration.

The other identified compound was 5-O-Methylembelin, which can be classified as quinone. These findings are like a study by Khairulmazmi & Tijjani (2019:454–458), where the natural compounds were extracted from *Moringa oleifera* leaves. They concluded that Moringa synthesises multiple secondary metabolites, among which are quinones. These compounds have been reported to have potent antioxidant activity by scavenging free radicals (Kou *et al.*, 2018:1).

3.5.2 The antioxidant activity of the *Moringa oleifera* leaf extracts

The *Moringa oleifera* leaf extracts were investigated through four complementary tests: DPPH, FRAP, ABTS, and ORAC. The methods mentioned earlier are based on different dynamics, which involve the transfer of electrons and hydrogen atom transfer, which can be considered an accurate representation of the mechanism of the antioxidant activity of the bioactive components (Rocchetti *et al.*, 2019:321–323). The antioxidant activity of the *M. oleifera* leaf extracts, namely acetone, aqueous, ethanol and methanol, are demonstrated in Figure 3.1. All the extracts showed positive results in terminating free radicals and the different types of antioxidant activities of the *M. oleifera* leaf powder extracts are detailed in sections a to d below.

a) 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) activity of the *M. oleifera* leaf powder extracts

The results of the antioxidant activity of the *M. oleifera* extracts evaluated by the DPPH radical scavenging assay are presented in Figure 3.1. All solvent extracts (acetone, aqueous, ethanol, and methanol) of the dried leaf powder showed a dose-dependent increase in antioxidant activity at concentrations ranging from 31.25 to 1000 μg/ml. In the DPPH essay, all extracts had high radical scavenging activity; however, ethanol and methanol revealed significantly (p = 0.001) higher antioxidant activity than the acetone and aqueous extracts. Both ethanol and methanol extracts of the dried *M. oleifera* leaf powder extracts demonstrated the highest antioxidant activities, 254.24, and 208.08 μmol TE/L, respectively, while the acetone and ethanol extract demonstrated 224.47 μmol TE/L, 208.08 μmol TE/L

respectively. Numerous studies on the antioxidant activity of *M. oleifera* leaves have been reported in the literature (Sohaimy *et al.*, 2015:195; Vázquez-León *et al.*, 2017:1595; Cabanac *et al.*, 2021:6). These findings are consistent with Sohaimy *et al.* (2015:197) for dried *M. oleifera* leaf extracts, where the methanol extract produced the highest antioxidant activity and the aqueous showed the lowest antioxidant activity. Siddhuraju & Becker (2003:2147) studied the antioxidant activity of freeze-dried *M. oleifera* extracts (aqueous, 80% methanol, and 70% ethanol), and they concluded that the antioxidant activity increased significantly (p = 0.001) with the concentration of all the extracts; however, ethanol and methanol extract demonstrated higher antioxidant activity. Therefore, the phenolic compounds in *M. oleifera* leaves are excellent electron donors, and they could create more stable products by terminating the radical chain reaction. In theory, higher antioxidant activity is related to higher polyphenol extraction because of the ability to donate hydrogen atoms (Jahan *et al.*, 2018:300). On another note, solvents can be divided into polar and non-polar, which can be determined by their dielectric constants, which may affect a solvent's total antioxidant and reducing properties (Nawaz *et al.*, 2020:4–6). The free radical scavenging ability of the *Moringa oleifera* leaf extracts may be related to the presence of different bioactive components in the plant. In theory, the antioxidant activity of a plant extract is associated with the presence of antioxidant compounds and polyphenols. Therefore, the high DPPH activity of the extracts may be due to the presence of flavonoids in *M. oleifera* leaves (Nobossé *et al.*, 2018:2196).

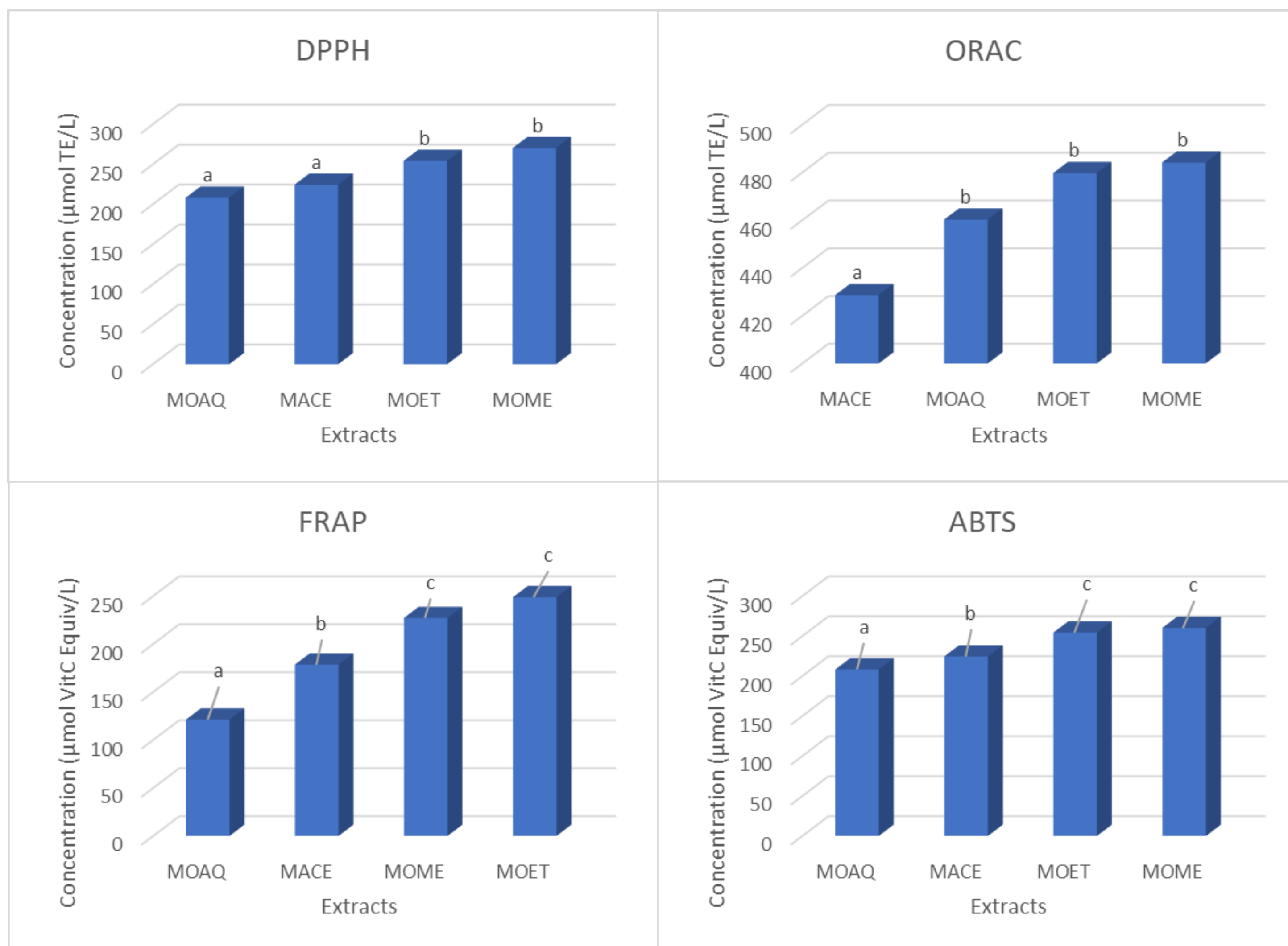
b) Ferric-reducing antioxidant power assay (FRAP) of the *M. oleifera* leaf powder extracts

The FRAP values for the different solvent extracts of *M. oleifera* leaf are demonstrated in Figure 3.1. The reducing ability is directly related to the antioxidant potential of each extract. The FRAP mechanism entails the presence of an antioxidant probe accepting electrons from peptides and converting them into coloured analytes. As expected, the FRAP assay showed a dose-dependent increase in antioxidant activity with a significant (p = 0.001) difference among the samples. The FRAP antioxidant power of the ethanol and methanol was significantly (p = 0.001) higher than the aqueous and acetone extract. In addition, the acetone and aqueous extracts showed significantly (p = 0.001) different FRAP activity of 178.52, and 121.23 µmol vit C equiv/L, respectively. These results imply that the ethanolic and methanolic extracts released comparatively high amounts of amino acids with the ability to donate electrons to reduce Fe^{3+}. Similar findings were observed (Omede, 2016:2458) where the results of the FRAP assay *Moringa oleifera* leaf extracts were dependent on the concentration when the FRAP values were determined. Omede, (2016: 2458) concluded that the ferric-reducing power of the crude methanolic extract was significantly (p = 0.002) higher than that of the aqueous extract. Similar findings were reported by Nobossé *et al.*

(2018:2192), where the ethanol and methanol extracts of 45-day-old *Moringa oleifera* leaves had the most relevant FRAP activity. Baldisserotto *et al.* (2023:8) reported similar findings where the hydroalcoholic extracts of *M. oleifera* leaf powder showed a higher antioxidant (FRAP) activity. According to Dai & Mumper (2010:7327), analysing the FRAP is an excellent method of quantifying the antioxidant capabilities of polyphenol compounds in fruits and vegetable extracts. Thus, the findings of this section highlight that the antioxidant activity of the methanol and ethanol extracts is directly related to their quantities of phenolic acids.

c) ABTS (2,2'-azino-di-3-ethylbenzthialozine sulphonate) radical cation scavenging ability of the *M. oleifera* leaf powder extracts

In addition to the analysis of the antioxidant behaviour of *Moringa oleifera* leaf extracts, the ABTS assay was used to measure the ability of the extracts to scavenge antioxidant capacities in food. In this assay, the relative ability of the extracts to scavenge the ABTS is compared with the Trolox standard. The radical scavenging activity of the *Moringa oleifera* leaf extracts is shown in Figure 3.1. ABTS was inhibited by the aqueous, acetone, methanol, and ethanol extracts at 208.08, 224.47, 26.170, and 254.24 μmol Vit C equiv/L respectively. The ethanolic and methanolic extracts had a significantly (p = 0.01) higher activity against ABTS, among other extracts. In addition, the aqueous extract had a significantly (p = 0.001) lower ABTS activity. The scavenging of the ABTS radical could result from the availability of proton-scavenging radicals. Similar results were reported by Rocchetti *et al.* (2020:6–7), where the 50% methanol extract showed higher ABTS activity of 45 mgTE/g among other solvents. Similarly, Xu *et al.* (2021:701) reported that ethanol extracts of 15 *Moringa oleifera* leaves collected from different locations had a high antioxidant capacity in ABTS essay.



Antioxidant activity of the MOAQ, MACE, MOET, MOME extracts by DPPH, ORAC, FRAP, ABTS. All bars indicate the mean ± standard deviation of triplicate experiments. Bars followed by the same superscript are not significantly ($p > 0.05$) different.

Figure 3.1 Antioxidant activity of the *Moringa oleifera* leaf extracts

These results are consistent with Avilés-Gaxiola *et al.* (2021:469), where the *Moringa oleifera* leaves protein and hydrolysate exhibited a potent ABTS radical scavenging activity. The ethanolic extract of *Moringa oleifera* leaf powder showed a ABTS (antioxidant capacity) which was related to the extract having a strong free radical scavenging capacity. It was also reported that the strong radical scavenging activity of the *M. oleifera* extract was related to the total phytochemical content of the extract (Ogundipe et al., 2022:1). Thus, the ability of the extracts to eliminate free radicals validates the use of *M. oleifera* leaf powder extracts as an antioxidant in food processing.

d) Oxygen radical absorbance capacity (ORAC) scavenging activity of the *M. oleifera* leaf powder extracts

To accurately assess the antioxidant activity of the extracts, the ORAC antioxidant essay was also applied to measure the peroxyl radical absorbing capacity. The methanol, ethanol, and aqueous extracts presented significantly (p = 0.002) higher antioxidant activity than the acetone extract. Similarly, Rocchetti *et al.* (2019:326) studied the antioxidant activity of 100% methanol and methanol/water (50:50) *Moringa oleifera* leaf extracts using different extraction methods. They reported that the homogeniser-assisted 100% methanol extract produced a significantly (p = 0.005) higher oxygen radical absorbance capacity of 536.24 µmol TE/g. Considering corresponding work in the literature, Castro-López *et al.* (2017:1143–1144) reported an ORAC estimate of 168.5 µmol TE/g after analysing *M. oleifera* leaves from Mexico. Thus, the ORAC activity reported in our study is 2 times higher (e.g., 168.5 µmol TE/g vs 484.14 µmol TE/g). They also reported a high antioxidant capacity of about 154.71-182.31 µmol TE/g ORAC activity in *M. oleifera* methanolic extract.

The four different methods presented consistent results in determining the antioxidant capacity of *M. oleifera* leaf powder extracts. Compared with other solvent extracts, the methanolic extract possesses comparatively higher antioxidant activity in DPPH, ABTS, FRAP, and ORAC antioxidant essays. Agrawal *et al.* (2017:145) reported similar findings that the methanolic extracts of plant extracts had high antioxidant activity. Methanol has the highest polarity amongst the solvents, which extracts more polyphenol compounds. Therefore, methanol is the best solvent to obtain significant antioxidant activity. The DPPH, ORAC, ABTS, and FRAP are dependent on the extraction solvent, and due to different polarities and solubilities, different compounds are extracted. Smuda *et al.* (2018:1139–1140) reported that the methanol extract was comparatively rich in bioactive components such as flavonoids, which have been proven to have potent antioxidants and intense free radical scavenging activity. The antioxidant activity of the *M. oleifera* leaf extracts may be due to their free radical trapping power by metal chelation and the donation of hydrogen atoms or electrons (Nile *et al.*, 2018:5).

3.5.3 Anti-tyrosinase activity of *Moringa oleifera* leaf powder extracts

This section aims to determine the inhibitory effect of *Moringa oleifera* leaf powder extracts against tyrosinase, an enzyme responsible for melanin production. The half-maximal (IC_{50}) inhibitory concentration indicates the ability of a treatment to prevent 50% of a biological process. All the extracts of *M. oleifera* leaf powder demonstrated inhibitory activity against tyrosinase, and the findings were presented as the IC_{50} (Table 3.2). The IC_{50} of the *M. oleifera* leaf powder extracts were between 34.66 to 71.29 µg/mL. The MOAQ (*M. oleifera* leaf aqueous extract) showed a significantly (p = 0.001) stronger inhibition on tyrosinase activity with an IC_{50} value of 34.66 µg/mL. There was no significant (p = 0.06) difference between the IC_{50} of the MOME (*M. oleifera* leaf methanol extract) and MOAC (*M. oleifera* leaf acetone extract). However, the MOME (IC_{50} = 58.26 µg/mL) and MOAC (IC_{50} = 60.60 µg/mL) extracts showed a significantly (p = 0.06) stronger inhibition against tyrosinase activity than the MOET (*M. oleifera* leaf methanol extract. The strong inhibition of the methanol extract may be related to the dissolution efficiency of methanol as reported by Vergara-Jimenez *et al*. (2017:4). In contrast, the MOET had a significantly (p = 0.08) weak inhibition against tyrosinase activity IC_{50} than all extracts with an IC_{50} value of 71.29 µg/mL.

Previous work has shown the tyrosinase inhibitory activity of plant extracts in vitro by measuring the ortho-quinones from the oxidation of L-tyrosine in the apparatus of melanogenesis. For example, in a study conducted by maceration extraction and ethanol as a solvent, Abidin *et al*. (2019:56) observed that the crude ethanol extract of the Moringa leaves was less active against tyrosinase than its purified extract. In another study, the uncompetitive inhibition mechanism of *M. oleifera* leaf extract against mushroom tyrosinase. Hashim *et al*. (2021:3) reported an IC_{50} of 121.3 ± 0.4 µg/mL and a good negative correlation between the total phenolic content and IC_{50}. They further noted that many compounds, such as flavonoids were recognised as active tyrosinase inhibitors.

Table 3.3 Tyrosinase inhibitory activity (IC_{50}) of *Moringa oleifera* leaf powder extracts

Moringa extract	IC_{50} (µg/mL)
MOME	58.26 ± 1.50^{a}
MOAC	60.60 ± 2.45^{a}
MOET	71.29 ± 1.33^{b}
MOAQ	34.66 ± 3.03^{c}

Values are mean ± standard deviation, means within a column followed by different superscripts are significantly (p > 0.05) different. MOME = *Moringa oleifera* leaf methanol extract, MOAC = *Moringa oleifera* leaf Acetone extract, MOET = *Moringa oleifera* leaf ethanol extract, MOAQ = *Moringa oleifera* leaf aqueous extract.

Moringa oleifera leaf extracts have been proven to be one of many plants with antioxidant properties (Paliwal *et al*., 2011:8). Hwang *et al.* (2022:13–15) stated that compounds with strong antioxidant activity also show powerful activity against tyrosinase. Multiple studies have been conducted to characterise and determine the relationship between the inhibitory activity and structure of flavonoids from natural sources (El-Nashar *et al*., 2021:4–6; Jiang *et al*., 2012:1222; Yener *et al*., 2020:8). It was established that flavonoids with an α-keto group showed a notable tyrosinase inhibitory activity (Chang, 2009:2446). Abdul Karim *et al.* (2014:9) reported that most natural compounds with a resorcinol and catechol functional group, such as flavonoids, were known to be potent tyrosinase inhibitors. The mechanism can be explained by the similar structure of the α-keto group in flavonoids and L-dopa, a substrate that forms melanin during melanogenesis. According to Kubo *et al*., (2000:1750), some flavonoids suppress diphenolase activity using the metal chelation mechanism. The mechanism is specific for flavonoids such as quercetin, morin, and kaempferol, where the tyrosinase activity is inhibited by their ability to chelate copper in the enzyme, leading to an irreversible inactivation of tyrosinase. The mechanism was further supported by the chelation of tyrosinase where there was a noticeable spectral shift when flavonoids such as quercetin and kaempferol were incubated with tyrosinase. Thus, the results imply that the tyrosinase inhibition could be due to the phenolic compounds, especially flavonoids, in the extracts. The intense anti-tyrosinase activity of the extracts suggests that the MOAQ, MOET, MOAC and MOME can be applied as a potent anti-browning agent in food processing due to their activity against tyrosinase.

3.6 Conclusion

This study examined the bioactive components, antioxidant and anti-tyrosinase activity of *M. oleifera* leaf powder extracts. A total of 23 phytocompounds such as phenolic acids, flavonoids, amino acids, fatty acids and quinones were identified in all MOLP extracts. Flavonoids such as orientin, rutin, and hyperoside were identified. Neochlorogenic acid and dicaffeoylquinic acid were among the identified phenolic acids in the *M. oleifera* leaf powder extracts. The highest antioxidant activity was shown by ethanol (MOET) and methanol (MOME) extracts compared to the aqueous (MOAQ) and acetone (MACE) extracts. The aqueous extract showed a strong anti-tyrosinase activity while the ethanol extract showed low inhibition. The findings of this chapter suggest that the *Moringa oleifera* leaf powder extracts are rich sources of bioactive components and are potent tyrosinase inhibitors. Therefore, the extracts can be incorporated in food formulations as functional foods, and they can be used as anti-browning agents.

3.7 References

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CHAPTER 4:

EFFECT OF THE *MORINGA OLEIFERA* LEAF POWDER EXTRACTS ON THE COLOUR, NUTRITIONAL CONTENT AND STORAGE STABILITY OF THE DRIED APPLE SLICES

ABSTRACT

Dried apple slices are popular and healthy snacks despite the limited information on natural preservatives and how they affect their phytochemical and proximate composition. The aim of this chapter was to investigate the influence of the different *Moringa oleifera* leaf powder (MOLP) extracts, MOET (*Moringa oleifera* leaf ethanolic extract), MOAC (*Moringa oleifera* leaf acetone extract), MOAQ (*Moringa oleifera* aqueous extract) and MOME (*Moringa oleifera* methanolic extract) at low (0.003%), mid (0.03%) and upper (0.3%), concentrations in combination with 2% citric acid to create an anti-browning treatment. The effect of the treatments was investigated on the colour, browning index. The 0.003% *M. oleifera* leaf powder aqueous extract (MOAQ) anti-browning mixture was further used to treat the dried apple slices and the proximate composition was analysed. The shelf-life of the dried apple slices treated with the 0.003% MOAQ mixture was studied through the determination of physicochemical properties (moisture, water activity, extensibility, and colour). Notable differences in colour of the dried apple slices treated with the different anti-browning mixtures were observed. The 0.003% *M. oleifera* leaf powder aqueous (MOAQ) extract anti-browning mixture had an outstanding effect on the colour of the dried apple slices. The dried apple slices treated with the 0.003% MOAQ anti-browning mixture were lighter, yellower, less green and had a low browning index (BI). The 0.003% MOAQ anti-browning mixture further improved the proximate content of the dried apple slices such as higher ash, vitamin C and total fat content. The increase in moisture and water activity increased the extensibility of the dried apple slices during storage. These apple slices also maintained a lighter colour while the control was significantly ($p = 0.001$) browner. The estimated shelf-life based on lightness (L*) of the treated apple slices was 153 days compared to the control (126 days). Therefore, the 0.003% MOAQ treatment increased the shelf-life of the dried apple slices, and it can be used in the food industry to reduce browning in dried fruits.

4.1 Introduction

Consumers have been increasingly calling on manufacturers for healthy and nutritious snacks. As the consumers demand healthy snacks increases, new product developers, researchers and manufacturers have responded by exploring various alternatives such as apples which are rich in essential nutrients. Apples are rich in fibre, vitamins, polyphenolic compounds, and antioxidants, which are important and essential in nutrition (Cichowska-Bogusz *et al.*, 2020:1). The nutritional content of fruits such as apples depends on the variety, size, and ripeness. However, the nutritional content depends on weather conditions, soil types, post-harvesting processes and technologies, handling, transportation, and storage (Campeanu *et al.*, 2009:162). Apples are typically consumed fresh, but processing is mandatory to extend the shelf life. Hot air drying is commonly used to extend the shelf life of fruits; however, the process causes irreparable physicochemical and nutritional changes, such as textural colour variations and deteriorates the nutritional value. In addition, hot air drying of fruits results in textural damages such as low rehydration, shrinkage, and firmness (Yadav & Singh, 2014:1660). Enzymatic browning, ascorbic acid oxidation and non-enzymatic browning result in colour changes in dried apple products. A synthetic antioxidant, sulphur dioxide, is usually used to preserve the colour of vitamin C and phenolic compounds. However, the Food and Drug Administration (FDA) restricts its use because of its hazardous effects such as stomach issues, headache and diarrhea on asthmatic individuals (Mlček *et al.*, 2023:2; Pruteanu *et al.*, 2023:13–15). Thus, the strategies for developing new anti-browning agents are moving towards natural plant extracts to replace chemical treatments and synthetic additives.

Multiple studies have reported using natural anti-browning extracts, namely green tea (Salminen & Russotti, 2017:4) and pineapple (Sarkar *et al.*, 2017:56). Abidin *et al.* (2019:52) conducted a study on the ability of purified and crude *M. oleifera* extract to prevent melanin formation. They concluded that the Moringa leaves contain flavonoids that can avert tyrosinase activity, an enzyme in melanin formation during browning. Vhangani & Van Wyk (2021:5–7) also explored plant extracts as natural inhibitors in the browning of fruits and vegetables. Furthermore, Arendse & Jideani (2022a:10) investigated the combination of some weak acids, such as ascorbic acid, citric acid, and potassium sorbate, with *Moringa oleifera* leaf powder extract to determine how it would avert discolouration of the dried apple slices. They further investigated the dried apple slices' microbial safety, storage stability, and consumer acceptability, such as taste and texture. These findings have motivated researchers to develop potential natural additives to extend the shelf life and maintain the quality of fruits and vegetables. Thus, treatment with *Moringa oleifera* leaf extract complexes can effectively reduce the application of sulphites before drying. This can be achieved by coating or treating fruits and vegetables with edible plant extracts before drying, reducing

oxidation during hot air drying and extending the shelf life by controlling the growth of microorganisms. Furthermore, the treatment can improve the overall nutritional content and reduce the deterioration of the colour of fruits during the drying process. In the previous chapter, the IC_{50} for each *M. oleifera* leaf powder extract was obtained. Even though previous studies have reported on using plant extracts to preserve fruits and vegetables and their effect on their shelf life, no study has investigated the effect of aqueous, acetone, ethanol, and methanol extracts of *M. oleifera* leaf powder with citric acid as anti-browning agents at the estimated concentrations.

In Chapter 3, the anti-tyrosinase activity of the *M. oleifera* leaf powder (MOLP) extracts were determined and the *M. oleifera* leaf powder aqueous extract (MOAQ) was chosen as the potential candidate for further treatment in the determination of shelf-life due to its strong anti-tyrosinase activity. Thus, this chapter aims to use the different extracts of MOLP at estimated IC_{50} concentrations for the pretreatment of apple slices as anti-browning agents.

4.2 Materials and methods

4.2.1 Source of chemical reagents and equipment

The apples were purchased from a local supermarket in Cape Town, South Africa and stored at 4°C until use in the experiments. Citric acid (anhydrous powder) was obtained from CJP Chemicals Pty Ltd, Cape Town. The polyethylene glycol (PEG-6000) was purchased from Sigma Aldrich Co, Johannesburg. The milli-Q water purification system (Millipore, Microsep, Bellville, South Africa) was used to purify the water used in this study.

4.2.2 Preparation of the fresh apple slices

Apples with a uniform colour, size and shape were carefully selected, washed in a 0.002% NaCl solution, and rinsed to prevent surface contamination. The apples were accurately weighed before processing to assist with calculating the average yield loss after processing. Thereafter, the apples were peeled and cored using a stainless-steel multifunctional peeler. A kitchen mandoline slicer was used to uniformly cut the apples into 4 mm slices at ambient temperatures, and the average weight was randomly recorded.

4.2.3 Preparation of anti-browning solutions

The anti-browning complexes were prepared using *M. oleifera* leaf powder extracts and citric acid to treat the apple slices before drying. The MOET (*Moringa oleifera* leaf ethanolic extract), MOAC (*Moringa oleifera* leaf acetone extract), MOAQ (*Moringa oleifera* leaf aqueous extract) and MOME (*Moringa oleifera* leaf methanolic extract) were individually combined with polyethylene glycol (PEG-6000) at a ratio of 1:1 to ensure complete dissolution of the extracts in distilled water. Thereafter, MOET, MOAC, MOAQ and MOME

extract solutions were prepared separately at 0.003, 0.03 and 0.3% with 2% citric acid to make anti-browning mixtures. Each extract was individually weighed with 2% citric and dissolved in 1000 ml distilled water at the concentrations mentioned to prepare the treatment solutions. The solutions were continuously stirred at room temperature to dissolve all extracts thoroughly. A separate treatment solution of 2% citric acid was also prepared to determine the influence of the weak acid as a positive control.

4.2.4 Treatment of the fresh apple slices

The fresh apple slices were sub-merged into each anti-browning solution for 5 min and placed in a vegetable spinning dryer (Smartlife, South Africa) to remove the excess water. Thereafter, the apple slices were separately placed in a single-layer tray with the untreated apple slices as the control. The trays were carefully labelled and placed in a food dehydrator (Excalibur EXC10, 50 Hz, 600W) at 70°C for approximately 2 hours until the apple slices lost a constant weight. Thereafter, the apple slices were allowed to cool, placed in pouches with an aluminium foil lining and stored in a cool, dry place at room temperature. The screening of the best browning inhibitor was performed subject to colour and proximate composition (moisture, ash, protein, fibre, carbohydrates) and vitamin B1, B2 and C.

4.2.5 The colour and browning index of the dried apple slices

The colour of the dried apple slices was measured using a Konica Minolta Spectrophotometer CM5. The instrument was calibrated using black and white tiles, respectively. The samples were placed on a sample dish, and the reflectance was measured for CIELAB and LCh colour scales. The L* coordinate represents the lightness, with 100 representing white and closer to 0 representing black. The measurements for each sample were performed in triplicate at three different rotated positions (each reading was recorded as the average reading per rotated position) (Nielsen, 2007).

The values were recorded as L* (lightness), a* (chromaticity coordinate +a* = red and -a* = green) b* (chromaticity coordinate +b* = yellow and -b* = blue), C* (chroma) and h (hue angle 0°, 90°, 180° and 270°). The total colour difference (ΔE) of the apple slices was determined using equation 3:

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (3)$$

The browning index of each sample was estimated using equations 4 and 5.

$$\text{Browning index (BI)} = 100\ (\mathrm{x} - 0.31) \times 0.17 \quad (4)$$

$$X = \frac{(a* + 1.75L*)}{(5.645L* + a* - 0.3012b*)} \quad (5)$$

Where L* = lightness, a* = greenness or redness, b* = yellowness or blueness

4.2.6 Determination of the proximate composition of the apple slices

The samples' moisture content was determined using a vacuum oven AOAC method 926.12 (Nielsen, 2007:90–91). An amount of 5 g of the sample was weighed into crucibles and placed in a vacuum oven at 105°C for 3 hours until a constant mass while the ash content was determined using a muffle furnace as described by Yameogo *et al.* (2011:265). An amount of 2 g of the sample was accurately placed in crucibles and allowed char on an open flame under a fume hood. Thereafter, the charred samples were allowed to mineralise at high temperatures of 500°C in the muffle furnace overnight. The crucibles were withdrawn and cooled in the desiccator before weighing. The protein was determined using the Dumas combustion method. A Gas chromatograph 6890 – Mass Spectrometer 5973N (GC-MS) determined the total fat and fatty acid content using the AOAC, method 996.06. The fibre was determined using the AOAC methods 985.29, and carbohydrates were determined by difference.

4.2.7 Thiamine (vitamin B1) and riboflavin (vitamin B2) analysis of dried apple slices

To prepare an extract, 2 g of the homogenised dried apple slices were weighed into an Erlenmeyer flask, 30 ml of 0.1 M hydrochloric acid (HCl) was added, and the mixture was heated for 5 minutes at 40°C. Thereafter, an ash-less filter paper was used to filter the mixture, and it was centrifuged (Thermo electron Industries SAS, 500 W) at 1800 rpm for 10 minutes (Otemuyiwa & Adewusi, 2013). Riboflavin and thiamine standards were used as external standards (Sigma-Aldrich, Germany). The standard solutions were prepared as Otemuyiwa *et al.* (2013:416) described. The standards were stored at -20 ˚C for 2 weeks to prevent degradation.

The samples and standards were separated using an HPLC (Agilent 1100 HPLC system). The system was equipped with a G1322A vacuum degassing unit, a G1311A quaternary pump, a thermostat column compartment, an autosampler, G1315C Diode Array Detector (DAD), and a Fluorescence Detector (FLD). The Agilent Chemstation14 software (Agilent Technologies, Waldbron, Germany) integrated peak areas to record and store data. The chromatographic separation column consisted of stainless steel (4.6 x 150 mm) Eclipse XDD and a 5 µm Bondapak C18 column. The HPLC has an integrated Ultraviolet detector set at 254 nm wavelength to monitor the column elution. The elution was isocratic, and the flow rate was 0.60 $Ml.min^{-1}$ (Sunarić *et al.*, 2020:1730–1732; Otemuyiwa *et al.*, 2013:416).

4.2.8 Effect of MOAQ anti-browning solution on the shelf-life stability of the dried apple slices

a) Water activity and moisture of the dried apple slices

The water activity of the apple slices was measured using a Karl Fisher Titrator (787 Titrino Plus Metrohm). An amount of 5 g of the homogenised sample was transferred into a sample dish inside the analyser. After 60 seconds, a_w (water activity) a reading was provided, all tests were conducted in triplicates and the average was taken as the a_w of the dried apple slices (Wolfe & Liu, 2003:1677). The moisture content of the samples was determined using a vacuum oven AOAC method 926.12 (Nielsen, 2007:90–91). An amount of 5 g of the sample was weighed into crucibles and placed in a vacuum oven at 105°C for 3 hours until a constant mass was obtained.

b) Texture of the dried apple slices

The method of Gujral & Brar (2003:3) was used to determine the extensibility of the dried apple slices on the Instron 3344 testing machine. Each dried apple slice was placed between 2 clamps aligned at approximately 40 mm apart, with one clamp connected to the Instron's platform and the second one was attached to the moving arm. Using a 100 N load, the test was conducted by puling each apple slice apart at a speed of 40 mm/min until it was broken apart and the results were presented as the percentage of the initial length. An average of ten replications was presented for each reading to ensure consistency of the texture (Kahraman *et al.*, 2021:5).

c) Colour of the dried apple slices

The colour of the dried apple slices was measured using a Konica Minolta Spectrophotometer CM5 as described in section 4.2.4.

d) Determination of the accelerated shelf-life of the MOAQ-treated dried apple slices

Accelerated shelf-life test (ASLT) was used to determine the shelf-life of the dried apple slices as described by Darniadia *et al.* (2021:99) with some modifications. Accelerated shelf-life involves storing the samples under extreme temperature conditions and determining a corresponding shelf-life at room temperature. In this context, dried apple slices were incubated at 35°C for 4 weeks in aluminium pouches (250 g each). The physicochemical properties such as water activity, moisture, extensibility, and change in colour of the MOAQ-treated dried apple slices and control were evaluated on day 0, 7, 14, 21. The moisture and lightness of the dried apple slices treated with 0.003% MOAQ + citric acid was modelled using the first order reaction kinetics in equation 6. Thereafter, the lightness of the dried apple slices was used to estimate the shelf life of the dried apple slices when stored at room temperature (25°C) due to its higher correlation co-efficient (r^2).

$$\ln A_t = \ln A_0 - k_t \quad (6)$$

Where $\ln A_t$ = Concentration at time (t); $\ln A_0$ = Initial concentration; $-k_t$ (day^{-1}) = degradation rate constant.

4.3 Results and Discussion

4.3.1 Colour of the dried apple slices treated with the *M. oleifera* leaf powder extracts

Lightness measures the luminous intensity of colour from a scale of 0 (indicating black) to 100 (indicating white). The lightness of the dried apple slices observed after treatment with 2% citric acid only, MOET, MOAC, MOAQ, and MOME complexes at 0.003, 0.03, and 0.3% with 2% citric acid for each extract as per IC_{50} obtained in chapter 3 are presented from Tables 4.1. The lightness of the untreated dried apple slices was not significantly (p = 0.06) different from those treated with 2% citric acid only. Similarly, the MOAC (*M. oleifera* acetone leaf extract) anti-browning mixture had no significant (p = 0.051) effect on the lightness of the dried apple slices for all concentrations. The dried apple slices treated with the 0.003% MOET (*M. oleifera* ethanol leaf extract) anti-browning mixture were significantly (p = 0.001) lighter than those treated at higher concentrations. There was no significant (p = 0.082) difference on the lightness of the dried apple slices treated at 0.03 & 0.3% MOET anti-browning mixture. Moreover, the lightness of the dried apple slices treated with the MOET anti-browning mixture at high concentrations was not significantly (p = 0.063) different from the untreated samples. Thus, this suggests that the combination of 2% citric acid and the MOET extract reduced the lightness of the dried apple slices when a low concentration of the MOET extract (0.003% + citric acid) was added.

Table 4.1 The L* (lightness) of the dried apple slices treated with different *Moringa oleifera* leaf extract complexes based on extract.

	Extract			
Concentration	MOET	MOAC	MOAQ	MOME
No Treatment	71.35 ± 1.50^{a}	71.35 ± 1.50^{a}	71.35 ± 1.50^{a}	71.35 ± 1.50^{a}
2%Citric	71.86 ± 5.15^{a}	72.27 ± 4.79^{a}	77.25 ± 0.87^{a}	79.99 ± 1.92^{a}
0.003% + 2% CA	78.56 ± 0.61^{b}	73.13 ± 3.85^{a}	80.11 ± 0.87^{b}	76.09 ± 4.10^{b}
0.03% + 2% CA	71.70 ± 4.76^{a}	72.43 ± 6.47^{a}	78.97 ± 0.87^{c}	74.33 ± 4.00^{b}
0.3% + 2% CA	69.20 ± 5.42^{a}	75.23 ± 2.26^{a}	79.15 ± 0.87^{c}	77.45 ± 3.13^{a}

[1]Values are mean ± standard deviation of triplicate measurements. Means within a column followed by the same superscript are not significantly (p > 0.05) different. MOET = *Moringa oleifera* leaf ethanolic extract, MOAC = *Moringa oleifera* leaf acetone extract, MOAQ = *Moringa oleifera* aqueous extract, MOME= *Moringa oleifera* methanolic extract and CA = citric acid.

The different concentrations of the MOAQ (*M. oleifera* aqueous leaf extract) anti-browning agent influenced the lightness of the dried apple slices. The anti-browning mixture with 0.003% MOAQ and citric acid resulted in significantly ($p = 0.001$) lighter dried apple slices. In contrast, the was no significant ($p = 0.052$) difference on the lightness of the apple slices treated with 0.03 and 0.3% MOAQ complexes. The findings further suggest that when the MOAQ extract is combined with 2% citric acid at 0.003%, the lightness of the dried apple slices improves. However, the lightness of the dried apple slices treated with MOME (*M. oleifera* methanol leaf extract) anti-browning mixture containing 0.03% MOME did not differ from the 0.003% MOME-treated apple slices.

The (a* redness, or -a* greenness) is also an essential attribute, which may affect the acceptability of the dried apple slices (Table 4.2). The treatment with 2% citric acid only did not significantly ($p = 0.239$) influence the redness of the dried apple slices. Furthermore, there was no significant ($p = 0.07$) difference on the redness of the dried apple slices treated with different concentrations of the MOET anti-browning mixture. Similarly, the different concentrations of the MOAC, MOAQ and MOME in the anti-browning mixtures did not have a significant ($p = 0.05$) effect on the redness of the dried apple slices. However, the dried apple slices treated with the MOAQ anti-browning mixture were greener than all others.

Table 4.2 The a* (redness or greenness) of the dried apple slices treated with different *Moringa oleifera* leaf extract complexes based on extract.

Concentration	Extract			
	MOET	MOAC	MOAQ	MOME
No Treatment	3.64 ± 1.09^{a}	3.64 ± 1.09^{a}	3.64 ± 1.09^{a}	3.64 ± 1.09^{a}
2%Citric	3.62 ± 1.49^{a}	3.09 ± 1.04^{a}	1.40 ± 0.87^{a}	0.89 ± 0.96^{a}
0.003% + 2% CA	1.57 ± 0.33^{b}	2.06 ± 0.52^{a}	-0.75 ± 0.87^{c}	1.02 ± 1.63^{a}
0.03% + 2% CA	1.60 ± 0.41^{b}	1.58 ± 1.50^{a}	-0.86 ± 0.87^{c}	1.98 ± 2.20^{a}
0.3% + 2% CA	0.34 ± 1.35^{b}	2.80 ± 2.54^{a}	0.01 ± 0.87^{c}	1.57 ± 1.26^{a}

[1]Values are mean ± standard deviation of triplicate measurements. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different. MOET = *Moringa oleifera* leaf ethanolic extract, MOAC = *Moringa oleifera* leaf acetone extract, MOAQ = *Moringa oleifera* aqueous extract, MOME= *Moringa oleifera* methanolic extract, CA = citric acid.

The yellowness or blueness of the pre-treated dried apple slices are presented in Table 4.3. The untreated dried apple slices had a significantly ($p = 0.001$) yellower tint than those treated with 2% citric acid, MOET, MOAC, MOAQ anti-browning complexes. However, there was no significant ($p = 0.009$) difference between the yellowness of the untreated dried apple slices and those treated with 0.03% MOME ant-browning mixture. The treatment at 0.3% (upper), 0.03% (mid) and 0.003% (low) with the MOET anti-browning mixture had no

significant (p = 0.001) difference on the yellowness of the dried apple slices. In contrast, the dried apple slices treated with the 0.03% MOAC anti-browning mixture were less than those treated at the lower (0.003%) and upper (0.3%). Similarly, the dried apple slices treated with the 0.03% MOAQ anti-browning agent were significantly (p = 0.001) yellower than those treated at 0.003 % and 0.3% MOAQ. In contrast, the dried apple slices treated with the 0.3% MOME anti-browning mixture were yellower than those treated at low (0.003%) and mid (0.03%) concentrations. Moreover, there was no significant (p = 0.082) difference in the yellowness of the dried apple slices treated at low (0.003%) and mid (0.03%) concentrations of the MOME anti-browning mixture.

Table 4.3 The b* (yellowness and blueness) of dried apple slices treated with different *Moringa oleifera* leaf extract complexes based on extract.

Concentration	Extract			
	MOET	MOAC	MOAQ	MOME
No Treatment	24.91 ± 0.56^{a}	24.91 ± 0.56^{a}	24.91 ± 0.56^{a}	24.91 ± 0.56^{a}
2%Citric	22.69 ± 1.02^{b}	22.02 ± 1.64^{b}	23.33 ± 0.87^{b}	22.15 ± 1.52^{b}
0.003% + 2% CA	22.56 ± 1.47^{b}	20.61 ± 3.13^{b}	21.93 ± 0.87^{c}	22.53 ± 2.38^{b}
0.03% + 2% CA	22.48 ± 2.57^{b}	19.74 ± 1.24^{c}	19.43 ± 0.87^{d}	21.65 ± 1.34^{b}
0.3% + 2% CA	20.05 ± 4.00^{b}	23.08 ± 0.52^{b}	21.01 ± 0.87^{c}	24.03 ± 1.08^{a}

[1]Values are mean ± standard deviation of triplicate measurements. Means within a column followed by the same superscript are not significantly (p > 0.05) different. MOET = *Moringa oleifera* leaf ethanolic extract, MOAC = *Moringa oleifera* leaf acetone extract, MOAQ = *Moringa oleifera* aqueous extract, MOME= *Moringa oleifera* methanolic extract, CA = citric acid.

The chroma, also referring to the intensity/purity of colour of the dried apple slices, is also demonstrated in Table 4.4. There was no significant (p = 0.07) difference in colour intensity between the 2% citric acid and MOET-treated dried apple slices at different concentrations. However, the colour of the untreated dried apple slices was significantly (p = 0.001) more intense than that of the MOET-treated samples. Similarly, the apple slices treated with the MOAC anti-browning mixture had a significantly (p = 0.001) higher clarity at 0.03% MOAC than 0.003%, 0.3% and 2% citric acid (p = 0.001). The concentration did not significantly (p = 0.05) affect any colour attributes of the dried apple slices treated with MOME (*M. oleifera* leaf powder methanol extract) complex. Additionally, treatment with different MOET (*M. oleifera* leaf powder ethanol extract) complex concentrations only significantly (p = 0.001) affected the lightness of the dried slices. The different concentrations of the MOAC (*M. oleifera* leaf powder acetone extract) complex significantly (p = 0.001) affected the redness and intensity of the dried apple slices. Conversely, for the MOAQ (*M. oleifera* leaf powder aqueous extract) complex, the concentration significantly (p = 0.003) affected all the colour attributes of the dried apple slices (p = 0.05).

Table 4.4 The chroma (purity) of the dried apple slices treated with different *Moringa oleifera* leaf extract complexes based on extract.

Concentration	Extract			
	MOET	MOAC	MOAQ	MOME
No Treatment	25.10 ± 0.60[a]	25.10 ± 0.60[a]	25.10 ± 0.60[a]	25.10 ± 0.60[a]
2%Citric	22.68 ± 1.17[b]	22.23 ± 1.53[b]	23.41 ± 0.87[b]	22.18 ± 1.57[b]
0.003% + 2% CA	22.43 ± 1.65[c]	20.70 ± 3.17[b]	21.93 ± 0.87[c]	22.57 ± 2.41[b]
0.03% + 2% CA	22.35 ± 2.73[c]	19.82 ± 1.18[c]	19.44 ± 0.87[d]	21.78 ± 1.44[b]
0.3% + 2% CA	19.36 ± 4.63[c]	23.31 ± 0.69[b]	21.02 ± 0.87[c]	24.08 ± 1.12[a]

[1]Values are mean ± standard deviation of triplicate measurements. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different. MOET = *Moringaoleifera* leaf ethanolic extract, MOAC = *Moringa oleifera* leaf acetone extract, MOAQ = *Moringa oleifera* aqueous extract, MOME= *Moringa oleifera* methanolic extract, CA = citric acid.

The findings suggest that the dried apple slices treated with the lowest concentration of 0.003% MOAQ + 2% citric were lighter, greener, and yellower. The findings agree with the strong activity of the aqueous extract against tyrosinase with significantly ($p = 0.001$) lower IC_{50} as described in chapter 3. Moreover, the ability of all the *M. oleifera* leaf powder anti-browning complexes reported in this chapter may be connected to the presence of bioactive components identified in chapter 3. The bioactive compounds such as phenolic acids and flavonoids were reported to have a strong activity against polyphenol oxidase, enzyme responsible for browning in fruits and vegetables (Moon *et al.*, 2020:6). In addition to this, the presence of 2% citric acid also influenced the lightness of the dried apple slices as previously described by Dite Hunjek *et al.* (2020:9–10). Siddiq *et al.* (2005:154–155) reported that 0.06% *Moringa oleifera* methanolic extract was enough to extend the shelf life of sunflower oil under accelerated storage. This agrees with a study by Rodríguez *et al.* (2020:6) using a low dosage of *Moringa oleifera* in edible films to preserve minimally processed papaya. Therefore, this chapter supports the application of the 0.003% MOAQ (*M. oleifera* leaf powder extract) anti-browning mixture in preservation of fruits because of its influence on the colour attributes.

4.3.2 Browning index (BI) of the dried apple slices

The browning index (BI) of the concentrations and extracts of interest are demonstrated in Table 4.5. The BI of the dried apple slices treated at 0.03% was 26.35, 38.51, and 45.21 for MOAQ, MOET and non-treated dried apple slices, respectively. In addition, the BI of MOAQ, MOET and non-treated dried apple slices at 0.3% was 29.28, 39.32, and 45.21, respectively. The browning index (BI) is an essential parameter in assessing colour during drying of apple slices where non-enzymatic and enzymatic browning may occur. BI indicates the general change in the browning of products with high sugar content, such as fruits ranging from 0 to

100 (Kahraman *et al.*,2021:3; Bal *et al.*, 2011:828). The MOAQ-treated samples had a significantly (p = 0.001) lower browning index than MOET and non-treated dried apple slices at 0.3 and 0.03%. Conversely, the non-treated apple slices had a significantly (p = 0.001) higher BI than MOET and MOAQ at 0.03 and 0.3%. A significant (p = 0.001) decrease in the BI after treatment at 0.03 and 0.3% indicates that both MOAC and MOAQ anti-browning mixtures decreased the browning of the dried apple slices. Similar observations were reported by Arendse & Jideani (2022a:7–10), where an extract of *Moringa oleifera* leaf powder and 2% citric acid reduced the discolouration of dried apple slices after drying at 70°C for 7 hours.

Table 4.5 The browning index of dried apple slices treated with different *Moring oleifera* leaf extract complexes at different concentrations.

Extract	Browning index	
	0.03%	**0.30%**
No treatment	45.21 ± 5.12[a]	45.21 ± 5.12[a]
MOAQ + 2% CA	26.35 ± 3.72[b]	29.28 ± 3.58[b]
MOET + 2% CA	38.51 ± 1.65[c]	39.32 ± 0.95[c]

Values are mean ± standard deviation of centre point triplicates measures. Means within a column followed by the same superscript are not significant (p > 0.05). MOET = *Moringa oleifera* leaf ethanolic extract, MOAQ = *Moringa oleifera* aqueous extract, CA = citric acid.

The effect of citric acid against browning can be justified by its Copper (Cu) chelating and acidifying activities (Moon *et al.*, 2020:6). According to Nairn *et al.* (2015:372–373), tyrosinase is active at alkaline conditions (optimum pH = 7) in the presence of copper and substrates. The copper chelating mechanism is related to the ability of citric acid to form complex structures with Cu in the active sites of tyrosinase. The pH of MOET, MOAC, MOME and MOME antibrowning mixtures was between 2.2 and 3.6, favouring the chelation of Cu at the active sites of the enzyme, therefore retarding browning. Similarly, Putnik *et al.* (2017:5–6) reported that citric and ascorbic acid treatments reduced the browning of fresh-cut apples during storage with ultrasound treatment in modified atmospheric packaging. They further explained the effectiveness of the treatment could also be associated with improved access to the interior cells, decreasing the monophenolase activity. These findings agree with (Tinello *et al.* (2018:2279), where an anti-browning solution containing ascorbic acid, citric acid and unripe grape juice significantly (p = 0.001) reduced the browning of golden delicious, dried apple slices.

M. oleifera leaf is a superfood and an excellent source of phenolic compounds such as flavonoids (Singh *et al.*, 2017:109). Multiple studies reported that *M. oleifera* leaf is an excellent antioxidant and can extend the shelf life of foods (Rahman *et al.*, 2020:38; Mukumbo *et al.*, 2014:323). The antioxidant activity of *M. oleifera* leaf is associated with the

abundant group of polyphenols, especially flavonoids. According to the literature, these flavonoids are categorised into groups depending on their structural arrangements (Brodowska, 2017:108). Polyphenol oxidase accepts flavonoids with an alpha and keto group as substrates, inhibiting the enzyme from functioning (Huang *et al.*, 2021:646). Therefore, the significant (p = 0.001) decrease in browning of apple slices treated with *Moringa oleifera* leaf complexes of the *Moringa oleifera* dipping solutions can be explained by various polyphenols present in the extracts, including flavonoids.

4.3.3 The proximate composition of *M. oleifera* extract-treated dried apple slices

The proximate composition of the dried apple slices was evaluated after treatment with the MOAQ with 2% citric acid mixture and minimally processing. The dried apple slices' protein, fat, moisture, ash, fibre, carbohydrates, and micronutrients such as vitamins B1, B2 and C were evaluated. The macro and micronutrients of the dried apple slices are demonstrated in Table 4.6. The carbohydrates comprise most of the macronutrients determined in the dried apple slices for treated and non-treated samples at 50.30 and 54.71 %, respectively. However, the carbohydrates in the treated apple slices were significantly (p = 0.01) lower than in the non-treated samples. These findings are like the carbohydrate content reported by Akubot *et al.* (2013:64) in dried kernels of African star apple flour. Moreover, the overall carbohydrates and other nutritional components are influenced by storage, type, and variety.

The moisture observed for treated apple slices was 7.37 and 6.16 % for non-treated samples. The drying reduced the moisture content directly related to the water activity of the apple slices. This is favourable for the stability of the apple slices to increase the shelf life by preventing enzymatic and microbial storage at below 10% moisture content or 0.6 water activity (Tinello *et al.*, 2018:2281).

The protein of the non-treated samples was significantly (p = 0.01) higher than that of treated apple slices. Furthermore, the fat content of both treated and non-treated samples was very low, 0.26 and 0.16 %, respectively. The ash content is an essential component of the proximate composition. It refers to the remaining inorganic minerals that remain after the combustion of organic matter in food products (Afify *et al.*, 2017:56). In this context, the ash content in the treated apple slices was higher than in the non-treated samples (p = 0.001). The range of the micronutrients agrees with these findings, where Vitamin C of the treated samples is significantly (p = 0.01) higher than the non-treated apple slices. Vitamin C was the highest macronutrient in the dried apple slices. Authors have reported on the effect of pre-treatments and drying on the proximate composition and quality of dried apples (Olalusi & Erinle, 2019:99; Ropelewska *et al.*, 2023:6–8). This can be explained by findings reported by Makule *et al.* (2020:425), where ascorbic acid was determined as a principal nutrient in cashew apples. In contrast, no significant (p = 0.05) difference existed between the treated and non-treated samples' in Vitamin B1, B2 and nitrogen content. *M. oleifera* leaf has been used in food fortification in many food products and is famous for its medicinal and nutritional

qualities (Ma *et al.*, 2020:429; Castillo-López *et al.*, 2017:164). Therefore, the significantly (p = 0.01) high proximate properties observed in the Vitamin C and fat may be associated with addition of the aqueous *M. oleifera* leaf extract during the pre-treatment stages.

Table 4.6 The proximate macro, and micronutrients composition of the dried apple slices before and after treatment.

Nutritional Component	Untreated	MOAQ-treated
Proximate (%)		
Protein	1.48 ± 0.01[a]	1.32 ± 0.01[b]
Total fat	0.16 ± 0.10[a]	0.26 ± 0.01[b]
Moisture	6.16 ± 0.28[a]	7.37 ± 0.64[b]
Ash	1.54 ± 0.07[a]	1.73 ± 0.02[b]
Fibre	3.28 ± 0.96[a]	3.12 ± 0.06[a]
Carbohydrates	54.71 ± 3.64[a]	50.30 ± 2.33[b]
Micronutrients (mg/100g)		
Vitamin B1	0.16 ± 0.01[a]	0.15 ± 0.01[b]
Vitamin B2	<0.003[a]	<0.003[a]
Vitamin C	15.54 ± 0.93[a]	18.78 ± 0.77[b]
Nitrogen	0.22 ± 0.01[a]	0.22 ± 0.01[a]

Values are mean ± standard deviation of centre point triplicates. Means within a row followed by the same superscript are not significant (p > 0.05).

4.3.4 Accelerated shelf-life of the MOAQ-treated dried apple slices

a) Effect of storage duration on the water activity and moisture

The water activity demonstrates the energy state and the amount of free water in a sample (Van Der Hoeven-Hangoor *et al.*, 2014:1783). Most enzymatic reactions are slowed down when the water activity is less than 0.8 (low a_w). The general target value of died food products is a_w = 0.6, which is against the optimum aw for the growth of pathogenic bacteria, yeasts, and moulds (Xie *et al.*, 2021:2). Table 4.7 illustrates the a_w values and moisture content for the treated and untreated dried apple slices. The water activity of the dried apple slices was analysed over time during storage at 35˚C. The a_w of both the treated samples and the control was between 0.47 to 0.57. The a_w of the treated samples in day 0, 14 and 21 was significantly (p = 0.001) higher than the control. The mean values of the water activity for the treated samples were slightly lower than the control. Conversely, there was no significant (p = 0.001) difference between a_w of the control and treated samples on day 7. The a_w was higher than 0.6 (targeted value for dried food products). It was also observed that the a_w of the dried apple slices increases by an average of 10% during storage for 21 days. These

findings are like the study of Ghinea *et al.* (2022:5–7) where the a_w of dehydrator/oven dried apple chips was between 0.374 and 0.650 with a slight increase during storage.

Table 4.7 Effect of storage duration on the physical characteristics of the apple slices during storage at 35°C

Quality parameter		Storage duration (days)			
		0	7	14	21
Water activity	Control	0.47 ± 0.02^{a}	0.5 3 ± 0.03^{a}	0.56 ± 0.01^{a}	0.56 ± 0.00^{a}
	Treated	0.51 ± 0.01^{b}	0.51 ± 0.01^{a}	0.57 ± 0.00^{b}	0.57 ± 0.01^{b}
Moisture (%)	Control	5.40 ± 0.35^{a}	7.76 ± 0.24^{a}	10.24 ± 0.30^{a}	11.91 ± 0.31^{a}
	Treated	6.34 ± 0.29^{b}	5.75 ± 1.11^{b}	10.38 ± 0.23^{b}	16.46 ± 0.60^{b}
Texture (%)	Control	26.53 ± 3.59^{a}	28.10 ± 2.09^{a}	31.50 ± 2.51^{a}	36.40 ± 1.76^{a}
	Treated	27.03 ± 3.59^{b}	47.44 ± 5.18^{b}	44.37 ± 4.03^{b}	52.91 ± 5.99^{b}
L*	Control	69.98 ± 0.02^{a}	45.22 ± 0.03^{a}	43.95 ± 0.06^{a}	37.26 ± 0.01^{a}
	Treated	76.29 ± 1.88^{b}	55.03 ± 0.02^{b}	49.08 ± 0.20^{b}	44.33 ± 1.81^{b}
a*	Control	4.45 ± 0.10^{a}	10.02 ± 0.05^{a}	10.80 ± 0.01^{a}	37.26 ± 0.01^{a}
	Treated	2.77 ± 1.00^{b}	9.67 ± 0.05^{b}	10.79 ± 0.06^{b}	8.25 ± 0.02^{b}
b*	Control	27.54 ± 0.22^{a}	19.89 ± 0.04^{a}	21.61 ±0.05^{a}	11.24 ± 0.03^{a}
	Treated	18.97 ± 0.36^{b}	25.41 ± 0.03^{b}	25.63 ± 0.13^{b}	15.58 ± 0.08^{b}
BI	Control	9.39 ± 0.13^{a}	19.67 ± 0.08^{a}	21.82 ± 0.01^{a}	15.88 ± 0.02^{a}
	Treated	5.03 ± 1.01^{b}	16.82 ± 0.07^{b}	20.42 ± 0.04^{b}	16.40 ± 0.59^{b}

[1]Values are mean ± standard deviation of triplicate measurements. Means within a column for each dependent variable for each storage time followed by the same superscript are not significant ($p > 0.05$).

The moisture content of the treated samples was in the range of 6.34–16.46%. In the case of the untreated samples, the moisture content was between 5.40–11.91%. These findings are like findings reported by Ghinea *et al.* (2022:6) who reported a moisture content of 5.55% for dried Starkrimson apple cultivator chips after dehydration. There was a significant (p = 0.001) difference on the moisture content of the treated and untreated samples during storage at accelerated conditions. The moisture content of the treated samples was significantly (p = 0.001) higher than the untreated samples during storage at accelerated conditions. The moisture content of the control (5.40%) was initially (day 0) significantly (p = 0.01) lower than the treated sample (6.34%) (p = 0.001). The moisture content of the treated, dried apple slices increased by 61.48% while the moisture content of the control significantly (p = 0.001) increased by 54.65%. These results are like findings reported by Hossain *et al.* (2023:359) where the moisture content of dried jackfruit bulb slices treated with 1% citric acid initially showed a higher moisture content during storage when compared to the control and samples treated with 0.5% citric acid. According to Doymaz (2020:4–5), citric acid increases the permeability of the fruit cell walls, therefore increasing the diffusivity of moisture. Similarly, Arendse & Jideani (2022:10–11) reported that dried apple slices treated with citric acid had a high moisture diffusivity than the control. Therefore, the significantly (p = 0.002) higher increase in moisture of the treated samples may be connected to the treatment of samples with 2% citric acid and how it affects the permeability of the dried apple slices. Moreover, the increase in the moisture content during storage is connected to the environmental changes which results in modification of the relative humidity in the packaging system (Hussain *et al.*, 2021:4).

b) Effect of treatment and storage duration on the extensibility of the dried apple slices

The extensibility of the pre-treated, dried apple slices during storage at accelerated conditions is demonstrated in Table 4.7. At day 0, the extensibility of the treated apple slices (6.34%) was significantly (p = 0.001) than the control (26.53%). It was further noted that the extensibility of the treated samples was significantly (p = 0.001) higher than the control for day 7, 14 and 21. Thus, this means that the overall extensibility of the dried apple slices over time was significant (p = 0.001). The extensibility of the control increased by 27.11% during storage while the treated samples showed an increase in extensibility by 48.91%. Thus, the samples treated with the *Moringa oleifera* leaf aqueous extract and 2% citric acid became soft and soggy during storage whereas the control was less chewy. Therefore, the increase in extensibility of the dried apple slices may be due to the increase in moisture of the dried apple slices over time. Similarly, Arendse & Jideani (2022:10–11) reported that an increase in moisture content resulting in a less crispy and chewy texture of pre-treated dried apple slices.

Research by Miranda *et al.* (2014:569–570) investigated the relationship between the moisture content and texture of dried apricot. They reported that samples stored at 35°C in glass containers showed an increase in moisture content and decrease in the breaking force during storage. The significant (p = 0.002) increase in extensibility in the pre-treated dried apple slices could be possibly due to the modifications in the cell structures during osmotic dehydration thus resulting in softer pre-treated tissues than the control (Sette *et al.*, 2016:161). Thus, the increase in extensibility of the dried apple slices can be linked to the presence of citric acid and water in the treatment solution used for treatment thus affecting the permeability of the samples to moisture.

c) Effect of storage duration on the colour attributes and browning index

The effect of the pre-treatment on the colour attributes of the dried apple slices during storage are presented in Table 4.7. At day 0, the lightness of the pre-treated samples (L^* = 76.29) was significantly (p = 0.001) higher than the control (L^* = 69.98). Likewise, the pre-treated dried apple slices were significantly (p = 0.001) lighter than the control at day 7, 14 and 21 (p = 0.001). However, there was a significant (p = 0.001) decrease in the lightness of both the control and pre-treated samples over time during storage at 35°C. The treated samples showed a significant (p = 0.001) decrease in lightness by 72%. Similarly, the lightness of the control rapidly decreased by 73.81%. In contrast, the redness of the control (a^* = 4.45) was significantly (p = 0.001) higher than the pre-treated samples. Likewise, the control was significantly (p = 0.002) redder than the pre-treated samples at day 7, 14 and 21. The redness of both the control and pre-treated samples increased during storage at accelerated conditions. Even though the redness of both samples increased over time, the control was significantly (p = 0.01) redder than the pre-treated apple slices over time.

It was further observed that the control was significantly (p = 0.001) yellower than the pre-treated samples at day 0. In contrast, the yellowness of the pre-treated (b^* = 25.41) dried apple slices were significantly (p = 0.001) higher than the control (b^* = 19.89). Thus, this means there was an increase in the yellowness of the pre-treated samples after storage for 7 days. The yellowness of the pre-treated samples remained significantly (p = 0.001) higher than the control at day 14 and 21. After storage for 7 days, there was a significant (p = 0.001) decrease in the yellowness of the pre-treated samples and the control. Thus, it can be concluded that the pre-treated dried apple slices were significantly (p = 0.004) lighter, less red, and yellower than the control.

Table 4.7 also demonstrates the browning index (BI) of the dried apple slices. There was a significant (p = 0.008) difference on the BI of the pre-treated samples and the control during the storage time (p = 0.001). The browning index of the control was significantly (p = 0.001) higher than the pre-treated samples at day 0 (p = 0.001). Similarly, a significantly (p = 0.001)

higher BI at day 7, 14 and 21 for the control was observed. Thus, the pre-treated dried apple slices were less brown than the control over storage at 35°C.

The findings of this study are like those observed by Arendse & Jideani (2022:5–7) on the colour of dried apple slices treated with potassium sorbate, citric acid and *Moringa oleifera* leaf powder extract. They also reported that dried apple slices treated with *Moringa oleifera* leaf extract and some weak acids was less brown than the control. Similarly, Gupta *et al.* (2015:50) investigated the effect of anti-browning solutions containing ascorbic and citric acid on the browning of Red Chief apples. They reported that minimal colour changes, and less browning were observed in the dried apple slices that were pre-treated with 1% citric acid and ascorbic acid. Shrestha *et al.* (2020:12–15) also reported that a combination of 1% citric acid and ascorbic acid was the best inhibitor of polyphenol oxidase activity in fresh cut Golden Delicious apples therefore controlling discolouration. Citric acid is an acidic, copper chelating agent, which means it possesses the ability to bind to the metal cofactors of Polyphenol oxidase with the aim of supressing its activity (Moon *et al.*, 2020:5–6). Therefore, the effectiveness of the *Moringa oleifera* leaf powder extract and 2% citric acid against browning may be connected to the presence of citric acid.

Additionally, Wessels *et al.* (2014:22) investigated the anti-browning effect of 36 plant extracts on minimally processed apple slices. They concluded that the bioactive compounds in the plant extracts influenced the activity of Polyphenol oxidase, responsible for browning by reacting as competitive inhibitors. It was further reported that the inhibitory activity of the plant extracts against browning was linked to the presence of secondary metabolites such as phenolic compounds. Therefore, the significant difference in browning and colour attributes of the pre-treated samples may be connected to the presence of phenolic acids in *the Moringa oleifera* leaf extract. This agrees with Karim *et al.* (2018:155) who reported on the antioxidant activity of *Moringa oleifera* leaf extracts due to high contents of flavonoids and phenolic acids.

4.3.5 The shelf-life of the dried apple slices based on the lightness

The parameters of the first order reaction kinetics were used to model the accelerated shelf-life. The results of the dried apple slices pre-treated with the 0.003% MOAQ (*M. oleifera* leaf powder aqueous extract) anti-browning mixture and control are summarised in Table 4.8. The first order reaction kinetics is a chemical reaction that is dependent on the concentration of a single reactant. The degradation rate constant of the untreated apple slices ($k = 4.2683$ day^{-1}) was higher than the pre-treated dried apple slices ($k = 0.0249$ day^{-1}). Similarly, the initial lightness (L*) of the control was higher than the pre-treated dried apple slices. The differences in the degradation rate constant (k) and initial lightness (L*) values are connected to the significant ($p < 0.05$) differences in the lightness of the control and dried apple slices reported earlier in section 4.3.1. In contrast, the lightness at time t for both the control and

pre-treated apple slices are similar because the acceptable lightness used to indicate shelf-life at end of life is 2.996. The lightness of the dried apple slices has an impact on the consumer acceptability; hence lightness was used to determine the accelerated shelf-life. Moreover, the lightness was used to determine the shelf-life of the dried apple slices because of its high correlation co-efficient (r^2). Ghinea *et al.* (2022:9) also reported that one of the major elements that influence how consumers accept the quality of dried apple slices is colour. The shelf life of the apples treated with the MOAQ anti-browning agent at accelerated temperature (35°C) was 51 days and 42 days for the control. A kinetic value of Q_{10} = 3 as reported by Grizotto *et al.* (2006:709) for dried fruits from papaya pulp permitted an estimation of shelf-life of 153 days for pre-treated and 126 days for untreated apple slices when stored at room temperature (25°C). Arendse & Jideani (2022:5–7) also reported thar dried apple slices treated with 0.1% *M. oleifera leaf* extract had an acceptable texture, taste, and colour for 120 days. Similarly, Richter Reis *et al.* (2017:122) reported on the accelerated shelf-life of dried litchi where he assumed the common Q_{10} for dried fruits as 3. The Q_{10} refers to the quotient separating the rates of reaction at given temperature (Germer *et al.*, 2014:10). The major cause of expiry was caused by browning of the product which may be due to enzymatic browning since there was an increase in the water activity and moisture content during storage. The major reason for expiry may also be related to related to non-enzymatic browning since the dried samples became brown and polyphenol oxidase activity was controlled by low water activity (Moon et al., 2020:2-3).

Table 4.8 Shelf-life of treated & untreated samples bases on the lightness of the dried apple slices

	0.003% MOAQ & 2% CA	**Control**
k (day^{-1})	0.0249	4.2683
Lightness at time t	2.996	2.996
Initial Lightness	0.0247	4.1531
Shelf-life at 35 °C (days)	51	42
Shelf-life at 25°C (days)	153	126

[1]MOAQ (*M. oleifera* leaf powder aqueous extract), CA = citric acid.

4.4 Conclusion

The aim of this chapter was to establish the effectiveness of the *M. oleifera* leaf powder extracts when combined with 2% citric acid as anti-browning agents in dried apple slices. This chapter also aimed at establishing the shelf-life stability of the dried apple slices treated with the *M. oleifera* leaf powder extracts. The colour attributes were influenced by lower concentrations of some anti-browning mixtures. However, the 0.003% MOAQ (*M. oleifera* leaf powder aqueous extract) anti-browning complex had an outstanding effect on the colour

of the dried apple slices. The dried apple slices were lighter, yellower, and less green which aligns with the low 50% inhibitory concentration (IC_{50}) and strong anti-tyrosinase activity of the MOAQ extract. Moreover, the browning index indicated that there was less browning activity in the apple slices treated with the 0003% MOAQ anti-browning mixture. The 0.003% MOAQ anti-browning mixture further improved the nutritional content of the dried apple slices by improving the protein, fat and vitamin C. These findings agree with the hypothesis that the physicochemical properties and nutritional content of the dried apple slices will be retained after treatment with the *M. oleifera* leaf powder extract based anti-browning agents. The ability of citric acid (added in the anti-browning mixture) to chelate copper and reducing the pH also contributed to the effect of the anti-browning mixtures on the physicochemical properties of the dried apple slices. The dried apple slices treated with the 0.003% MOAQ anti-browning agent had an estimated shelf-life of 153 days at 25°C (room temperature) whereas the control had an acceptable lightness for 126 days. These findings suggest that the *M. oleifera* leaf powder extract (MOLP) anti-browning mixture can improve the shelf-life and storage quality of the dried apple slices. Therefore, this section identified the MOAQ anti-browning mixture as a potent preservative for fruits by highlighting its capability for sustaining acceptable colour properties and extending the shelf-life of dried apple slices.

4.5 References

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CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

The anti-browning effect of *Moringa oleifera* leaf extract on the nutritional, physicochemical and storage quality of dried apple (*Malus domestica*) slices was investigated in this study. The following objectives were identified in this study:

1. Characterize and determine the phenolic composition of the acetone, aqueous, ethanol and methanol *Moringa oleifera* leaf extracts based on the structure and functionality.
2. Determine the antioxidant activity and establish the 50% inhibitory concentration (IC_{50}) of the *Moringa oleifera* leaf powder extracts (acetone, aqueous, ethanol and methanol).
3. Establish the effectiveness of the most effective *Moringa oleifera* extract and citric acid as an anti-browning agent in dried apple slices.
4. Establish dried apples treated with the anti-browning mixture's physicochemical, nutritional, and shelf-life stability.

The first objective was obtained by dissolving the *M. oleifera* leaf powder in different solvents namely MOME (*M. oleifera* leaf powder methanol extract), MOET (*M. oleifera* leaf powder ethanol extract), MOAQ *(M. oleifera* leaf powder aqueous extract), MACE (*M. oleifera* leaf powder acetone extract) and determining the bioactive components using a Gas chromatograph – Mass Spectrometer (GC-MS) based on the structure and functionality. Bioactive compounds such as flavonoids, phenolic acids, fatty acids, amino acids and quinones were identified in the all the *M. oleifera* leaf powder extracts. The hypothesis that the *M. oleifera* leaf powder extracts contained bioactive compounds such as flavonoids and phenolic compounds was accepted. The second objective was achieved by determining the anti-tyrosinase activity of the *M. oleifera* leaf powder extracts in terms of IC_{50} (50% inhibitory concentration). It was also achieved by determining the antioxidant activity in terms of DPPH (,1-Diphenyl-2-picryl-hydrazyl), FRAP (ferric-reducing antioxidant power), ABTS (2,2'-azino-di-3-ethylbenzthialozine sulphonate), and ORAC (oxygen radical absorbance capacity) essays. The MOAQ extract had the strongest activity against tyrosinase because of its low IC_{50} value. The *M. oleifera* leaf powder extracts all showed a dose-dependent antioxidant activity. The MOME (*M. oleifera* leaf powder methanol extract) had the highest antioxidant activity against the FRAP, ABTS, ORAC and DPPH antioxidant essays. The third objective was achieved by establishing the most effective *M. oleifera* leaf powder extract with citric acid as an anti-browning agent in dried apple slices. To make anti-browning agents, The *M. oleifera* leaf powder extracts were individually combined with 2% citric acid with each extract added at upper (0.3%), mid (0.03%) and lower (0.003%) based on the strong Inhibitory concentration against tyrosinase. The dried apple slices were pre-treated, dried and colour attributes were measured for CIELAB and LCh colour scales. The various anti-browning

treatments affected the colour attributes with 0.003% MOAQ-treated samples revealing a lighter colour, L*. The other colour attributes, a* (redness), and b* (yellowness) varied among the different treatment complexes. Moreover, the dried apple slices treated with the 0.003% MOAQ anti-browning mixture had a lower browning index than the untreated ones. Moreover, the dried apple slices treated with the 0.003% MOAQ anti-browning mixture had a higher ash, vitamin C and total fat content than the untreated apple slices. The hypothesis that the dried apple slices treated with the *M. oleifera* leaf powder anti-browning mixtures will retain their physicochemical and nutritional properties was accepted. The fourth objective was achieved by using the most effective anti-browning mixture, 0.003% *M. oleifera* leaf powder aqueous anti-browning agent to determine the shelf-life of the dried apple slices under accelerated storage conditions (35°C) for 4 weeks. The storage quality of the dried apple slices was analysed by determining the change in moisture, water activity, extensibility, and colour on day 0, 7, 14 and 21. The water activity, moisture, and extensibility of the dried apple slices increased with an increase in storage time. The accelerated shelf-life of the dried apple slices treated with the 0.003% MOAQ anti-browning mixture was modelled in terms of acceptable lightness (L*) and moisture using the first order reaction kinetics. The lightness (L*) was used to determine the shelf-life because of its strong correlation co-efficient. The shelf life of the dried apple slices treated with the 0.003% MOAQ anti-browning agent at 25°C was longer (153 days) than the control (126 days) when a $Q_{10} = 3$ was estimated for dried fruits. The accelerated shelf-life test could be improved by incubating the dried apple slices at different temperatures to determine the Q_{10} of the experiments. The hypothesis that the storage quality and shelf-life of the dried apple slices treated with the *M. oleifera* leaf powder anti-browning solution will be improved was accepted.

Conclusions that can be obtained from this study:

1. The *Moringa oleifera* leaf powder (MOLP) extracts contain bioactive compounds such as flavonoids, phenolic acids, amino acids, fatty acids, amino acids and quinones.
2. The MOLP extracts have the potential to reduce tyrosinase and antioxidant activity.
3. The MOAQ (*M. oleifera* leaf powder extract) is a strong potent tyrosinase inhibitor. This study provided an explanation as to why the MOAQ extract was used to treat the apple was used to treat the dried apple slices at low (0.003%), mid (0.03%) and upper (0.3%) concentrations.
4. The 0.003% MOAQ anti-browning mixture was effective in improving the colour attributes and reducing browning of the dried apple slices. The presence of bioactive compounds could be responsible for how the aqueous extract (MOAQ) reduced browning.
5. The MOAQ anti-browning mixture maintained the colour and effectively reduced browning of the dried apple slices for 153 days.

Future research can be directed towards extracting the bioactive compounds suspected to have influential activity on the browning. The following could be of interest in the future:

1. Future studies can expand on the changes in colour attributes and anti-browning activity by determining the mechanism of inhibition demonstrated by the bioactive compounds in enzymatic browning reactions.
2. The impact of a different storage conditions such as higher temperatures, relative humidity on the moisture, water activity, and extensibility could be investigated to determine optimal storage conditions that can maintain the shelf-life and quality of the product.
3. Consumer acceptability tests would provide insight into the desirability and quality of the dried apple slices.